and an increased sensitivity to EAE (Watanabe et al, Nat. Immunol, 4:670-679, 2003). If observed until old age BTLA knock-out mice spontaneously develop autoantibodies, an auto-immune hepatitis like disease and inflammatory cell infiltrates in multiple organs (Oya et al., Arthritis Rheum 58: 2498-2510, 2008). This evidence indicates that the BTLA inhibitory receptor plays a crucial role in maintaining immune homeostasis and inhibiting autoimmunity. Furthermore, HVEM-BTLA signaling is involved in the regulation of mucosal inflammation and infection immunity (Shui et al., J Leukoc Biol. 89:517-523, 2011).

Therapeutic agents that are capable of modulating BTLA function to inhibit autoreactive lymphocytes in the context of autoimmune disorders would be highly desirable.

It has previously been shown that monoclonal antibodies binding to mouse BTLA can act as agonists, inducing signaling through the receptor to inhibit immune cell responses. In the presence of agonist anti-BTLA antibody (mAb), anti-CD3 and anti-CD28 activated T-cells show reduced IL-2 production and proliferation (Kreig et al., J. Immunol., 175, 6420-6472, 2005).

Furthermore, anti-mouse-BTLA agonist antibodies have been shown to ameliorate disease in murine models of graft-versus-host disease (Sakoda et al., Blood. 117:2506-2514; Albring et al., J Exp Med. 207:2551-9, 2010). Agonist antibodies targeting the human BTLA receptor have been shown to inhibit T cell responses *ex-vivo* (see Otsuki et al., Biochem Biophys Res Commun 344:1121-7, 2006; and WO2011/014438), but have not yet been translated to the clinic.

There is a need in the art to discover new and useful agents, such as antibodies or antigen-binding antibody fragments, capable of modulating BTLA.

SUMMARY

The present disclosure relates to anti-human BTLA agonist antibodies or antibody fragments thereof having one or more desirable properties, including high binding affinities, high agonist potency, high agonist efficacy, good pharmacokinetics and low antigenicity in human subjects. The disclosure also relates to use of the antibodies or antibody fragments of the disclosure in the treatment of disease.

In one aspect, the present disclosure refers to an isolated human antibody or an antigen-binding fragment thereof that specifically binds B and T lymphocyte attenuator (BTLA), comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising three CDRs: CDRH1, CDRH2 and CDRH3, wherein CDRH1, CDRH2, CDRH3 have an amino acid sequence as set forth in SEQ ID NO: 205, 387, or 207, with from 0 to 3 amino acid modifications.

In another aspect, the present disclosure refers to an isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising three CDRs: CDRL1, CDRL2 and CDRL3, wherein CDRL1, CDRL2, and CDRL3 have an amino acid sequence as set forth in SEQ ID NO: 142, 143, or 210, respectively, with from 0 to 3 amino acid modifications.

In another aspect, the present disclosure refers to an isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising three CDRs: CDRH1, CDRH2 and CDRH3, wherein CDRH1, CDRH2, CDRH3 have an amino acid sequence as set forth in SEQ ID NO: 205, SEQ ID NO: 387, and SEQ ID NO: 207, respectively, with from 0 to 3 amino acid modification, and the light chain comprises a light chain variable region comprising three CDRs CDRL1, CDRL2, and CDRL3 have an amino acid sequence as set forth in SEQ ID NO: 142, SEQ ID NO: 143, and SEQ ID NO: 210, respectively, with from 0 to 3 amino acid modification.

In another aspect, the present disclosure refers to an isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 390 or a sequence with at least 90% identity thereto.

In yet another aspect, the present disclosure refers to an isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 378or a sequence with at least 90% identity thereto.

In yet another aspect, the present disclosure refers to an isolated nucleic acid which comprises one or more nucleotide sequences encoding polypeptides capable of forming an antibody or antigen-binding fragment as disclosed herein.

In yet another aspect, the present disclosure refers to a host cell comprising the nucleic acid sequence as disclosed herein.

In yet another aspect, the present disclosure refers to a method of producing an antibody or an antigen-binding fragment thereof that binds to BTLA, comprising the step of culturing the host cell as disclosed herein, under conditions for production of said antibody or an antigen-binding fragment thereof, optionally further comprising isolating and/or purifying said antibody or an antigen-binding fragment thereof.

In yet another aspect, the present disclosure refers to a method for preparing a human antibody or antigen-binding fragment thereof that specifically binds BTLA, the method comprising the steps of: 1) providing a host cell comprising one or more nucleic acid molecules encoding the amino acid sequence of a heavy chain and a light chain which when expressed are capable of combining to create an antibody or antigen-binding molecule; 2) culturing the host cell expressing the encoded amino acid sequence; and 3) isolating the antibody or antigen-binding molecule as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a therapeutically effective amount of the antibody or antigen-binding fragment thereof as disclosed herein and at least one pharmaceutically acceptable excipient.

In yet another aspect, the present disclosure refers to an antibody or antigen-binding fragment thereof as disclosed herein, or the pharmaceutical composition as disclosed herein, for use in therapy.

In yet another aspect, the present disclosure refers to the use of an antibody or antigen-binding fragment thereof as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment or prevention of inflammatory or autoimmune diseases, and disorders of excessive immune cell proliferation.

According to a first example of the disclosure there is provided an isolated antibody or an antigen-binding fragment thereof that specifically binds BTLA, wherein the antibody has a heavy chain and/or light chain with at least one complementarity-determining region (CDR) as present in an antibody selected from the group consisting of 11.5.1, 2.8.6, 12F11, 14D4, 15B6, 15C6, 16E1, 16F10, 16H2, 1H6, 21C7, 24H7, 26B1, 26F3, 27G9, 3A9, 3E8, 4B1, 4D3, 4D5, 4E8, 4H4, 6G8, 7A1, 8B4, 8C4, 6.2 and 831, as identified in Table 1 and described herein.

According to a second example of the disclosure there is provided an isolated nucleic acid comprising a nucleotide sequence that encodes a heavy chain polypeptide or a light chain polypeptide of the isolated antibody or an antigen-binding fragment thereof of the first example of the disclosure.

According to a third example of the disclosure there is provided a vector comprising the nucleic acid of the second example of the disclosure.

According to a fourth example of the disclosure there is provided a host cell comprising the nucleic acid sequence according to the second example of the disclosure or the vector according to the third example of the disclosure.

According to a fifth example of the disclosure there is provided a method of producing an antibody or an antigen-binding fragment thereof according to the first example of the disclosure, comprising the step of culturing the host cell of the fourth example of the disclosure under conditions for production of said antibody or an antigen-binding fragment thereof, and optionally isolating and/or purifying said antibody or an antigen-binding fragment thereof.

According to a sixth example of the disclosure there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the antibody or antigen-binding fragment thereof of the first example of the disclosure, or that produced by the fifth example of the disclosure.

According to a seventh example of the disclosure there is provided a method of preparing a pharmaceutical composition, the method comprising formulating antibody or an antigen- binding fragment thereof in accordance with the first example of the disclosure, or one produced in accordance with the fifth example of the disclosure into a composition including at least one

additional component. In a particular example, the at least one additional component is a pharmaceutically acceptable excipient.

According to an eighth example of the disclosure there is provided a method of treating a BTLA-related disease in a patient, comprising administering to the patient a therapeutically effective amount of the antibody or antigen-binding fragment thereof of the first example of the disclosure or the pharmaceutical composition of the sixth example of the disclosure.

DETAILED DESCRIPTION

The inventors have identified particularly strong agonist antibodies to BTLA which are predicted to be more efficacious than current antibodies at suppressing T cell responses and thus be particularly useful in the treatment of immune mediated disorders.

As used in this specification and the appended claims, the singular forms "a", “an” and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

It is to be understood that one, some, or all of the properties of the various embodiments described herein may be applied to any aspect unless the content clearly dictates otherwise. Furthermore, that the various embodiments may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

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 is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press;

CLAIMS:

What is claimed is:

1. An isolated human antibody or an antigen-binding fragment thereof that specifically binds B and T lymphocyte attenuator (BTLA), comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising three CDRs: CDRH1, CDRH2 and CDRH3, wherein CDRH1, CDRH2, CDRH3 have an amino acid sequence as set forth in SEQ ID NO: 205, 387, or 207, with from 0 to 3 amino acid modifications.
2. An isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising three CDRs: CDRL1, CDRL2 and CDRL3, wherein CDRL1, CDRL2, and CDRL3 have an amino acid sequence as set forth in SEQ ID NO: 142, 143, or 210, respectively, with from 0 to 3 amino acid modifications.
3. An isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising three CDRs: CDRH1, CDRH2 and CDRH3, wherein CDRH1, CDRH2, CDRH3 have an amino acid sequence as set forth in SEQ ID NO: 205, SEQ ID NO: 387, and SEQ ID NO: 207, respectively, with from 0 to 3 amino acid modification, and the light chain comprises a light chain variable region comprising three CDRs CDRL1, CDRL2, and CDRL3 have an amino acid sequence as set forth in SEQ ID NO: 142, SEQ ID NO: 143, and SEQ ID NO: 210, respectively, with from 0 to 3 amino acid modification.
4. An isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 390 or a sequence with at least 90% identity thereto.
5. An isolated human antibody or an antigen-binding fragment thereof that specifically binds BTLA, comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 378or a sequence with at least 90% identity thereto.
6. The antibody antigen-binding fragment thereof of any of the preceding claims, wherein: the heavy chain comprises a heavy chain variable region comprising an amino acid

sequence as set forth in SEQ ID NO: 390, and the light chain comprises a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 378, and wherein the antibody binds to BTLA.

7. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein the heavy chain or light chain also comprise a constant region.
8. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein the heavy chain and light chain are connected by a flexible linker to form a single- chain antibody.
9. The antibody or antigen-binding fragment thereof of any of the preceding claims, which binds a residue of BTLA selected from: D52, P53, E55, E57, E83, Q86, E103, L106 and E92.
10. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein CDRL2 has no amino acid modifications as compared to SEQ ID NO: 143.
11. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein CDRH2 has an amino acid modification at position 5, position 7, or both, numbered according to SEQ ID NO: 387.
12. The antibody or antigen-binding fragment thereof of claim 11, wherein CDRH2 has an aspartic acid (D) at position 5 numbered according to SEQ ID NO: 387.
13. The antibody or antigen-binding fragment thereof of claim 11, wherein CDRH2 has Aspartic acid (D) at position 5, and Asparagine (N) at position 7, numbered according to SEQ ID NO: 387.
14. The antibody or antigen-binding fragment thereof of any of the preceding claims, which is an IgG1, IgG2 or IgG4 antibody.
15. The antibody of any of the preceding claims, which is selected from the group consisting of: a human antibody, a humanised antibody, a chimeric antibody and a multispecific antibody (such as a bispecific antibody).
16. The antigen-binding fragment according to any of claims 1 to 14, which is selected from the group consisting of: scFv, sc(Fv)2, dsFv, Fab, Fab', (Fab')2 and a diabody.
17. The antibody or antigen-binding fragment thereof of any of the preceding claims, which is monoclonal.
18. The antibody or antigen-binding fragment thereof of any preceding claim, wherein said antibody or antigen-binding fragment thereof agonizes human BTLA expressed on the surface of an immune cell, wherein said immune cell is optionally a T cell.

19. The antibody or antigen-binding fragment thereof of any preceding claim, wherein binding of said antibody or antigen-binding fragment thereof to human BTLA expressed on the surface of an immune cell decreases proliferation of said cell relative to a comparable immune cell not bound by said antibody or antigen-binding fragment thereof, and wherein said cell is optionally a T cell.
20. The antibody or antigen-binding fragment thereof of claim 19, wherein said decrease in cell proliferation is measured by an assay described in Example 8 or Example 9.
21. The antibody or antigen-binding fragment thereof of claim 19 or 20, wherein said decrease in cell proliferation is measured in vitro or in vivo.
22. The antibody or antigen-binding fragment thereof of any one of claims 19-21, wherein said decrease in cell proliferation is at least about 10%, 15%, 20%, 25%, 30%, 40%, or 50%.
23. The antibody or antigen-binding fragment thereof of any one of claims 19-21, wherein said decrease in cell proliferation is from about 10% to 50%, 10% to 40%, 10% to 30%, 10% to 20%, 10% to 15%, 20% to 50%, 20% to 40%, or 20% to 30%.
24. The antibody or antigen-binding fragment thereof of any preceding claim, wherein said antibody or antigen-binding fragment thereof comprises a domain that binds to an Fc receptor.
25. The antibody or antigen-binding fragment thereof of claim 24, wherein said Fc receptor is expressed on the surface of an immune cell.
26. The antibody or antigen-binding fragment thereof of claim 25, wherein said immune cell is an antigen presenting cell.
27. The antibody or antigen-binding fragment thereof of claim 26, wherein said antigen presenting cell is a dendritic cell, macrophage, monocyte, or neutrophil.
28. The antibody or antigen-binding fragment thereof of any one of claims 24-27, wherein said antibody or antigen-binding fragment thereof binds to human BTLA expressed on the surface of a T cell.
29. The antibody or antigen-binding fragment thereof of any one of claims 24-28, wherein said Fc receptor is FcγRIIB.
30. The antibody or antigen-binding fragment thereof of any preceding claim, wherein binding of said antibody or antigen-binding fragment thereof to human BTLA expressed on the surface of an immune cell decreases NFκB signaling of said immune cell relative

to a comparable immune cell not bound by said antibody or antigen-binding fragment thereof, and wherein said immune cell is optionally a T cell.

31. The antibody or antigen-binding fragment thereof of claim 30, wherein said decrease in NFκB signaling of said immune cell is measured by an assay described in Example 10.

32. The antibody or antigen-binding fragment thereof of claim 30 or 31, wherein said decrease in NFκB signaling of said immune cell is at least about 10%, 15%, 20%, 25%, 30%, or 40%.

33. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein said antibody or antigen-binding fragment thereof specifically binds human B and T Lymphocyte Attenuator (BTLA) with a KD of less than 10 nM, each as determined by surface plasmon resonance (SPR) at 37°C,

34. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein said antibody or antigen-binding fragment thereof binds cynomolgus BTLA with a KD of less than 20 nM, as determined by surface plasmon resonance (SPR) at 37°C;

35. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein said antibody or antigen-binding fragment thereof specifically binds human B and T Lymphocyte Attenuator (BTLA) with a KD of less than 10 nM, each as determined by surface plasmon resonance (SPR) at 37°C, and wherein said antibody binds cynomolgus BTLA with a KD of less than 20 nM, as determined by surface plasmon resonance (SPR) at 37°C; does not inhibit binding of BTLA to herpes virus entry mediator (HVEM); and inhibits proliferation of T cells in vitro, as determined by a mixed lymphocyte reaction assay.

36. The antibody or antigen-binding fragment thereof of claim 35, wherein said antibody or antigen-binding fragment thereof binds human B and T Lymphocyte Attenuator (BTLA) with an on rate of at least 5.0×10^5 (1/Ms) at 37°C.

37. The antibody or antigen-binding fragment thereof of any of the preceding claims, wherein said antibody or antigen-binding fragment thereof specifically binds human B and T Lymphocyte Attenuator (BTLA) with an on rate of at least 5.0×10^5 (1/Ms) , as determined by surface plasmon resonance (SPR) at 37°C, wherein said antibody does not inhibit binding of BTLA to herpes virus entry mediator (HVEM); and wherein said antibody inhibits proliferation of T cells in vitro, as determined by a mixed lymphocyte reaction assay.

38. The antibody or antigen-binding fragment thereof of claim 37, wherein said antibody or antigen-binding fragment thereof binds human B and T Lymphocyte Attenuator (BTLA) with a KD of less than 10 nM, as determined by surface plasmon resonance (SPR) at 37°C.

39. The antibody or antigen-binding fragment thereof of claim 37 or 38, wherein said antibody or antigen-binding fragment thereof wherein said antibody binds cynomolgus BTLA with a KD of less than 20 nM, as determined by surface plasmon resonance (SPR) at 37°C.

40. An isolated nucleic acid which comprises one or more nucleotide sequences encoding polypeptides capable of forming an antibody or antigen-binding fragment as claimed in any of claims 1 to 39.

41. A host cell comprising the nucleic acid sequence according to claim 40.

42. A method of producing an antibody or an antigen-binding fragment thereof that binds to BTLA, comprising the step of culturing the host cell of claim 41, under conditions for production of said antibody or an antigen-binding fragment thereof, optionally further comprising isolating and/or purifying said antibody or an antigen-binding fragment thereof.

43. A method for preparing a human antibody or antigen-binding fragment thereof that specifically binds BTLA, the method comprising the steps of:

1) providing a host cell comprising one or more nucleic acid molecules encoding the amino acid sequence of a heavy chain and a light chain which when expressed are capable of combining to create an antibody or antigen-binding molecule;
2) culturing the host cell expressing the encoded amino acid sequence; and
3) isolating the antibody or antigen-binding molecule of any of claims 1 to 39.

44. A pharmaceutical composition comprising a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any of claims 1 to 39 and at least one pharmaceutically acceptable excipient.

45. An antibody or antigen-binding fragment thereof in accordance with any of claims 1 to 39, or the pharmaceutical composition in accordance with claim 44, for use in therapy.

46. Use of an antibody or antigen-binding fragment thereof in accordance with any of claims 1 to 39, or the pharmaceutical composition in accordance with claim 44 in the manufacture of a medicament for the treatment or prevention of inflammatory or autoimmune diseases, and disorders of excessive immune cell proliferation.

47. The use in accordance with claim 46, wherein the disease or condition comprises Addison's disease, allergy, alopecia areata, amyotrophic lateral sclerosis, ankylosing spondylitis, anti-phospholipid syndrome, asthma, autoimmune haemolytic anaemia, autoimmune hepatitis, autoimmune pancreatitis, autoimmune polyendocrine syndrome, Behcet’s disease, bullous pemphigoid, cerebral malaria, chronic inflammatory demyelinating polyneuropathy, coeliac disease, Crohn's disease, Cushing's Syndrome, dermatomyositis, diabetes mellitus type 1, eosinophilic granulomatosis with polyangiitis, graft versus host disease, Graves' disease, Guillain-Barre syndrome, Hashimoto’s thyroiditis, Hidradenitis Suppurativa, inflammatory fibrosis, inflammatory bowel disease, juvenile arthritis, Kawasaki disease, leukemia, lymphoma, lymphoproliferative disorders, multiple sclerosis, myasthenia gravis, myeloma, neuromyelitis optica, pemphigus, polymyositis, primary biliary cholangitis, primary sclerosing cholangitis, psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, Sjögren's syndrome, systemic lupus erythematosus, Takayasu’s arteritis, temporal arteritis, transplant rejection, transverse myelitis, ulcerative colitis, uveitis, vasculitis, vitiligo, or Vogt-Koyanagi-Harada Disease.

48. The use in accordance with claim 46, wherein the disease or condition comprises vasculitis, systemic lupus erythematosus, ulcerative colitis, inflammatory bowel disease, or graft versus host disease.