

ANTI-NTB-A ANTIBODIES AND RELATED COMPOSITIONS AND METHODS

SEQUENCE LISTING

[0001] A sequence listing designated NTBA-00303PR_ST25.txt, and has a size of 29 kilobytes, is incorporated by reference.

BACKGROUND

[0002] NTB-A, a single-pass type I membrane glycoprotein also referred to as SLAMF6, is an immunoglobulin superfamily (Ig-SF) member belonging to the CD2/SLAM subfamily. *See, e.g., Bottino et al., J. Exp. Med.* 194:235-246, 2001. NTB-A is characterized, in its extracellular portion, by an N-terminal V-type domain followed by a C2-type domain, while the intracytoplasmic portion contains three tyrosine-based motifs: two immunoreceptor tyrosine-based switch motifs (ITSM; TxYxxV/I) and a classical immunoreceptor tyrosine-based inhibition motif (ITIM; I/V/L/SxYxxL). *See id.* Through its ITSM motifs, NTB-A associates with the SH2 domain of the SLAM-associated protein SH2D1A and the related Ewing's sarcoma activated transcript (EAT) 2. *See Bottino et al., supra; Falco et al., Eur. J. Immunol.* 34:1663–1672, 2004; Flaig et al., *J. Immunol.* 172:6524–6527, 2004.

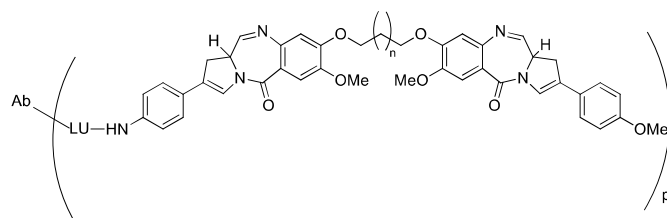
[0003] NTB-A is expressed on natural killer (NK) cells, NK-like T-cells, T-cells, monocytes, dendritic cells, B-cells, and eosinophils. *See Salort JD. et al., Immunology Letters* 129-136, 2011; Matesanz-Isabel et al., *Immunology Letters* 104-112, 2011; Munitz et al., *Journal of Immunology* 174:110-118, 2005; Bottino et al., *Journal of Experimental Medicine* 194(3):235-246; 2001. NTB-A can function through homotypic interactions (*i.e.*, as a self-ligand), and has been shown to act as a positive regulator of NK cell functions via signaling, inducing NK cell cytotoxicity. *See, e.g., See Bottino et al., supra; Falco et al., supra; Flaig et al., supra.* NTB-A has also been shown to be expressed on B-cells from chronic lymphocytic leukemia (CLL) and B-cell lymphoma patients. *See Korver et al., British Journal of Haematology* 137:307-318, 2007.

SUMMARY

[0003a] In one aspect, the present disclosure provides an antibody that specifically binds to the human NTB-A protein wherein the antibody comprises a mature heavy chain variable region and a mature light chain variable region, wherein: (a) the mature heavy chain variable region comprises three heavy chain complementarity determining regions (CDRs) as defined by Kabat: heavy chain CDR1 consisting of SEQ ID NO:10, heavy chain CDR2 consisting of SEQ ID NO:11, and heavy chain CDR3 consisting of SEQ ID NO:12; and the mature light

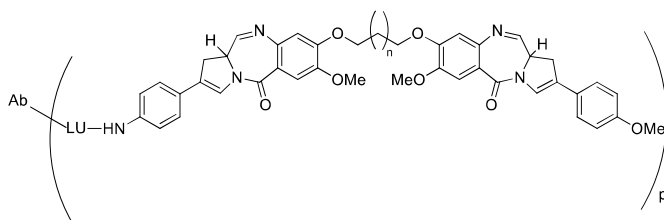
chain variable region comprises three light chain CDRs as defined by Kabat: light chain CDR1 consisting of SEQ ID NO:19, light chain CDR2 consisting of SEQ ID NO:20, and light chain CDR3 consisting of SEQ ID NO:21; or (b) the mature heavy chain variable region comprises three heavy chain complementarity determining regions (CDRs) as defined by IMGT: heavy chain CDR1 consisting of SEQ ID NO:22, heavy chain CDR2 consisting of SEQ ID NO:23, and heavy chain CDR3 consisting of SEQ ID NO:24; and the mature light chain variable region comprises three light chain CDRs as defined by IMGT: light chain CDR1 consisting of SEQ ID NO:25, light chain CDR2 consisting of the amino acid sequence ATS, and light chain CDR3 consisting of SEQ ID NO:26.

[0003b] In another aspect, the present disclosure provides an anti-NTB-A antibody-drug conjugate compound having the formula



wherein: the subscript n is 1 or 3; LU is a cleavable Linker Unit; Ab is an antibody as described herein; and the subscript p is an integer from 1 to 4.

[0003c] In another aspect, the present disclosure provides an antibody-drug conjugate composition comprising a population of anti-NTB-A antibody-drug conjugate molecules having the formula:



wherein: the subscript n is 1 to 3; LU is a cleavable Linker Unit; Ab is an antibody as described herein; and the subscript p is an integer from 1 to 4; wherein the average drug load of the composition is about 2.

BRIEF DESCRIPTION OF THE FIGURES

[0004] Fig. 1 shows a sequence alignment of the mouse 20F3 heavy chain variable region, the humanized sequence with mouse CDRs (boxed) in a human acceptor without backmutations, and humanized variants, HA-HE. CDRs are as defined by Kabat.

[0005] Fig. 2 shows a sequence alignment of the mouse 20F3 light chain variable region, the humanized sequence with mouse CDRs (boxed) in a human acceptor without backmutations and humanized variants, LA-LD. CDRs are as defined by Kabat.

WHAT IS CLAIMED IS:

1. An antibody that specifically binds to the human NTB-A protein wherein the antibody comprises a mature heavy chain variable region and a mature light chain variable region, wherein:

(a) the mature heavy chain variable region comprises three heavy chain complementarity determining regions (CDRs) as defined by Kabat: heavy chain CDR1 consisting of SEQ ID NO:10, heavy chain CDR2 consisting of SEQ ID NO:11, and heavy chain CDR3 consisting of SEQ ID NO:12; and the mature light chain variable region comprises three light chain CDRs as defined by Kabat: light chain CDR1 consisting of SEQ ID NO:19, light chain CDR2 consisting of SEQ ID NO:20, and light chain CDR3 consisting of SEQ ID NO:21; or

(b) the mature heavy chain variable region comprises three heavy chain complementarity determining regions (CDRs) as defined by IMGT: heavy chain CDR1 consisting of SEQ ID NO:22, heavy chain CDR2 consisting of SEQ ID NO:23, and heavy chain CDR3 consisting of SEQ ID NO:24; and the mature light chain variable region comprises three light chain CDRs as defined by IMGT: light chain CDR1 consisting of SEQ ID NO:25, light chain CDR2 consisting of the amino acid sequence ATS, and light chain CDR3 consisting of SEQ ID NO:26.

2. The antibody of claim 1, wherein the antibody is a humanized, chimeric or veneered antibody.

3. The antibody of claim 1, wherein the mature heavy chain region comprises an amino acid sequence as set forth in SEQ ID NO:8 and wherein the mature light chain variable region comprises an amino acid sequence as set forth in SEQ ID NO:18.

4. The antibody of claim 1, wherein the mature heavy chain and the mature light chain comprise amino acid sequences selected from the groups consisting of:

SEQ ID NO:5 and SEQ ID NO:15, respectively;

SEQ ID NO:5 and SEQ ID NO:16, respectively;

SEQ ID NO:5 and SEQ ID NO:17, respectively;

SEQ ID NO:5 and SEQ ID NO:18, respectively;

SEQ ID NO:6 and SEQ ID NO:15, respectively;

SEQ ID NO:6 and SEQ ID NO:16, respectively;
SEQ ID NO:6 and SEQ ID NO:17, respectively;
SEQ ID NO:6 and SEQ ID NO:18, respectively;
SEQ ID NO:7 and SEQ ID NO:15, respectively;
SEQ ID NO:7 and SEQ ID NO:16, respectively;
SEQ ID NO:7 and SEQ ID NO:17, respectively;
SEQ ID NO:7 and SEQ ID NO:18, respectively;
SEQ ID NO:8 and SEQ ID NO:15, respectively;
SEQ ID NO:8 and SEQ ID NO:16, respectively;
SEQ ID NO:8 and SEQ ID NO:17, respectively;
SEQ ID NO:8 and SEQ ID NO: 18, respectively;
SEQ ID NO:9 and SEQ ID NO:15, respectively;
SEQ ID NO:9 and SEQ ID NO:16, respectively;
SEQ ID NO:9 and SEQ ID NO:17, respectively; and
SEQ ID NO:9 and SEQ ID NO:18, respectively.

5. The antibody of claim 1, wherein the mature heavy chain variable region is fused to a heavy chain constant region and the mature light chain variable region is fused to a light chain constant region.

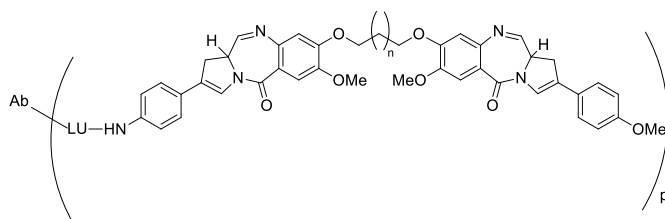
6. The antibody of claim 5, wherein the heavy chain constant region is a mutant form of natural human constant region, and wherein the heavy chain constant region has reduced binding to an Fcγ receptor relative to the natural human constant region.

7. The antibody of claim 5, wherein the heavy chain constant region is of IgG1 isotype.

8. The antibody of claim 5, wherein the heavy chain constant region has an amino acid sequence comprising SEQ ID NO: 28 or SEQ ID NO: 29 and the light chain constant region has an amino acid sequence comprising SEQ ID NO: 27.

9. The antibody of claim 1, wherein the antibody is conjugated to a cytotoxic or cytostatic agent.

10. An anti-NTB-A antibody-drug conjugate compound having the formula



wherein:

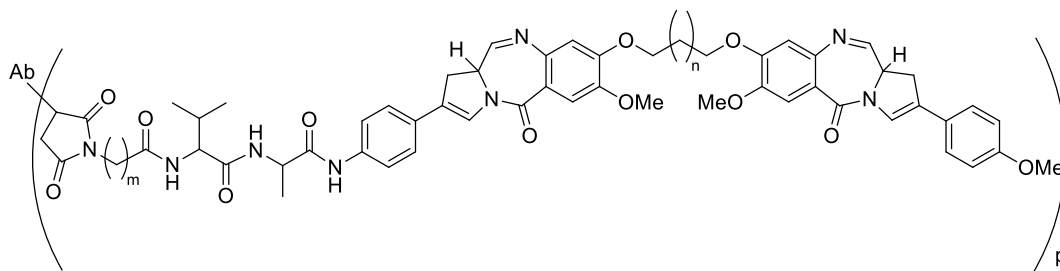
the subscript n is 1 or 3;

LU is a cleavable Linker Unit;

Ab is an antibody as set forth in claim 1; and

the subscript p is an integer from 1 to 4.

11. An anti-NTB-A antibody-drug conjugate compound of claim 10 having the formula



wherein:

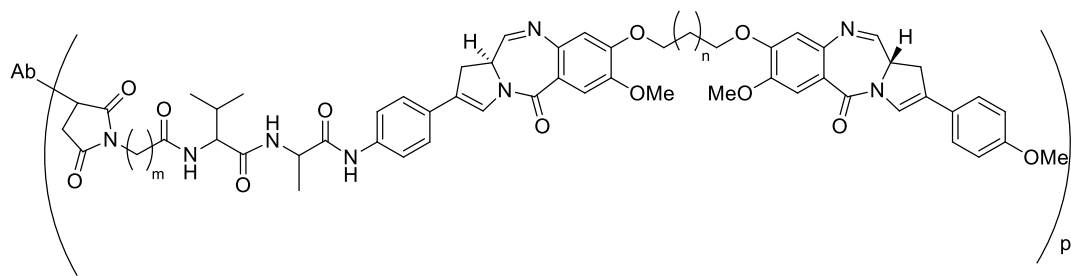
the subscript n is 1 or 3;

the subscript m is 2 to 5

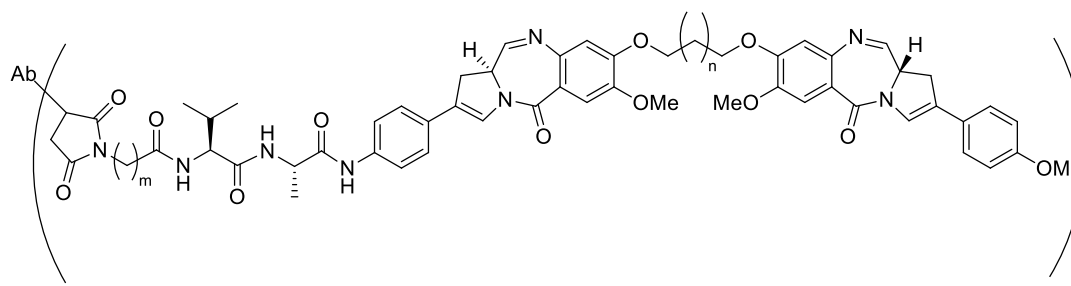
Ab is an antibody as set forth in claim 1; and

the subscript p is an integer from 1 to 4.

12. The antibody-drug conjugate compound of claim 11 having the formula:



13. The antibody-drug conjugate compound of claim 11 having the formula:

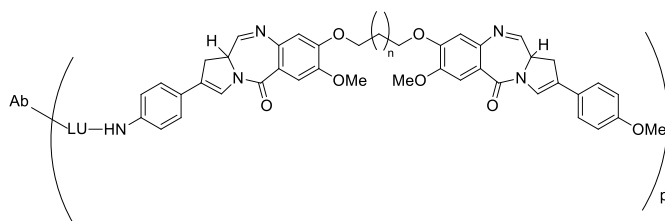


14. The antibody-drug conjugate compound of claim 11, wherein attachment to Ab is via a sulfur atom of an engineered cysteine residue of Ab.

15. The antibody-drug conjugate compound of claim 11, wherein attachment to Ab is via a sulfur atom or an engineered cysteine residue at position 239 of the heavy chain constant region, according to the EU index system of numbering.

16. The antibody-drug conjugate compound of claim 10, wherein p is 2.

17. An antibody-drug conjugate composition comprising a population of anti-NTB-A antibody-drug conjugate molecules having the formula:



wherein:

the subscript n is 1 to 3;

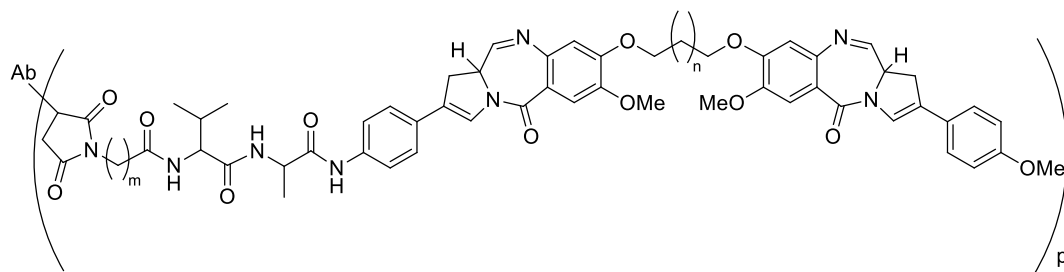
LU is a cleavable Linker Unit;

Ab is an antibody as set forth in claim 1; and

the subscript p is an integer from 1 to 4;

wherein the average drug load of the composition is about 2.

18. An antibody-drug conjugate composition of claim 17, comprising a population of anti-NTB-A antibody-drug conjugate molecules having the formula:



wherein

the subscript n is 1 to 3;

the subscript m is 2 to 5

Ab is an antibody as set forth in claim 1; and

wherein the average drug load of the composition is about 2.

The chemical structure shows a dendritic polymer with a dendritic core and a dendritic periphery. The core consists of a central benzimidazole ring system. The periphery is composed of a dendritic chain of amide and ester linkages, including a chiral center (a chiral amine). The structure is enclosed in large parentheses with a subscript 'p' indicating the polymer repeat unit. The dendritic chain includes a chiral amine (a chiral amine) and a chiral amine (a chiral amine).