

BRIEF SUMMARY ~~OF THE INVENTION~~

**[0009]** Provided herein are BTLA-binding agents comprising immunoglobulin heavy and light chains polypeptides. Also provided herein is a method of using the BTLA-binding agents to modulate interaction between HVEM and BTLA and/or T cell response in a mammal.

**[0010]** Related compositions and methods also are provided, as will be apparent from the following detailed description.

**[0010a]** In one aspect, the present disclosure provides a BTLA-binding agent comprising: an immunoglobulin heavy chain variable region comprising:

(a) a CDRH1 comprising  $X^1SX^2MN$  (SEQ ID NO: 195), wherein  $X^1$  is N or T, and  $X^2$  is W, F, H, G, P, R, K, D, S, L, V, N, or Y

(b) a CDRH2 comprising  $RIYPX^1GX^2X^3DTNYX^4GKFK$  (SEQ ID NO: 196), wherein:  
 $X^1$  is absent or A;  
 $X^2$  is D, Y, Q, G, L, F, H, S, P, R, or T;  
 $X^3$  is G, Y, A, F, S, D, V, T, E, K, or R; and  
 $X^4$  is N, V, Q, R, A, F, Y, S, G, P, or T;

and

(c) a CDRH3 comprising  $X^1SGTFX^2X^3GNYX^4X^5YFDV$  (SEQ ID NO: 197),  
wherein:

$X^1$  is K or R;  
 $X^2$  is N or D;  
 $X^3$  is D, S, F, Y, F, V, S, G, T, R, I, L, or E;  
 $X^4$  is R or H; and  
 $X^5$  is W, R, F, L, N, Y, P, I, V, A, S, G, R, or K;

or comprising the immunoglobulin heavy chain variable region of SEQ ID NO: 144 or any one of SEQ ID NOs: 43-143 or 145-156, or at least the CDRs thereof; or an amino acid sequence with at least 90% sequence identity thereto;

and

an immunoglobulin light chain variable region comprising:

(a) a CDRL1 comprising  $RX^1SENIYX^2X^3LA$  (SEQ ID NO: 198), wherein  
 $X^1$  is A or V;

X<sup>2</sup> is S or N; and

X<sup>3</sup> is H, N, or Y;

(b) a CDRL2 comprising X<sup>1</sup>AX<sup>2</sup>NLAX<sup>3</sup> (SEQ ID NO: 199), wherein

X<sup>1</sup> is A or N;

X<sup>2</sup> is T or K; and

X<sup>3</sup> is N, L, Q, G, F, V, K, S, R, T, H, or P; and

(c) a CDRL3 comprising QX<sup>1</sup>FX<sup>2</sup>GPPLT (SEQ ID NO: 200), wherein

X<sup>1</sup> is L or H; and

X<sup>2</sup> is W, F, Y, P, N, V, K, M, L, G, or S;

or comprising the immunoglobulin light chain variable region of SEQ ID NO: 174 or any of SEQ ID NOs: 157-173 or 175-192, or at least the CDRs thereof; or an amino acid sequence with at least 90% sequence identity thereto;

or a BTLA binding agent as set forth in Table 3.

**[0010b]** In another aspect, the present disclosure provides a BTLA-binding agent comprising:

a heavy chain immunoglobulin variable region comprising:

(a) a CDRH1 comprising Asp Tyr Thr Ile His (SEQ ID NO: 27),

(b) a CDRH2 comprising Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Asp Lys Phe Lys Xaa (SEQ ID NO: 30), wherein Xaa is aspartic acid (Asp) or valine (Val);

(c) a CDRH3 comprising Arg Xaa1 Xaa2 Tyr Xaa3 Met Xaa4 Tyr (SEQ ID NO: 32),

wherein:

Xaa1 is asparagine (Asn) or serine (Ser),

Xaa2 is tyrosine (Tyr) or histidine (His),

Xaa3 is alanine (Ala) or valine (Val), and

Xaa4 is glutamic acid (Glu) or aspartic acid (Asp);

or comprising a heavy chain variable region comprising any one of SEQ ID NOs: 1-15, 207, 208, 217, or 218, or at least the CDRs thereof, or an amino acid sequence with at least 90% sequence identity thereto;

and

a light chain immunoglobulin variable region comprising:

(a) a CDRL1 comprising Lys Ala Ser Gln Asn Val Phe Thr Asn Val Ala (SEQ ID NO: 36);

(b) a CDRL2 comprising Ser Ala Ser Tyr Arg Xaa Ser (SEQ ID NO: 39), wherein Xaa is tyrosine (Tyr) or serine (Ser); and

(c) a CDRL3 comprising Gln Gln Tyr Xaa1 Xaa2 Tyr Pro Tyr Thr (SEQ ID NO: 41), wherein:

Xaa1 is serine (Ser) or asparagine (Asn), and

Xaa2 is threonine (Thr) or serine (Ser);

or comprising a light chain variable region comprising any one of SEQ ID NOs: 16-25, 209, 210, 219, or 220, or at least the CDRs thereof, or an amino acid sequence with at least 90% sequence identity thereto;

or

a BTLA binding agent as set forth in Table 2.

**[0010c]** In another aspect, the present disclosure provides a nucleic acid sequence encoding the immunoglobulin heavy chain and/or light chain polypeptide of the BTLA-binding agent as described herein, optionally in a vector. In another aspect, the present disclosure provides a cell comprising the nucleic acid as described herein. In another aspect, the present disclosure provides a composition comprising (a) the BTLA-binding agent as described herein or nucleic acid encoding same and (b) a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a method of preparing a BTLA-binding agent as described herein, the method comprising expressing a nucleotide sequence encoding the immunoglobulin heavy chain polypeptide and a nucleic acid encoding the immunoglobulin light chain polypeptide in a cell.

**[0010d]** In another aspect, the present disclosure provides a method of detecting soluble BTLA in blood, plasma, serum, or tissue comprising contacting a blood, plasma, serum, or tissue sample with an antibody as described herein, optionally wherein the soluble BTLA in the blood, plasma, serum, or tissue sample is bound to an antibody as described herein, or wherein the blood, plasma, serum, or tissue sample is contacted with an antibody as described herein and an antibody as described herein.

[0010e] In another aspect, the present disclosure provides a method of detecting, measuring, or monitoring the pharmacological activity of a BTLA binding agent in a subject, or selecting a subject for treatment with a BTLA binding agent, the method comprising detecting soluble BTLA (sBTLA) in a sample of blood, plasma, serum, or tissue from a subject to whom a BTLA binding agent has been administered.

[0010f] In yet another aspect, the present disclosure provides a composition comprising a BTLA binding agent as described herein, a BTLA binding agent as described herein, or both, for use in a method as described herein. In another aspect, the present disclosure provides a use of a BTLA binding agent as described herein in the manufacture of a medicament for treating a disorder that is responsive to BTLA modulation in a mammal. In another aspect, the present disclosure provides a BTLA binding agent as described herein for use in therapy.

#### BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0011] Figure 1 depicts the results of surface plasmon resonance binding kinetics of the 6G3 antibody to human BTLA and cynomolgus monkey BTLA extracellular domain.

[0012] Figure 2 is a graph depicting the results of Kinetic Exclusion Assay binding kinetics of the 6G3 antibody to human BTLA and cynomolgus monkey BTLA extracellular domain.

[0013] Figure 3 is a graph depicting the results of binding of the 6G3 antibody to 293c18 cells stably transfected with human BTLA and cynomolgus monkey BTLA.

[0014] Figure 4 is a graph depicting the results of binding of the 6G3 antibody to normal donor human peripheral blood CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and CD20<sup>+</sup> B cells.

[0015] Figure 5A is a graph depicting the results of binding of the 6G3 antibody to normal cynomolgus monkey peripheral blood CD3<sup>+</sup> T cells.

[0016] Figure 5B is a graph depicting the results of binding of the 6G3 antibody to normal cynomolgus monkey peripheral blood CD20<sup>+</sup> B cells.

[0017] Figure 5C is a flow cytometry dot plot depicting the results of binding of a reference anti-BTLA antibody to normal cynomolgus monkey peripheral blood CD3<sup>+</sup> and CD3<sup>-</sup> cells.

[0018] Figure 5D is a flow cytometry dot plot depicting the results of binding of the 6G3 antibody to normal cynomolgus monkey peripheral blood CD3<sup>+</sup> and CD3<sup>-</sup> cells.

**[0019]** Figure 6 is a graph depicting the results of a competition assay, which illustrates the ability of anti-BTLA antibodies to compete with HVEM-Fc and a pre-formed HVEM-Fc/trimeric LIGHT complex for binding to 293c18 cells stably transfected with human BTLA.

**[0020]** Figure 7A is a ribbon-model illustration of the crystal structure of human BTLA extracellular domain (black) docked with a space-filling model of the crystal structure of human HVEM extracellular binding domain (gray). The model depicts the results of a hydrogen-

## CLAIMS:

1. A BTLA-binding agent comprising:  
an immunoglobulin heavy chain variable region comprising:
  - (a) a CDRH1 comprising  $X^1SX^2MN$  (SEQ ID NO: 195), wherein  $X^1$  is N or T, and  $X^2$  is W, F, H, G, P, R, K, D, S, L, V, N, or Y
  - (b) a CDRH2 comprising  $RIYPX^1GX^2X^3DTNYX^4GKFK$  (SEQ ID NO: 196), wherein:
    - $X^1$  is absent or A;
    - $X^2$  is D, Y, Q, G, L, F, H, S, P, R, or T;
    - $X^3$  is G, Y, A, F, S, D, V, T, E, K, or R; and
    - $X^4$  is N, V, Q, R, A, F, Y, S, G, P, or T;
 and
  - (c) a CDRH3 comprising  $X^1SGTFX^2X^3GNYX^4X^5YFDV$  (SEQ ID NO: 197), wherein:
    - $X^1$  is K or R;
    - $X^2$  is N or D;
    - $X^3$  is D, S, F, Y, F, V, S, G, T, R, I, L, or E;
    - $X^4$  is R or H; and
    - $X^5$  is W, R, F, L, N, Y, P, I, V, A, S, G, R, or K;
 or comprising the immunoglobulin heavy chain variable region of SEQ ID NO: 144 or any one of SEQ ID NOs: 43-143 or 145-156, or at least the CDRs thereof; or an amino acid sequence with at least 90% sequence identity thereto;
- and
  - an immunoglobulin light chain variable region comprising:
    - (a) a CDRL1 comprising  $RX^1SENIYX^2X^3LA$  (SEQ ID NO: 198), wherein
      - $X^1$  is A or V;
      - $X^2$  is S or N; and
      - $X^3$  is H, N, or Y;
    - (b) a CDRL2 comprising  $X^1AX^2NLAX^3$  (SEQ ID NO: 199), wherein
      - $X^1$  is A or N;
      - $X^2$  is T or K; and

X<sup>3</sup> is N, L, Q, G, F, V, K, S, R, T, H, or P; and

(c) a CDRL3 comprising QX<sup>1</sup>FX<sup>2</sup>GPPLT (SEQ ID NO: 200), wherein

X<sup>1</sup> is L or H; and

X<sup>2</sup> is W, F, Y, P, N, V, K, M, L, G, or S;

or comprising the immunoglobulin light chain variable region of SEQ ID NO: 174 or any of SEQ ID NOs: 157-173 or 175-192, or at least the CDRs thereof; or an amino acid sequence with at least 90% sequence identity thereto;

or a BTLA binding agent as set forth in Table 3.

2. The BTLA-binding agent of claim 1, wherein the immunoglobulin heavy chain polypeptide comprises the sequence:

QVQLVQSGAEVKKPGSSVKVSCKASGYX<sup>1</sup>FSX<sup>2</sup>SX<sup>3</sup>MNWVRQAPGQGLEWMGRIYPX<sup>4</sup>  
GX<sup>5</sup>X<sup>6</sup>DTNYX<sup>7</sup>GKFKGRVTITADKX<sup>8</sup>TX<sup>9</sup>TAYMELX<sup>10</sup>SLRSEX<sup>11</sup>TAVX<sup>12</sup>YX<sup>13</sup>CAX<sup>14</sup>SGTF  
X<sup>15</sup>X<sup>16</sup>GNYX<sup>17</sup>X<sup>18</sup>YFDVWGKGTTVTVSSA (SEQ ID NO: 193), wherein

X<sup>1</sup> is A or V;

X<sup>2</sup> is N or T;

X<sup>3</sup> is W, F, H, G, P, R, K, D, S, L, V, N, or Y;

X<sup>4</sup> is absent or A;

X<sup>5</sup> is D, Y, Q, G, L, F, H, S, P, R, or T;

X<sup>6</sup> is G, Y, A, F, S, D, V, T, E, K, or R;

X<sup>7</sup> is N, V, Q, R, A, F, Y, S, G, P, or T;

X<sup>8</sup> is S or F;

X<sup>9</sup> is S, T, or N;

X<sup>10</sup> is S or R;

X<sup>11</sup> is D or V;

X<sup>12</sup> is absent or Y;

X<sup>13</sup> is Y or F;

X<sup>14</sup> is K or R;

X<sup>15</sup> is N or D;

X<sup>16</sup> is D, S, F, Y, F, V, S, G, T, R, I, L, or E;

X<sup>17</sup> is R or H; and

X<sup>18</sup> is W, R, F, L, N, Y, P, I, V, A, S, G, R, or K.

3. The BTLA-binding agent of claim 1 or 2, wherein the immunoglobulin heavy chain polypeptide comprises any one of SEQ ID NOs: 43-156, or at least the CDRs thereof; or an amino acid sequence with at least 90% sequence identity thereto.

4. The BTLA-binding agent of any of claims 1-3, wherein the immunoglobulin light chain polypeptide comprises the sequence:

X<sup>1</sup>IQX<sup>2</sup>TQSPSSLSASVGDRVTITCRX<sup>3</sup>SENIYX<sup>4</sup>X<sup>5</sup>LAWYQQKX<sup>6</sup>GKAPKLLIYX<sup>7</sup>AX<sup>8</sup>NLA  
X<sup>9</sup>GVPSRFSGSGSGTDX<sup>10</sup>TLTISSLQPEDFATYYCQX<sup>11</sup>FX<sup>12</sup>GPPLTFGGGTKVEIKR (SEQ  
ID NO: 194), wherein

X<sup>1</sup> is A or D;

X<sup>2</sup> is L or M;

X<sup>3</sup> is A or V;

X<sup>4</sup> is S or N;

X<sup>5</sup> is H, N, or Y;

X<sup>6</sup> is P or Q;

X<sup>7</sup> is A or N;

X<sup>8</sup> is T or K;

X<sup>9</sup> is N, L, Q, G, F, V, K, S, R, T, H, or P;

X<sup>10</sup> is F or Y;

X<sup>11</sup> is L or H;

X<sup>12</sup> is W, F, Y, P, N, V, K, M, L, G, S.

5. The BTLA binding agent of any of claims 1-4, wherein the immunoglobulin light chain polypeptide comprises any of SEQ ID NOs: 157-192, or at least the CDRs thereof; or an amino acid sequence with at least 90% sequence identity thereto.

6. The BTLA binding agent of any of claims 1-5 comprising

- (a) a CDRH1 comprising SEQ ID NO: 201;
- (b) a CDRH2 comprising SEQ ID NO: 202;
- (c) a CDRH3 comprising SEQ ID NO: 203;
- (d) a CDRL1 comprising SEQ ID NO: 204;
- (e) a CDRL2 comprising SEQ ID NO: 205; and
- (f) a CDRL3 comprising SEQ ID NO: 206.

7. The BTLA binding agent of any of claims 1-5 comprising an immunoglobulin heavy chain variable region of SEQ ID NO: 144, or at least the CDRs thereof; and an immunoglobulin light chain variable region of SEQ ID NO: 174, or at least the CDRs thereof; or comprising an immunoglobulin heavy chain variable region with 90% or more sequence identity to SEQ ID NO: 144, and an immunoglobulin light chain variable region of SEQ ID NO: 174.

8. A BTLA-binding agent comprising:

a heavy chain immunoglobulin variable region comprising:

- (a) a CDRH1 comprising Asp Tyr Thr Ile His (SEQ ID NO: 27),
- (b) a CDRH2 comprising Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Asp Lys Phe Lys Xaa (SEQ ID NO: 30), wherein Xaa is aspartic acid (Asp) or valine (Val);
- (c) a CDRH3 comprising Arg Xaa1 Xaa2 Tyr Xaa3 Met Xaa4 Tyr (SEQ ID NO: 32),

wherein:

- Xaa1 is asparagine (Asn) or serine (Ser),
- Xaa2 is tyrosine (Tyr) or histidine (His),
- Xaa3 is alanine (Ala) or valine (Val), and
- Xaa4 is glutamic acid (Glu) or aspartic acid (Asp);

or comprising a heavy chain variable region comprising any one of SEQ ID NOs: 1-15, 207, 208, 217, or 218, or at least the CDRs thereof, or an amino acid sequence with at least 90% sequence identity thereto;

and

a light chain immunoglobulin variable region comprising:

- (a) a CDRL1 comprising Lys Ala Ser Gln Asn Val Phe Thr Asn Val Ala (SEQ ID NO: 36);

(b) a CDRL2 comprising Ser Ala Ser Tyr Arg Xaa Ser (SEQ ID NO: 39), wherein Xaa is tyrosine (Tyr) or serine (Ser); and

(c) a CDRL3 comprising Gln Gln Tyr Xaa1 Xaa2 Tyr Pro Tyr Thr (SEQ ID NO: 41), wherein:

Xaa1 is serine (Ser) or asparagine (Asn), and

Xaa2 is threonine (Thr) or serine (Ser);

or comprising a light chain variable region comprising any one of SEQ ID NOs: 16-25, 209, 210, 219, or 220, or at least the CDRs thereof, or an amino acid sequence with at least 90% sequence identity thereto;

or

a BTLA binding agent as set forth in Table 2.

9. The BTLA-binding agent of claim 87, wherein the CDRH2 comprises SEQ ID NO: 31; and CDRH3 comprises SEQ ID NO: 33 or 34.

10. The BTLA-binding agent of any of claims 8 or 9, wherein the CDRL2 comprises SEQ ID NO: 40; and the CDRL3 comprises SEQ ID NO: 42.

11. The BTLA-binding agent of any of claims 8-10, wherein the binding agent comprises a heavy chain variable region comprising the amino acid sequence Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Xaa1 Thr Xaa2 Thr Asp Tyr Thr Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met Gly Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Asp Lys Phe Lys Xaa3 Arg Val Thr Ile Thr Xaa4 Asp Xaa5 Ser Xaa6 Xaa7 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Xaa8 Cys Ala Arg Arg Xaa9 Xaa10 Tyr Xaa11 Met Xaa12 Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala (SEQ ID NO: 26), wherein:

Xaa1 is phenylalanine (Phe) or tyrosine (Tyr),

Xaa2 is phenylalanine (Phe) or leucine (Leu),

Xaa3 is aspartic acid (Asp) or valine (Val),

Xaa4 is alanine (Ala) or arginine (Arg),

Xaa5 is lysine (Lys) or threonine (Thr),

Xaa6 is alanine (Ala) or serine (Ser),

Xaa7 is serine (Ser) or threonine (Thr),  
 Xaa8 is tyrosine (Tyr) or phenylalanine (Phe),  
 Xaa9 is asparagine (Asn) or serine (Ser),  
 Xaa10 is tyrosine (Tyr) or histidine (His),  
 Xaa11 is alanine (Ala) or valine (Val), and  
 Xaa12 is glutamic acid (Glu) or aspartic acid (Asp).

12. The BTLA-binding agent of any of claims 8-11, wherein the binding agent comprises a heavy chain variable region comprising any one of SEQ ID NOs: 1-15, 207, 208, 217, or 218, or at least the CDRs thereof, or an amino acid sequence with at least 90% sequence identity thereto.

13. The BTLA-binding agent of any of claims 8-12, wherein the binding agent comprises a light chain variable region comprising the amino acid sequence Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ala Ser Gln Asn Val Phe Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Xaa Pro Lys Xaa Leu Ile Tyr Ser Ala Ser Tyr Arg Xaa Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Xaa Cys Gln Gln Tyr Xaa Xaa Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg (SEQ ID NO: 35), wherein

Xaa1 is serine (Ser) or proline (Pro),  
 Xaa2 is proline (Pro) or leucine (Leu),  
 Xaa3 is tyrosine (Tyr) or serine (Ser),  
 Xaa4 is tyrosine (Tyr) or phenylalanine (Phe),  
 Xaa5 is serine (Ser) or asparagine (Asn), and  
 Xaa6 is threonine (Thr) or serine (Ser).

14. The BTLA-binding agent of any of claims 8-13, wherein the binding agent comprises a light chain variable region comprising any one of SEQ ID NOs: 16-25, 209, 210, 219, or 220, or at least the CDRs thereof, or an amino acid sequence with at least 90% sequence identity thereto.

15. The BTLA-binding agent of any one of claims 1-14, which is an antibody, an antibody conjugate, or an antigen-binding fragment thereof, optionally an IgG1 or IgG4 antibody.

16. The BTLA-binding agent of claim 15, which is a F(ab')<sub>2</sub> fragment, a Fab' fragment, a Fab fragment, a Fv fragment, a scFv fragment, a dsFv fragment, a dAb fragment, or a single chain binding polypeptide.

17. A nucleic acid sequence encoding the immunoglobulin heavy chain and/or light chain polypeptide of the BTLA-binding agent of any one of claims 1-16, optionally in a vector.

18. A cell comprising the nucleic acid of claim 17.

19. A composition comprising (a) the BTLA-binding agent of any one of claims 1-16 or nucleic acid encoding same and (b) a pharmaceutically acceptable carrier.

~~20. — A method of modulating BTLA signaling in a mammal, which method comprises administering the BTLA binding agent of any one of claims 1-16, a nucleic acid encoding same, or composition comprising same, to the mammal.~~

~~21. — The method of claim 20, wherein the mammal has a disorder that is responsive to BTLA modulation, and the disorder is thereby treated.~~

~~22. — The method of claim 21, wherein the disorder is an autoimmune or inflammatory disease.~~

~~23. — The method of claim 21 or 22, wherein the disease is rheumatoid arthritis, graft vs host disease, psoriasis, or inflammatory bowel disease.~~

204. A method of preparing a BTLA-binding agent according to any of claims 1-16, the method comprising expressing a nucleotide sequence encoding the immunoglobulin heavy chain polypeptide and a nucleic acid encoding the immunoglobulin light chain polypeptide in a cell.

215. A method of detecting soluble BTLA in blood, plasma, serum, or tissue comprising contacting a blood, plasma, serum, or tissue sample with an antibody of any of

claims 8-14, optionally wherein the soluble BTLA in the blood, plasma, serum, or tissue sample is bound to an antibody of any of claims 1-7, or wherein the blood, plasma, serum, or tissue sample is contacted with an antibody of any of claims 8-14 and an antibody of any of claims 1-7.

**226.** A method of detecting, measuring, or monitoring the pharmacological activity of a BTLA binding agent in a subject, or selecting a subject for treatment with a BTLA binding agent, the method comprising detecting soluble BTLA (sBTLA) in a sample of blood, plasma, serum, or tissue from a subject to whom a BTLA binding agent has been administered.

**237.** The method of claim **226**, wherein the BTLA binding agent is an anti-BTLA antibody.

**248.** The method of claim **226** or **237**, wherein the BTLA binding agent is an anti-BTLA antibody that binds BTLA without substantially inhibiting BTLA binding to HVEM, or that binds BTLA and enhances BTLA signaling.

**259.** The method of any of claims **226-248**, wherein the BTLA binding agent is a BTLA binding agent of any of claims 1-7.

**2630.** The method of any of claims **226-259**, wherein the method comprises detecting sBTLA in the blood, plasma, serum, or tissue sample by contacting the blood, plasma, serum, or tissue sample or fraction thereof with an anti-BTLA capture antibody that binds to sBTLA.

**2731.** The method of claim **2630**, wherein the anti-BTLA capture antibody is an antibody of any of claims 8-14.

**2832.** The method of any of claims **226-2731**, wherein the method further comprises comparing the concentration of sBTLA in the blood, plasma, serum, or tissue sample to a reference sBTLA concentration.

**2933.** The method of claim **2832**, wherein the reference sBTLA concentration is the concentration of sBTLA in a blood, plasma, serum, or tissue sample from the same patient prior to administration of the BTLA binding agent; or the concentration of sBTLA in the blood, plasma, serum, or tissue of another normal, non-diseased subject of the same type that has not received administration of a BTLA binding agent, or a reference sBTLA concentration

established by the statistical analysis of the sBTLA concentration in the blood, plasma, serum, or tissue of a population of such subjects.

~~34. — The method of any of claims 226-2933, further comprising a step of administering a BTLA binding agent to the subject.~~

~~3530.~~ The method of any of claims ~~226-3429~~, wherein the method comprises comparing the concentration of sBTLA in sample of blood, plasma, serum, or tissue from a subject to whom a BTLA binding agent has been administered with the concentration of sBTLA in a sample of blood, plasma, serum, or tissue from the same subject at a different point in time, either before or after the BTLA binding agent had been administered to the subject.

~~3631.~~ The method of any of claims ~~226-3630~~, wherein the sBTLA detected in the blood, plasma, serum, or tissue sample of the subject is sBTLA bound to the BTLA binding agent.

~~3732.~~ The method of claim ~~2832~~, wherein the reference sBTLA concentration is established by a method comprising contacting a blood, plasma, serum, or tissue sample of the subject or blood, plasma, serum, or tissue samples from a population of subjects with a BTLA binding agent of any of claims 1-7 and a capture antibody of any of claims 8-14.

~~3833.~~ A composition comprising a BTLA binding agent of any of claims 1-7, a BTLA binding agent of any of claims 8-14, or both, for use in a method according to any of claims 20-327.

~~3934.~~ Use of a BTLA binding agent of any of claims 8-14 in the manufacture of a medicament for treating a disorder that is responsive to BTLA modulation in a mammal.

~~3540.~~ Use of a BTLA binding agent of any of claims 1-7 in the manufacture of a medicament for treating a disorder that is responsive to BTLA modulation in a mammal.

~~41. — The BTLA binding agent of claim 40, wherein the BTLA binding agent comprises an immunoglobulin heavy chain variable region of SEQ ID NO: 144, or at least the CDRs thereof; and an immunoglobulin heavy light chain variable region of SEQ ID NO: 174, or at least the CDRs thereof; or comprises an immunoglobulin heavy chain variable region with~~

~~90% or more sequence identity to SEQ ID NO: 144, and an immunoglobulin light chain variable region of SEQ ID NO: 174.~~

3642. The ~~method-use~~ of any of claims ~~3934 or 35-41~~, wherein the disorder is an autoimmune or inflammatory disease.

3743. The ~~method-use~~ of ~~any of~~ claims ~~39-41~~36, wherein the disease is rheumatoid arthritis, graft vs host disease, psoriasis, or inflammatory bowel disease.

38. A BTLA binding agent of any of claims 1-14 for use in therapy.