

ANTI-TIGIT ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 62/722,063, filed August 23, 2018; US Provisional Application No. 62/734,130, filed September 20, 2018; and US Provisional Application No. 62/822,674, filed March 22, 2019; each of which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] Provided herein are antibodies that bind to human TIGIT (T-cell immunoreceptor with Ig and ITIM domains), and uses thereof.

BACKGROUND

[0003] TIGIT (“T-cell immunoreceptor with Ig and ITIM domains”) is an immune receptor that is expressed on subsets of T cells, such as activated, memory, and regulatory T cells and natural killer (NK) cells. TIGIT is a member of the CD28 family within the Ig superfamily of proteins, and serves as a co-inhibitory molecule that limits T cell proliferation and activation and NK cell function. TIGIT mediates its immunosuppressive effect by competing with CD226 (also known as DNAX Accessory Molecule-1, or “DNAM-1”) for the same set of ligands: CD155 (also known as poliovirus receptor or “PVR”) and CD112 (also known as poliovirus receptor-related 2 or “PVRL2”). *See*, Levin et al., *Eur. J. Immunol.*, 2011, 41:902-915. Because the affinity of CD155 for TIGIT is higher than its affinity for CD226, in the presence of TIGIT CD226 signaling is inhibited, thereby limiting T cell proliferation and activation.

[0004] In patients with melanoma, TIGIT expression is upregulated on tumor antigen (TA)-specific CD8⁺ T cells and CD8⁺ tumor-infiltrating lymphocytes (TILs). Blockade of TIGIT in the presence of TIGIT ligand (CD155)-expressing cells increased the proliferation, cytokine production, and degranulation of both TA-specific CD8⁺ T cells and CD8⁺ TILs *See*, Chauvin et al., *J Clin Invest.*, 2015, 125:2046-2058. Thus, TIGIT represents a potential therapeutic target for stimulating anti-tumor T cell responses in patients, although there remains a need for improved methods of blocking TIGIT and promoting anti-tumor responses.

BRIEF SUMMARY

Embodiment 1. A composition comprising isolated antibodies that bind to human TIGIT (T-cell immunoreceptor with Ig and ITIM domains), wherein the antibodies have a

binding affinity (K_D) for human TIGIT of less than 5 nM, and wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.

Embodiment 2. The composition of embodiment 1, wherein the antibodies have a K_D for human TIGIT of less than 1 nM.

Embodiment 3. The composition of embodiment 1, wherein the antibodies have a K_D for human TIGIT of less than 100 pM.

Embodiment 4. The composition of any one of embodiments 1 to 3, wherein the antibodies exhibit cross-reactivity with cynomolgus monkey TIGIT and/or mouse TIGIT.

Embodiment 5. The composition of embodiment 4, wherein the antibodies exhibit cross-reactivity with both cynomolgus monkey TIGIT and mouse TIGIT.

Embodiment 6. The composition of any one of embodiments 1 to 5, wherein the antibodies block binding of CD155 to TIGIT.

Embodiment 7. The composition of any one of embodiments 1 to 5, wherein the antibodies block binding of CD112 to TIGIT.

Embodiment 8. The composition of any one of embodiments 1 to 5, wherein the antibodies block binding of both CD155 and CD112 to TIGIT.

Embodiment 9. The composition of any one of embodiments 1 to 8, wherein the antibodies compete for binding to human TIGIT with an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 64.

Embodiment 10. The composition of any one of embodiments 1 to 9, wherein the antibodies when bound to human TIGIT bind one or both of amino acid positions 81 and 82.

Embodiment 11. The composition of embodiment 10, wherein the antibodies bind both of amino acid positions 81 and 82.

Embodiment 12. The composition of embodiment 10 or embodiment 11, wherein amino acid positions 81 and 82 are Phe81 and Lys82.

Embodiment 13. The composition of any one of embodiments 1 to 12, wherein the antibodies bind to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82.

Embodiment 14. A composition comprising isolated antibodies that binds to human TIGIT, wherein the antibodies when bound to human TIGIT bind one or both of amino acid positions 81 and 82, and wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.

Embodiment 15. The composition of embodiment 14, wherein the antibodies bind both of amino acid positions 81 and 82.

Embodiment 16. The composition of embodiment 14 or embodiment 15, wherein amino acid positions 81 and 82 are Phe81 and Lys82.

Embodiment 17. A composition comprising isolated antibodies that binds to human TIGIT, wherein the antibodies bind to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82, and wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.

Embodiment 18. The composition of embodiment 13 or embodiment 17, wherein the epitope comprises Phe at position 81.

Embodiment 19. The composition of any one of embodiments 13, 17, and 18, wherein the epitope comprises Lys or Ser at position 82.

Embodiment 20. The composition of any one of embodiments 13, and 17 to 19, wherein the epitope comprises Phe at position 81 and Lys or Ser at position 82.

Embodiment 21. The composition of embodiment 20, wherein the epitope comprises Phe81 and Lys82.

Embodiment 22. The composition of any one of embodiments 13 and 17 to 21, wherein the epitope is a discontinuous epitope.

Embodiment 23. The composition of any one of embodiments 13 and 17 to 22, wherein the antibodies bind to an epitope on human TIGIT that further comprises one or more of

amino acid positions 51, 52, 53, 54, 55, 73, 74, 75, 76, 77, 79, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93.

Embodiment 24. The composition of embodiment 23, wherein the epitope further comprises one or more amino acid residues selected from the group consisting of Thr51, Ala52, Gln53, Val54, Thr55, Leu73, Gly74, Trp75, His76, Ile77, Pro79, Asp83, Arg84, Val85, Ala86, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

Embodiment 25. The composition of embodiment 24, wherein the epitope comprises the amino acid residues Thr51, Ala52, Gln53, Val54, Thr55, Gly74, Trp75, His76, Ile77, Phe81, Lys82, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

Embodiment 26. The composition of embodiment 24, wherein the epitope comprises the amino acid residues Ala52, Gln53, Leu73, Gly74, Trp75, Pro79, Phe81, Lys82, Asp83, Arg84, Val85, and Ala86.

Embodiment 27. The composition of any one of embodiments 13 and 17 to 26, wherein the epitope comprises the sequence ICNADLGWHISPSFK (SEQ ID NO: 258).

Embodiment 28. The composition of any one of embodiments 1 to 27, wherein human TIGIT comprises the sequence of SEQ ID NO: 218.

Embodiment 29. The composition of any one of embodiments 1 to 28, wherein each of the antibodies comprises one or more of:

(a) a heavy chain CDR1 comprising a sequence selected from SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 comprising a sequence selected from SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186, SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, and SEQ ID NO:295;

(c) a heavy chain CDR3 comprising a sequence selected from SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134, SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, and SEQ ID NO:296;

(d) a light chain CDR1 comprising a sequence selected from SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, and SEQ ID NO:287;

(e) a light chain CDR2 comprising a sequence selected from SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, and SEQ ID NO:288; or

(f) a light chain CDR3 comprising a sequence selected from SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, and SEQ ID NO:215.

Embodiment 30. The composition of embodiment 29, wherein each of the antibodies comprises:

(a) a heavy chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, and SEQ ID NO:295;

(c) a heavy chain CDR3 sequence comprising an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:286, SEQ ID NO:228, SEQ ID NO:292, SEQ ID NO:230, and SEQ ID NO:296;

(d) a light chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:67 and SEQ ID NO:287;

(e) a light chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:69 and SEQ ID NO:288; and/or

(f) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

Embodiment 31. The composition of embodiment 29 or embodiment 30, wherein each of the antibodies comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 4, 6, 8, 13, 15, and 17, respectively; or
- (b) SEQ ID NOs: 22, 24, 26, 31, 33, and 35, respectively; or
- (c) SEQ ID NOs: 40, 42, 44, 49, 51, and 53, respectively; or
- (d) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (e) SEQ ID NOs: 283, 285, 62, 287, 288, and 71, respectively; or
- (f) SEQ ID NOs: 284, 60, 286, 67, 69, and 71, respectively; or
- (g) SEQ ID NOs: 76, 78, 80, 85, 87, and 89, respectively; or
- (h) SEQ ID NOs: 94, 96, 98, 103, 105, and 107, respectively; or
- (i) SEQ ID NOs: 112, 114, 116, 121, 123, and 125, respectively; or
- (j) SEQ ID NOs: 130, 132, 134, 139, 141, and 143, respectively; or
- (k) SEQ ID NOs: 148, 150, 152, 157, 159, and 161, respectively; or
- (l) SEQ ID NOs: 166, 168, 170, 175, 177, and 179, respectively; or
- (m) SEQ ID NOs: 184, 186, 188, 193, 195, and 197, respectively; or
- (n) SEQ ID NOs: 202, 204, 206, 211, 213, and 215, respectively; or
- (o) SEQ ID NOs: 221, 222, 223, 13, 15, and 17, respectively; or
- (p) SEQ ID NOs: 224, 225, 62, 67, 69, and 71, respectively; or
- (q) SEQ ID NOs: 293, 297, 62, 287, 288, and 71, respectively; or
- (r) SEQ ID NOs: 294, 225, 286, 67, 69, and 71, respectively; or
- (s) SEQ ID NOs: 226, 227, 228, 67, 69, and 71, respectively; or
- (t) SEQ ID NOs: 289, 291, 228, 287, 288, and 71, respectively; or
- (u) SEQ ID NOs: 290, 227, 292, 67, 69, and 71, respectively; or
- (v) SEQ ID NOs: 224, 229, 230, 67, 69, and 71, respectively; or
- (w) SEQ ID NOs: 293, 295, 230, 287, 288, and 71, respectively; or
- (x) SEQ ID NOs: 294, 229, 296, 67, 69, and 71, respectively; or
- (y) SEQ ID NOs: 224, 227, 230, 67, 69, and 71, respectively; or
- (z) SEQ ID NOs: 293, 290, 230, 287, 288, and 71, respectively; or
- (aa) SEQ ID NOs: 294, 290, 230, 67, 69, and 71, respectively; or
- (bb) SEQ ID NOs: 231, 232, 235, 103, 105, and 107, respectively; or
- (cc) SEQ ID NOs: 233, 234, 236, 103, 105, and 107, respectively; or

- (dd) SEQ ID NOs: 233, 234, 237, 103, 105, and 107, respectively; or
- (ee) SEQ ID NOs: 166, 238, 170, 175, 177, and 179, respectively; or
- (ff) SEQ ID NOs: 239, 240, 170, 175, 177, and 179, respectively; or
- (gg) SEQ ID NOs: 239, 240, 241, 175, 177, and 179, respectively; or
- (hh) SEQ ID NOs: 239, 240, 242, 175, 177, and 179, respectively; or
- (ii) SEQ ID NOs: 243, 168, 244, 175, 177, and 179, respectively.

Embodiment 32. The composition of any one of embodiments 1 to 31, wherein each of the antibodies comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (b) SEQ ID NOs: 283, 285, 62, 287, 288, and 71, respectively; or
- (c) SEQ ID NOs: 284, 60, 286, 67, 69, and 71, respectively.

Embodiment 33. The composition of any one of embodiments 1 to 32, wherein each of the antibodies comprises:

(a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257; and/or

(b) a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208.

Embodiment 34. The composition of any one of embodiments 1 to 33, wherein each of the antibodies comprises:

(a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10; or

- (b) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:19 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:28; or
- (c) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:37 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:46; or
- (d) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:64; or
- (e) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:73 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:82; or
- (f) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:100; or
- (g) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:109 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:118; or
- (h) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:127 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:136; or
- (i) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:145 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:154; or
- (j) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:172; or
- (k) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:181 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:190; or

(l) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:199 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:208.

Embodiment 35. The composition of embodiment 34, wherein each of the antibodies comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:28; or

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:46; or

(d) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64; or

(e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:73 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:82; or

(f) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:100; or

(g) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:109 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:118; or

(h) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:127 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:136; or

(i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:154; or

(j) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256,

or SEQ ID NO:257 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:172; or

(k) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:190; or

(l) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:199 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:208.

Embodiment 36. The composition of any one of embodiments 1 to 35, wherein each of the antibodies comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:55, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64.

Embodiment 37. The composition of any one of embodiments 1 to 36, wherein the antibodies are IgG antibodies.

Embodiment 38. The composition of embodiment 37, wherein the antibodies are IgG1 antibodies or IgG3 antibodies.

Embodiment 39. The composition of any one of embodiments 1 to 38, wherein each of the antibodies comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 262, 264, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 40. The composition of embodiment 39, wherein each of the antibodies comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 41. The composition of embodiment 39, wherein each of the antibodies comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

Embodiment 42. A composition comprising isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, and wherein each of the antibodies comprises a heavy chain comprising the amino

acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 43. A composition comprising isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, and wherein each of the antibodies comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

Embodiment 44. The composition of any one of embodiments 1 to 43, wherein the antibodies exhibit synergy with an anti-PD-1 antibody or an anti-PD-L1 antibody.

Embodiment 45. The composition of embodiment 44, wherein synergy is determined using an assay that comprises contacting a co-culture comprising (i) Jurkat effector cells that express PD-1, TIGIT and CD226, wherein the Jurkat effector cells comprise a luciferase reporter gene driven by the IL-2 promoter; and (ii) CHO-K1 artificial antigen presenting cells (aAPCs) expressing a TCR activator, PD-L1 and CD155; with the composition and an anti-PD-1 antibody or an anti-PD-L1 antibody.

Embodiment 46. The composition of any one of embodiments 1 to 45, wherein regulatory T (Treg) cells are depleted from human PBMCs contacted with the composition.

Embodiment 47. The composition of any one of embodiments 1 to 46, expression of MCP1, IL-8, and MIP1 α is increased in human PBMCs contacted with the composition.

Embodiment 48. The composition of any one of embodiments 1 to 47, wherein monocyte/macrophages are activated when contacted with the composition.

Embodiment 49. The composition of any one of embodiments 1 to 48, wherein CD86 and MHCII are upregulated when CD14⁺ monocyte/macrophages are contacted with the composition.

Embodiment 50. The composition of any one of embodiments 1 to 49, wherein CD14⁺ monocyte/macrophages contacted with the composition mature into antigen presenting cells.

Embodiment 51. The composition of any one of embodiments 1 to 50, wherein the memory T cells contacted with the composition show increased IFN γ production in response to antigen.

Embodiment 52. The composition of any one of embodiments 1 to 51, wherein the memory T cells contacted with the composition demonstrate enhanced response to antigen.

Embodiment 53. The composition of any one of embodiments 1 to 52, wherein effector memory CD8⁺ T cells and/or effector memory CD4⁺ T cells are increased in tumors contacted with the composition.

Embodiment 54. The composition of any one of embodiments 1 to 53, wherein the composition enhances a Th1 response in an animal administered the composition.

Embodiment 55. The composition of any one of embodiments 1 to 54, wherein the antibodies of the composition have lower binding affinity for Fc γ RIIa and/or Fc γ RIIb compared to the same anti-TIGIT antibodies that are not afucosylated.

Embodiment 56. The composition of any one of embodiments 1 to 55, wherein the composition mediates antibody-dependent cellular phagocytosis (ADCP) of cells that express TIGIT in the presence of monocyte macrophages.

Embodiment 57. The composition of any one of embodiments 1 to 56, wherein the antibodies are monoclonal.

Embodiment 58. The composition of any one of embodiments 1 to 57, wherein the antibodies are fully human antibodies.

Embodiment 59. The composition of any one of embodiments 1 to 58, wherein the antibodies are chimeric antibodies.

Embodiment 60. The composition of any one of embodiments 1 to 28 and 44 to 59, wherein the antibodies are humanized.

Embodiment 61. The composition of any one of embodiments 1 to 60, wherein the antibodies are antibody fragments.

Embodiment 62. The composition of embodiment 61, wherein the antibody fragments are Fab, Fab', F(ab')₂, scFv, or diabodies.

Embodiment 63. The composition of any one of embodiments 1 to 62, wherein the antibodies are bispecific antibodies.

Embodiment 64. The composition of any one of embodiments 1 to 63, wherein the antibodies are antibody-drug conjugates.

Embodiment 65. An antibody that binds to human TIGIT (T-cell immunoreceptor with Ig and ITIM domains), wherein the antibody has a binding affinity (K_D) for human TIGIT of less than 5 nM, and wherein the antibody is afucosylated.

Embodiment 66. The antibody of embodiment 65, wherein the antibody has a K_D for human TIGIT of less than 1 nM.

Embodiment 67. The antibody of embodiment 66, wherein antibody has a K_D for human TIGIT of less than 100 pM.

Embodiment 68. The antibody of any one of embodiments 65 to 67, wherein antibody exhibits cross-reactivity with cynomolgus monkey TIGIT and/or mouse TIGIT.

Embodiment 69. The antibody of embodiment 68, wherein the antibody exhibits cross-reactivity with both cynomolgus monkey TIGIT and mouse TIGIT.

Embodiment 70. The antibody of any one of embodiments 65 to 69, wherein the antibody blocks binding of CD155 to TIGIT.

Embodiment 71. The antibody of any one of embodiments 65 to 70, wherein the antibody blocks binding of CD112 to TIGIT.

Embodiment 72. The antibody of any one of embodiments 65 to 71, wherein the antibody blocks binding of both CD155 and CD112 to TIGIT.

Embodiment 73. The antibody of any one of embodiments 65 to 72, wherein the antibody competes for binding to human TIGIT with an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 64.

Embodiment 74. The antibody of any one of embodiments 65 to 73, wherein when bound to human TIGIT binds one or both of amino acid positions 81 and 82.

Embodiment 75. The antibody of embodiment 74, wherein the antibody binds both of amino acid positions 81 and 82.

Embodiment 76. The antibody of embodiment 74 or embodiment 75, wherein amino acid positions 81 and 82 are Phe81 and Lys82.

Embodiment 77. The antibody of any one of embodiments 65 to 76, wherein the antibody binds to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82.

Embodiment 78. An antibody that binds to human TIGIT, wherein when bound to human TIGIT binds one or both of amino acid positions 81 and 82, and wherein the antibody is afucosylated.

Embodiment 79. The antibody of embodiment 78, wherein the antibody binds both of amino acid positions 81 and 82.

Embodiment 80. The antibody of embodiment 78 or embodiment 79, wherein amino acid positions 81 and 82 are Phe81 and Lys82.

Embodiment 81. An antibody that binds to human TIGIT, wherein the antibody binds to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82, and wherein the antibody is afucosylated.

Embodiment 82. The antibody of embodiment 77 or embodiment 81, wherein the epitope comprises Phe at position 81.

Embodiment 83. The antibody of any one of embodiments 77, 81, and 82, wherein the epitope comprises Lys or Ser at position 82.

Embodiment 84. The antibody of any one of embodiments 77 and 81 to 83, wherein the epitope comprises Phe at position 81 and Lys or Ser at position 82.

Embodiment 85. The antibody of embodiment 84, wherein the epitope comprises Phe81 and Lys82.

Embodiment 86. The antibody of any one of embodiments 77 and 81 to 85, wherein the epitope is a discontinuous epitope.

Embodiment 87. The antibody of any one of embodiments 77 and 81 to 86, wherein the antibody binds to an epitope on human TIGIT that further comprises one or more of amino acid positions 51, 52, 53, 54, 55, 73, 74, 75, 76, 77, 79, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93.

Embodiment 88. The antibody of embodiment 87, wherein the epitope further comprises one or more amino acid residues selected from the group consisting of Thr51, Ala52, Gln53, Val54, Thr55, Leu73, Gly74, Trp75, His76, Ile77, Pro79, Asp83, Arg84, Val85, Ala86, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

Embodiment 89. The antibody of embodiment 88, wherein the epitope comprises the amino acid residues Thr51, Ala52, Gln53, Val54, Thr55, Gly74, Trp75, His76, Ile77, Phe81, Lys82, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

Embodiment 90. The antibody of embodiment 88, wherein the epitope comprises the amino acid residues Ala52, Gln53, Leu73, Gly74, Trp75, Pro79, Phe81, Lys82, Asp83, Arg84, Val85, and Ala86.

Embodiment 91. The antibody of any one of embodiments 77 and 81 to 90, wherein the epitope comprises the sequence ICNADLGWHISPSFK (SEQ ID NO: 258).

Embodiment 92. The antibody of any one of embodiments 65 to 91, wherein human TIGIT comprises the sequence of SEQ ID NO: 218.

Embodiment 93. The antibody of any one of embodiments 65 to 92, wherein the antibody comprises one or more of:

(a) a heavy chain CDR1 comprising a sequence selected from SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 comprising a sequence selected from SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186, SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID

NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, and SEQ ID NO:295;

(c) a heavy chain CDR3 comprising a sequence selected from SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134, SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, and SEQ ID NO:296;

(d) a light chain CDR1 comprising a sequence selected from SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, and SEQ ID NO:287;

(e) a light chain CDR2 comprising a sequence selected from SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, and SEQ ID NO:288; or

(f) a light chain CDR3 comprising a sequence selected from SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, and SEQ ID NO:215.

Embodiment 94. The antibody of embodiment 93, wherein the antibody comprises:

(d) a heavy chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, and SEQ ID NO:290;

(e) a heavy chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, and SEQ ID NO:295;

(f) a heavy chain CDR3 sequence comprising an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:286, SEQ ID NO:228, SEQ ID NO:292, SEQ ID NO:230, and SEQ ID NO:296;

(g) a light chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:67 and SEQ ID NO:287;

(h) a light chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:69 and SEQ ID NO:288; and/or

(i) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

Embodiment 95. The antibody of embodiment 94, wherein the antibody comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 4, 6, 8, 13, 15, and 17, respectively; or
- (b) SEQ ID NOs: 22, 24, 26, 31, 33, and 35, respectively; or
- (c) SEQ ID NOs: 40, 42, 44, 49, 51, and 53, respectively; or
- (d) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (e) SEQ ID NOs: 76, 78, 80, 85, 87, and 89, respectively; or
- (f) SEQ ID NOs: 94, 96, 98, 103, 105, and 107, respectively; or
- (g) SEQ ID NOs: 112, 114, 116, 121, 123, and 125, respectively; or
- (h) SEQ ID NOs: 130, 132, 134, 139, 141, and 143, respectively; or
- (i) SEQ ID NOs: 148, 150, 152, 157, 159, and 161, respectively; or
- (j) SEQ ID NOs: 166, 168, 170, 175, 177, and 179, respectively; or
- (k) SEQ ID NOs: 184, 186, 188, 193, 195, and 197, respectively; or
- (l) SEQ ID NOs: 202, 204, 206, 211, 213, and 215, respectively; or
- (m) SEQ ID NOs: 221, 222, 223, 13, 15, and 17, respectively; or
- (n) SEQ ID NOs: 224, 225, 62, 67, 69, and 71, respectively; or
- (o) SEQ ID NOs: 226, 227, 228, 67, 69, and 71, respectively; or
- (p) SEQ ID NOs: 224, 229, 230, 67, 69, and 71, respectively; or
- (q) SEQ ID NOs: 224, 227, 230, 67, 69, and 71, respectively; or
- (r) SEQ ID NOs: 231, 232, 235, 103, 105, and 107, respectively; or
- (s) SEQ ID NOs: 233, 234, 236, 103, 105, and 107, respectively; or
- (t) SEQ ID NOs: 233, 234, 237, 103, 105, and 107, respectively; or
- (u) SEQ ID NOs: 166, 238, 170, 175, 177, and 179, respectively; or
- (v) SEQ ID NOs: 239, 240, 170, 175, 177, and 179, respectively; or
- (w) SEQ ID NOs: 239, 240, 241, 175, 177, and 179, respectively; or
- (x) SEQ ID NOs: 239, 240, 242, 175, 177, and 179, respectively; or
- (y) SEQ ID NOs: 243, 168, 244, 175, 177, and 179, respectively.

Embodiment 96. The antibody of any one of embodiments 65 to 95, wherein the antibody comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (j) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or

- (k) SEQ ID NOs: 283, 285, 62, 287, 288, and 71, respectively; or
- (l) SEQ ID NOs: 284, 60, 286, 67, 69, and 71, respectively.

Embodiment 97. The antibody of any one of embodiments 65 to 96, wherein the antibody comprises:

- (a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257; and/or
- (b) a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208.

Embodiment 98. The antibody of any one of embodiments 65 to 97, wherein the antibody comprises:

- (a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10; or
- (b) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:19 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:28; or
- (c) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:37 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:46; or
- (d) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:64; or
- (e) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:73 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:82; or

(f) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:100; or

(g) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:109 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:118; or

(h) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:127 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:136; or

(i) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:145 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:154; or

(j) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:172; or

(k) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:181 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:190; or

(l) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:199 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:208.

Embodiment 99. The antibody of embodiment 98, wherein the antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:28; or

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:46; or

(d) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64; or

(e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:73 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:82; or

(f) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:100; or

(g) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:109 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:118; or

(h) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:127 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:136; or

(i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:154; or

(j) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:172; or

(k) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:190; or

(l) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:199 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:208.

Embodiment 100. The antibody of any one of embodiments 65 to 99, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:55, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64.

Embodiment 101. The antibody of any one of embodiments 65 to 100, wherein the antibody is an IgG antibody.

Embodiment 102. The antibody of embodiment 101, wherein the antibody is an IgG1 antibody or an IgG3 antibody.

Embodiment 103. The antibody of any one of embodiments 65 to 102, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 262, 264, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 104. The antibody of embodiment 103, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 105. The antibody of embodiment 103, wherein the antibody comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

Embodiment 106. An antibody that binds to human TIGIT, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 262, 264, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 107. An antibody that binds to human TIGIT, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

Embodiment 108. An antibody that binds to human TIGIT, wherein the antibody comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

Embodiment 109. The antibody of any one of embodiments 65 to 108, wherein the antibody exhibits synergy with an anti-PD-1 antibody or an anti-PD-L1 antibody.

Embodiment 110. The antibody of embodiment 109, wherein synergy is determined using an assay that comprises contacting a co-culture comprising (i) Jurkat effector cells that express PD-1, TIGIT and CD226, wherein the Jurkat effector cells comprise a luciferase reporter gene driven by the IL-2 promoter; and (ii) CHO-K1 artificial antigen presenting cells (aAPCs) expressing a TCR activator, PD-L1 and CD155; with the composition and an anti-PD-1 antibody or an anti-PD-L1 antibody.

Embodiment 111. The antibody of any one of embodiments 65 to 110, wherein regulatory T (Treg) cells are depleted from human PBMCs contacted with the antibody.

Embodiment 112. The antibody of any one of embodiments 65 to 111, expression of MCP1, IL-8, and MIP1 α is increased in human PBMCs contacted with the antibody.

Embodiment 113. The antibody of any one of embodiments 65 to 112, wherein monocyte/macrophages are activated when contacted with the antibody.

Embodiment 114. The antibody of any one of embodiments 65 to 113, wherein CD86 and MHCII are upregulated when CD14⁺ monocyte/macrophages are contacted with the antibody.

Embodiment 115. The antibody of any one of embodiments 65 to 114, wherein CD14⁺ monocyte/macrophages contacted with the antibody mature into antigen presenting cells.

Embodiment 116. The antibody of any one of embodiments 65 to 115, wherein the memory T cells contacted with the antibody show increased IFN γ production in response to antigen.

Embodiment 117. The antibody of any one of embodiments 65 to 116, wherein the memory T cells contacted with the antibody demonstrate enhanced response to antigen.

Embodiment 118. The antibody of any one of embodiments 65 to 117, wherein effector memory CD8⁺ T cells and/or effector memory CD4⁺ T cells are increased in tumors contacted with the antibody.

Embodiment 119. The antibody of any one of embodiments 65 to 118, wherein the antibody enhances a Th1 response in an animal administered the antibody.

Embodiment 120. The antibody of any one of embodiments 65 to 119, wherein the antibody has lower binding affinity for Fc γ RIIa and/or Fc γ RIIb compared to the same anti-TIGIT antibody that is not afucosylated.

Embodiment 121. The antibody of any one of embodiments 65 to 120, wherein the composition mediates antibody-dependent cellular phagocytosis (ADCP) of cells that express TIGIT in the presence of monocyte macrophages.

Embodiment 122. The antibody of any one of embodiments 65 to 121, wherein the antibody is monoclonal.

Embodiment 123. The antibody of any one of embodiments 65 to 122, wherein the antibody is a fully human antibody.

Embodiment 124. The antibody of any one of embodiments 65 to 123, wherein the antibody is a chimeric antibody.

Embodiment 125. The antibody of any one of embodiments 65 to 92 and 109 to 124, wherein the antibody is humanized.

Embodiment 126. The antibody of any one of embodiments 65 to 125, wherein the antibody is an antibody fragment.

Embodiment 127. The antibody of embodiment 126, wherein the antibody fragment is a Fab, a Fab', a F(ab')₂, a scFv, or a diabody.

Embodiment 128. The antibody of any one of embodiments 65 to 127, wherein the antibody is a bispecific antibody.

Embodiment 129. The antibody of any one of embodiments 65 to 128, wherein the antibody is an antibody-drug conjugate.

Embodiment 130. A pharmaceutical formulation comprising the composition of any one of embodiments 1 to 64 or the antibody of any one of embodiments 65 to 129 and a pharmaceutically acceptable carrier.

Embodiment 131. An isolated polynucleotide that encodes (i) the heavy chain of the antibody of any one of embodiments 106 to 108; (ii) the light chain of the antibody of any one of embodiments 106 to 108; or (iii) the heavy chain and the light chain of the antibody of any one of embodiments 106 to 108.

Embodiment 132. The isolated polynucleotide of embodiment 131, wherein the polynucleotide comprises (i) a nucleotide sequence selected from SEQ ID NOs: 259, 261, 163, 265, 267, 269, and 271; or (ii) a nucleotide sequence of SEQ ID NO: 273; or (iii) a nucleotide sequence selected from SEQ ID NOs: 259, 261, 263, 265, 267, 269, and 271, and a nucleotide sequence of SEQ ID NO: 273.

Embodiment 133. A vector comprising the polynucleotide of embodiment 131 or embodiment 132.

Embodiment 134. An isolated host cell comprising the isolated polynucleotide of embodiment 131 or embodiment 132, or the vector of embodiment 133.

Embodiment 135. An isolated host cell that expresses the antibody of any one of embodiments 106-108.

Embodiment 136. The host cell of embodiment 134 or embodiment 135, which is engineered to produce afucosylated antibodies.

Embodiment 137. A method of producing an antibody that binds to human TIGIT, comprising incubating the host cell of any one of embodiments 134 to 136 under conditions suitable for producing the antibody.

Embodiment 138. The method of embodiment 137, wherein the host cell is engineered to produce afucosylated antibodies.

Embodiment 139. The method of embodiment 137, wherein the host cell is cultured in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies.

Embodiment 140. The method of any one of embodiments 137 to 139, further comprising isolating the antibodies.

Embodiment 141. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by the method of any one of embodiments 138 to 140.

Embodiment 142. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by the method comprising incubating the host cell of embodiment 134 or embodiment 135 under conditions suitable for producing afucosylated antibodies, and isolating the antibodies to form the composition of isolated antibodies.

Embodiment 143. The composition of embodiment 142, wherein the host cell is engineered to produce afucosylated antibodies.

Embodiment 144. The composition of embodiment 142, wherein the host cell is cultured in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies.

Embodiment 145. A host cell comprising a polynucleotide comprising a nucleotide sequence encoding the (i) antibodies of the composition of any one of embodiments 1 to 63, or (ii) the antibody of any one of embodiments 65 to 128, wherein the host cell is engineered to produce afucosylated antibodies.

Embodiment 146. The host cell of embodiment 145, wherein the polynucleotide is a vector.

Embodiment 147. A host cell that expresses (i) the antibodies of the composition of any one of embodiments 1 to 63, or (ii) the antibody of any one of embodiments 65 to 128, wherein the host cell is engineered to produce afucosylated antibodies.

Embodiment 148. The host cell of any one of embodiments 145 to 147, wherein the host cell comprises:

(a) the nucleotide sequence of SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, SEQ ID NO:74, SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, SEQ ID NO:182, SEQ ID NO:200, SEQ ID NO:259, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, or SEQ ID NO:271; and/or

(b) the nucleotide sequence of SEQ ID NO:11, SEQ ID NO:29, SEQ ID NO:47, SEQ ID NO:65, SEQ ID NO:83, SEQ ID NO:101, SEQ ID NO:119, SEQ ID NO:137, SEQ ID NO:155, SEQ ID NO:173, SEQ ID NO:191, SEQ ID NO:209, or SEQ ID NO:273.

Embodiment 149. A method of producing afucosylated antibodies that bind TIGIT, comprising culturing the host cell of any one of embodiments 145 to 148 under conditions suitable for producing the afucosylated antibodies.

Embodiment 150. A method of producing afucosylated antibodies that bind TIGIT, comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies, wherein the host cell comprises a polynucleotide comprising a nucleotide sequence encoding the (i) antibodies of the composition of any one of embodiments 1 to 63, or (ii) the antibody of any one of embodiments 65 to 128.

Embodiment 151. The method of embodiment 150, wherein the polynucleotide is a vector.

Embodiment 152. A method of producing afucosylated antibodies that bind TIGIT, comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies, wherein the host cell expresses (i) the antibodies of the composition of any one of embodiments 1 to 63, or (ii) the antibody of any one of embodiments 65 to 128.

Embodiment 153. The method of any one of embodiments 150 to 152, wherein the host cell comprises:

(a) the nucleotide sequence of SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, SEQ ID NO:74, SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, SEQ ID NO:182, SEQ ID NO:200, SEQ ID NO:259, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, or SEQ ID NO:271; and/or

(b) the nucleotide sequence of SEQ ID NO:11, SEQ ID NO:29, SEQ ID NO:47, SEQ ID NO:65, SEQ ID NO:83, SEQ ID NO:101, SEQ ID NO:119, SEQ ID NO:137, SEQ ID NO:155, SEQ ID NO:173, SEQ ID NO:191, SEQ ID NO:209, or SEQ ID NO:273.

Embodiment 154. The method of any one of embodiments 150 to 153, wherein the fucose analogue is 2-fluorofucose.

Embodiment 155. The method of any one of embodiments 149 to 154, further comprising isolating the afucosylated antibodies.

Embodiment 156. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by the method of any one of embodiments 149 to 155.

Embodiment 157. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by a method comprising incubating the host cell of any one of embodiments 145 to 147 under conditions suitable for producing afucosylated antibodies, and isolating the antibodies to form the composition of isolated antibodies.

Embodiment 158. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by a method comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies, wherein the host cell comprises a polynucleotide comprising a nucleotide sequence encoding the (i) antibodies of the composition of any one of embodiments 1 to 63, or (ii) the antibody of any one of embodiments 65 to 128.

Embodiment 159. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by a method comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies, wherein the host cell expresses (i) the antibodies of the composition of any one of embodiments 1 to 63, or (ii) the antibody of any one of embodiments 65 to 128.

Embodiment 160. A kit comprising:
the composition of any one of embodiments 1 to 64, the antibody of any one of embodiments 65 to 129, or the pharmaceutical composition of embodiment 130; and
an additional therapeutic agent.

Embodiment 161. The kit of embodiment 160, wherein the additional therapeutic is an anti-cancer agent.

Embodiment 162. The kit of embodiment 160 or embodiment 161, wherein the additional therapeutic agent is an antibody.

Embodiment 163. The kit of any one of embodiments 160 to 162, wherein the additional therapeutic agent is an antagonist or an inhibitor of a T cell coinhibitor; an agonist of a T cell coactivator; an immune stimulatory cytokine; or SGN-2FF.

Embodiment 164. The kit of any one of embodiments 160 to 163, wherein the additional therapeutic agent binds a protein selected from CD25, PD-1, PD-L1, Tim3, Lag3, CTLA4, 41BB, OX40, CD3, CD40, CD47M, GM-CSF, CSF1R, TLR, STING, RIGI, TAM receptor kinase, NKG2A, NKG2D, GD2, HER2, EGFR, PDGFR α , SLAMF7, VEGF, CTLA-4, CD20, cCLB8, KIR, and CD52.

Embodiment 165. The kit of embodiment any one of embodiments 160 to 164, wherein the additional therapeutic agent is selected from an anti-CD25 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-Tim3 antibody, anti-Lag3 antibody, anti-CTLA4 antibody, anti-41BB antibody, anti-OX40 antibody, anti-CD3 antibody, anti-CD40 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-STING antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-HER2 antibody, an anti-EGFR antibody, an anti-PDGFR- α -antibody, an anti-SLAMF7 antibody, an anti-VEGF antibody, an anti-CTLA-4 antibody, an anti-CD20 antibody, an anti-cCLB8 antibody, an anti-KIR antibody, and an anti-CD52 antibody.

Embodiment 166. The kit of any one of embodiments 160 to 165, wherein the additional therapeutic agent comprises a cytokine selected from IL-15, IL-21, IL-2, GM-CSF, M-CSF, G-CSF, IL-1, IL-3, IL-12, and IFN γ .

Embodiment 167. The kit of any one of embodiments 160 to 165, wherein the additional therapeutic agent is selected from SEA-CD40, avelumab, durvalumab, nivolumab, pembrolizumab, pidilizumab, atezolizumab, Hul4.18K322A, Hu3F8, dinituximab, trastuzumab, cetuximab, olaratumab, necitumumab, elotuzumab, ramucirumab, pertuzumab, ipilimumab, bevacizumab, rituximab, obinutuzumab, siltuximab, ofatumumab, lirilumab, and alemtuzumab.

Embodiment 168. The kit of embodiment 160 or embodiment 161, wherein the additional therapeutic agent is selected from an alkylating agent (*e.g.*, cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepe, nitrosoureas, or temozolomide), an anthracycline (*e.g.*, doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (*e.g.*, paclitaxel or docetaxel), a histone deacetylase inhibitor (*e.g.*, vorinostat or romidepsin), an inhibitor of topoisomerase (*e.g.*, irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (*e.g.*, bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (*e.g.*, azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (*e.g.*, actinomycin or bleomycin), a platinum-based agent (*e.g.*, cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (*e.g.*, vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, or docetaxel), galardin, thalidomide, lenalidomide, and pomalidomide.

Embodiment 169. A method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of the composition of any one of

embodiments 1 to 64, the antibody of any one of embodiments 65 to 129, or the pharmaceutical composition of embodiment 130.

Embodiment 170. The method of embodiment 169, wherein the cancer is a cancer that is enriched for expression of CD112 or CD155.

Embodiment 171. The method of embodiment 169 or embodiment 170, wherein the cancer is a cancer that is enriched for T cells or natural killer (NK) cells that express TIGIT.

Embodiment 172. The method of any one of embodiments 169 to 171, wherein the cancer is bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, gastric cancer, pancreatic cancer, colorectal cancer, colon cancer, kidney cancer, clear cell renal carcinoma, head and neck cancer, lung cancer, lung adenocarcinoma, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, melanoma, neoplasm of the central nervous system, mesothelioma, lymphoma, leukemia, chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, Hodgkin lymphoma, myeloma, or sarcoma.

Embodiment 173. The method of embodiment 172, wherein the cancer is lymphoma, leukemia, chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, or Hodgkin lymphoma.

Embodiment 174. The method of any one of embodiments 169 to 173, further comprising administering to the subject a therapeutically effective amount of an additional therapeutic agent.

Embodiment 175. The method of embodiment 174, wherein the additional therapeutic is an anti-cancer agent.

Embodiment 176. The method of embodiment 174 or embodiment 175, wherein the additional therapeutic agent is an antibody.

Embodiment 177. The method of any one of embodiments 174 to 176, wherein the additional therapeutic agent is an antagonist or an inhibitor of a T cell coinhibitor; an agonist of a T cell coactivator; or an immune stimulatory cytokine; or SGN-2FF.

Embodiment 178. The method of any one of embodiments 174 to 177, wherein the additional therapeutic agent binds a protein selected from CD25, PD-1, PD-L1, Tim3, Lag3, CTLA4, 41BB, OX40, CD3, CD40, CD47M, GM-CSF, CSF1R, TLR, STING, RIGI, TAM

receptor kinase, NKG2A, NKG2D, GD2, HER2, EGFR, PDGFR α , SLAMF7, VEGF, CTLA-4, CD20, cCLB8, KIR, and CD52.

Embodiment 179. The method of embodiment 178, wherein the additional therapeutic agent is selected from an anti-CD25 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-Tim3 antibody, anti-Lag3 antibody, anti-CTLA4 antibody, anti-41BB antibody, anti-OX40 antibody, anti-CD3 antibody, anti-CD40 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-STING antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-HER2 antibody, an anti-EGFR antibody, an anti-PDGFR- α -antibody, an anti-SLAMF7 antibody, an anti-VEGF antibody, an anti-CTLA-4 antibody, an anti-CD20 antibody, an anti-cCLB8 antibody, an anti-KIR antibody, and an anti-CD52 antibody.

Embodiment 180. The method of embodiment 174 or embodiment 175, wherein the additional therapeutic agent comprises a cytokine selected from IL-15, IL-21, IL-2, GM-CSF, M-CSF, G-CSF, IL-1, IL-3, IL-12, and IFN γ .

Embodiment 181. The method of any one of embodiments 174 to 179, wherein the additional therapeutic agent is selected from SEA-CD40, avelumab, durvalumab, nivolumab, pembrolizumab, pidilizumab, atezolizumab, Hul4.18K322A, Hu3F8, dinituximab, trastuzumab, cetuximab, olaratumab, necitumumab, elotuzumab, ramucirumab, pertuzumab, ipilimumab, bevacizumab, rituximab, obinutuzumab, siltuximab, ofatumumab, lirilumab, and alemtuzumab.

Embodiment 182. The method of embodiment 174 or embodiment 175, wherein the additional therapeutic agent is selected from an alkylating agent (*e.g.*, cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepea, nitrosoureas, or temozolomide), an anthracycline (*e.g.*, doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (*e.g.*, paclitaxel or docetaxel), a histone deacetylase inhibitor (*e.g.*, vorinostat or romidepsin), an inhibitor of topoisomerase (*e.g.*, irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (*e.g.*, bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (*e.g.*, azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (*e.g.*, actinomycin or bleomycin), a platinum-based agent (*e.g.*, cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (*e.g.*, vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, or docetaxel), galardin, thalidomide, lenalidomide, and pomalidomide.

Embodiment 183. The method of any one of embodiments 174 to 182, wherein the composition, the antibody, or the pharmaceutical composition is administered concurrently with the additional therapeutic agent.

Embodiment 184. The method of any one of embodiments 174 to 183, wherein the composition, the antibody, or the pharmaceutical composition is administered sequentially to the additional therapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] **FIG. 1.** Binding of 65 anti-TIGIT antibody clones and an irrelevant isotype control antibody to HEK 293 cells engineered to express human TIGIT (top panel), cynomolgus monkey TIGIT (middle panel), and mouse TIGIT (bottom panel).

[0006] **FIG. 2.** Binding of 65 anti-TIGIT antibody clones and an irrelevant isotype control antibody to primary human T cells (top panel), cynomolgus monkey T cells (middle panel), and mouse T cells (bottom panel). For the bottom panel, 35 of 65 clones were evaluated. Of the 35 clones evaluated, 5 of the 35 did not bind mTIGIT-Fc protein (clones 20, 27, 55, 56, and 60), as indicated by the light green bars.

[0007] **FIG. 3A-3D.** (A-C) Binding titration values of eight anti-TIGIT antibody clones (clones 2, 5, 13, 16, 17, 20, 25, and 54) to human (A), mouse (B), and cynomolgus monkey (C) TIGIT expressed on HEK 293 cells. Results are shown for singlicate wells. (D) EC₅₀ values of eight anti-TIGIT antibody clones (clones 2, 5, 13, 16, 17, 20, 25, and 54) to human, mouse, and cynomolgus monkey TIGIT expressed on HEK 293 cells.

[0008] **FIG. 4.** Binding titration of anti-TIGIT antibody clones 13 and 25 to activated mouse splenic T cells. Results are shown for singlicate wells. Clone 13 had an EC₅₀ of 0.24 µg/mL. Clone 25 had an EC₅₀ of 2.28 µg/mL.

[0009] **FIG. 5A-5B.** Anti-TIGIT antibodies blocked CD155 interaction with TIGIT expressed on HEK 293 cells, for both human CD155 binding to HEK 293 cells expressing human TIGIT (A) and mouse CD155 binding to HEK 293 cells expressing mouse TIGIT (B). Results are shown for singlicate wells.

[0010] **FIG. 6.** Anti-TIGIT antibodies blocked human CD112 interaction with human TIGIT expressed on HEK 293 cells. Results are shown for singlicate wells.

[0011] **FIG. 7A-7B.** (A) Upper panel: Select anti-TIGIT antibodies effectively blocked TIGIT-CD155 engagement, resulting in T cell activation, as measured by a > 1.5-fold induction in luciferase activity. About 12 clones showed >1.5-fold induction in the bioassay. Two clones did not block TIGIT-CD155 interaction in ForteBio assay (pink bars). Fold induction was

measured over no Ab control. Mean and SD are of duplicate experiments; antibodies were at 20 $\mu\text{g/mL}$. Gray bar = hIgG1 isotype control. Black bar = no antibody control (defined as baseline). Lower panel: Correlation plot of TIGIT/CD155 blockade bioassay versus TIGIT-Fc affinity. The activity in the bioassay correlated with affinity for recombinant protein. (B) Dose response of 12 selected anti-TIGIT clones in TIGIT/CD155 blockade bioassay. Clones 13 and 25, which showed strong binding to all three species, showed good activity in the bioassay. Mean and SD are of triplicate wells.

[0012] FIG. 8. Select anti-TIGIT antibodies synergized with anti-PD-1, resulting in T cell activation. Mean and SD are of triplicate wells. Both clone 13 and clone 25 showed synergy with anti-PD-1 in combination bioassay.

[0013] FIG. 9A-9H. (A-D) Binding titration (A-C) and EC50 values (D) for binding to human (A), mouse (B), and cynomolgus monkey (C) TIGIT expressed on HEK 293 cells for fully human anti-TIGIT clone 13 ("c13 hIgG1") and mouse IgG1 ("c13 mIgG1") and mouse IgG2a ("c13 mIgG2a") chimeras of clone 13. Mean and SD are of duplicate wells. (E-F) Antibodies c13 hIgG1, c13 mIgG1, and c13 mIgG2a blocked CD155 interaction with TIGIT expressed on HEK 293 cells, for both human CD155 binding to HEK 293 cells expressing human TIGIT (E) and mouse CD155 binding to HEK 293 cells expressing mouse TIGIT (F). Results are for singlicate wells. (G) Antibodies c13 hIgG1, c13 mIgG1, and c13 mIgG2a blocked human CD112 interaction with human TIGIT expressed on HEK 293 cells. Results are for singlicate wells. (H) Dose response of parental and chimeric anti-TIGIT antibody clones c13 hIgG1, c13 mIgG1, and c13 mIgG2a in TIGIT/CD155 blockade bioassay. Mean and SD are of triplicate wells.

[0014] FIG. 10A-10K. Anti-TIGIT antibodies that can engage activating Fc γ receptors mediated anti-tumor efficacy in a CT26 syngeneic tumor model in mice. (A) Group mean tumor volume. (B-K) Individual animal tumor volume for groups 1 through 10. PR = Partial Response (tumor volume is 50% or less of its day 1 volume for three consecutive measurements and equal to or greater than 13.5 mm³ for one or more of these three measurements). CR = Complete Response (tumor volume is less than 13.5 mm³ for three consecutive measurements).

[0015] FIG. 11. Sensorgrams of human Fc γ RIIIa 158V binding titrated anti-TIGIT antibodies by BLI. The results are shown for anti-TIGIT clone 13 hIgG1 wild-type, afucosylated, and LALA-PG (left panels), anti-TIGIT clone 13 mIgG2a wild-type, afucosylated, and LALA-PG (center panels), and anti-TIGIT clone 13C hIgG1 wild-type (right panel).

[0016] FIG. 12. Binding of anti-TIGIT mIgG2a antibodies to CHO cells expressing mCD16 (the murine form of Fc γ RIVa). Afucosylated clone 13 mIgG2a (black squares) bound

with significantly greater affinity compared to wild-type mIgG2a (black circles) or mIgG2a LALA-PG (black triangles). Binding data is summarized in the table below.

[0017] **FIG. 13.** Expression of TIGIT (top panel), CD226 (middle panel) and CD155 (bottom panel) by T-cell subsets from healthy donors. Tregs expressed the highest levels of TIGIT, while all T cell subsets tested expressed the activating receptor CD226. Expression of CD155 was mostly absent.

[0018] **FIG. 14.** Depletion of TIGIT-positive Treg cells by clone 13 IgG1 wild-type (black circles), clone 13 IgG1 afucosylated (black squares), clone 13 IgG1 LALA-PG (black triangles), and a human IgG1 isotype control (gray inverted triangles), at various concentrations.

[0019] **FIG. 15.** Depletion of Tregs in allogeneic NK/Treg co-culture experiments by clone 13 IgG1 wild-type (black circles), clone 13 IgG1 afucosylated (black squares), and clone 13 IgG1 LALA-PG (black triangles). Afucosylated OKT9 antibody (open circles) was included as a positive control.

[0020] **FIG. 16.** Production of inflammatory cytokines MCP-1 (top panel), IL-8 (middle panel), and MIP1 α (bottom panel) by PBMCs after treatment with clone 13 IgG1 wild-type antibody (black squares), clone 13 IgG1 afucosylated antibody (black diamond), or clone 13 IgG1 LALA-PG antibody (black circles).

[0021] **FIG. 17.** Dose-dependent upregulation of expression of CD86 (left panel) and MHC class II (right panel) on CD14⁺ monocytes after treatment with clone 13 IgG1 wild-type antibody (gray circles), clone 13 IgG1 afucosylated antibody (gray squares), and clone 13 IgG1 LALA-PG antibody (black triangles).

[0022] **FIG. 18A-18C.** *In vivo* activity of anti-TIGIT antibodies in three different syngeneic tumor models. FIG. 8A shows the effect of clone 13 IgG2a wild-type, clone 13 IgG2a afucosylated, and clone 13 IgG2a LALA-PG on the tumor volume in the A20 syngeneic lymphoma model. FIG. 8B shows the effect of clone 13 IgG2a wild-type, clone 13 IgG2a afucosylated, and clone 13 IgG2a LALA-PG on the tumor volume in CT26 syngeneic colon cancer model. FIG. 8C shows the effect of clone 13 IgG2a wild-type, clone 13 IgG2a afucosylated, and clone 13 IgG2a LALA-PG on the tumor volume in MC38 syngeneic colon cancer model. In all 3 models, clone 13 mIgG2a wild-type (black circles) and clone 13 mIgG2a afucosylated (black diamonds) were more effective at controlling tumor size when compared to clone 13 IgG2a LALPG (black squares) or untreated animals (gray triangles).

[0023] **FIG. 19.** ADCC activity against A549 cells contacted with cetuximab alone (open squares), the combination of cetuximab with anti-TIGIT antibody clone 13 IgG1 (black squares), and an IgG1 isotype control antibody (open triangles).

[0024] **FIG. 20.** CMV-induced IFN γ expression in PBMCs exposed to CMV recall antigen and treated with anti-PD-1 antibody (pembrolizumab treatment is shown with gray squares, nivolumab treatment is shown in gray diamonds) and increasing concentrations of anti-TIGIT antibody clone 13 IgG1. Treatment with anti-TIGIT antibody clone 13 IgG1 alone is also shown (black squares).

[0025] **FIG. 21.** CMV-induced IFN γ expression in PBMCs exposed to CMB recall antigen and treated with increasing concentrations of anti-TIGIT antibodies clone 13 IgG1 wild-type ("Clone 13 IgG1"), afucosylated clone 13 IgG1 ("Clone 13 SEA IgG1"), and clone 13 IgG1 LALA-PG ("Clone 13 LALA IgG1").

[0026] **FIG. 22.** IL2 expression in HLA mismatched co-cultured PBMCs treated with increasing concentrations of anti-TIGIT antibodies clone 13 IgG1 wild-type ("Clone 13 IgG1"), afucosylated clone 13 IgG1 ("Clone 13 SEA IgG1"), and clone 13 IgG1 LALA-PG ("Clone 13 LALA IgG1").

[0027] **FIG. 23.** Cytokine induction in plasma and tissues from CT26 colon cancer xenograft mice in response to anti-TIGIT antibodies. Mice implanted with CT26 colon cancer cells were treated with 1 mg/kg clone 13 IgG2a wild type ("WT"), afucosylated clone 13 IgG1 ("SEA"), or clone 13 IgG2a LALA-PG ("LALA"), and cytokine levels were measured in plasma, spleen, and tumor lysates.

[0028] **FIG. 24.** Changes in intratumoral and peripheral CD8⁺ T cell subsets in CT26 colon cancer xenograft mice following treatment with anti-TIGIT antibody clone 13 IgG2a wild type ("WT TIGIT"), afucosylated clone 13 IgG2a ("SEA TIGIT"), or clone 13 IgG2a LALA-PG ("LALA TIGIT").

[0029] **FIG. 25.** Changes in intratumoral and peripheral CD4⁺ T cell subsets in CT26 colon cancer xenograft mice treated with anti-TIGIT antibody clone 13 IgG2a wild type ("WT TIGIT"), afucosylated clone 13 IgG2a ("SEA TIGIT"), or clone 13 IgG2a LALA-PG ("LALA TIGIT").

[0030] **FIG. 26A-B.** Changes in splenic CD8⁺ (A) and CD4⁺ (B) T cell subsets in CT26 colon cancer xenograft mice following six doses of anti-TIGIT antibody clone 13 IgG2a wild type ("WT TIGIT"), afucosylated clone 13 IgG2a ("SEA TIGIT"), or clone 13 IgG2a LALA-PG ("LALA TIGIT"). Results from live CD45⁺ cells gated on CD8⁺ or CD4⁺ are shown.

[0031] **FIG. 27.** Cytokine induction in plasma and spleen of CT26 colon cancer xenograft mice treated with anti-TIGIT antibody clone 13 IgG2a wild type ("WT TIGIT"), afucosylated clone 13 IgG2a ("SEA TIGIT"), or clone 13 IgG2a LALA-PG ("LALA TIGIT").

[0032] **FIG. 28.** Induction of antigen-specific T cell responses in mice treated with anti-TIGIT antibody clone 13 IgG2a wild type ("WT TIGIT"), afucosylated clone 13 IgG2a ("SEA TIGIT"), or clone 13 IgG2a LALA-PG ("LALA TIGIT").

[0033] **FIG. 29A-C.** *In vivo* activity of anti-TIGIT antibodies in three different syngeneic tumor models. FIG. 29A shows the effect of 5 mg/kg clone 13 IgG2a wild-type ("TIGIT") and 0.1 mg/kg, 1 mg/kg, and 5 mg/kg clone 13 IgG2a afucosylated ("SEA TIGIT") on the tumor volume in the EMT6 syngeneic breast cancer model. FIG. 29B shows the effect of 5 mg/kg clone 13 IgG2a wild-type ("TIGIT") and 0.1 mg/kg, 1 mg/kg, and 5 mg/kg clone 13 IgG2a afucosylated ("SEA TIGIT") on the tumor volume in E0771 syngeneic breast cancer model. FIG. 29C shows the effect of 1 mg/ml clone 13 IgG2a wild-type on the tumor volume in an externally maintained CT26 syngeneic colon cancer model.

[0034] **FIG. 30.** Correlation of NK cell (left) and activated CD8 T cell (right) molecular signatures with syngeneic tumor model response to anti-TIGIT antibody treatment.

[0035] **FIG. 31.** Correlation of TIGIT (left) and CD155 (right) expression with syngeneic tumor model response to anti-TIGIT antibody treatment.

[0036] **FIG. 32.** Anti-OVA IgG1 (left panel) and IgG2a (right panel) levels in mice following vaccination with OVA and treatment with 1 mg/kg clone 13 IgG2a wild-type ("WT TIGIT"), clone 13 mIgG2a afucosylated ("SEA TIGIT"), or clone 13 mIgG2a LALA-PG ("LALA TIGIT") antibody.

[0037] **FIG. 33.** Cytokine expression following re-stimulation of splenocytes *ex vivo* from mice treated with OVA vaccination followed by 1 mg/kg clone 13 IgG2a wild-type ("WT TIGIT"), clone 13 mIgG2a afucosylated ("SEA TIGIT"), or clone 13 mIgG2a LALA-PG ("LALA TIGIT") antibody.

[0038] **FIG. 34.** Re-challenge of mice that showed a complete response in the CT26 syngeneic tumor model following treatment with clone 13 mIgG2a afucosylated antibody.

[0039] **FIG. 35.** Treg depletion by anti-TIGIT antibodies clone 13 IgG1 wild-type ("WT"), clone 13 IgG1 afucosylated ("SEA Clone 13 IgG1"), or clone 13 IgG1 DLE ("DLE").

[0040] **FIG. 36.** Treg depletion by anti-TIGIT antibodies clone 13 IgG1 wild-type ("WT Clone 13"), clone 13 IgG1 afucosylated ("SEA Clone 13 IgG1"), or clone H5/L4 IgG1.

[0041] **FIG. 37.** Efficacy of anti-TIGIT antibodies clone 13 IgG1 wild-type ("WT Clone 13" or "WT TIGIT"), clone 13 IgG1 afucosylated ("SEA Clone 13" or "SGN-TGT"), anti-PD-1 antibody, and combinations of anti-PD-1 antibody and clone 13 IgG1 wild-type or clone 13 IgG1 afucosylated in an MC38 mouse tumor model.

[0042] **FIG. 38A-38B.** FIG. 38A IL-2 expression (FIG. 38A) or IL-10 expression (FIG. 38B) following contact with superantigen staphylococcal enterotoxin B peptide and anti-TIGIT

antibodies clone 13 IgG1 wild-type (“WT Clone 13”), clone 13 IgG1 afucosylated (“SEA clone 13”), clone 13 IgG1 DLE (“DLE”), or clone H5/L4 IgG1.

[0043] FIG. 39A-39D. Binding of anti-TIGIT antibodies clone 13 IgG1 wild-type (“Clone 13 IgG1”), clone 13 IgG1 afucosylated (“SEA Clone 13”), clone 13 LALA-PG (“Clone 13 LALA”), clone 13 IgG1 DLE (“Clone 13 DLE”), or clone H5/L4 IgG1 to FcγRI (FIG. 39A), FcγRIIIa V/V (FIG. 39B), FcγRIIb (FIG. 39C), or FcγRIIa (FIG. 39D) expressed on the surface of CHO cells.

[0044] FIG. 40. Phagocytosis of TIGIT-positive Jurkat cells mediated by anti-TIGIT antibodies clone 13 IgG1 wild-type (“Clone 13”), clone 13 IgG1 afucosylated (“SEA Clone 13”), and clone 13 IgG1 DLE (“Clone 13 DLE”).

[0045] FIG. 41. CDC of TIGIT-positive Jurkat cells mediated by anti-TIGIT antibodies clone 13 IgG1 wild-type (“Clone 13 IgG1”), clone 13 IgG1 afucosylated (“SEA Clone 13”), clone 13 LALA-PG (“Clone 13 LALA-PG”), clone 13 IgG1 DLE (“Clone 13 DLE”), or clone H5/L4 IgG1; and by anti-CD47 antibody hB6h12.3.

DETAILED DESCRIPTION

I. Introduction

[0046] Provided herein are antibodies having high affinity for human TIGIT (T-cell immunoreceptor with Ig and ITIM domains), and further having cross-reactivity with either or both of mouse TIGIT and cynomolgus monkey TIGIT, have been identified that inhibit the interaction between TIGIT and CD155. These antibodies also exhibit synergy with anti-PD-1 antibodies. Thus, the anti-TIGIT antibodies described herein may be used in a number of therapeutic applications, such as for the treatment of various cancers, either as a single agent or in combination with another therapeutic agent. In some embodiments, the anti-TIGIT antibodies are afucosylated.

[0047] Accordingly, in some embodiments, the present invention provides compositions, kits, and methods of treatment comprising an antibody that binds to human TIGIT, wherein the antibody is afucosylated.

II. Definitions

[0048] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, *e.g.*, Lackie, *DICTIONARY OF CELL AND MOLECULAR BIOLOGY*, Elsevier (4th ed. 2007); Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, Cold Springs Harbor Press (Cold Springs

Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention.

[0049] As used herein, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “an antibody” optionally includes a combination of two or more such molecules, and the like.

[0050] The term “about,” as used herein, refers to the usual error range for the respective value readily known to the skilled person in this technical field.

[0051] As used herein, the term “TIGIT” refers to “T-cell immunoreceptor with Ig and ITIM domains.” The protein encoded by the TIGIT gene is a member of the CD28 family within the Ig superfamily of proteins. TIGIT is expressed on several classes of T cells and on natural killer (NK) cells and mediates its immunosuppressive effect by competing with CD226 for the ligands CD155 and CD112. See, Levin et al., *Eur. J. Immunol.*, 2011, 41:902-915. TIGIT is also referred to in the art as WUCAM (Washington University Cell Adhesion Molecule) and VSTM3 (HUGO designation). See, Levin et al., *Eur J Immunol*, 2011, 41:902-915. Accordingly, reference to “TIGIT” throughout this application also includes a reference to WUCAM and/or VSTM3 unless otherwise stated or apparent from context. Human TIGIT nucleotide and protein sequences are set forth in, e.g., Genbank Accession Nos. NM173799 (SEQ ID NO:217) and NP776160 (SEQ ID NO:218), respectively.

[0052] The term “antibody” includes intact antibodies and antigen-binding fragments thereof, wherein the antigen-binding fragments comprise the antigen-binding region and at least a portion of the heavy chain constant region comprising asparagine (N) 297, located in CH2. Typically, the “variable region” contains the antigen-binding region of the antibody and is involved in specificity and affinity of binding. See, *Fundamental Immunology 7th Edition*, Paul, ed., Wolters Kluwer Health/Lippincott Williams & Wilkins (2013). Light chains are typically classified as either kappa or lambda. Heavy chains are typically classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0053] The term “antibody” also includes bivalent or bispecific molecules, diabodies, triabodies, and tetrabodies. Bivalent and bispecific molecules are described in, e.g., Kostelny *et al.* (1992) *J. Immunol.* 148:1547, Pack and Pluckthun (1992) *Biochemistry* 31:1579, Hollinger *et al.* (1993), *PNAS. USA* 90:6444, Gruber *et al.* (1994) *J Immunol.* 152:5368, Zhu *et al.* (1997) *Protein Sci.* 6:781, Hu *et al.* (1996) *Cancer Res.* 56:3055, Adams *et al.* (1993) *Cancer Res.* 53:4026, and McCartney, *et al.* (1995) *Protein Eng.* 8:301.

[0054] The term “antibody” includes an antibody by itself (naked antibody) or an antibody conjugated to a cytotoxic or cytostatic drug.

[0055] A “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al. (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, for example, U.S. Patent No. 4816567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson et al. (1991) *Nature*, 352:624-628 and Marks et al. (1991) *J. Mol. Biol.*, 222:581-597, for example or may be made by other methods. The antibodies described herein are monoclonal antibodies.

[0056] Specific binding of a monoclonal antibody to its target antigen means an affinity of at least 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} M⁻¹. Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (e.g., lock and key type) whereas nonspecific binding is usually the result of van der Waals forces.

[0057] The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region, means a light chain variable region without the light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

[0058] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a “J” region of about 12 or more amino acids, with the heavy chain also including a “D” region of about 10 or more amino acids. (See generally, *Fundamental Immunology* (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989, Ch. 7, incorporated by reference in its entirety for all purposes).

[0059] The mature variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD, 1987 and 1991), or Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987); Chothia et al., Nature 342:878-883 (1989), or a composite of Kabat and Chothia, or IMGT (ImMunoGeneTics information system), AbM or Contact or other conventional definition of CDRs. Kabat also provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number. Unless otherwise apparent from the context, Kabat numbering is used to designate the position of amino acids in the variable regions. Unless otherwise apparent from the context EU numbering is used to designated positions in constant regions.

[0060] A “humanized” antibody is an antibody that retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody with their human counterparts. See, e.g., Morrison *et al.*, *PNAS USA*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988); Padlan, *Molec. Immun.*, 28:489-498 (1991); Padlan, *Molec. Immun.*, 31(3):169-217 (1994).

[0061] As used herein, the term “chimeric antibody” refers to an antibody molecule in which (a) the constant region, or a portion thereof, is replaced so that the antigen binding site (variable region, CDR, or portion thereof) is linked to a constant region of a different species.

[0062] The term “epitope” refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, in *Methods in Molecular Biology*, Vol. 66, Glenn E. Morris, Ed. (1996).

[0063] Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined by X-ray crystallography of the antibody bound to its antigen to identify contact residues. Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0064] Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody to a common antigen (see, e.g., Junghans et al., *Cancer Res.* 50:1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (e.g., at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50% but preferably 75%, 90% or 99% as measured in a competitive binding assay. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[0065] The phrase “specifically binds” refers to a molecule (e.g., antibody or antibody fragment) that binds to a target with greater affinity, avidity, more readily, and/or with greater duration to that target in a sample than it binds to a non-target compound. In some embodiments, an antibody that specifically binds a target is an antibody that binds to the target with at least 2-fold greater affinity than non-target compounds, such as, for example, at least 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 25-fold, 50-fold, or 100-fold greater affinity. For example, an antibody that specifically binds TIGIT will typically bind to TIGIT with at least a 2-fold greater affinity than to a non-TIGIT target. It will be understood by a person of ordinary skill in the art reading this definition, for example, that an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” does not necessarily require (although it can include) exclusive binding.

[0066] The term “binding affinity” is herein used as a measure of the strength of a non-covalent interaction between two molecules, e.g., an antibody, or fragment thereof, and an antigen. The term “binding affinity” is used to describe monovalent interactions (intrinsic activity).

[0067] Binding affinity between two molecules, e.g. an antibody, or fragment thereof, and an antigen, through a monovalent interaction may be quantified by determination of the

dissociation constant (K_D). In turn, K_D can be determined by measurement of the kinetics of complex formation and dissociation using, as a nonlimiting example, the surface plasmon resonance (SPR) method (Biacore™). The rate constants corresponding to the association and the dissociation of a monovalent complex are referred to as the association rate constants k_a (or k_{on}) and dissociation rate constant k_d (or k_{off}), respectively. K_D is related to k_a and k_d through the equation $K_D = k_d / k_a$. The value of the dissociation constant can be determined directly by well-known methods, and can be computed even for complex mixtures by methods such as those, for example, set forth in Caceci *et al.* (1984, *Byte* 9: 340-362). For example, the K_D may be established using a double-filter nitrocellulose filter binding assay such as that disclosed by Wong & Lohman (1993, *Proc. Natl. Acad. Sci. USA* 90: 5428-5432). Other standard assays to evaluate the binding ability of ligands such as antibodies towards target antigens are known in the art, including for example, ELISAs, Western blots, RIAs, and flow cytometry analysis, and other assays exemplified elsewhere herein. The binding kinetics and binding affinity of the antibody also can be assessed by standard assays known in the art or as described in the Examples section below, such as Surface Plasmon Resonance (SPR), e.g. by using a Biacore™ system; kinetic exclusion assays such as KinExA®; and BioLayer interferometry (e.g., using the ForteBio® Octet platform). In some embodiments, binding affinity is determined using a BioLayer interferometry assay. See, e.g., Wilson *et al.*, *Biochemistry and Molecular Biology Education*, 38:400-407 (2010); Dysinger *et al.*, *J. Immunol. Methods*, 379:30-41 (2012); and Estep *et al.*, *Mabs*, 2013, 5:270-278.

[0068] The term “cross-reacts,” as used herein, refers to the ability of an antibody to bind to an antigen other than the antigen against which the antibody was raised. In some embodiments, cross-reactivity refers to the ability of an antibody to bind to an antigen from another species than the antigen against which the antibody was raised. As a non-limiting example, an anti-TIGIT antibody as described herein that is raised against a human TIGIT antigen can exhibit cross-reactivity with TIGIT from a different species (e.g., mouse or monkey).

[0069] An “isolated” antibody refers to an antibody that has been identified and separated and/or recovered from components of its natural environment and/or an antibody that is recombinantly produced. A “purified antibody” is an antibody that is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the monoclonal antibody is combined with an excess of pharmaceutical acceptable carrier(s) or other vehicle intended to facilitate its use. Interfering proteins and other contaminants can include, for example, cellular components of the cells from which an antibody is isolated or recombinantly produced. Sometimes monoclonal antibodies are at least 60%, 70%, 80%, 90%, 95 or 99% w/w pure of interfering proteins and contaminants

from production or purification. The antibodies described herein, including rat, chimeric, veneered and humanized antibodies can be provided in isolated and/or purified form.

[0070] The term “immuno-oncology agent” refers to an agent that enhances, stimulates, or upregulates an immune response against a cancer in a subject (e.g., in stimulating an immune response for inhibiting tumor growth). In some embodiments, an immuno-oncology agent is a small molecule, antibody, peptide, protein, circular peptide, peptidomimetic, polynucleotide, inhibitory RNA, aptamer, drug compound, or other compound. In some embodiments, an immuno-oncology agent is an antagonist or inhibitor of PD-1 or the PD-1 pathway.

[0071] “Subject,” “patient,” “individual” and like terms are used interchangeably and refer to, except where indicated, mammals such as humans and non-human primates, as well as rabbits, rats, mice, goats, pigs, and other mammalian species. The term does not necessarily indicate that the subject has been diagnosed with a particular disease, but typically refers to an individual under medical supervision.

[0072] The terms “therapy,” “treatment,” and “amelioration” refer to any reduction in the severity of symptoms. In the case of treating cancer, treatment can refer to reducing, e.g., tumor size, number of cancer cells, growth rate, metastatic activity, cell death of non-cancer cells, etc. As used herein, the terms “treat” and “prevent” are not intended to be absolute terms. Treatment and prevention can refer to any delay in onset, amelioration of symptoms, improvement in patient survival, increase in survival time or rate, etc. Treatment and prevention can be complete (no detectable symptoms remaining) or partial, such that symptoms are less frequent or severe than in a patient without the treatment described herein. The effect of treatment can be compared to an individual or pool of individuals not receiving the treatment, or to the same patient prior to treatment or at a different time during treatment. In some aspects, the severity of disease is reduced by at least 10%, as compared, e.g., to the individual before administration or to a control individual not undergoing treatment. In some aspects, the severity of disease is reduced by at least 25%, 50%, 75%, 80%, or 90%, or in some cases, no longer detectable using standard diagnostic techniques.

[0073] As used herein, a “therapeutic amount” or “therapeutically effective amount” of an agent (e.g., an antibody as described herein) is an amount of the agent that prevents, alleviates, abates, ameliorates, or reduces the severity of symptoms of a disease (e.g., a cancer) in a subject.

[0074] The terms “administer,” “administered,” or “administering” refer to methods of delivering agents, compounds, or compositions to the desired site of biological action. These methods include, but are not limited to, topical delivery, parenteral delivery, intravenous delivery, intradermal delivery, intramuscular delivery, colonic delivery, rectal delivery, or intraperitoneal delivery. Administration techniques that are optionally employed with the agents

and methods described herein, include *e.g.*, as discussed in Goodman and Gilman, The Pharmacological Basis of Therapeutics, current ed.; Pergamon; and Remington's, Pharmaceutical Sciences (current edition), Mack Publishing Co., Easton, PA.

III. Antibodies Against TIGIT

[0075] In one aspect, antibodies that bind to human TIGIT (T-cell immunoreceptor with Ig and ITIM domains) are provided. As described herein, in some embodiments, the anti-TIGIT antibody inhibits interaction between TIGIT and one or both of the ligands CD155 and CD112. In some embodiments, the anti-TIGIT antibody inhibits the interaction between TIGIT and CD155 in a functional bioassay, allowing CD155-CD226 signaling to occur. In some embodiments, the anti-TIGIT antibody exhibits synergy with an anti-PD-1 agent (*e.g.*, an anti-PD-1 antibody) or an anti-PD-L1 agent (*e.g.*, an anti-PD-L1 antibody).

[0076] The present inventors found that, surprisingly, anti-TIGIT antibodies with enhanced effector function, such as may be achieved with afucosylated IgG1 antibodies, deplete Treg cells and show improved efficacy *in vivo*. Accordingly, in various embodiments, afucosylated anti-TIGIT antibodies are provided.

Exemplary Characteristics of Anti-TIGIT Antibodies

[0077] In some embodiments, an anti-TIGIT antibody, such as an afucosylated anti-TIGIT antibody, binds to human TIGIT protein (SEQ ID NO:218) or a portion thereof with high affinity. In some embodiments, the antibody has a binding affinity (K_D) for human TIGIT of less than 5 nM, less than 1 nM, less than 500 pM, less than 250 pM, less than 150 pM, less than 100 pM, less than 50 pM, less than 40 pM, less than 30 pM, less than 20 pM, or less than about 10 pM. In some embodiments, the antibody has a binding affinity (K_D) for human TIGIT of less than 50 pM. In some embodiments, the antibody has a K_D for human TIGIT in the range of about 1 pM to about 5 nM, *e.g.*, about 1 pM to about 1 nM, about 1 pM to about 500 pM, about 5 pM to about 250 pM, or about 10 pM to about 100 pM.

[0078] In some embodiments, in addition to binding to human TIGIT with high affinity, an afucosylated anti-TIGIT antibody exhibits cross-reactivity with cynomolgus monkey (“cyno”) TIGIT (*e.g.*, a cyno TIGIT protein having the sequence of SEQ ID NO:219) and/or mouse TIGIT (*e.g.*, a mouse TIGIT protein having the sequence of SEQ ID NO:220). In some embodiments, the anti-TIGIT antibody binds to mouse TIGIT (*e.g.*, a mouse TIGIT having the sequence of SEQ ID NO:220) with a binding affinity (K_D) of 100 nM or less. In some embodiments, the anti-TIGIT antibody binds to human TIGIT with a K_D of 5 nM or less, and cross-reacts with mouse TIGIT with a K_D of 100 nM or less. In some embodiments, an anti-

TIGIT antibody that binds to a human TIGIT also exhibits cross-reactivity with both cynomolgus monkey TIGIT and mouse TIGIT.

[0079] In some embodiments, antibody cross-reactivity is determined by detecting specific binding of the anti-TIGIT antibody to TIGIT that is expressed on a cell (e.g., a cell line that expresses human TIGIT, cyno TIGIT, or mouse TIGIT, or a primary cell that endogenously expresses TIGIT, e.g., primary T cells that endogenously express human TIGIT, cyno TIGIT, or mouse TIGIT). In some embodiments, antibody binding and antibody cross-reactivity is determined by detecting specific binding of the anti-TIGIT antibody to purified or recombinant TIGIT (e.g., purified or recombinant human TIGIT, purified or recombinant cyno TIGIT, or purified or recombinant mouse TIGIT) or a chimeric protein comprising TIGIT (e.g., an Fc-fusion protein comprising human TIGIT, cyno TIGIT, or mouse TIGIT, or a His-tagged protein comprising human TIGIT, cyno TIGIT, or mouse TIGIT).

[0080] Methods for analyzing binding affinity, binding kinetics, and cross-reactivity are known in the art. See, e.g., Ernst *et al.*, Determination of Equilibrium Dissociation Constants, *Therapeutic Monoclonal Antibodies* (Wiley & Sons ed. 2009). These methods include, but are not limited to, solid-phase binding assays (e.g., ELISA assay), immunoprecipitation, surface plasmon resonance (SPR, e.g., Biacore™ (GE Healthcare, Piscataway, NJ)), kinetic exclusion assays (e.g. KinExA®), flow cytometry, fluorescence-activated cell sorting (FACS), BioLayer interferometry (e.g., Octet™ (FortéBio, Inc., Menlo Park, CA)), and Western blot analysis. SPR techniques are reviewed, e.g., in Hahnfeld *et al.* Determination of Kinetic Data Using SPR Biosensors, *Molecular Diagnosis of Infectious Diseases* (2004). In a typical SPR experiment, one interactant (target or targeting agent) is immobilized on an SPR-active, gold-coated glass slide in a flow cell, and a sample containing the other interactant is introduced to flow across the surface. When light of a given wavelength is shined on the surface, the changes to the optical reflectivity of the gold indicate binding, and the kinetics of binding. In some embodiments, kinetic exclusion assays are used to determine affinity. This technique is described, e.g., in Darling *et al.*, *Assay and Drug Development Technologies* Vol. 2, number 6 647-657 (2004). In some embodiments, BioLayer interferometry assays are used to determine affinity. This technique is described, e.g., in Wilson *et al.*, *Biochemistry and Molecular Biology Education*, 38:400-407 (2010); Dysinger *et al.*, *J. Immunol. Methods*, 379:30-41 (2012).

[0081] In some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction between TIGIT and the ligand CD155. In some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction between TIGIT and the ligand CD112. In some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction between TIGIT and both of the ligands CD155 and CD112.

[0082] In some embodiments, the ability of an anti-TIGIT antibody to inhibit interactions between TIGIT and CD155 and/or CD112 is evaluated by measuring whether physical interactions between TIGIT and CD155 or CD112 decrease in a binding assay. In some embodiments, the binding assay is a competitive binding assay. The assay may be performed in various formats, such as but not limited to an ELISA assay, flow cytometry, a surface plasmon resonance (SPR) assay (e.g., Biacore™), or BioLayer interferometry (e.g., ForteBio Octet™). See, e.g., Duff et al., *Biochem J.*, 2009, 419:577-584; Dysinger et al., *J. Immunol. Methods*, 379:30-41 (2012); and Estep et al, *Mabs*, 2013, 5:270-278.

[0083] In some embodiments, the anti-TIGIT antibody inhibits the interaction between TIGIT and CD155 in a functional bioassay, such as a functional cellular assay in which inhibition of TIGIT/CD155 interaction is evaluated by measuring activation of CD155-CD226 signaling in the cell (e.g., via activation of a downstream reporter). A non-limiting exemplary functional cellular assay is described in the Examples section below. In this exemplary functional assay, luciferase expression requires TCR engagement and a co-stimulatory signal from CD155-CD226. A first cell (also referred to as a “T effector cell”) expresses a TCR complex, TIGIT, and CD226 on the cell surface and contains a luciferase gene. A second cell (also referred to as an “artificial antigen presenting cell”) expresses a TCR activator and CD155. Co-culture of the cells in the absence of anti-TIGIT antibody results in a TIGIT-CD155 interaction that inhibits co-stimulation of the effector cell by CD155-CD226, preventing expression of luciferase by the effector cell. In the presence of an anti-TIGIT antibody that inhibits the interaction between TIGIT and CD155, CD155 and CD226 are able to interact and produce a co-stimulatory signal that drives luciferase expression in the first cell. Such functional cellular assays are described in the art, e.g., Cong et al., *Genetic Engineering and Biotechnology News*, 2015, 35(10):16-17, and are also commercially available (e.g., TIGIT/CD155 Blockade Bioassay Kit, Promega Corp., Madison, WI). In some embodiments, an anti-TIGIT antibody that inhibits the interaction between TIGIT and CD155 increases the level or amount of activation of CD155-CD226 signaling (e.g., as measured in a cellular assay such as the TIGIT/CD155 Blockade Bioassay Kit) by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to the level or amount of CD155-CD226 signaling in the absence of the anti-TIGIT antibody. In some embodiments, an anti-TIGIT antibody that inhibits the interaction between TIGIT and CD155 increases the level or amount of activation of CD155-CD226 signaling (e.g., as measured in a cellular assay such as the TIGIT/CD155 Blockade Bioassay Kit) by at least about 1.2-fold, at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold or

more as compared to the level or amount of CD155-CD226 signaling in the absence of the anti-TIGIT antibody.

[0084] In some embodiments, an anti-TIGIT antibody that binds to human TIGIT (and optionally exhibits cross-reactivity with cynomolgus monkey and/or mouse TIGIT and/or optionally inhibits interaction between TIGIT and CD155 and/or CD112) exhibits synergy with an anti-PD-1 agent (e.g., an anti-PD-1 antibody). In some embodiments, the anti-TIGIT antibody enhances the effect of the anti-PD-1 agent (e.g., anti-PD-1 antibody) by at least about 1.2-fold, at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold or more.

[0085] In some embodiments, the anti-TIGIT antibody exhibits synergy with an anti-PD-1 agent (e.g., an anti-PD-1 antibody) in a functional bioassay, such as a functional cellular assay in which inhibition of TIGIT signaling and inhibition of PD-1 signaling is evaluated by measuring the activation of signaling in an effector cell. A non-limiting exemplary functional cellular assay is described in the Examples section below. In this exemplary functional assay, a first cell (also referred to as a “T effector cell”) expresses a TCR complex, TIGIT, CD226, and PD-1 on the cell surface and contains a luciferase gene. A second cell (also referred to as an “artificial antigen presenting cell”) expresses a TCR activator, CD155, and PD-L1. Expression of the luciferase gene by the effector cell is activated by either or both of (1) blockade of TIGIT-CD155 interaction, thereby allowing CD155-CD226 interaction and subsequent co-stimulation of luciferase expression by the effector cell, or (2) blockade of PD-1/PD-L1 interaction, thereby relieving the inhibition of luciferase expression by the effector cell. The level of luciferase expression in the absence or presence of anti-TIGIT antibodies and anti-PD-1 agents or anti-PD-L1 agents can be measured and quantified for determining whether an anti-TIGIT antibody exhibits synergy with the anti-PD-1 agent or the anti-PD-L1 agent. Such functional cellular assays are described in the art (e.g., Cong et al., *Genetic Engineering and Biotechnology News*, 2015, 35(10):16-17), and are also commercially available (e.g., PD-1/TIGIT Combination Bioassay Kit, Promega Corp., Madison, WI).

[0086] In some embodiments, the efficacy of an anti-TIGIT antibody, as well as whether the anti-TIGIT antibody inhibits synergistically with an anti-PD-1 agent (e.g., an anti-PD-1 antibody) or an anti-PD-L1 agent (e.g., an anti-PD-L1 antibody), can be measured using an *in vivo* model, e.g., an *in vivo* tumor model. For example, the efficacy of an anti-TIGIT antibody as described herein, or the efficacy of an anti-TIGIT antibody as described herein when administered in combination with an anti-PD-1 agent or an anti-PD-L1 agent can be evaluated using a syngeneic mouse tumor model. Suitable syngeneic tumor models are described in the art.

See, e.g., Rios-Doria et al., *Neoplasia*, 2015, 17:661-670; and Moynihan et al., *Nature Medicine*, 2016, doi:10.1038/nm.4200. In some embodiments, an anti-TIGIT antibody reduces the size of a tumor or the overall number of tumors in an *in vivo* model by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more as compared to a control or reference value (e.g., as compared to tumor size or overall number of tumors in an untreated control).

[0087] In some embodiments, an anti-TIGIT antibody recognizes an epitope of human TIGIT that comprises one or both of amino acid positions 81 and 82, as numbered with reference to SEQ ID NO:218. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Lys or Ser at position 82. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys at position 82. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Ser at position 82.

[0088] In some embodiments, an anti-TIGIT antibody recognizes a linear epitope that comprises one or both of amino acid positions 81 and 82 (e.g., a linear epitope that comprises Phe at position 81 and Lys or Ser at position 82). In some embodiments, an anti-TIGIT antibody recognizes a discontinuous epitope that comprises one or both of amino acid positions 81 and 82 (e.g., a discontinuous epitope that comprises Phe at position 81 and Lys or Ser at position 82).

[0089] In some embodiments, an anti-TIGIT antibody binds to an epitope on human TIGIT that further comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or more) of amino acid positions 51, 52, 53, 54, 55, 73, 74, 75, 76, 77, 79, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93. In some embodiments, an anti-TIGIT antibody binds to an epitope on human TIGIT that further comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or more) of the following: Thr at position 51, Ala at position 52, Glu or Gln at position 53, Val at position 54, Thr at position 55, Leu at position 73, Gly at position 74, Trp at position 75, His at position 76, Val or Ile at position 77, Ser or Pro at position 79, Asp at position 83, Arg at position 84, Val at position 85, Val or Ala at position 86, Pro at position 87, Gly at position 88, Pro at position 89, Ser or Gly at position 90, Leu at position 91, Gly at position 92, or Leu at position 93. In some embodiments, an anti-TIGIT antibody binds to an epitope on human TIGIT that further comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or more) of the amino acid residues Thr51, Ala52, Gln53, Val54, Thr55, Leu73, Gly74, Trp75, His76, Ile77, Pro79, Asp83, Arg84, Val85, Ala86, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

[0090] In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys or Ser at position 82, and further comprises Thr at position 51, Ala at

position 52, Glu or Gln at position 53, Val at position 54, and/or Thr at position 55. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys or Ser at position 82, and further comprises Gly at position 74, Trp at position 75, His at position 76, and/or Val or Ile at position 77. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys or Ser at position 82, and further comprises Pro at position 87, Gly at position 88, Pro at position 89, Ser or Gly at position 90, Leu at position 91, Gly at position 92, and/or Leu at position 93. In some embodiments, an anti-TIGIT antibody recognizes an epitope comprising the amino acid residues Thr51, Ala52, Gln53, Val54, Thr55, Gly74, Trp75, His76, Ile77, Phe81, Lys82, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

[0091] In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys or Ser at position 82, and further comprises Ala at position 52 and/or Glu or Gln at position 53. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys or Ser at position 82, and further comprises Leu at position 73, Gly at position 74, and/or Trp at position 75. In some embodiments, an anti-TIGIT antibody recognizes an epitope that comprises Phe at position 81 and Lys or Ser at position 82, and further comprises Asp at position 83, Arg at position 84, Val at position 85, and/or Val or Ala at position 86. In some embodiments, an anti-TIGIT antibody recognizes an epitope comprising the amino acid residues Ala52, Gln53, Leu73, Gly74, Trp75, Pro79, Phe81, Lys82, Asp83, Arg84, Val85, and Ala86.

[0092] In some embodiments, an anti-TIGIT antibody recognizes an epitope of human TIGIT comprising the sequence ICNADLGWHISPSFK (SEQ ID NO:258), which corresponds to residues 68-82 of human TIGIT (SEQ ID NO:218). In some embodiments, an anti-TIGIT antibody recognizes an epitope of human TIGIT consisting of the sequence ICNADLGWHISPSFK (SEQ ID NO:258).

Certain Anti-TIGIT Antibody Sequences

[0093] In some embodiments, an anti-TIGIT antibody that binds to human TIGIT and that optionally exhibits cross-reactivity with cynomolgus monkey TIGIT and/or mouse TIGIT comprises a light chain variable region sequence, or a portion thereof, and/or a heavy chain variable region sequence, or a portion thereof, derived from any of the following antibodies described herein: Clone 2, Clone 2C, Clone 3, Clone 5, Clone 13, Clone 13A, Clone 13B, Clone 13C, Clone 13D, Clone 14, Clone 16, Clone 16C, Clone 16D, Clone 16E, Clone 18, Clone 21, Clone 22, Clone 25, Clone 25A, Clone 25B, Clone 25C, Clone 25D, Clone 25E, Clone 27, or Clone 54. The amino acid sequences of the CDR, light chain variable domain (VL), and heavy chain variable domain (VH) of the anti-TIGIT antibodies Clone 2, Clone 2C, Clone 3, Clone 5,

Clone 13, Clone 13A, Clone 13B, Clone 13C, Clone 13D, Clone 14, Clone 16, Clone 16C, Clone 16D, Clone 16E, Clone 18, Clone 21, Clone 22, Clone 25, Clone 25A, Clone 25B, Clone 25C, Clone 25D, Clone 25E, Clone 27, and Clone 54 are set forth in the Sequence Table below.

[0094] In some embodiments, an anti-TIGIT antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257. In some embodiments, an anti-TIGIT antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257. In some embodiments, a VH sequence having at least 90% sequence identity to a reference sequence (e.g., SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257) contains one, two, three, four, five, six, seven, eight, nine, ten or more substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence but retains the ability to bind to human TIGIT and optionally, retains the ability to block binding of CD155 and/or CD112 to TIGIT.

[0095] In some embodiments, an anti-TIGIT antibody comprises a light chain variable region (VL) comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208. In some embodiments, an anti-TIGIT antibody comprises a VL comprising the amino acid sequence of SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID

NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208. In some embodiments, a VL sequence having at least 90% sequence identity to a reference sequence (e.g., SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208) contains one, two, three, four, five, six, seven, eight, nine, ten or more substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence but retains the ability to bind to human TIGIT and optionally, retains the ability to block binding of CD155 and/or CD112 to TIGIT.

[0096] In some embodiments, an anti-TIGIT antibody comprises a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257, and further comprises a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208. In some embodiments, an anti-TIGIT antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and further comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208.

[0097] In some embodiments, an anti-TIGIT antibody comprises:

(a) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:1 or SEQ ID NO:245 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:10;

(b) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:19 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:28;

(c) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:37 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:46;

(d) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:64;

(e) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:73 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:82;

(f) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:100;

(g) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:109 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:118;

(h) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:127 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:136;

(i) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:145 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:154;

(j) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:172;

(k) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:181 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:190; or

(l) a VH comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:199 and a VL comprising an amino acid sequence that has at least 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to SEQ ID NO:208.

[0098] In some embodiments, an anti-TIGIT antibody comprises:

- (a) a VH comprising the amino acid sequence of SEQ ID NO:1 and a VL comprising the amino acid sequence of SEQ ID NO:10;
- (b) a VH comprising the amino acid sequence of SEQ ID NO:19 and a VL comprising the amino acid sequence of SEQ ID NO:28;
- (c) a VH comprising the amino acid sequence of SEQ ID NO:37 and a VL comprising the amino acid sequence of SEQ ID NO:46;
- (d) a VH comprising the amino acid sequence of SEQ ID NO:55 and a VL comprising the amino acid sequence of SEQ ID NO:64;
- (e) a VH comprising the amino acid sequence of SEQ ID NO:73 and a VL comprising the amino acid sequence of SEQ ID NO:82;
- (f) a VH comprising the amino acid sequence of SEQ ID NO:91 and a VL comprising the amino acid sequence of SEQ ID NO:100;
- (g) a VH comprising the amino acid sequence of SEQ ID NO:109 and a VL comprising the amino acid sequence of SEQ ID NO:118;
- (h) a VH comprising the amino acid sequence of SEQ ID NO:127 and a VL comprising the amino acid sequence of SEQ ID NO:136;
- (i) a VH comprising the amino acid sequence of SEQ ID NO:145 and a VL comprising the amino acid sequence of SEQ ID NO:154;
- (j) a VH comprising the amino acid sequence of SEQ ID NO:163 and a VL comprising the amino acid sequence of SEQ ID NO:172;
- (k) a VH comprising the amino acid sequence of SEQ ID NO:181 and a VL comprising the amino acid sequence of SEQ ID NO:190;
- (l) a VH comprising the amino acid sequence of SEQ ID NO:199 and a VL comprising the amino acid sequence of SEQ ID NO:208; or
- (m) a VH comprising the amino acid sequence of SEQ ID NO:245 and a VL comprising the amino acid sequence of SEQ ID NO:10; or
- (n) a VH comprising the amino acid sequence of SEQ ID NO:246 and a VL comprising the amino acid sequence of SEQ ID NO:64; or

- (o) a VH comprising the amino acid sequence of SEQ ID NO:247 and a VL comprising the amino acid sequence of SEQ ID NO:64; or
- (p) a VH comprising the amino acid sequence of SEQ ID NO:248 and a VL comprising the amino acid sequence of SEQ ID NO:64;
- (q) a VH comprising the amino acid sequence of SEQ ID NO:249 and a VL comprising the amino acid sequence of SEQ ID NO:64; or
- (r) a VH comprising the amino acid sequence of SEQ ID NO:250 and a VL comprising the amino acid sequence of SEQ ID NO:100; or
- (s) a VH comprising the amino acid sequence of SEQ ID NO:251 and a VL comprising the amino acid sequence of SEQ ID NO:100; or
- (t) a VH comprising the amino acid sequence of SEQ ID NO:252 and a VL comprising the amino acid sequence of SEQ ID NO:100; or
- (u) a VH comprising the amino acid sequence of SEQ ID NO:253 and a VL comprising the amino acid sequence of SEQ ID NO:172; or
- (v) a VH comprising the amino acid sequence of SEQ ID NO:254 and a VL comprising the amino acid sequence of SEQ ID NO:172; or
- (w) a VH comprising the amino acid sequence of SEQ ID NO:255 and a VL comprising the amino acid sequence of SEQ ID NO:172; or
- (x) a VH comprising the amino acid sequence of SEQ ID NO:256 and a VL comprising the amino acid sequence of SEQ ID NO:172; or
- (y) a VH comprising the amino acid sequence of SEQ ID NO:257 and a VL comprising the amino acid sequence of SEQ ID NO:172.

[0099] In some embodiments, an anti-TIGIT antibody comprises heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3, wherein one or more (e.g., one, two, three, four, five, or six) of the CDRs are selected from the heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 shown in Tables A, B, C, D, and E.

Table A: Clone 13 alternative CDR definitions

CDR definition	Heavy chain (HC) CDR1	SEQ ID NO	HC CDR2	SEQ ID NO	HC CDR3	SEQ ID NO
Composite	GTFSSY AIS	58	SIIPFGTANYAQKFQG	60	ARGPSEVGAILGYVWFDP	62
IMGT	GGTFSSYA	283	IIPFGTA	285	ARGPSEVGAILGYVWFDP	62
Kabat	SYAIS	284	SIIPFGTANYAQKFQG	60	GPSEVGAILGYVWFDP	286

CDR definition	Light chain (LC) CDR1	SEQ ID NO	LC CDR2	SEQ ID NO	LC CDR3	SEQ ID NO
Composite	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71
IMGT	QSLHLSNGYNY	287	LGS	288	MQARRIPIT	71
Kabat	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71

Table B: Clone 13A alternative CDR definitions

CDR definition	Heavy chain (HC) CDR1	SEQ ID NO	HC CDR2	SEQ ID NO	HC CDR3	SEQ ID NO
Composite	GTFLLSAIS	224	SLIPYFGTANYAQKFQG	225	ARGPSEVGAILGYVWFDP	62
IMGT	GGTFLLSA	293	LIPYFGTA	297	ARGPSEVGAILGYVWFDP	62
Kabat	SSAIS	294	SLIPYFGTANYAQKFQG	225	GPSEVGAILGYVWFDP	286

CDR definition	Light chain (LC) CDR1	SEQ ID NO	LC CDR2	SEQ ID NO	LC CDR3	SEQ ID NO
Composite	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71
IMGT	QSLHLSNGYNY	287	LGS	288	MQARRIPIT	71
Kabat	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71

Table C: Clone 13B alternative CDR definitions

CDR definition	Heavy chain (HC) CDR1	SEQ ID NO	HC CDR2	SEQ ID NO	HC CDR3	SEQ ID NO
Composite	GTFSAWAIS	226	SIIPYFGKANYAQKFQG	227	ARGPSEVSGILGYVWFDP	228
IMGT	GGTFSAWA	289	IIPYFGKA	291	ARGPSEVSGILGYVWFDP	228
Kabat	AWAIS	290	SIIPYFGKANYAQKFQG	227	GPSEVSGILGYVWFDP	292

CDR definition	Light chain (LC) CDR1	SEQ ID NO	LC CDR2	SEQ ID NO	LC CDR3	SEQ ID NO
Composite	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71
IMGT	QSLHLSNGYNY	287	LGS	288	MQARRIPIT	71
Kabat	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71

Table D: Clone 13C alternative CDR definitions

CDR definition	Heavy chain (HC) CDR1	SEQ ID NO	HC CDR2	SEQ ID NO	HC CDR3	SEQ ID NO
Composite	GTFLLSAIS	224	SIPLFGKANYAQKFQG	229	ARGPSEVKGILGYVWFDP	230
IMGT	GGTFLLSA	293	IPLFGKA	295	ARGPSEVKGILGYVWFDP	230
Kabat	SSAIS	294	SIPLFGKANYAQKFQG	229	GPSEVKGILGYVWFDP	296

CDR definition	Light chain (LC) CDR1	SEQ ID NO	LC CDR2	SEQ ID NO	LC CDR3	SEQ ID NO
Composite	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71
IMGT	QSLHLSNGYNY	287	LGS	288	MQARRIPIT	71
Kabat	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71

Table E: Clone 13D alternative CDR definitions

CDR definition	Heavy chain (HC) CDR1	SEQ ID NO	HC CDR2	SEQ ID NO	HC CDR3	SEQ ID NO
Composite	GTFLSSAIS	224	SIIPYFGKANYAQKFQG	227	ARGPSEVKGILGYVWFDP	230
IMGT	GGTFLSSA	293	IIPYFGKA	290	ARGPSEVKGILGYVWFDP	230
Kabat	SSAIS	294	SIIPYFGKANYAQKFQG	227	GPSEVKGILGYVWFDP	296
CDR definition	Light chain (LC) CDR1	SEQ ID NO	LC CDR2	SEQ ID NO	LC CDR3	SEQ ID NO
Composite	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71
IMGT	QSLHLSNGYNY	287	LGS	288	MQARRIPIT	71
Kabat	RSSQSLHLSNGYNYLD	67	LGSNRAS	69	MQARRIPIT	71

[00100] In some embodiments, an anti-TIGIT antibody comprises one or more (e.g., one, two, three, four, five, or six) of:

a heavy chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, and SEQ ID NO:290;

a heavy chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, and SEQ ID NO:295;

a heavy chain CDR3 sequence comprising an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:286, SEQ ID NO:228, SEQ ID NO:292, SEQ ID NO:230, and SEQ ID NO:296;

a light chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:67 and SEQ ID NO:287;

a light chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:69 and SEQ ID NO:288; and/or

a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00101] In some embodiments, an anti-TIGIT antibody comprises a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, or SEQ ID NO:290; a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, or SEQ ID NO:295; a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:62, SEQ ID NO:286, SEQ

ID NO:228, SEQ ID NO:292, SEQ ID NO:230, or SEQ ID NO:296; a light chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:67 or SEQ ID NO:287; a light chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:69 or SEQ ID NO:288; and a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00102] In some embodiments, an anti-TIGIT antibody comprises a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, or SEQ ID NO:290; a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, or SEQ ID NO:295; and a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:62, SEQ ID NO:286, SEQ ID NO:228, SEQ ID NO:292, SEQ ID NO:230, or SEQ ID NO:296.

[00103] In some embodiments, an anti-TIGIT antibody comprises a light chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:67 or SEQ ID NO:287; a light chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:69 or SEQ ID NO:288; and a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00104] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:58, SEQ ID NO:283, or SEQ ID NO:284; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:60 or SEQ ID NO:285; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:62 or SEQ ID NO:286; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:67 or SEQ ID NO:287; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:69 or SEQ ID NO:288; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00105] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:224, SEQ ID NO:293, or SEQ ID NO:294; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:225 or SEQ ID NO:297; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:62 or SEQ ID NO:286; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:67 or SEQ ID NO:287; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:69 or SEQ ID NO:288; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00106] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:226, SEQ ID NO:289, or SEQ ID NO:290; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:227 or SEQ ID NO:291; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:228 or SEQ ID NO:292; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:67 or SEQ ID NO:287; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:69 or SEQ ID NO:288; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00107] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:224, SEQ ID NO:293, or SEQ ID NO:294; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:229 or SEQ ID NO:295; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:230 or SEQ ID NO:296; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:67 or SEQ ID NO:287; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:69 or SEQ ID NO:288; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00108] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:224, SEQ ID NO:293, or SEQ ID NO:294; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:227 or SEQ ID NO:290; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:230 or SEQ ID NO:296; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:67 or SEQ ID NO:287; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:69 or SEQ ID NO:288; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00109] In some embodiments, an anti-TIGIT antibody comprises one or more (e.g., one, two, three, four, five, or six) of:

a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, or SEQ ID NO:290;

a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186,

SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, or SEQ ID NO:295;

a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134, SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, or SEQ ID NO:296;

a light chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, or SEQ ID NO:287;

a light chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, or SEQ ID NO:288; and/or

a light chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, or SEQ ID NO:215.

[00110] In some embodiments, an anti-TIGIT antibody comprises a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, or SEQ ID NO:290; a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186, SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, or SEQ ID NO:295; and a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134,

SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, or SEQ ID NO:296.

[00111] In some embodiments, an anti-TIGIT antibody comprises a light chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, or SEQ ID NO:287; a light chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, or SEQ ID NO:288; and a light chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, or SEQ ID NO:215.

[00112] In some embodiments, an anti-TIGIT antibody comprises:

(i) a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, or SEQ ID NO:290; and

(ii) a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186, SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, or SEQ ID NO:295; and

(iii) a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134, SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, or SEQ ID NO:296; and

(iv) a light chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, or SEQ ID NO:287; and

(v) a light chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, or SEQ ID NO:288; and

(vi) a light chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, or SEQ ID NO:215.

[00113] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:221; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:222; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:223; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:13; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:15; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:17.

[00114] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:58, SEQ ID NO:224, or SEQ ID NO:226; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:60, SEQ ID NO:225, SEQ ID NO:227, or SEQ ID NO:229; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:62, SEQ ID NO:228, or SEQ ID NO:230; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:67; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:69; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

[00115] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:94, SEQ ID NO:231, or SEQ ID NO:233; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:96, SEQ ID NO:232, or SEQ ID NO:234; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:98, SEQ ID NO:235, SEQ ID NO:236, or SEQ ID NO:237; (iv) a light chain CDR1 sequence comprising the amino acid

sequence of SEQ ID NO:103; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:105; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:107.

[00116] In some embodiments, an anti-TIGIT antibody comprises: (i) a heavy chain CDR1 sequence comprising the amino acid sequence of any of SEQ ID NO:166, SEQ ID NO:239, or SEQ ID NO:243; (ii) a heavy chain CDR2 sequence comprising the amino acid sequence of any of SEQ ID NO:168, SEQ ID NO:238, or SEQ ID NO:240; (iii) a heavy chain CDR3 sequence comprising the amino acid sequence of any of SEQ ID NO:170, SEQ ID NO:241, SEQ ID NO:242, or SEQ ID NO:244; (iv) a light chain CDR1 sequence comprising the amino acid sequence of SEQ ID NO:175; (v) a light chain CDR2 sequence comprising the amino acid sequence of SEQ ID NO:177; and (vi) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:179.

[00117] In some embodiments, an anti-TIGIT antibody comprises a heavy chain CDR1-3 and a light chain CDR1-3 comprising the amino acid sequences of:

- (a) SEQ ID NOs: 4, 6, 8, 13, 15, and 17, respectively;
- (b) SEQ ID NOs: 22, 24, 26, 31, 33, and 35, respectively;
- (c) SEQ ID NOs: 40, 42, 44, 49, 51, and 53, respectively;
- (d) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively;
- (e) SEQ ID NOs: 76, 78, 80, 85, 87, and 89, respectively;
- (f) SEQ ID NOs: 94, 96, 98, 103, 105, and 107, respectively;
- (g) SEQ ID NOs: 112, 114, 116, 121, 123, and 125, respectively;
- (h) SEQ ID NOs: 130, 132, 134, 139, 141, and 143, respectively;
- (i) SEQ ID NOs: 148, 150, 152, 157, 159, and 161, respectively;
- (j) SEQ ID NOs: 166, 168, 170, 175, 177, and 179, respectively;
- (k) SEQ ID NOs: 184, 186, 188, 193, 195, and 197, respectively;
- (l) SEQ ID NOs: 202, 204, 206, 211, 213, and 215, respectively; or
- (m) SEQ ID NOs: 221, 222, 223, 13, 15, and 17, respectively; or
- (n) SEQ ID NOs: 224, 225, 62, 67, 69, and 71, respectively; or
- (o) SEQ ID NOs: 226, 227, 228, 67, 69, and 71, respectively; or
- (p) SEQ ID NOs: 224, 229, 230, 67, 69, and 71, respectively; or
- (q) SEQ ID NOs: 224, 227, 230, 67, 69, and 71, respectively; or
- (r) SEQ ID NOs: 231, 232, 235, 103, 105, and 107, respectively; or
- (s) SEQ ID NOs: 233, 234, 236, 103, 105, and 107, respectively; or
- (t) SEQ ID NOs: 233, 234, 237, 103, 105, and 107, respectively; or
- (u) SEQ ID NOs: 166, 238, 170, 175, 177, and 179, respectively; or

- (v) SEQ ID NOs: 239, 240, 170, 175, 177, and 179, respectively; or
- (w) SEQ ID NOs: 239, 240, 241, 175, 177, and 179, respectively; or
- (x) SEQ ID NOs: 239, 240, 242, 175, 177, and 179, respectively; or
- (y) SEQ ID NOs: 243, 168, 244, 175, 177, and 179, respectively.

[00118] In some embodiments, an anti-TIGIT antibody comprises one or more heavy chain framework regions (FR1, FR2, FR3, and/or FR4) comprising an amino acid sequence of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, or SEQ ID NO:207.

[00119] In some embodiments, an anti-TIGIT antibody comprises one or more light chain framework regions (FR1, FR2, FR3, and/or FR4) comprising an amino acid sequence of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:138, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, or SEQ ID NO:216.

[00120] In some embodiments, an anti-TIGIT antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

[00121] In some embodiments, the anti-TIGIT antibodies of the instant disclosure do not compete for binding with the antibodies described in US 2009/0258013, US 2016/0176963, US 2016/0376365, or WO 2016/028656. In some embodiments, the anti-TIGIT antibodies of the instant disclosure do not bind to the same epitope as antibodies described in US 2009/0258013, US 2016/0176963, US 2016/0376365, or WO 2016/028656.

Preparation of Antibodies

[00122] For preparing an antibody that binds to TIGIT, many techniques known in the art can be used. *See, e.g., Kohler & Milstein, Nature 256:495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985); Coligan, Current Protocols in Immunology (1991); Harlow & Lane, Antibodies, A Laboratory Manual (1988); and Goding, Monoclonal Antibodies: Principles and Practice (2nd ed. 1986)).*

[00123] The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, *e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma that expresses the antibody and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Additionally, phage or yeast display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., Nature 348:552-554 (1990); Marks et al., Biotechnology 10:779-783 (1992); Lou et al. (2010) PEDS 23:311; and Chao et al., Nature Protocols, 1:755-768 (2006)). Alternatively, antibodies and antibody sequences may be isolated and/or identified using a yeast-based antibody presentation system, such as that disclosed in, e.g., Xu et al., Protein Eng Des Sel, 2013, 26:663-670; WO 2009/036379; WO 2010/105256; and WO 2012/009568. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, Immunology (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can also be adapted to produce antibodies. Antibodies can also be made bispecific, i.e., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker et al., EMBO J. 10:3655-3659 (1991); and Suresh et al., Methods in Enzymology 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or antibodies covalently bound to immunotoxins (see, e.g., U.S. Patent No. 4,676,980, WO 91/00360; and WO 92/200373).*

[00124] Antibodies can be produced using any number of expression systems, including prokaryotic and eukaryotic expression systems. In some embodiments, the expression system is a mammalian cell, such as a hybridoma, or a CHO cell. Many such systems are widely available from commercial suppliers. In embodiments in which an antibody comprises both a heavy chain and light chain, the heavy chain and heavy chain and light chain may be expressed using a single vector, *e.g., in a di-cistronic expression unit, or be under the control of different promoters. In other embodiments, the heavy chain and light chain region may be expressed using separate*

vectors. Heavy chains and light chains as described herein may optionally comprise a methionine at the N-terminus.

[00125] In some embodiments, antibody fragments (such as a Fab, a Fab', a F(ab')₂, a scFv, or a diabody) are generated. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g., Morimoto et al., J. Biochem. Biophys. Meth.*, 24:107-117 (1992); and Brennan *et al., Science*, 229:81 (1985)). However, these fragments can now be produced directly using recombinant host cells. For example, antibody fragments can be isolated from antibody phage libraries. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* cells and chemically coupled to form F(ab')₂ fragments (*see, e.g., Carter et al., BioTechnology*, 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to those skilled in the art. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). *See, e.g.,* PCT Publication No. WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. The antibody fragment may also be a linear antibody as described, *e.g.,* in U.S. Patent No. 5,641,870.

[00126] In some embodiments, the antibody or antibody fragment can be conjugated to another molecule, *e.g.,* polyethylene glycol (PEGylation) or serum albumin, to provide an extended half-life *in vivo*. Examples of PEGylation of antibody fragments are provided in Knight *et al. Platelets* 15:409, 2004 (for abciximab); Pedley *et al., Br. J. Cancer* 70:1126, 1994 (for an anti-CEA antibody); Chapman *et al., Nature Biotech.* 17:780, 1999; and Humphreys, *et al., Protein Eng. Des.* 20: 227, 2007).

[00127] In some embodiments, multispecific antibodies comprising an anti-TIGIT antibody as described herein are provided, *e.g.,* a bispecific antibody. Multispecific antibodies are antibodies that have binding specificities for at least two different sites. In some embodiments, the multispecific antibody has a binding specificity for TIGIT (*e.g.,* human TIGIT) and has a binding specificity for at least one other antigen. Methods for making multispecific antibodies include, but are not limited to, recombinant co-expression of two pairs of heavy chain and light chain in a host cell (*see, e.g., Zuo et al., Protein Eng Des Sel*, 2000, 13:361-367); "knobs-into-holes" engineering (*see, e.g., Ridgway et al., Protein Eng Des Sel*, 1996, 9:617-721); "diabody" technology (*see, e.g., Hollinger et al., PNAS (USA)*, 1993, 90:6444-6448); and intramolecular trimerization (*see, e.g., Alvarez-Cienfuegos et al., Scientific Reports*, 2016, doi:10.1038/srep28643); *See also, Spiess et al., Molecular Immunology*, 2015, 67(2), Part A:95-106.

[00128] In some embodiments, antibody-drug conjugates comprising an anti-TIGIT antibody as described herein are provided. In antibody-drug conjugates, a monoclonal antibody having a binding specificity for an antigen (e.g., TIGIT) is covalently linked to a cytotoxic drug. Methods for making antibody-drug conjugates are described, e.g., in Chudasama et al., *Nature Chemistry*, 2016, 8:114-119; WO 2013/068874; and US 8,535,678.

Selection of Constant Region

[00129] Heavy and light chain variable regions of the anti-TIGIT antibodies described herein can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent cell-mediated cytotoxicity, antibody dependent cellular phagocytosis and/or complement dependent cytotoxicity are desired. For example, human isotopes IgG1 and IgG3 have strong complement-dependent cytotoxicity, human isotype IgG2 weak complement-dependent cytotoxicity and human IgG4 lacks complement-dependent cytotoxicity. Human IgG1 and IgG3 also induce stronger cell mediated effector functions than human IgG2 and IgG4. Light chain constant regions can be lambda or kappa. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')₂, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

[00130] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype binds to a non-polymorphic region of one or more other isotypes.

[00131] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (see, e.g., Winter et al., US Patent No. 5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005, 2006), or to prolong half-life in humans (see, e.g., Hinton et al., J. Biol. Chem. 279:6213, 2004).

[00132] Exemplary substitution include the amino acid substitution of the native amino acid to a cysteine residue is introduced at amino acid position 234, 235, 237, 239, 267, 298, 299, 326, 330, or 332, preferably an S239C mutation in a human IgG1 isotype (numbering is according to the EU index (Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991); see US 20100158909, which is herein incorporated reference). The presence of an additional cysteine residue may allow interchain disulfide bond formation. Such interchain disulfide bond formation can cause steric hindrance, thereby

reducing the affinity of the Fc region-Fc γ R binding interaction. The cysteine residue(s) introduced in or in proximity to the Fc region of an IgG constant region can also serve as sites for conjugation to therapeutic agents (i.e., coupling cytotoxic drugs using thiol specific reagents such as maleimide derivatives of drugs. The presence of a therapeutic agent causes steric hindrance, thereby further reducing the affinity of the Fc region-Fc γ R binding interaction. Other substitutions at any of positions 234, 235, 236 and/or 237 reduce affinity for Fc γ receptors, particularly Fc γ RI receptor (*see, e.g.*, US 6,624,821, US 5,624,821.) A preferred combination of mutations is S239D, A330L and I332E, which increases the affinity of the Fc domain for Fc γ RIIIA and consequently increases ADCC.

[00133] The in vivo half-life of an antibody can also impact its effector functions. The half-life of an antibody can be increased or decreased to modify its therapeutic activities. FcRn is a receptor that is structurally similar to MHC Class I antigen that non-covalently associates with β 2-microglobulin. FcRn regulates the catabolism of IgGs and their transcytosis across tissues (Ghetie and Ward, 2000, *Annu. Rev. Immunol.* 18:739-766; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113). The IgG-FcRn interaction takes place at pH 6.0 (pH of intracellular vesicles) but not at pH 7.4 (pH of blood); this interaction enables IgGs to be recycled back to the circulation (Ghetie and Ward, 2000, *Ann. Rev. Immunol.* 18:739-766; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113). The region on human IgG1 involved in FcRn binding has been mapped (Shields *et al.*, 2001, *J. Biol. Chem.* 276:6591-604). Alanine substitutions at positions Pro238, Thr256, Thr307, Gln311, Asp312, Glu380, Glu382, or Asn434 of human IgG1 enhance FcRn binding (Shields *et al.*, 2001, *J. Biol. Chem.* 276:6591-604). IgG1 molecules harboring these substitutions have longer serum half-lives. Consequently, these modified IgG1 molecules may be able to carry out their effector functions, and hence exert their therapeutic efficacies, over a longer period of time compared to unmodified IgG1. Other exemplary substitutions for increasing binding to FcRn include a Gln at position 250 and/or a Leu at position 428. EU numbering is used for all positions in the constant region.

[00134] Oligosaccharides covalently attached to the conserved Asn297 are involved in the ability of the Fc region of an IgG to bind Fc γ R (Lund *et al.*, 1996, *J. Immunol.* 157:4963-69; Wright and Morrison, 1997, *Trends Biotechnol.* 15:26-31). Engineering of this glycoform on IgG can significantly improve IgG-mediated ADCC. Addition of bisecting N-acetylglucosamine modifications (Umana *et al.*, 1999, *Nat. Biotechnol.* 17:176-180; Davies *et al.*, 2001, *Biotech. Bioeng.* 74:288-94) to this glycoform or removal of fucose (Shields *et al.*, 2002, *J. Biol. Chem.* 277:26733-40; Shinkawa *et al.*, 2003, *J. Biol. Chem.* 278:6591-604; Niwa *et al.*, 2004, *Cancer Res.* 64:2127-33) from this glycoform are two examples of IgG Fc

engineering that improves the binding between IgG Fc and FcγR, thereby enhancing Ig-mediated ADCC activity.

[00135] A systemic substitution of solvent-exposed amino acids of human IgG1 Fc region has generated IgG variants with altered FcγR binding affinities (Shields *et al.*, 2001, *J. Biol. Chem.* 276:6591-604). When compared to parental IgG1, a subset of these variants involving substitutions at Thr256/Ser298, Ser298/Glu333, Ser298/Lys334, or Ser298/Glu333/Lys334 to Ala demonstrate increased in both binding affinity toward FcγR and ADCC activity (Shields *et al.*, 2001, *J. Biol. Chem.* 276:6591-604; Okazaki *et al.*, 2004, *J. Mol. Biol.* 336:1239-49).

[00136] Complement fixation activity of antibodies (both C1q binding and CDC activity) can be improved by substitutions at Lys326 and Glu333 (Idusogie *et al.*, 2001, *J. Immunol.* 166:2571-2575). The same substitutions on a human IgG2 backbone can convert an antibody isotype that binds poorly to C1q and is severely deficient in complement activation activity to one that can both bind C1q and mediate CDC (Idusogie *et al.*, 2001, *J. Immunol.* 166:2571-75). Several other methods have also been applied to improve complement fixation activity of antibodies. For example, the grafting of an 18-amino acid carboxyl-terminal tail piece of IgM to the carboxyl-termini of IgG greatly enhances their CDC activity. This is observed even with IgG4, which normally has no detectable CDC activity (Smith *et al.*, 1995, *J. Immunol.* 154:2226-36). Also, substituting Ser444 located close to the carboxy-terminal of IgG1 heavy chain with Cys induced tail-to-tail dimerization of IgG1 with a 200-fold increase of CDC activity over monomeric IgG1 (Shopes *et al.*, 1992, *J. Immunol.* 148:2918-22). In addition, a bispecific diabody construct with specificity for C1q also confers CDC activity (Kontermann *et al.*, 1997, *Nat. Biotech.* 15:629-31).

[00137] Complement activity can be reduced by mutating at least one of the amino acid residues 318, 320, and 322 of the heavy chain to a residue having a different side chain, such as Ala. Other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or Val, or such aromatic non-polar residues as Phe, Tyr, Trp and Pro in place of any one of the three residues also reduce or abolish C1q binding. Ser, Thr, Cys, and Met can be used at residues 320 and 322, but not 318, to reduce or abolish C1q binding activity. Replacement of the 318 (Glu) residue by a polar residue may modify but not abolish C1q binding activity. Replacing residue 297 (Asn) with Ala results in removal of lytic activity but only slightly reduces (about three fold weaker) affinity for C1q. This alteration destroys the glycosylation site and the presence of carbohydrate that is required for complement activation. Any other substitution at this site also destroys the glycosylation site. The following mutations and any combination thereof also reduce C1q binding: D270A, K322A, P329A, and P311S (see WO 06/036291).

[00138] Reference to a human constant region includes a constant region with any natural allotype or any permutation of residues occupying polymorphic positions in natural allotypes. Also, up to 1, 2, 5, or 10 mutations may be present relative to a natural human constant region, such as those indicated above to reduce Fcγ receptor binding or increase binding to FcRN.

Nucleic Acids, Vectors, and Host Cells

[00139] In some embodiments, the anti-TIGIT antibodies as described herein are prepared using recombinant methods. Accordingly, in some aspects, the invention provides isolated nucleic acids comprising a nucleic acid sequence encoding any of the anti-TIGIT antibodies as described herein (e.g., any one or more of the CDRs described herein); vectors comprising such nucleic acids; and host cells into which the nucleic acids are introduced that are used to replicate the antibody-encoding nucleic acids and/or to express the antibodies. In some embodiments, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell; or a human cell.

[00140] In some embodiments, a polynucleotide (e.g., an isolated polynucleotide) comprises a nucleotide sequence encoding an antibody described herein. In some embodiments, the polynucleotide comprises a nucleotide sequence encoding one or more amino acid sequences (e.g., CDR, heavy chain, light chain, and/or framework regions) disclosed in the Sequence Table. In some embodiments, the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence having at least 85% sequence identity (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a sequence (e.g., a CDR, heavy chain, light chain, or framework region sequence) disclosed in the Sequence Table below.

[00141] In some embodiments, a polynucleotide (e.g., an isolated polynucleotide) comprises a nucleotide sequence encoding a heavy chain variable region as described herein. In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257. In some embodiments, the polynucleotide comprises a nucleotide sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, SEQ ID NO:74, SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, SEQ ID NO:182, SEQ ID NO:200, SEQ ID NO:259, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, or SEQ ID NO:271.

[00142] In some embodiments, a polynucleotide (e.g., an isolated polynucleotide) comprises a nucleotide sequence encoding a light chain variable region as described herein. In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208. In some embodiments, the polynucleotide comprises a nucleotide sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:11, SEQ ID NO:29, SEQ ID NO:47, SEQ ID NO:65, SEQ ID NO:83, SEQ ID NO:101, SEQ ID NO:119, SEQ ID NO:137, SEQ ID NO:155, SEQ ID NO:173, SEQ ID NO:191, SEQ ID NO:209, or SEQ ID NO: 273.

[00143] In some embodiments, the polynucleotide comprises a nucleotide sequence encoding comprises a nucleotide sequence encoding a heavy chain variable region and a light chain variable region as described herein. In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and encoding a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208. In some embodiments, the polynucleotide comprises a nucleotide sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, SEQ ID NO:74, SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, SEQ ID NO:182, SEQ ID NO:200, SEQ ID NO:259, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, or SEQ ID NO:271, and further comprises a nucleotide sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:11, SEQ ID NO:29, SEQ ID NO:47, SEQ ID NO:65, SEQ ID NO:83, SEQ ID NO:101, SEQ ID NO:119, SEQ ID NO:137, SEQ ID NO:155, SEQ ID NO:173, SEQ ID NO:191, SEQ ID NO:209, or SEQ ID NO: 273.

[00144] In a further aspect, methods of making an anti-TIGIT antibody as described herein are provided. In some embodiments, the method includes culturing a host cell as described

herein (e.g., a host cell expressing a polynucleotide or vector as described herein) under conditions suitable for expression of the antibody. In some embodiments, the antibody is subsequently recovered from the host cell (or host cell culture medium).

[00145] Suitable vectors containing polynucleotides encoding antibodies of the present disclosure, or fragments thereof, include cloning vectors and expression vectors. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mpl8, mpl9, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. Cloning vectors are available from commercial vendors such as BioRad, Stratagene, and Invitrogen.

[00146] Expression vectors generally are replicable polynucleotide constructs that contain a nucleic acid of the present disclosure. The expression vector may replicate in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, and any other vector.

Expression of Recombinant Antibodies

[00147] Antibodies are typically produced by recombinant expression. Recombinant polynucleotide constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies.

[00148] Mammalian cells are a preferred host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, *From Genes to Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines (e.g., DG44), various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and

transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See Co et al., *J. Immunol.* 148:1149 (1992).

[00149] Once expressed, antibodies can be purified according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis and the like (see generally, Scopes, *Protein Purification* (Springer-Verlag, NY, 1982)).

Glycosylation Variants

[00150] Antibodies may be glycosylated at conserved positions in their constant regions (Jefferis and Lund, (1997) *Chem. Immunol.* 65:111-128; Wright and Morrison, (1997) *TibTECH* 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd *et al.*, (1996) *Mol. Immunol.* 32:1311-1318; Wittwe and Howard, (1990) *Biochem.* 29:4175-4180), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner, (1996) *Current Opin. Biotech.* 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra *et al.*, (1995) *Nature Med.* 1:237-243). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd *et al.*, (1996) *Mol. Immunol.* 32:1311-1318), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana *et al.* (1999) *Mature Biotech.* 17:176-180).

[00151] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino

acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[00152] Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc.

[00153] Addition of glycosylation sites to the antibody can be accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of the antibody.

[00154] The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. These methods include isolation from a natural source (in the case of naturally-occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

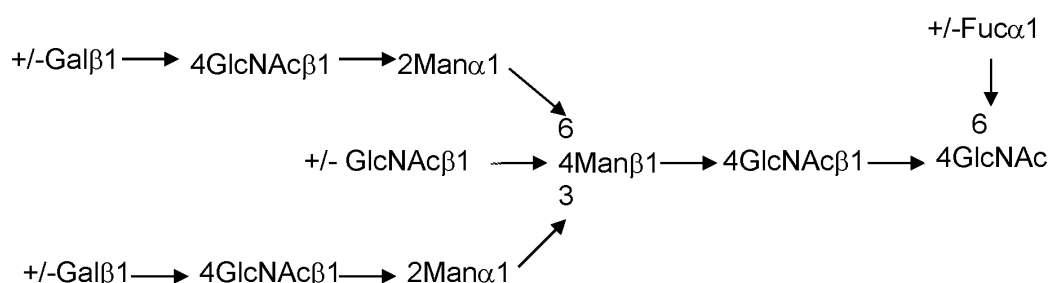
[00155] The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the amino acid sequence or the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, *e.g.*, antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected. See, *e.g.*, Hse *et al.*, (1997) J. Biol. Chem. 272:9062-9070. In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Patent Nos. 5047335; 5510261; 5278299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, *e.g.*, make defective in processing certain types of polysaccharides. These and similar techniques are known in the art.

[00156] The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic

digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo- β -galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides

[00157] A preferred form of modification of glycosylation of antibodies is reduced core fucosylation. “Core fucosylation” refers to addition of fucose (“fucosylation”) to N-acetylglucosamine (“GlcNAc”) at the reducing terminal of an N-linked glycan.

[00158] A “complex N-glycoside-linked sugar chain” is typically bound to asparagine 297 (according to the number of Kabat). As used herein, the complex N-glycoside-linked sugar chain has a biantennary composite sugar chain, mainly having the following structure:



where $\pm$ indicates the sugar molecule can be present or absent, and the numbers indicate the position of linkages between the sugar molecules. In the above structure, the sugar chain terminal which binds to asparagine is called a reducing terminal (at right), and the opposite side is called a non-reducing terminal. Fucose is usually bound to N-acetylglucosamine (“GlcNAc”) of the reducing terminal, typically by an $\alpha 1,6$ bond (the 6-position of GlcNAc is linked to the 1-position of fucose). “Gal” refers to galactose, and “Man” refers to mannose.

[00159] A “complex N-glycoside-linked sugar chain” includes 1) a complex type, in which the non-reducing terminal side of the core structure has zero, one or more branches of galactose-N-acetylglucosamine (also referred to as “gal-GlcNAc”) and the non-reducing terminal side of gal-GlcNAc optionally has a sialic acid, bisecting N-acetylglucosamine or the like; and 2) a hybrid type, in which the non-reducing terminal side of the core structure has both branches of a high mannose N-glycoside-linked sugar chain and complex N-glycoside-linked sugar chain.

[00160] According to the present methods, typically only a minor amount of fucose is incorporated into the complex N-glycoside-linked sugar chain(s) of the anti-TIGIT antibodies. For example, in various embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less

than about 5%, or less than about 3% of the antibodies in a composition have core fucosylation by fucose. In some embodiments, about 2% of the antibodies in the composition have core fucosylation by fucose. In various embodiments, when less than 60% of the antibodies in a composition have core fucosylation by fucose, the antibodies of the composition may be referred to as “nonfucosylated” or “afucosylated.” In some embodiments, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.

[00161] In certain embodiments, only a minor amount of a fucose analog (or a metabolite or product of the fucose analog) is incorporated into the complex N-glycoside-linked sugar chain(s). For example, in various embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3% of anti-TIGIT antibodies have core fucosylation by a fucose analog or a metabolite or product of the fucose analog. In some embodiments, about 2% of the anti-TIGIT antibodies have core fucosylation by a fucose analog or a metabolite or product of the fucose analog.

[00162] In some embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3% of the antibodies in a composition have a fucose residue on a G0, G1, or G2 glycan structure. (*See, e.g.*, Raju et al., 2012, MAbs 4: 385-391, Figure 3.) In some embodiments, about 2% of the antibodies in the composition have a fucose residue on a G0, G1, or G2 glycan structure. In various embodiments, when less than 60% of the antibodies in a composition have a fucose residue on a G0, G1, or G2 glycan structure, the antibodies of the composition may be referred to as “afucosylated.” In some embodiments, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition lack fucose on a G0, G1, or G2 glycan structure. It should be noted that G0 glycans include G0-GN glycans. G0-GN glycans are monoantennary glycans with one terminal GlcNAc residue. G1 glycans include G1-GN glycans. G1-GN glycans are monoantennary glycans with one terminal galactose residue. G0-GN and G1-GN glycans can be fucosylated or non-fucosylated.

[00163] Methods of making afucosylated antibodies by incubating antibody-producing cells with a fucose analogue are described, e.g., in WO2009/135181. Briefly, cells that have been engineered to express anti-TIGIT antibodies are incubated in the presence of a fucose analogue or an intracellular metabolite or product of the fucose analog. An intracellular metabolite can be, for example, a GDP-modified analog or a fully or partially de-esterified analog. A product can be, for example, a fully or partially de-esterified analog. In some embodiments, a fucose

analogue can inhibit an enzyme(s) in the fucose salvage pathway. For example, a fucose analog (or an intracellular metabolite or product of the fucose analog) can inhibit the activity of fucokinase, or GDP-fucose-pyrophosphorylase. In some embodiments, a fucose analog (or an intracellular metabolite or product of the fucose analog) inhibits fucosyltransferase (preferably a 1,6-fucosyltransferase, *e.g.*, the FUT8 protein). In some embodiments, a fucose analog (or an intracellular metabolite or product of the fucose analog) can inhibit the activity of an enzyme in the de novo synthetic pathway for fucose. For example, a fucose analog (or an intracellular metabolite or product of the fucose analog) can inhibit the activity of GDP-mannose 4,6-dehydratase or/or GDP-fucose synthetase. In some embodiments, the fucose analog (or an intracellular metabolite or product of the fucose analog) can inhibit a fucose transporter (*e.g.*, GDP-fucose transporter).

[00164] In one embodiment, the fucose analogue is 2-fluorofucose. Methods of using fucose analogues in growth medium and other fucose analogues are disclosed, *e.g.*, in WO 2009/135181, which is herein incorporated by reference.

[00165] Other methods for engineering cell lines to reduce core fucosylation included gene knock-outs, gene knock-ins and RNA interference (RNAi). In gene knock-outs, the gene encoding FUT8 (alpha 1,6- fucosyltransferase enzyme) is inactivated. FUT8 catalyzes the transfer of a fucosyl residue from GDP-fucose to position 6 of Asn-linked (N-linked) GlcNAc of an N-glycan. FUT8 is reported to be the only enzyme responsible for adding fucose to the N-linked biantennary carbohydrate at Asn297. Gene knock-ins add genes encoding enzymes such as GNTIII or a golgi alpha mannosidase II. An increase in the levels of such enzymes in cells diverts monoclonal antibodies from the fucosylation pathway (leading to decreased core fucosylation), and having increased amount of bisecting N-acetylglucosamines. RNAi typically also targets FUT8 gene expression, leading to decreased mRNA transcript levels or knocking out gene expression entirely. Any of these methods can be used to generate a cell line that would be able to produce an afucosylated antibody, *e.g.*, a anti-TIGIT antibody.

[00166] Many methods are available to determine the amount of fucosylation on an antibody. Methods include, *e.g.*, LC-MS via PLRP-S chromatography, electrospray ionization quadrupole TOF MS, Capillary Electrophoresis with Laser-Induced Fluorescence (*CE-LIF*) and , Hydrophilic Interaction Chromatography with Fluorescence Detection (HILIC).

IV. Therapeutic Methods Using Anti-TIGIT Antibodies

[00167] In some embodiments, methods for treating or preventing a cancer in a subject are provided. In some embodiments, the method comprises administering to the subject a therapeutic amount of an anti-TIGIT antibody. In some embodiments, the anti-TIGIT antibody

is afucosylated. In some embodiments, the method comprises administering to the subject a therapeutic amount of a pharmaceutical composition comprising anti-TIGIT antibodies, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated. In some embodiments, the subject is a human.

[00168] In some embodiments, the cancer is a cancer or cancer cell that is enriched for expression of CD112 and/or CD155. In some embodiments, CD112- and/or CD155-enriched cancers are identified by immunohistochemistry assessment of tumor samples using antibodies specific for CD112 or CD155. In some embodiments, CD112 or CD155 expression is enriched or increased in tumor cells or in tumor infiltrating leukocytes. In some embodiments, the cancer is identified based on the assessment of CD112 and/or CD155 mRNA levels in tumor samples (e.g., by methods known in the art such as quantitative RT-PCR). In some embodiments, measurements of soluble CD112 or CD155 in blood samples obtained from cancer patients may be used to identify a cancer that is enriched for expression of CD112 and/or CD155. In some embodiments, the method comprises obtaining a sample from a subject (e.g., a tumor sample or a blood sample), measuring the level of CD112 and/or CD155 in the sample from the subject, and comparing the level of CD112 and/or CD155 in the sample from the subject to a control value (e.g., a sample from a healthy control subject or a level of CD112 and/or CD155 expression determined for a population of healthy controls). In some embodiments, the method comprises determining that the level of CD112 and/or CD155 in the sample from the subject is higher than a control value, and subsequently administering to the subject an anti-TIGIT antibody as described herein.

[00169] In some embodiments, the cancer is a cancer or cancer cell that is enriched for T cells or natural killer (NK) cells that express TIGIT. In some embodiments, TIGIT-enriched cancers are identified by immunohistochemistry assessment of tumor samples using antibodies specific for TIGIT. In some embodiments, an antibody that is specific for T cells or NK cells (e.g., anti-CD3, anti-CD4, anti-CD8, anti-CD25, or anti-CD56) is used to determine a subset or subsets of tumor infiltrating cells that express TIGIT. In some embodiments, the cancer is identified based on the assessment of TIGIT mRNA levels in tumor samples. In some embodiments, measurements of soluble TIGIT in blood samples obtained from cancer patients may be used (optionally in combination with an antibody that is specific for T cells or NK cells) to identify a cancer that is enriched for T cells or NK cells that express TIGIT. In some embodiments, the method comprises obtaining a sample from a subject (e.g., a tumor sample or a blood sample), measuring the level of TIGIT in the sample from the subject, optionally detecting the presence of T cells or NK cells (e.g., using an antibody that is specific for T cells or NK cells such as anti-

CD3, anti-CD4, anti-CD8, anti-CD25, or anti-CD56) and comparing the level of TIGIT in the sample from the subject to a control value (e.g., a sample from a healthy control subject or a level of TIGIT expression determined for a population of healthy controls). In some embodiments, the method comprises determining that the level of TIGIT in the sample from the subject is higher than a control value, and subsequently administering to the subject an anti-TIGIT antibody as described herein.

[00170] In some embodiments, the cancer is bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, gastric cancer, pancreatic cancer, colorectal cancer, colon cancer, kidney cancer, clear cell renal carcinoma, head and neck cancer, lung cancer, lung adenocarcinoma, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, melanoma, neoplasm of the central nervous system, mesothelioma, lymphoma, leukemia, chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, Hodgkin lymphoma, myeloma, or sarcoma. In some embodiments, the cancer is selected from gastric cancer, testicular cancer, pancreatic cancer, lung adenocarcinoma, bladder cancer, head and neck cancer, prostate cancer, breast cancer, mesothelioma, and clear cell renal carcinoma. In some embodiments, the cancer is a lymphoma or a leukemia, including but not limited to acute myeloid, chronic myeloid, acute lymphocytic or chronic lymphocytic leukemia, diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, small lymphocytic lymphoma, primary mediastinal large B-cell lymphoma, splenic marginal zone B-cell lymphoma, or extranodal marginal zone B-cell lymphoma. In some embodiments, the cancer is selected from chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, and Hodgkin lymphoma. In some embodiments, the cancer is a metastatic cancer.

[00171] In some embodiments, the method further comprises administering to the subject a therapeutic amount of an additional therapeutic agent. In some embodiments, the additional therapeutic agent is an immuno-oncology agent. In some embodiments, the immuno-oncology agent is an agent (e.g., an antibody, small molecule, or peptide) that antagonizes or inhibits a component of an immune checkpoint pathway, such as the PD-1 pathway, the CTLA-4 pathway, the Lag3 pathway, or the TIM-3 pathway. In some embodiments, the immuno-oncology agent is an agonist of a T cell coactivator (i.e., an agonist of a protein that stimulates T cell activation) by targeting the OX-40 pathway, the 4-1BB (CD137) pathway, the CD27 pathway, the ICOS pathway, or the GITR pathway.

[00172] In some embodiments, the immuno-oncology agent is a PD-1 pathway inhibitor. In some embodiments, the PD-1 pathway inhibitor is an anti-PD-1 antibody or anti-PD-L1 antibody, such as but not limited to pembrolizumab, nivolumab, durvalumab, pidilizumab,

avelumab, or atezolizumab. PD-1 pathway inhibitors are described in the art. See, e.g., Dolan et al., *Cancer Control*, 2014, 21:231-237; Luke et al., *Oncotarget*, 2014, 6:3479-3492; US 2016/0222113; US 2016/0272708; US 2016/0272712; and US 2016/0319019.

[00173] In some embodiments, the immuno-oncology agent is an agonist of a T cell coactivator. In some embodiments, the immuno-oncology agent is an agonist of CD28, CD28H, CD3, 4-1BB (CD137), ICOS, OX40, GITR, CD27, or CD40. In some embodiments, the immuno-oncology agent is an immune stimulatory cytokine. In some embodiments, the immune stimulatory cytokine is granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 3 (IL-3), interleukin 12 (IL-12), interleukin 15 (IL-15), or interferon gamma (IFN- γ). In some embodiments, the immuno-oncology agent is SGN-2FF (Seattle Genetics; *see, e.g.*, WO 2009/135181 A2, WO 2012/019165 A2, and WO 2017/096274 A1).

[00174] In some embodiments, the additional therapeutic agent is selected from an anti-CD25 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-Tim3 antibody, anti-Lag3 antibody, anti-CTLA4 antibody, anti-41BB antibody, anti-OX40 antibody, anti-CD3 antibody, anti-CD40 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-STING antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-HER2 antibody, an anti-EGFR antibody, an anti-PDGFR- α -antibody, an anti-SLAMF7 antibody, an anti-VEGF antibody, an anti-CTLA-4 antibody, an anti-CD20 antibody, an anti-cCLB8 antibody, an anti-KIR antibody, and an anti-CD52 antibody. In some embodiments, the additional therapeutic agent is selected from SEA-CD40 (Seattle Genetics; *see, e.g.*, WO 2006/128103 A2 and WO 2016/069919 A1), avelumab, durvalumab, nivolumab, pembrolizumab, pidilizumab, atezolizumab, Hul4.18K322A (anti-GD2 antibody, St. Jude), Hu3F8 (anti-GD2 antibody, MSKCC), dinituximab, trastuzumab, cetuximab, olaratumab, necitumumab, elotuzumab, ramucirumab, pertuzumab, ipilimumab, bevacizumab, rituximab, obinutuzumab, siltuximab, ofatumumab, and alemtuzumab.

[00175] In some embodiments, treatment with an anti-TIGIT antibody as described herein is combined with one or more other anti-cancer treatments, such as surgery or radiation. In some embodiments, treatment with an anti-TIGIT antibody as described herein is combined with one or more other anti-cancer agents, such as chemotherapeutic agents. Nonlimiting exemplary chemotherapeutic agents include an alkylating agent (*e.g.*, cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, or temozolomide), an anthracycline (*e.g.*, doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (*e.g.*, paclitaxel or docetaxel), a histone deacetylase

inhibitor (*e.g.*, vorinostat or romidepsin), an inhibitor of topoisomerase (*e.g.*, irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (*e.g.*, bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (*e.g.*, azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (*e.g.*, actinomycin or bleomycin), a platinum-based agent (*e.g.*, cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (*e.g.*, vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, or docetaxel), galardin, thalidomide, lenalidomide, and pomalidomide.

[00176] In some embodiments, the anti-TIGIT antibody (and optionally an additional therapeutic agent) is administered at a therapeutically effective amount or dose. A daily dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied according to several factors, including the chosen route of administration, the formulation of the composition, patient response, the severity of the condition, the subject's weight, and the judgment of the prescribing physician. The dosage can be increased or decreased over time, as required by an individual patient. In certain instances, a patient initially is given a low dose, which is then increased to an efficacious dosage tolerable to the patient. Determination of an effective amount is well within the capability of those skilled in the art.

[00177] The route of administration of an anti-TIGIT antibody or pharmaceutical composition comprising an anti-TIGIT antibody (and optionally an immuno-oncology agent or other therapeutic treatment) can be oral, intraperitoneal, transdermal, subcutaneous, intravenous, intramuscular, inhalational, topical, intralesional, rectal, intrabronchial, nasal, transmucosal, intestinal, ocular or otic delivery, or any other methods known in the art. In some embodiments, the anti-TIGIT antibody (and optionally an immuno-oncology agent) is administered orally, intravenously, or intraperitoneally.

[00178] Co-administered therapeutic agents (*e.g.*, the anti-TIGIT antibody and an additional therapeutic agent) can be administered together or separately, simultaneously or at different times. When administered, the therapeutic agents independently can be administered once, twice, three, four times daily or more or less often, as needed. In some embodiments, the administered therapeutic agents are administered once daily. In some embodiments, the administered therapeutic agents are administered at the same time or times, for instance as an admixture. In some embodiments, one or more of the therapeutic agents is administered in a sustained-release formulation.

[00179] In some embodiments, the anti-TIGIT antibody and an additional therapeutic agent are administered concurrently. In some embodiments, the anti-TIGIT antibody and an additional therapeutic agent are administered sequentially. For example, in some embodiments, an anti-TIGIT antibody is administered first, for example for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 days or more prior to administering an additional therapeutic agent. In some embodiments, an additional therapeutic agent is administered first, for example for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 days or more prior to administering an anti-TIGIT antibody.

[00180] In some embodiments, the anti-TIGIT antibody (and optionally the additional therapeutic agent) is administered to the subject over an extended period of time, e.g., for at least 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350 days or longer.

V. Compositions and Kits

[00181] In another aspect, compositions and kits comprising an anti-TIGIT antibody for use in treating or preventing a cancer in a subject are provided.

Pharmaceutical Compositions

[00182] In some embodiments, pharmaceutical compositions comprising an anti-TIGIT antibody for use in administering to a subject having a cancer are provided. In some embodiments, the anti-TIGIT antibody is as described herein, e.g., an anti-TIGIT antibody having a binding affinity, activity, cross-reactivity, epitope recognition, and/or one or more CDR, VH, and/or VL sequences as disclosed herein. In some embodiments, the anti-TIGIT antibody is afucosylated.

[00183] In some embodiments, an anti-TIGIT antibody and an additional therapeutic agent are formulated into pharmaceutical compositions, together or separately, as described herein. In some embodiments, the additional therapeutic agent is an immuno-oncology agent, such as a PD-1 pathway inhibitor or a CTLA-4 pathway inhibitor. In some embodiments, the immuno-oncology agent is an agonist of a T cell coactivator. In some embodiments, the PD-1 pathway inhibitor is an anti-PD-1 antibody or anti-PD-L1 antibody, such as but not limited to pembrolizumab, nivolumab, durvalumab, pidilizumab, or atezolizumab.

[00184] Guidance for preparing formulations for use in the present invention is found in, for example, *Remington: The Science and Practice of Pharmacy*, 21st Ed., 2006, *supra*; *Martindale: The Complete Drug Reference*, Sweetman, 2005, London: Pharmaceutical Press; Niazi, *Handbook of Pharmaceutical Manufacturing Formulations*, 2004, CRC Press; and Gibson, *Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug*

Selection to Commercial Dosage Form, 2001, Interpharm Press, which are hereby incorporated herein by reference. The pharmaceutical compositions described herein can be manufactured in a manner that is known to those of skill in the art, *i.e.*, by means of conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping or lyophilizing processes. The following methods and excipients are merely exemplary and are in no way limiting.

[00185] In some embodiments, an anti-TIGIT antibody (and optionally an additional therapeutic agent) is prepared for delivery in a sustained-release, controlled release, extended-release, timed-release or delayed-release formulation, for example, in semi-permeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those skilled in the art. Current extended-release formulations include film-coated tablets, multiparticulate or pellet systems, matrix technologies using hydrophilic or lipophilic materials and wax-based tablets with pore-forming excipients (*see*, for example, Huang, *et al. Drug Dev. Ind. Pharm.* 29:79 (2003); Pearnchob, *et al. Drug Dev. Ind. Pharm.* 29:925 (2003); Maggi, *et al. Eur. J. Pharm. Biopharm.* 55:99 (2003); Khanvilkar, *et al., Drug Dev. Ind. Pharm.* 228:601 (2002); and Schmidt, *et al., Int. J. Pharm.* 216:9 (2001)). Sustained-release delivery systems can, depending on their design, release the compounds over the course of hours or days, for instance, over 4, 6, 8, 10, 12, 16, 20, 24 hours or more. Usually, sustained release formulations can be prepared using naturally-occurring or synthetic polymers, for instance, polymeric vinyl pyrrolidones, such as polyvinyl pyrrolidone (PVP); carboxyvinyl hydrophilic polymers; hydrophobic and/or hydrophilic hydrocolloids, such as methylcellulose, ethylcellulose, hydroxypropylcellulose, and hydroxypropylmethylcellulose; and carboxypolymethylene.

[00186] For oral administration, an anti-TIGIT antibody (and optionally an additional therapeutic agent) can be formulated readily by combining with pharmaceutically acceptable carriers that are well known in the art. Such carriers enable the compounds to be formulated as tablets, pills, dragees, capsules, emulsions, lipophilic and hydrophilic suspensions, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by mixing the compounds with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents

can be added, such as a cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[00187] The anti-TIGIT antibody (and optionally the additional therapeutic agent) can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. For injection, the compound or compounds can be formulated into preparations by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In some embodiments, compounds can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[00188] The anti-TIGIT antibody (and optionally the additional therapeutic agent) can be administered systemically by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. For topical administration, the agents are formulated into ointments, creams, salves, powders and gels. In one embodiment, the transdermal delivery agent can be DMSO. Transdermal delivery systems can include, *e.g.*, patches. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. Exemplary transdermal delivery formulations include those described in U.S. Patent Nos. 6,589,549; 6,544,548; 6,517,864; 6,512,010; 6,465,006; 6,379,696; 6,312,717 and 6,310,177, each of which are hereby incorporated herein by reference.

[00189] In some embodiments, a pharmaceutical composition comprises an acceptable carrier and/or excipients. A pharmaceutically acceptable carrier includes any solvents, dispersion media, or coatings that are physiologically compatible and that preferably does not interfere with or otherwise inhibit the activity of the therapeutic agent. In some embodiments, the carrier is suitable for intravenous, intramuscular, oral, intraperitoneal, transdermal, topical, or subcutaneous administration. Pharmaceutically acceptable carriers can contain one or more physiologically acceptable compound(s) that act, for example, to stabilize the composition or to increase or decrease the absorption of the active agent(s). Physiologically acceptable compounds can include, for example, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins,

compositions that reduce the clearance or hydrolysis of the active agents, or excipients or other stabilizers and/or buffers. Other pharmaceutically acceptable carriers and their formulations are well-known and generally described in, for example, *Remington: The Science and Practice of Pharmacy*, 21st Edition, Philadelphia, PA. Lippincott Williams & Wilkins, 2005. Various pharmaceutically acceptable excipients are well-known in the art and can be found in, for example, *Handbook of Pharmaceutical Excipients* (5th ed., Ed. Rowe *et al.*, Pharmaceutical Press, Washington, D.C.).

[00190] Dosages and desired drug concentration of pharmaceutical compositions of the disclosure may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of one in the art. Suitable dosages are also described herein.

Kits

[00191] In some embodiments, kits for use in treating a subject having a cancer are provided. In some embodiments, the kit comprises:

an anti-TIGIT antibody; and
an additional therapeutic agent.

[00192] In some embodiments, anti-TIGIT antibody is as described herein, e.g., an anti-TIGIT antibody having a binding affinity, activity, cross-reactivity, epitope recognition, and/or one or more CDR, VH, and/or VL sequences as disclosed herein. In some embodiments, the anti-TIGIT antibody is afucosylated. In some embodiments, the additional therapeutic agent is an immuno-oncology agent, such as a PD-1 pathway inhibitor or a CTLA-4 pathway inhibitor. In some embodiments, the immuno-oncology agent is an agonist of a T cell coactivator. In some embodiments, the PD-1 pathway inhibitor is an anti-PD-1 antibody or anti-PD-L1 antibody. In some embodiments, the immuno-oncology agent is pembrolizumab, nivolumab, durvalumab, pidilizumab, or atezolizumab.

[00193] In some embodiments, the kits can further comprise instructional materials containing directions (*i.e.*, protocols) for the practice of the methods of this invention (e.g., instructions for using the kit for treating a cancer). While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (*e.g.*, magnetic discs, tapes, cartridges, chips), optical media (*e.g.*, CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

VI. Examples

[00194] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Generation of anti-TIGIT Antibodies

[00195] Fully human anti-TIGIT monoclonal antibodies were generated using yeast-based antibody presentation system (see, e.g., Xu et al, “Addressing polyspecificity of antibodies selected from an in vitro yeast presentation system: a FACS-based, high-throughput selection and analytical tool,” *PEDS*, 2013, 26:663-670; WO 2009/036379; WO 2010/105256; and WO 2012/009568). Eight naïve human synthetic yeast libraries each of $\sim 10^9$ diversity were screened. For the first two rounds of selection, a magnetic bead sorting technique utilizing the Miltenyi MACS system was performed, as previously described (see, e.g., Siegel et al, “High efficiency recovery and epitope-specific sorting of an scFv yeast display library,” *J Immunol Methods*, 2004, 286:141-153). Briefly, yeast cells ($\sim 10^{10}$ cells/library) were incubated with 5 mL of 10 nM biotinylated Fc-fusion antigen for 30 minutes at 30°C in wash buffer (phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA)). After washing once with 40 mL ice-cold wash buffer, the cell pellet was resuspended in 20 mL wash buffer, and Streptavidin MicroBeads (500 μ L) were added to the yeast and incubated for 15 minutes at 4°C. Next, the yeast were pelleted, resuspended in 20 mL wash buffer, and loaded onto a Miltenyi LS column. After the 20 mL were loaded, the column was washed 3 times with 3 mL wash buffer. The column was then removed from the magnetic field, and the yeast were eluted with 5 mL of growth media and then grown overnight. The following rounds of selection were performed using flow cytometry. Approximately 2×10^7 yeast were pelleted, washed three times with wash buffer, and incubated at 30°C with 10 nM Fc-fusion antigen and decreasing concentrations of biotinylated monomeric antigen (100 to 1 nM) under equilibrium conditions, 10 nM biotinylated Fc-fusion antigens or 100 nM monomeric antigens of different species in order to obtain species cross-reactivity, or with a poly-specificity depletion reagent (PSR) to remove non-specific antibodies from the selection. For the PSR depletion, the libraries were incubated with a 1:10 dilution of biotinylated PSR reagent as previously described (see, e.g., Xu et al, *supra*). Yeast were then washed twice with wash buffer and stained with LC-FITC (diluted 1:100) and either

SA-633 (diluted 1:500) or EA-PE (extravidin-R-PE, diluted 1:50) secondary reagents for 15 minutes at 4°C. After washing twice with wash buffer, the cell pellets were resuspended in 0.3 mL wash buffer and transferred to strainer-capped sort tubes. Sorting was performed using a FACS ARIA sorter (BD Biosciences) and sort gates were determined to select for antibodies with desired characteristics. Selection rounds were repeated until a population with all of the desired characteristics was obtained. After the final round of sorting, yeast were plated and individual colonies were picked for characterization.

[00196] Antigens included recombinant dimeric human TIGIT-Fc (Acro Biosystems TIT-H5254), monomeric human TIGIT (Sino Biological 10917-H08H), dimeric mouse TIGIT-Fc (R&D Systems, 7267-TG), and monomeric mouse TIGIT (Sino Biologics 50939-M08H).

[00197] *Naïve campaign:* 744 clones were sequenced yielding 345 unique clones (unique CDRH3). 18 VH germlines were represented in the clones.

[00198] *Light chain batch diversification campaign:* Heavy chain (VH) plasmids from an enriched binder pool from round six of the naïve discovery selections were extracted from the yeast via smash and grab, propagated in and subsequently purified from E. Coli, and then transformed into a light chain library with a diversity of 10^7 .

[00199] Selections were performed under essentially the same conditions as that for the naïve discovery. Briefly, one round of magnetic bead enrichment was followed by three rounds of selections by flow cytometry. In the magnetic bead enrichment round, 10 nM biotinylated Fc-fusion antigen was used. The first round on the flow cytometer consisted of a positive selection round using 100 nM biotinylated monovalent antigen. This was followed by a second round, which consisted of a negative selection round for PSR depletion. The final (third) round consisted of a positive selection round, in which the monovalent antigen was titrated at 100 nM, 10 nM, 1 nM. For all libraries, the yeasts from the 1 nM sorts from this third round were plated, and individual colonies were picked and characterized. In total, 728 clones were sequenced, yielding 350 unique HC/LC combinations (93 unique CDRH3s).

[00200] A total of 695 unique clones were identified between the naïve and the light chain batch shuffle campaigns.

Example 2: Characterization of anti-TIGIT Antibodies

[00201] 65 clones were selected for production and further evaluation, representing 12 VH germlines and 9 VL germlines.

Antibody production and purification

[00202] Yeast clones were grown to saturation and then induced for 48 h at 30°C with shaking. After induction, yeast cells were pelleted and the supernatants were harvested for

purification. IgGs were purified using a Protein A column and eluted with acetic acid, pH 2.0. Fab fragments were generated by papain digestion and purified over KappaSelect (GE Healthcare LifeSciences).

Binding of anti-TIGIT antibodies to recombinant human and mouse protein

[00203] ForteBio affinity measurements were performed on an Octet RED384 generally as previously described (see, e.g., Estep et al., “High throughput solution-based measurement of antibody-antigen affinity and epitope binning,” *Mabs*, 2013, 5:270-278). Briefly, ForteBio affinity measurements were performed by loading IgGs on-line onto AHQ sensors. Sensors were equilibrated off-line in assay buffer for 30 minutes and then monitored on-line for 60 seconds for baseline establishment. Sensors with loaded IgGs were exposed to 100 nM antigen (dimeric Fc-fusion antigen or monomeric antigen) for 3 minutes, and afterwards were transferred to assay buffer for 3 minutes for off-rate measurement. All binding and dissociation kinetics were analyzed using the 1:1 binding model.

[00204] Of the 65 IgG clones, 43 had an affinity for TIGIT monomer of < 100 nM. Of the 65 IgG clones, 34 cross-reacted with mouse TIGIT-Fc. Binding affinity for selected clones is shown in Table 1 below.

Epitope binning/Ligand competition assay

[00205] Epitope binning/ligand blocking was performed using a standard sandwich format cross-blocking assay on the ForteBio Octet RED384 system. Control anti-target IgG was loaded onto AHQ sensors and unoccupied Fc-binding sites on the sensor were blocked with an irrelevant human IgG1 antibody. The sensors were then exposed to 100 nM target antigen followed by a second anti-target antibody or ligand (human CD155-Fc (Sino Biological, 10109-H02H)). Additional binding by the second antibody or ligand after antigen association indicates an unoccupied epitope (non-competitor), while no binding indicates epitope blocking (competitor or ligand blocking).

[00206] Four binning antibodies (not mutually exclusive) were used for bin assessment and five overlapping binning profiles were identified. 63 of the 65 anti-TIGIT antibodies competed with the ligand for binding to hTIGIT-Fc. Binning profiles and ligand competition results for selected clones are shown in Table 1 below.

Table 1. Epitope binning, ligand competition, and affinity data for selected anti-TIGIT clones

Clone	Bin Code	CD155 Competition	IgG KD Human TIGIT-Fc (M)	IgG KD Human TIGIT monomer (M)	IgG KD Mouse TIGIT-Fc (M)
2	1, 2, 3, 4	Yes	9.56E-10	1.01E-08	2.03E-09
3	1, 2, 3, 4	Yes	2.77E-09	7.36E-08	5.64E-09
5	1, 2, 3, 4	Yes	9.85E-10	1.41E-08	3.25E-09
13	1, 2, 3	Yes	5.43E-10	2.56E-09	1.16E-10
14	1, 2, 3	Yes	2.01E-09	5.87E-08	2.43E-09
16	1, 2, 3	Yes	6.90E-10	2.06E-09	1.05E-08
18	1, 2, 3	Yes	2.39E-09	5.08E-08	8.82E-09
21	1, 2, 3	Yes	5.85E-10	2.18E-09	N.B.
22	1, 2, 3	Yes	7.90E-10	1.38E-08	1.05E-08
25	1, 2, 3	Yes	6.20E-10	6.18E-10	1.10E-09
27	1, 2, 3	Yes	5.58E-10	2.32E-09	N.B.
54	1, 2, 3	Yes	6.89E-10	3.49E-09	N.B.

Notes:

N.B. = Non-Binder under the conditions of this assay

Bin code and CD155 competition data was generated on ForteBio Octet RED384 system using a standard sandwich format cross-blocking assay as described in Example 2.

KD affinity data was generated on ForteBio Octet RED384 system as described in Example 2.

Binding of anti-TIGIT antibodies to human, mouse, and cynomolgus monkey TIGIT overexpressed in HEK 293 cells

[00207] HEK 293 cells were engineered to stably express high levels of human, mouse or cynomolgus monkey TIGIT by lentiviral transduction. Approximately 100,000 parental HEK 293 (TIGIT-negative) cells or HEK 293 cells overexpressing human, mouse or cynomolgus monkey were stained with 100 nM of each anti-TIGIT antibody for 5 minutes at room temperature. Cells were then washed twice with wash buffer and incubated with anti-human IgG conjugated to PE for 15 minutes on ice. Cells were then washed twice with wash buffer and analyzed by flow cytometry on a FACS Canto II instrument (BD Biosciences). Fold over background (FOB) was calculated as the median fluorescence intensity (MFI) of the anti-TIGIT clone bound to target-positive cells divided by the MFI of the anti-TIGIT clone bound to target-negative cells.

[00208] As shown in FIG. 1, all 65 antibodies showed specific binding to the 293-hTIGIT line (FOB>10, as indicated by the horizontal black line in the chart). 53 clones specifically bound the 293-cyTIGIT line while 31 clones specifically bound the 293-mTIGIT line.

Polyspecificity Reagent (PSR) assay

[00209] Assessment of binding to a polyspecificity reagent was conducted to determine specificity for TIGIT as previously described (see, e.g., Xu et al, *supra*). Briefly, biotinylated PSR reagent diluted 1:10 from stock was incubated with IgG-presenting yeast for 20 minutes on ice. Cells were washed and labeled with EA-PE (extravidin-R-PE) and read on a FACS analyzer. Scoring of polyspecific binding is on a 0 to 1 scale and is correlated to control IgGs with low, medium and high non-specific binding with a score of 0 indicating no binding and a score of 1 indicating very high non-specific binding.

[00210] 62 of the 65 clones were scored as non-polyspecific binders with a PSR score of < 0.10. Three clones scored as low polyspecific binders (PSR score 0.10 – 0.33).

Hydrophobic Interaction Chromatography assay

[00211] Hydrophobic interaction chromatography (HIC) was performed as described previously (Estep et al., *supra*). Briefly, 5 µg IgG samples were spiked in with a mobile phase A solution (1.8 M ammonium sulfate and 0.1 M sodium phosphate at pH 6.6) to achieve a final ammonium sulfate concentration of about 1 M before analysis. A Sepax Proteomix HIC butyl-NP5 column was used with a linear gradient of mobile phase A and mobile phase B solution (0.1 M sodium phosphate, pH 6.5) over 20 minutes at a flow rate of 1 mL/minute with UV absorbance monitoring at 280 nM.

[00212] Increased retention of antibodies on hydrophobic columns has been correlated with increased hydrophobicity and a propensity for poor expression, aggregation or precipitation during purification. Five of the 65 clones had high HIC retention time of > 11.5 minutes, 10 clones had a medium HIC retention time of 10.5 – 11.5 minutes, and the remainder of the clones had low HIC retention times.

Example 3: Binding of anti-TIGIT antibodies to human, mouse, and cynomolgus monkey TIGIT endogenously expressed on primary T cells

[00213] 65 antibodies shown to be specific for human TIGIT recombinant protein and human TIGIT expressed on HEK 293 cells were evaluated for their ability to bind endogenous TIGIT on primary human peripheral blood T cells. Antibodies were also evaluated for cross reactivity to cynomolgus TIGIT on peripheral blood T cells and 35 of the 65 clones were evaluated for cross reactivity to mouse TIGIT on activated splenic T cells.

[00214] Human pan T cells were negatively isolated from leukapheresis product to 99% purity. 100,000 cells were stained at 4°C for 30 minutes with 20 µg/mL of each anti-TIGIT antibody. The anti-TIGIT antibodies were detected with polyclonal goat anti-human IgG conjugated to PE (Jackson ImmunoResearch 109-116-098). Samples were analyzed on a

CytoFLEX flow cytometer. Percent TIGIT⁺ of the FSC/SSC gated lymphocyte population was determined for each antibody using anti-human IgG-PE only staining to determine the threshold for positivity.

[00215] Cynomolgus white blood cells were isolated from whole blood by red blood cell lysis (eBioscience 00-4300). 200,000 cells were stained at 4°C for 30 minutes with 20 µg/mL of each anti-TIGIT antibody. The anti-TIGIT antibodies were detected with polyclonal goat anti-human IgG adsorbed against monkey immunoglobulins conjugated to AlexaFluor647 (SouthernBiotech 2049-31) and T cells were identified by counterstaining with FITC-conjugated anti-CD3 clone SP34 (BD Pharmingen 556611). Samples were analyzed on a CytoFLEX flow cytometer. Percent TIGIT⁺ of the CD3⁺ population was determined for each antibody using anti-human IgG-PE only staining to determine the threshold for positivity.

[00216] BALB/c mouse T cells were isolated from spleens by negative selection (Stem Cell Technologies 19851A) to >99% purity. The cells were activated for 24 hours with plate bound anti-CD3 clone 145-2C11 (BioLegend 100302) to upregulate TIGIT. 200,000 activated cells were stained at 4°C for 30 minutes with 20 µg/mL of each anti-TIGIT antibody (35 of 65 clones tested). The anti-TIGIT antibodies were detected with polyclonal goat anti-human IgG conjugated to PE (Jackson ImmunoResearch 109-116-098). Samples were analyzed on a FACSCalibur flow cytometer. Median fluorescence intensity of the FSC/SSC gated lymphocyte population was determined for each antibody.

[00217] FIG. 2 shows binding of 65 anti-TIGIT antibody clones and an irrelevant isotype control antibody to primary human, cynomolgus monkey and mouse T cells. Clones 13 and 25 both showed strong binding to all three species of T cells.

Titratable binding of anti-TIGIT antibodies to cell surface expressed TIGIT

[00218] HEK 293 cells were engineered to stably express high levels of human, mouse or cynomolgus monkey TIGIT by lentiviral transduction. 200,000 293-TIGIT cells were stained at 4°C for 30 minutes with a 10-point, 3-fold titration (30 to 0.002 µg/mL) of each anti-TIGIT antibody. The anti-TIGIT antibodies were detected with polyclonal goat anti-human IgG conjugated to PE (Jackson ImmunoResearch 109-116-098). Samples were analyzed on a CytoFLEX flow cytometer. Median fluorescence intensity of the FSC/SSC gated population was determined for each antibody concentration. NonLinear regression of Log(X) transformed data was used to generate EC50 values in GraphPad Prism 6. None of the anti-TIGIT antibodies showed binding to parental HEK 293 cells (TIGIT⁻) (data not shown). FIG. 3A-C shows the binding titration and FIG. 3D shows the EC50 of binding of eight anti-TIGIT antibody clones (clone 2, clone 5, clone 13, clone 16, clone 17, clone 20, clone 25, and clone 54) to human, cynomolgus monkey, and mouse TIGIT expressed on HEK 293 cells.

[00219] C57BL/6 mouse T cells were isolated from spleens by negative selection (Stem Cell Technologies 19851A) to >99% purity. The cells were activated for 24 hours with plate bound anti-CD3 clone 145-2C11 (BioLegend 100302) to upregulate TIGIT. 200,000 cells were stained at 4°C for 30 minutes with an 8-point, 3-fold titration (30 to 0.014 µg/mL) of each anti-TIGIT antibody. The anti-TIGIT antibodies were detected with polyclonal goat anti-human IgG conjugated to PE (Jackson ImmunoResearch 109-116-098). Samples were analyzed on a FACSCalibur flow cytometer. Median fluorescence intensity of the FSC/SSC gated lymphocyte population was determined for each antibody. NonLinear regression of Log(X) transformed data was used to generate EC50 values in GraphPad Prism 6. FIG. 4 shows the binding titration and EC50 of binding of anti-TIGIT clones 13 and 25 to activated mouse splenic T cells.

Example 4: Anti-TIGIT antibodies block binding of CD155 and CD112 ligand to cell surface-expressed TIGIT

[00220] HEK 293 cells were engineered to stably express high levels of human or mouse TIGIT by lentiviral transduction. hCD155-Fc (Sino Biological 10109-H02H), hCD112-Fc (Sino Biological 10005-H02H) and mCD155-Fc (Sino Biological 50259-M03H) were conjugated to AlexaFluor647 (ThermoFisher A30009). 200,000 293-hTIGIT or 293-mTIGIT cells were co-incubated with 1 µg/mL CD155-Fc-AlexaFluor647 or 5 µg/mL CD112-Fc-AlexaFluor647 and a 12-point, 2-fold titration (10 to 0.005 µg/mL) of each anti-TIGIT antibody or an isotype control antibody. Samples were analyzed on a CytoFLEX flow cytometer. Median fluorescence intensity of the FSC/SSC gated population was determined for each antibody concentration. Percent blockade was calculated relative to the MFI of the no antibody control. NonLinear regression of Log(X) transformed data was performed in GraphPad Prism 6.

[00221] As shown in FIG. 5A-B, six anti-TIGIT antibody clones (clone 2, clone 5, clone 13, clone 17, clone 25, and clone 55) were tested, and five of the six clones (clone 2, clone 5, clone 13, clone 17, and clone 25) significantly blocked CD155 interaction with TIGIT expressed on HEK 293 cells for both human CD155/human TIGIT and for mouse CD155/mouse TIGIT. Clone 55 specifically binds human TIGIT but did not compete with hCD155-Fc for binding to hTIGIT-Fc in the ForteBio Octet ligand competition assay. Similarly, clone 55 did not efficiently block hCD155 interaction with the 293-hTIGIT cell line. Clone 2, clone 5, clone 13, clone 17, and clone 25 were also able to interrupt binding of human CD112 to human TIGIT. As observed for CD155, clone 55 was much less effective at blocking the CD112-TIGIT interaction. See FIG. 6.

Example 5: In vitro activity of anti-TIGIT antibodies in a TIGIT/CD155 blockade bioassay

[00222] The activity of anti-TIGIT antibodies can be functionally characterized using a TIGIT/CD155 blockade bioassay (e.g., TIGIT/CD155 Blockade Bioassay Kit, Promega Corp., Madison, WI), in which expression of a reporter gene is induced or enhanced when an antibody blocks TIGIT/CD155 interaction. The TIGIT/CD155 blockade bioassay comprises two cell types: an effector cell expressing TIGIT, CD226, and a TCR complex on the cell surface and containing a luciferase reporter gene; and an artificial antigen presenting cell that expresses CD155 and a TCR activator on the cell surface. In this bioassay, luciferase expression requires TCR engagement plus a co-stimulatory signal. The CD155-TIGIT interaction has higher affinity than the CD155-CD226 interaction, resulting in net inhibitory signaling and no luciferase expression. Blockade of the CD155-TIGIT interaction allows CD155-CD226 co-stimulation driving luciferase expression.

[00223] Jurkat effector cells expressing both TIGIT and CD226 were co-cultured with CHO-K1 artificial antigen presenting cells (aAPCs) expressing a TCR activator and CD155. The Jurkat effector cells contain a luciferase reporter gene driven by the IL-2 promoter. In the absence of blocking anti-TIGIT antibodies, CD155-TIGIT engagement leads to T cell co-inhibition and no IL-2 promoter activity. Upon addition of anti-TIGIT antibodies, CD155-TIGIT interaction is interrupted allowing CD155 to associate with CD226 to send a co-stimulatory signal and drive luciferase expression.

[00224] aAPCs were plated in 96-well plates and allowed to adhere overnight. The following day, 20 µg/mL of each anti-TIGIT antibody or an isotype control antibody and Jurkat effector cells were added to the plate. After a 6 hour incubation at 37°C, cells were lysed and luciferase substrate was added. Luciferase activity was quantified on a plate reader. Luciferase activity was calculated as a fold over the signal in the no antibody control.

[00225] As shown in FIG. 7A-7B, 12 anti-TIGIT antibody clones demonstrated functional blockade in this bioassay.

Example 6: In vitro activity of anti-TIGIT antibodies in a TIGIT/PD-1 combination bioassay

[00226] The synergistic activity of anti-TIGIT antibodies in combination with anti-PD-1 agents (e.g., anti-PD-1 antibodies) can be functionally characterized using a TIGIT/PD-1 combination bioassay, in which expression of a reporter gene is enhanced when antibodies block both the TIGIT/CD155 interaction and the PD-1/PD-L1 interaction. The bioassay comprises two cell types: an effector cell expressing TIGIT, CD226, PD-1, and a TCR complex on the cell surface and containing a luciferase reporter gene; and an artificial antigen presenting cell that expresses CD155, PD-L1, and a TCR activator on the cell surface. In this bioassay, luciferase

expression requires TCR engagement plus a co-stimulatory signal. The CD155-TIGIT interaction has higher affinity than the CD155-CD226 interaction, resulting in net inhibitory signaling and no luciferase expression. Additionally, binding of PD-L1 to PD-1 inhibits luciferase expression. Blockade of both the CD155-TIGIT interaction and the PD-1/PD-L1 interaction relieves the inhibition and allows CD155-CD226 co-stimulation driving luciferase expression.

[00227] Jurkat effector cells expressing PD-1, TIGIT and CD226 were co-cultured with CHO-K1 artificial antigen presenting cells (aAPCs) expressing a TCR activator, PD-L1 and CD155. The Jurkat effector cells contain a luciferase reporter gene driven by the IL-2 promoter. In the absence of blocking anti-TIGIT antibodies, PD-L1-PD-1 and CD155-TIGIT engagement leads to T cell co-inhibition and no IL-2 promoter activity. Upon addition of anti-PD-1 and anti-TIGIT antibodies, PD-L1-PD-1 interaction is blocked, relieving one co-inhibitory signal, and CD155-TIGIT interaction is interrupted, allowing CD155 to associate with CD226 to send a co-stimulatory signal and drive luciferase production.

[00228] aAPCs were plated in 96-well plates and allowed to adhere overnight. The following day, a 10-point 2.5-fold titration (100 to 0.03 µg/mL) of each anti-TIGIT antibody alone, or anti-PD-1 antibody (clone EH12.2H7, BioLegend, San Diego, CA), or each anti-TIGIT antibody + anti-PD-1 antibody (1:1 ratio) and Jurkat effector cells were added to the plate. After a 6 hour incubation at 37°C, cells were lysed and luciferase substrate was added. Luciferase activity was quantified on a plate reader. Luciferase activity was calculated as a fold over the signal in the no antibody control. As shown in FIG. 8, neither anti-TIGIT nor anti-PD-1 alone led to dramatic Jurkat activation, however, the combination of either anti-TIGIT clone 13 or clone 25 with anti-PD-1 yielded strong activation.

Example 7: In vivo activity of anti-TIGIT antibodies in a CT26 syngeneic tumor model in BALB/c mice

[00229] Based on affinity for murine TIGIT, anti-TIGIT clone 13 was chosen for evaluation in a murine syngeneic tumor model. Mouse IgG1 and mouse IgG2a chimeras of the parental fully human anti-TIGIT clone 13 were generated for *in vivo* experiments in order to address the question of whether Fc isotype has an effect on *in vivo* efficacy of antagonistic TIGIT antibodies. *In vitro*, the chimeric antibodies showed similar activity to the parental hIgG1 antibody with regards to (1) binding to human, mouse and cynomolgus monkey TIGIT, (2) blockade of CD155 and CD112 ligand binding to cell-surface expressed TIGIT and (3) activity in the CD155-TIGIT blockade bioassay. See FIG. 9A-9H.

[00230] 8 week old BALB/c mice with an average body weight of 19 g were obtained from Charles River Laboratories. Mice were implanted subcutaneously with 300,000 CT26 colon

carcinoma cells on the right lateral flank. Tumors were allowed to progress until the group average tumor volume was 72 mm³ (range of 48 – 88 mm³) on day 7 after tumor inoculation. Animals were allocated into 10 treatment groups of n=10 by pair match such that the group mean tumor volume was similar across all treatment groups. Tumor length and width were measured and tumor volume was calculated using the formula $\text{Volume (mm}^3\text{)} = 0.5 * \text{Length} * \text{Width}^2$ where length is the longer dimension. Anti-TIGIT clone 13 mIgG1, anti-TIGIT clone 13 mIgG2a and anti-PD-1 clone RMP1-14 (BioXCell) were diluted to the appropriate concentration for dosing in sterile PBS. Sterile PBS was used as the vehicle control. TIGIT antibodies were dosed at 5 or 20 mg/kg via intraperitoneal injection twice weekly for 3 weeks (6 doses total). Anti-PD-1 antibody was dosed at 5 mg/kg via intraperitoneal injection twice weekly for 2 weeks (4 doses total). Dosing initiated on the day of allocation (study day 1). Tumor volume and body weight measurements were collected twice weekly until mice reached a tumor volume cutoff of 2000 mm³. None of the animals exhibited body weight loss relative to pre-dose weights indicating exceptional tolerability of all test agents.

[00231] As shown in FIG. 10A, anti-mPD-1 alone did not have any effect on tumor progression. The mIgG1 anti-TIGIT chimera of clone 13 (“13-1”), which does not efficiently engage activating Fcγ receptors, did not mediate any anti-tumor activity, either as a single agent or in combination with anti-PD-1. In contrast, the mIgG2a chimera of clone 13 (“13-2”), which is capable of binding activating Fcγ receptors, slowed tumor progression (86.5% (5 mg/kg) or 74.4% (20 mg/kg) tumor growth inhibition on day 18). Three of ten animals in the 5 mg/kg 13-2 single agent group showed complete tumor regressions that were stable through the end of the study (study day 46). In the 20 mg/kg 13-2 single agent group, two of ten animals showed partial tumor regressions (defined as tumor volume <50% of initial volume for three consecutive measurements). FIG. 10A shows that the addition of anti-PD-1 to the mIgG2a clone 13 chimera (13-2) did not increase efficacy relative to 13-2 alone (day 18 tumor growth inhibition of 53.8% (5 mg/kg anti-TIGIT + 5 mg/kg anti-PD-1) vs 86.5% (5 mg/kg anti-TIGIT alone) and 89.6% (20 mg/kg anti-TIGIT + 5 mg/kg anti-PD-1) vs 74.4% (20 mg/kg anti-TIGIT alone). Similar numbers of complete and partial responders were observed in the combination groups. See, e.g., FIG. 10B-10K.

Example 8: Antibody Optimization and Characterization of Optimized Antibodies

[00232] Antibody clones 2, 13, 16, and 25 from the primary discovery output were selected for further affinity maturation. Optimization of antibodies was performed via introducing diversities into the heavy chain variable region. Two cycles of optimization were applied to the above lineages. The first cycle was comprised of a CDRH1 and CDRH2 diversification approach, while in the second cycle a CDRH3 mutagenesis approach was applied.

[00233] CDRH1 and CDRH2 approach: The CDRH3 of a single antibody was recombined into a premade library with CDRH1 and CDRH2 variants of a diversity of 1×10^8 . Selections were then performed with one round of MACS and four rounds of FACS as described for the naïve discovery.

[00234] In the first FACS round, the libraries were sorted for 1 nM monomeric TIGIT binding. The second FACS round was a PSR depletion round to reduce poly-specificity. The final two rounds were positive selection rounds using the parental Fab or IgG to pressure for high affinity. Fab/IgG pressure was performed as follows: antigen was incubated with 10 fold parental Fab or IgG and then incubated with the yeast libraries. Selections enriched for IgGs with better affinities than the parental Fab or IgG. Species cross-reactivity was checked in the last two rounds of FACS.

[00235] CDRH3 mutagenesis: Libraries were generated with CDRH3 diversification by randomizing positions in CDRH3. Selections were performed with one round of MACS and three rounds of FACS as described previously. PSR negative selections, species cross-reactivity, affinity pressure, and sorting was performed in order to obtain a population with the desired characteristics.

MSD-SET K_D Measurements

[00236] Equilibrium affinity measurements were performed generally as previously described (Estep et al., *supra*). Briefly, solution equilibrium titrations (SET) were performed in PBS + 0.1% IgG-Free BSA (PBSF) with biotinylated human TIGIT-His monomer held constant at 50 pM and incubated with 3-to 5-fold serial dilutions of antibody starting at around 5 nM. Antibodies (20 nM in PBS) were coated onto standard bind MSD-ECL plates overnight at 4°C or at room temperature for 30 min. Plates were then blocked with 1% BSA for 30 min with shaking at 700 rpm, followed by three washes with wash buffer (PBSF + 0.05% Tween 20). SET samples were applied and incubated on the plates for 150s with shaking at 700 rpm followed by one wash. Antigen captured on a plate was detected with 250 ng/ml sulfotag-labeled streptavidin in PBSF by incubation on the plate for 3 min. The plates were washed three times with wash buffer and then read on the MSD Sector Imager 2400 instrument using 1x Read Buffer T with surfactant. The percent free antigen was plotted as a function of titrated antibody in Prism and fit to a quadratic equation to extract the K_D . To improve throughput, liquid handling robots were used throughout MSD-SET experiments, including SET sample preparation.

[00237] Binding of the optimized antibodies to His-tagged human TIGIT, cyno TIGIT-Fc, and mouse TIGIT-Fc was measured using the ForteBio system as described above. The optimized antibodies were also tested for ligand blocking in a CD155 ligand competition assay,

and for binding to human TIGIT HEK, cyno TIGIT HEK, mouse TIGIT HEK, and parental HEK cell lines, as described above.

[00238] The affinity data and cell binding data for the affinity optimized antibodies is shown in Table 2 below.

Table 2. Affinity and Cell Binding Data for Affinity Optimized Antibodies

Clone Index	ForteBio IgG K _D Human TIGIT-His (M) Monovalent	ForteBio IgG K _D Cyno TIGIT-Fc (M) Avid	ForteBio IgG K _D Murine TIGIT-Fc (M) Avid	MSD IgG K _D (M) Human TIGIT-His	Cell binding Human HEK Cell (FOB Fold Over Background)	Cell binding Cyno HEK Cell (FOB Fold Over Background)	Cell binding Mouse TIGIT HEK Cell (FOB Fold Over Background)
2	8.18E-09	1.34E-09	1.76E-09	NA	158	162	73
2C	5.18E-10	9.84E-10	3.92E-10	1.60E-11	193	224	100
13	2.63E-09	1.04E-09	3.41E-10	NA	212	224	119
13A	6.27E-10	1.12E-09	3.70E-10	2.50E-11	206	240	115
13B	6.10E-10	1.05E-09	3.30E-10	5.30E-12	201	235	102
13C	5.63E-10	1.07E-09	3.29E-10	8.60E-12	194	281	116
13D	5.71E-10	1.16E-09	3.64E-10	5.00E-12	190	245	116
16	2.52E-09	4.67E-09	9.07E-09	NA	192	27	19
16C	9.11E-10	4.25E-09	8.01E-10	6.30E-12	208	157	99
16D	5.96E-10	1.15E-09	2.63E-09	1.30E-11	199	241	63
16E	7.78E-10	1.36E-09	3.70E-09	1.10E-11	195	186	56
25	1.27E-09	1.50E-09	9.67E-10	NA	205	247	117
25A	1.10E-09	1.64E-09	8.23E-10	1.80E-11	207	238	119
25B	1.16E-09	1.40E-09	7.19E-10	2.20E-11	222	291	129
25C	6.97E-10	1.24E-09	4.94E-10	5.60E-12	216	286	124
25D	8.46E-10	1.18E-09	5.80E-10	2.70E-11	225	272	137
25E	8.51E-10	1.18E-09	5.66E-10	1.30E-11	204	252	116

Example 9: Epitope Mapping

[00239] The epitopes of two of the monoclonal antibodies disclosed herein, Clone 13 and Clone 25, were characterized by peptide array. To reconstruct epitopes of the target molecule a library of peptide based epitope mimics was synthesized using solid-phase Fmoc synthesis. An amino functionalized polypropylene support was obtained by grafting with a proprietary hydrophilic polymer formulation, followed by reaction with t-butyloxycarbonyl-hexamethylenediamine (BocHMDA) using dicyclohexylcarbodiimide (DCC) with Nhydroxybenzotriazole (HOBt) and subsequent cleavage of the Boc-groups using trifluoroacetic acid (TFA). Standard Fmoc-peptide synthesis was used to synthesize peptides on the amino-functionalized solid support by custom modified JANUS liquid handling stations (Perkin Elmer).

[00240] Synthesis of structural mimics was performed using proprietary Chemically Linked Peptides on Scaffolds (CLIPS) technology (Pepscan). CLIPS technology allows to structure peptides into single loops, double-loops, triple loops, sheet-like folds, helix-like folds and combinations thereof. CLIPS templates are coupled to cysteine residues. The side-chains of multiple cysteines in the peptides are coupled to one or two CLIPS templates. For example, a 0.5 mM solution of the P2 CLIPS (2,6-bis(bromomethyl)pyridine) is dissolved in ammonium bicarbonate (20 mM, pH 7.8)/acetonitrile (1:3(v/v)). This solution is added onto the peptide arrays. The CLIPS template will bind to side-chains of two cysteines as present in the solid-phase bound peptides of the peptide-arrays (455 wells plate with 3 µl wells). The peptide arrays are gently shaken in the solution for 30 to 60 minutes while completely covered in solution. Finally, the peptide arrays are washed extensively with excess of H₂O and sonicated in disrupt-buffer containing 1 % SDS/0.1 % beta-mercaptoethanol in PBS (pH 7.2) at 70°C for 30 minutes, followed by sonication in H₂O for another 45 minutes. The T3 CLIPS carrying peptides were made in a similar way but with three cysteines.

[00241] Different sets of peptides were synthesized according to the following designs. Set 1 comprised a set of linear peptides having a length of 15 amino acids derived from the target sequence of human TIGIT with an offset of one residue. Set 2 comprised a set of linear peptides of Set 1, but with residues on positions 10 and 11 replaced by Ala. When a native Ala would occur on either position, it was replaced by Gly. Set 3 comprised a set of linear peptides of Set 1, which contained Cys residues. In this set, native Cys were replaced by Cys-acetamidomethyl ("Cys-acm"). Set 4 comprised a set of linear peptides having a length of 17 amino acids derived from the target sequence of human TIGIT with an offset of one residue. On positions 1 and 17 were Cys residues used to create looped mimics by means of mP2 CLIPS. Native Cys were replaced with Cys-acm. Set 6 comprised a set of linear peptides having a length of 22 amino

acids derived from the target sequence of human TIGIT with an offset of one residue. Residues on positions 11 and 12 were replaced with “PG” motif, while Cys residues were placed on positions 1 and 22 to create a constrained mimic with mP2. Native Cys residues were replaced by Cys-acm. Set 7 contained a set of linear peptides having a length of 27 amino acids. On positions 1-11 and 17-27 were 11-mer peptide sequences derived from the target sequence and joined via “GGSGG” (SEQ ID NO: 309) linker. Combinations were made based on the UniProt info on disulfide bridging for human TIGIT. Set 8 comprised a set of combinatorial peptides having a length of 33 amino acids. On positions 2-16 and 18-32 were 15-mer peptides derived from the target sequence of human TIGIT. On positions 1, 17 and 33 were Cys residues used to create discontinuous mimics by means of T3 CLIPS.

[00242] The binding of antibody to each of the synthesized peptides was tested in a pepscan-based ELISA. The peptide arrays were incubated with primary antibody solution (overnight at 4°C). After washing, the peptide arrays were incubated with a 1/1000 dilution of a goat anti-human HRP conjugate (Southern Biotech) for one hour at 25°C. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 20 µl/ml of 3 percent H₂O₂ were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD) - camera and an image processing system. The values obtained from the CCD camera range from 0 to 3000 mAU, similar to a standard 96-well plate ELISA-reader.

[00243] To verify the quality of the synthesized peptides, a separate set of positive and negative control peptides was synthesized in parallel. These were screened with commercial antibodies 3C9 and 57.9 (Posthumus *et al.*, *J. Virol.*, 1990, 64:3304–3309).

[00244] For Clone 13, when tested under high stringency conditions Clone 13 weakly bound discontinuous epitope mimics. The antibody was also tested under moderate stringency conditions and detectable binding of the antibody was observed. The highest signal intensities were recorded with discontinuous epitope mimics containing the core stretches ₆₈ICNADLGWHISPSFK₈₂ (SEQ ID NO: 258), ₄₂ILQCHLSSTTAQV₅₄ (SEQ ID NO: 298), ₁₀₈CIYHTYPDGTGTGRI₁₂₂ (SEQ ID NO: 299). Additional, weaker binding was observed with peptides containing peptide stretch ₈₀SFKDRVAPGPG₉₀ (SEQ ID NO: 300). Binding of the antibody to linear and simple conformational epitope mimics was generally lower and was only observed for motifs ₆₈ICNADLGWHISPSFK₈₂ (SEQ ID NO: 258), ₁₀₈CIYHTYPDGTGTGRI₁₂₂ (SEQ ID NO: 299) and ₈₀SFKDRVAPGPG₉₀ (SEQ ID NO: 300).

[00245] For Clone 25, when tested under high stringency conditions Clone 25 detectably bound peptides from all sets. Strongest binding was observed with discontinuous epitope mimics. While binding to peptides containing residues within stretch ₆₈ICNADLGWHISPSFK₈₂

(SEQ ID NO: 258) was also observed in other sets, binding to peptide stretch ₅₀TTAQVTQ₅₆ (SEQ ID NO: 301) was only observed in combination with ₆₈ICNADLGWHISPSFK₈₂ (SEQ ID NO: 258). Additional, weaker binding was also observed with peptides containing peptide stretch ₈₀SFKDRVAPGPGLGL₉₃ (SEQ ID NO: 300).

[00246] Based on these epitope mapping results for Clone 13 and Clone 25, fine mapping of the epitopes of Clone 13 and Clone 25 was performed using the methods described above using the following sets of peptides. Set 1 comprised a library of single residue epitope mutants based on the sequence CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302). Residues ADHIQRY (SEQ ID NO: 304) were subjected to replacement. Positions 1, 17, 19, 30 and 33 were not replaced. Native Cys residues were replaced by Cys-acm (denoted “2” throughout). Set 2 comprised a library of walking double Ala mutants derived from the sequence CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302). Positions 1, 17 and 33 were not replaced. Native Cys residues were replaced by Cys-acm. Set 3 comprised a library of single residue epitope mutants based on the sequence CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303). Residues ADHIQRY (SEQ ID NO: 304) were used for the replacement. Positions 1, 2, 17, 19, 30 and 33 were not replaced. Set 4 comprised a library of walking double Ala mutants derived from sequence CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303). Positions 1, 17 and 33 were not replaced.

[00247] Clone 13 was tested with four series of discontinuous epitope mutants derived from peptides CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302) and CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303) under high and moderate stringency conditions. Data analysis indicated that in all instances, replacements of residues ₈₁FK₈₂ with either single residues or double Ala impaired binding of Clone 13. Single mutations of other residues within discontinuous epitope mimics did not have drastic effects on binding. On the contrary, double Ala epitope mutants displayed a more pronounced effect on binding when compared with the series of single residue mutants for the corresponding discontinuous mimics. It was also found that double Ala replacements of residues ₅₁TAQVT₅₅ (SEQ ID NO: 305) within CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302) notably impacted binding of Clone 13. Signal intensities recorded for Clone 13 with epitope mimics derived from sequence CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303) were lower than those recorded with CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302). It was further found that that in addition to ₈₁FK₈₂ double Ala replacements of ₇₄GWHI₇₇ (SEQ ID NO: 306) notably

reduce binding of Clone 13. In addition, double Ala mutations within the stretch ⁸⁷PGPGLGL⁹³ (SEQ ID NO: 307) somewhat weakened binding.

[00248] Clone 25 was tested on four series of discontinuous epitope mutants derived from peptides CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302) and CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303) under high and moderate stringency conditions. Analysis of data collected from individual sets of epitope mutants indicated that single or double replacements of residues ⁸¹FK⁸² drastically affected binding. Single residue replacements of other residues within CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302) and CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303) did not cause a notable decrease in signal intensities. A series of double walking Ala mutants displayed more pronounced effects on Clone 25 binding to the mimic. In addition to ⁸¹FK⁸², double Ala replacements of residues ⁵²AQ⁵³ and P79 also mildly affected binding of the antibody to the epitope mimic CILQ2HLSSTTAQVTQCI2NADLGWHISPSFKC (SEQ ID NO: 302). Analysis of binding of Clone 25 to double Ala mutant series derived from CKDRVAPGPGLGLTLQCI2NADLGWHISPSFKC (SEQ ID NO: 303) again confirmed the importance of ⁸¹FK⁸², but also indicated that double Ala replacements of residues ⁷³LGW⁷⁵ and ⁸²KDRVA⁸⁶ (SEQ ID NO: 308) moderately affected the binding.

[00249] In summary, for the monoclonal antibodies Clone 13 and Clone 25 it was found that residues ⁸¹FK⁸² were crucial for the binding of both antibodies to TIGIT epitope mimics. For Clone 13, the residues ⁵¹TAQVT⁵⁵ (SEQ ID NO: 305), ⁷⁴GWHI⁷⁷ (SEQ ID NO: 306), and ⁸⁷PGPGLGL⁹³ (SEQ ID NO: 307) were also found to contribute to binding. For Clone 25, the residues ⁵²AQ⁵³, ⁷³LGW⁷⁵, P79, and, ⁸²KDRVA⁸⁶ (SEQ ID NO: 308) were also found to contribute to binding.

Example 10: Backbone-altered anti-TIGIT antibodies

[00250] In order to potentially improve upon the current generation of anti-TIGIT antibodies, an afucosylated anti-TIGIT antibody and Fc mutated version of an anti-TIGIT antibody with reduced effector function (IgG1 LALA-PG) were developed. It is expected that the afucosylated and LALA-PG antibodies will still block the CD155 and CD112 interactions with TIGIT. Without intending to be bound by any particular theory, it was hypothesized that an afucosylated anti-TIGIT antibody may bind directly to tumor infiltrated Tregs and lead to greater Fc mediated ADCC and/or ADCP and ultimately elicit greater anti-tumor immune response, or alternatively, that effector function could lead to depletion of effector T cells, so an effector null antibody may allow for preservation of activated CD8 T cells.

DNA & Vector Generation (Genewiz):

[00251] Antibody variable and constant domains sequences are synthesized using non-template PCR. The virtual gene sequence is converted into oligonucleotide sequences using Genewiz's bioinformatics tool. Oligonucleotides are synthesized, pooled and amplified using PCR. Full length amplicon from the PCR reaction is cloned into the vector and the product is then transformed into *E. coli* and unique colonies are isolated. Colonies are grown up overnight in liquid media and plasmid DNA isolated, purified, and sequence verified using Sanger sequencing. Light chains and heavy chains are cloned into pcDNA3.4 vectors.

Antibody Expression (Seattle Genetics):

[00252] A 1:1 ratio of antibody heavy chain and light chain vectors are diluted into ThermoFisher OptiPRO SFM medium with ExpiFectamine CHO transfection reagent. The DNA/transfection reagent is then added to an ExpiCHO culture in ThermoFisher ExpiCHO Expression medium and cultured for nine days with ExpiCHO enhancer added on day one and ExpiCHO feeds added on days one and two. To make antibodies with reduced core fucosylation on the N297 glycan (afucosylated antibodies), fucose analog 2-fluorofucose is added to the ExpiCHO culture on the day before and the day of transfection. Culture is harvested by centrifugation and 0.2 μ m filtration.

Antibody Purification (Seattle Genetics):

[00253] GE HiTrap mAb Select SuRe columns are used for the purification of each IgG. Prior to elution, the resin is washed with 5CV PBS+0.1% Triton, 5CV PBS+0.5M NaCl, and 7.5CV of PBS. The IgG is eluted using 25 mM Acetic Acid pH3 Buffer. The sample is buffer exchanged using a 26/60 HiPrep Desalt columns into PBS. The sample is then filter sterilized before a sample is taken for the characterization. Characterization includes A280 concentration, aSEC HPLC, aHIC HPLC, and reduced glycosylated and deglycosylated PLRP-MS (QToF).

Example 11: Backbone-altered antibody characterization

[00254] *In vivo*, monocytes, macrophages, neutrophils, dendritic cells, and NK cells can mediate ADCP (antibody-dependent cell-mediated phagocytosis) and ADCC (antibody-dependent cell-mediated cytotoxicity via Fc γ RI, Fc γ RIIa, Fc γ RI and Fc γ RIIIa. While all three receptors can participate in ADCP, Fc γ RIIIa is believed to be the predominant Fc γ receptor involved in ADCC. Afucosylation of IgG₁ antibodies results in higher affinity binding to Fc γ RIIIa and b, and thus can increase ADCC and ADCP activity.

[00255] Biolayer interferometry (BLI) was utilized to assess the binding kinetics of human Fc γ RIIIa 158V to various anti-TIGIT antibodies to understand the effects of afucosylated antibodies and the LALA-PG mutations at 30°C. An N-hydroxysuccinimide (NHS)-ester biotinylated human Fc γ RIIIa 158V-mono Fc N297A fusion protein (expressed at Seattle Genetics) was loaded onto high precision streptavidin biosensors (Pall ForteBio) at 3 μ g/mL for

300 seconds following a 300 second sensor check. After a 200 second baseline, titrated anti-TIGIT antibodies were associated for 50 seconds and dissociated for 200 seconds. Prior to analysis, the references were subtracted in each assay. The data were corrected with a Y-axis alignment at the start of association, an inter-step dissociation correction and Savitzky-Golay filtering. A 1:1 Langmuir isotherm global fit model was used to fit the curves.

[00256] Representative data are shown in FIG. 10. The sensorgrams for the TIGIT hIgG1 variants were similar to what has typically been observed for hFcγRIIIa binding by BLI: wild-type IgG1 in the 100-200 nM range and afucosylated IgG1 about 10-20 nM due to a 10-fold increase in k_{on} caused by an increased enthalpy, or increased interactions between hFcγRIIIa and hIgG1. hIgG1 LALA-PG had no quantifiable binding, even at the highest concentration of 5000 nM. The results are summarized in Table 3.

[00257] **Table 3.** Global fit analysis of anti-TIGIT antibodies binding human FcγRIIIa 158V by BLI using a 1:1 Langmuir isotherm model.

Ab	K_D (M)	k_{on} (1/Ms)	k_{off} (1/s)	RMax	Req/ Rmax (%)	Full X ²	Full R ²
anti-TIGIT Clone13C hIgG1	2.53E-07	1.46E+05	3.71E-02	0.57	89-67	0.18	0.9931
anti-TIGIT Clone13 hIgG1	3.7E-07	1.29E+05	4.80E-02	0.47	84-5	0.16	0.9900
anti-TIGIT Clone13 afucosylated hIgG1	1.35E-08	7.31E+05	9.85E-03	0.62	88-6	0.024	0.9993
anti-TIGIT Clone13 hIgG1 LALA-PG	NB*	-	-	-	-	-	-

*NB (non-binding). No observable binding of hFcγRIIIa 158V to anti-TIGIT Clone13 hIgG1 LALA-PG at concentrations of 20 μM

[00258] To evaluate the impact of Fc modification of anti-TIGIT antibodies on anti-tumor activity, chimeric antibodies comprised of human CDRs and murine IgG2a backbone in wild-type, or with afucosylated or LALA-PG modifications were made. Afucosylation of murine IgG2a antibodies are similar to production of afucosylated human IgG1 backbone and result in increased binding to murine FcγRIV, the cognate receptor to human FcγRIV. To assess the extent of altered FcγRIV binding, antibody affinity to FcγRIV expressed on CHO cells was determined by FACS using anti-mouse IgG FITC. As shown in FIG. 12, unfucosylated clone 13 mIgG2a bound to a significantly greater extent than both the wild type or LALA-PG modified backbone. Afucosylated clone 13 mIgG2a bound with an affinity of K_D 2.37 nM compared to the wild-type clone 13 mIgG2a, which bound with a K_D of 33.4.

Example 12: TIGIT expression on T cell subsets in healthy donor T cells.

[00259] TIGIT expression on T cell subsets in healthy donors was evaluated by flow cytometry. Frozen PBMC from 12 healthy donors were purchased from Astarte Biologics (Bothell, Washington) and Folio Conversant (Huntsville, Alabama). Donor ages ranged from 21 to 74 with a median age of 48. Cells were stained with a viability dye and the following anti-human antibodies following Fc receptor blocking: CD3, CD96, CD226, CCR7, CD25, CD127, CD45RA, CD8, CD4, CD226, CD155 (Biolegend), and TIGIT (eBioscience). Stained cells were then analyzed on an Attune Nxt flow cytometer (Life Technologies). Treg subsets were defined by appropriate expression of CD4, CD25 and CD127 ($CD4^+CD25^+CD127^{lo}$). $CD4^+$ and $CD8^+$ effector memory (T_{EM} ; $CD45RA^+CCR7^-$), $CD45RA^+$ effector memory (T_{EMRA} ; $CD45RA^+CCR7^-$), central memory (T_{CM} ; $CD45RA^+CCR7^+$), and naïve (T_N ; $CD45RA^+CCR7^+$) cell subsets were defined according to CD45RA and CCR7 expression. As shown in FIG. 3, TIGIT expression is highest on Tregs, while the activating receptor CD226 expression is lower on both memory and naïve CD8 T cell subsets and shows similar levels to naïve CD4 T cell subsets. CD155 expression is mostly absent among T cells but was seen on antigen presenting cells.

Example 13: Anti-TIGIT antibody clone 13 drives depletion of Tregs

[00260] To evaluate the effect of anti-TIGIT antibodies on the absolute numbers of Tregs and other T cell subsets in normal PBMC, healthy donor PBMCs from a donor expressing the V/F alleles of the $FC\gamma RIII$ receptor (Astarte Biologics) were isolated. Cells were cultured in the presence the wild-type, LALA-PG, or afucosylated versions of clone 13 anti-TIGIT antibody or a human IgG1 isotype control (Biolegend) at various concentrations. Cryopreserved PBMC (Astarte Biologics) were incubated in RPMI 1640 with 10% FBS in a 96-well round-bottom plate at 37°C. 2.5×10^5 PBMC were plated per well in triplicate wells with a titration of anti-TIGIT or control antibodies. After 24 hours, cells were washed, Fc receptor blocking was performed, and cells were stained with a viability stain and the following anti-human antibodies: CD3, CD4, CD8, CD25, CD127 and CD45RA (Biolegend). Stained cells were analyzed on an Attune Nxt flow cytometer and T cell subsets were characterized as described above. As shown in FIG. 14, Tregs were 73% positive for TIGIT at the start of the assay. After 24 hours in culture, the wild-type version of clone 13 IgG1 antibody (black filled circle) elicited a dose dependent-decrease in TIGIT-positive Tregs, with maximal depletion occurring at 1 μ g/ml. The afucosylated version of clone 13 (black filled square) conferred greater activity with maximal Treg depletion occurring at 200 pg/ml. The LALA-PG version of clone 13 (black filled triangle)

and the human IgG1 isotype control (gray inverted triangle) showed no apparent activity in this assay.

[00261] In addition to evaluating the wild-type, afucosylated, and LALA-PG versions of clone 13 anti-TIGIT antibody, a version was made comprising an IgG1 constant region with S293D/A330L/I332E (“DLE”) mutations that was previously reported to enhance the affinity for Fcγ receptors. *See* Lazar, 2006, *PNAS* 103: 4005-4010. The wild-type, afucosylated, LALA-PG, and DLE version of clone 13 were assessed for depletion of Tregs substantially as described above in healthy donor PBMCs from a donor expressing the high-affinity V/V alleles of the FcγRIII receptor.

[00262] As shown in FIG. 35, the clone 13 IgG1 wild-type antibody (triangles) depleted Tregs with an IC₅₀ of ~ 0.024 μg/ml, while both the clone 13 IgG1 DLE antibody (squares) and clone 13 IgG1afucosylated antibody (circles) depleted Tregs with much greater potency, with IC₅₀s of 0.0006 μg/ml and 0.0005 μg/ml, respectively. (In FIG. 35, the IgG1 wild-type data is from a prior experiment using PBMCs from the same donor.)

[00263] Another anti-TIGIT antibody, H5/L4 IgG1 (*see* WO 2016/028656 A1), was also tested for its ability to deplete Tregs healthy donor PBMCs from a donor expressing the high-affinity V/V alleles of the FcγRIII receptor. As shown in FIG. 36, clone 13 IgG1 afucosylated antibody was most potent at inducing depletion of Tregs, while the two IgG1 wild-type antibodies showed very similar potencies and depletion curves. (In FIG. 36, the clone 13 IgG1 wild-type data is from a prior experiment using PBMCs from the same donor.)

Example 14: Assessment of Treg depletion in an allogeneic NK/Treg co-culture

[00264] To look for potential antibody dependent cell-mediated cytotoxicity (ADCC) in a Treg/NK cell co-culture, cryopreserved human CD4⁺CD25⁺ T cells (Stem Cell Technologies) were thawed and stimulated with CD3/CD28 MACS iBead particles (Miltenyi Biotec) at a 1:2 bead:cell ratio in in RPMI 10% FBS media supplemented with 20 ng/ml recombinant human IL-2 (R&D systems) for 3 days to increase TIGIT expression. After 3 days, Treg were assessed for cell surface TIGIT expression via flow cytometry and were found to be ~40% positive. The Treg were then washed and labeled with CFSE (Life Technologies) to distinguish them from NK cells following co-culture. That same day, purified human NK cells (Astarte Biologics) were thawed and pre-activated for 2 hours in the presence of 200ng/ml IL-2. A co-culture of NK cells and Treg at a 2.5:1 NK:Treg ratio was set up in a 96-well plate in the presence of 100ng/ml IL-2 in RPMI 1640 with 10% FBS. Cells were incubated at 37°C with wild-type, LALA-PG, or afucosylated versions of clone 13 anti-TIGIT antibody or a human IgG1 isotype control (Biolegend) in triplicate wells, or an afucosylated anti-OKT9 antibody, which served as a positive control. After 24 hours, the cells were washed, stained with a viability dye, and

analyzed via FACS on an Attune Nxt flow cytometer. Tregs were identified and enumerated according to viability dye and CFSE staining.

[00265] The results are shown in FIG. 15. Treg levels dropped with clone 13 IgG1 wild-type treatment (black filled circles), demonstrating that depletion can in part be due to direct NK-mediated ADCC. The ability of clone 13 IgG1 wild-type to deplete T cells appeared maximal at 1 μ g/ml of treatment. The proportion of Treg depletion was more substantial with the clone 13 IgG1 afucosylated version (black filled squares), with maximal activity appearing at 40 ng/ml. The effector-null LALA-PG version of clone 13 IgG1 (black filled triangles) and the human IgG1 isotype control (gray inverted triangles) showed no apparent effect on Treg numbers, while the positive control SEA OKT9 antibody (open circles) showed the largest depletion. The results are consistent with the TIGIT expression data (FIG. 4) that showed Tregs expressed high levels of TIGIT.

Example 15: Anti-TIGIT antibody induces cytokine production

[00266] In addition to binding with higher affinity to Fc γ RIIIa on NK cells and driving ADCC, afucosylated antibodies also bind to higher affinity to Fc γ RIIIa and Fc γ RIIIb on antigen-presenting cells, and neutrophils and can enhance ADCC and antigen-presenting cell activation.

[00267] To investigate the impact of anti-TIGIT antibody and Fc variants on antigen presenting cells, PBMCs were isolated as follows. Blood from 3 unique human donors was collected into heparin tubes and, within about four hours of collection, aliquoted into 50 ml conical tubes (Falcon) and spun at 200g in an Eppendorf 5810R (A-4-62 rotor) for 20 minutes at 25°C without brakes, in order to separate the platelet-rich fraction. Following centrifugation, three distinct layers were formed: bottom layer, red blood cells (accounting for 50-80% of the total volume); middle layer, very thin band of white blood cells (also called “buffy coat”); top layer, straw-colored platelet rich plasma (PRP). The upper straw colored layer, which is enriched in platelets, was removed with a 1 ml pipette and discarded. Once the platelet rich plasma was removed, the remaining fractions were diluted with equal volumes of sterile PBS (Gibco). 15 ml of Histopaque-1077 (Sigma) warmed to room temperature was underlayered below the diluted fractions. The samples were spun at 1500 rpm for 25 minutes at 25°C without brakes. Following centrifugation, three layers are formed again: bottom layer, red blood cells (accounting for 50-80% of the total volume); middle layer, thick band of white blood cells; top layer, PBS and remaining platelets. The upper PBS/platelet layer was removed with a 1 ml pipet and discarded. The thick band of white blood cells was gently removed and placed into a clean 50 ml sterile conical tube. Tubes were filled to 50 ml and cells spun at 800g for 10 minutes. Wash solution was removed and pellets were resuspended in 10 ml of ACK red blood lysis buffer (Gibco) for ten minutes. 35 ml sterile PBS was added and cells were spun at 800g for 10

minutes. The wash solution was removed and the pellet was resuspended in 50 ml PBS. 500 µl of sample was removed and PBMC were counted with a Vi-cell-XR (Beckman Coulter). Cells were spun again at 800g for ten minutes.

[00268] Cells were resuspended at 1 million cells/ml in complete DMEM containing 10% heat inactivated FBS (Atlantica Biologics) and 1X penicillin/strepA, and 1X glutamine and plated at 100,000 cells/well in a 96 well plate. PBMCs were exposed to increasing concentrations (10, 1.0, 0.1, 0.01, 0.001, 0.0001 or 0 µg/ml) of clone 13 IgG1 wild type, clone 13 IgG1 afucosylated, or the clone 13 IgG1 LALA-PG for 24 hours. Tissue culture supernatants were collected and processed for cytokine production using a Luminex multiplex (Millipore) per the manufacturer's instructions.

[00269] The results are shown in FIG. 16. Treatment of PBMCs with clone 13 IgG1 wild type antibody (black filled squares) and clone 13 IgG1 afucosylated antibody (black filled diamonds) resulted in inflammatory cytokine production, including those associated with monocyte/macrophages activation such as MCP1, IL-8, and MIP1α. Exposure of cells to clone 13 IgG1 afucosylated elicited a stronger cytokine response, while the LALA-PG Fc effector null form resulted in very minimal cytokine production.

Example 16: Anti-TIGIT antibody induces activation markers on APCs

[00270] To evaluate the effect of anti-TIGIT antibody on antigen-presenting cells present in the PBMCs, expression of co-stimulatory molecules was assessed on the cell pellets remaining from the cytokine analysis described above. Cell pellets were resuspended in 50 ml of BD FACS buffer and transferred to 96 well round-bottomed microtiter plates. Fc receptors were blocked with human 100 µg/ml Fc fragments (Millipore) for 30 minutes on ice. A master mix composed of anti-CD14 antibody (BD), anti-CD86 antibody (BD), and anti-MHCII antibody (pan anti-DR,DP,DQ antibody, BD) diluted at 1:100 was prepared in BD FACS buffer containing 100 mg/ml human Fc fragments. Five µl of the master mix was added to each well containing 90 µl of resuspended cells, and samples were incubated for one hour on ice. Cells were then spun at 400g in a pre-cooled Eppendorf 5810R centrifuge for five minutes. Supernatants were removed and cells washed with 200 µl of BD FACS buffer. Cells were washed twice and then resuspended in 200 µl of FACS buffer. Samples were collected on an LSRII flow cytometer (BD Biosciences) with DIVA software (BD biosciences). The mean fluorescence intensity (MFI) of CD86 and MHCII on CD14⁺ monocyte/macrophages was analyzed using FlowJo software.

[00271] The results of that experiment are shown in FIG. 17. Treatment of CD14⁺ monocyte/macrophages with anti-TIGIT antibody clone 13 IgG1 wild type or clone 13 IgG1 afucosylated resulted in upregulation of activation markers CD86 (left panel) and MHCII (right

panel), indicating maturation of antigen presenting cells. Treatment with clone 13 IgG1 LALA-PG did not increase expression of CD86 or MHCII by CD14⁺ cells.

Example 17: In Vivo Assessment of anti-TIGIT antibodies with differentiated Fc affinity in syngeneic tumor models.

[00272] The anti-tumor efficacy of afucosylated anti-TIGIT antibody versus effector function null anti-TIGIT antibody comprising the LALA-PG mutation was investigated. This experiment was designed to determine whether the effector function of the anti-TIGIT antibody (enhanced in the afucosylated version and abolished in the LALA-PG version) is involved in the anti-tumor mechanism of action. Murine versions of anti-TIGIT antibody clone 13 with three different Fc domains were generated: 1) wild-type mIgG2a; 2) afucosylated mIgG2a; 3) mIgG2a LALA-PG (reduced effector function). The antibodies were evaluated in CT26 (colon), A20 (B cell lymphoma), and MC38 (colon) syngeneic tumor models, with six mice per antibody group per tumor model. Each antibody was administered intraperitoneal (i.p.) at a dose of 5mg/kg given every third day for six doses once tumors reached 100 mm³. Subcutaneous tumor length and width was measured using a digital caliper, and tumor volume was calculated using the formula $V = (L \times W^2)/2$.

[00273] The results of that experiment are shown in FIG. 18. Each line represents median tumor volume (mm³) (N=6 per group). FIG. 18A shows that the treatment of the A20 (syngeneic lymphoma model) model with anti-TIGIT antibodies clone 13 mIgG2a afucosylated, clone 13 mIgG2a wild-type, and clone 13 mIgG2a LALA-PG resulted in 4/6, 2/6, and 0/6 complete responses, respectively. FIG. 18B shows that the treatment of the CT26WT (murine colon carcinoma) model with anti-TIGIT antibodies clone 13 mIgG2a afucosylated, clone 13 mIgG2a wild-type, and clone 13 mIgG2a LALA-PG resulted in 4/6, 3/6, and 0/6 complete responses, respectively. FIG. 18C shows that treatment of the MC38 (murine colon carcinoma) model with anti-TIGIT antibodies clone 13 mIgG2a afucosylated, clone 13 mIgG2a wild-type, and clone 13 mIgG2a LALA-PG resulted in 1/6, 1/6, and 0/6 complete responses, respectively.

[00274] The dichotomy in responses induced in the different syngeneic models is interesting in light of their reported differential immune profiles. The CT26 and A20 tumor models are reported to be more highly infiltrated with NK and T cells, while the MC38 model is known to be more infiltrated with myeloid-derived suppressor cells (Mosely, et al., 2017, Canc. Immunol. Res. 5(1): 29-41). The increased activity of the anti-TIGIT antibodies in the A20 and CT26 tumor models supports the T cell modulatory activity of these antibodies and suggests that infiltrated T cells may serve as a positive predictive biomarker for this therapy. Collectively this data demonstrates that the anti-tumor activity *in vivo* of murine anti-TIGIT antibodies is

dependent on Fc effector function, and that the afucosylated IgG2a has enhanced efficacy compared to wild-type.

Example 18: Combinatorial activity of anti-TIGIT antibodies.

[00275] TIGIT is expressed not only on T cells, but also NK and antigen-presenting cells. Given the pleotropic expression, TIGIT function can potentially drive multifactorial activity and anti-TIGIT antibodies may have impact on a multitude of cellular functions, including for example NK cell ADCC activity, T cell activation, and optimal antigen recall responses. In addition to single agent activity, anti-TIGIT antibodies may amplify the activity of T cells, NK, or antigen presenting cells in combination with other therapeutics. TIGIT and CD226 are expressed on NK cells and TIGIT expression may not only actively inhibit optimal NK activity and ADCC potential, but may also prevent CD226 mediated activation. Blockade of TIGIT may therefore amplify NK mediated ADCC of human IgG1 targeted agents and ADCC directed therapeutics may show enhanced activity when combined with anti-TIGIT antibodies.

[00276] To investigate this possibility, the non-small cell lung carcinoma cell line, A549, was radiolabeled with $\text{Na}_2[^{51}\text{Cr}]\text{O}_4$ (100 μCi added to cells), washed and mixed with titrations of cetuximab alone, or cetuximab combined with 10 $\mu\text{g/mL}$ anti-TIGIT antibody clone 13 hIgG1 wild-type. An isotype control at 10 $\mu\text{g/mL}$ was also included in the experiment. Effector cells were isolated from cryopreserved normal donor PBMC using the EasySep Human NK Cell Enrichment Kit (Stem Cell Technologies). The donor cells were of the $\text{Fc}\gamma\text{RIIIa}$ 158 V/V genotype. Effector cells were added at an effector-to-target cell ratio of 10:1 (50,000:5000). After a 4 hour incubation, the radioactivity released into the culture supernatant was measured and the percent specific cell lysis was calculated as $(\text{test sample cpm} - \text{spontaneous cpm}) / (\text{total cpm} - \text{spontaneous cpm}) \times 100$. Spontaneous and total cpm values were determined from the supernatants of target cells incubated in medium alone and from target cells lysed with 1% Triton X-100, respectively.

[00277] As shown in FIG. 19, treatment of A549 target cells with anti-TIGIT antibody clone 13 IgG1 antibody alone did not drive ADCC activity, but when A549 target cells were contacted with a combination of cetuximab and anti-TIGIT antibody clone 13 IgG1, the maximum lytic activity was increased over that observed with cetuximab alone.

[00278] In addition to modulating NK activity, TIGIT blockade may increase antigen specific memory responses in T cells. Antibodies targeting the checkpoint proteins present on T cells may therefore show enhanced activity when combined with an anti-TIGIT antibody.

[00279] To investigate this possibility, antigen recall assays in response to cytomegalovirus (CMV) were performed. Human PBMCs from a CMV reactive donor were purchased from Astarte Bio. Memory T cells from this donor can be reactivated *in vitro* with

CMV antigen to assess their antigen-specific response. PBMCs were resuspended in X-vivo 10 (Lonza) containing 10% FBS (Atlanta Biologics), and 100,000 cells were plated into round bottom 96 well plates. Cells were exposed to 10 µg/ml of CMV antigens in the presence or absence of anti-TIGIT antibodies and/or anti-PD-1 directed antibodies. To assess the combinatorial activity, PBMCs were treated with a suboptimal dose of anti-PD-1 antibodies (pembrolizumab or nivolumab) at 1 µg/ml and increasing concentrations of anti-TIGIT antibody clone 13 IgG1 wild-type (1 pg/ml to 1 µg/ml) were added. Memory recall response to the CMV antigen was allowed to proceed for 5 days, after which tissue culture supernatants were harvested and cytokine responses determined by Luminex assay assessment (Millipore) per the manufacturer's instructions.

[00280] As shown in FIG. 20, anti-TIGIT antibody clone 13 IgG1 boosted IFN γ secretion by PBMCs treated with anti-PD-1 antibodies and exposed to CMV antigens, in a dose-dependent manner.

[00281] To investigate the combinatorial activity *in vivo* a syngeneic mouse tumor model was used. C57BL/6 mice, 8 per group, were implanted subcutaneously with 1×10^6 MC38 tumor cells on the flank. When tumors reached $\sim 100 \text{ mm}^3$, animals were randomized into groups and treated every three days for 3 doses (Q3dx3) with either 0.3 mg/kg anti-TIGIT antibody clone 13 hIgG1 wild-type, afucosylated clone 13 IgG1, 1 mg/kg anti-PD-1 antibody (anti-mouse PD-1 antibody clone 29F.1A12), a combination of clone 13 hIgG1 wild-type/anti-PD-1 antibody, or a combination of afucosylated clone 13 IgG1/anti-PD-1 antibody. Tumor volume was monitored over time and animals were euthanized when tumor burden reached $>1000 \text{ mm}^3$.

[00282] In this experiment, both clone 13 hIgG1 wild-type and afucosylated clone 13 IgG1 demonstrated good tumor growth delay, with 4/8 and 3/8 complete responses for wild-type and afucosylated antibody, respectively. *See* FIG. 37, upper left. The anti-PD-1 antibody also showed good efficacy in this experiment. The combination therapies showed even faster tumor regression than the single agents alone, as evidenced by the tumor volumes measured on day 15. FIG. 37, upper right and bottom.

Example 19: Activity of anti-TIGIT antibodies on human T cell responses in vitro.

[00283] To investigate memory T cell reactivation antigen recall experiments were performed. Human PBMCs (100,000 cells) from an HLA-A2 donor previously shown to be reactive to CMV peptides were re-suspended in RPMI containing 10% FBS, 1X penicillin and 1X glutamine, and plated into round bottom wells of a 96 well plate. Cells were stimulated with CMV peptides in the presence or absence of increasing concentrations of anti-TIGIT antibodies clone 13 hIgG1 wild-type, afucosylated clone 13 IgG1, or clone 13 hIgG1 LALA-PG for five

days. Tissue culture supernatants were collected and cytokine production assessed by Luminex multiplex assays as per the manufacturer's instructions.

[00284] As shown in FIG. 21, anti-TIGIT antibody stimulated cultures showed an increased response to CMV antigen as seen by an increase in IFN γ production. Afucosylated clone 13 IgG1 resulted in an amplified antigen specific T-cell response down to 1 pg/ml and this activity was more robust than the response associated with clone 13 IgG1 wild-type. The effector-null clone 13 IgG1 LALA-PG antibody was inefficient at driving a memory T cell response.

[00285] To investigate induction of a naïve effector T cell response a one way mixed lymphocyte reaction (MLR) was performed. Human PBMCs (100,000 cells) from an HLA-A2 donor were re-suspended in RPMI containing 10% FBS, 1X penicillin, and 1X glutamine, and cultured at a 1:1 ratio with irradiated HLA mis-matched allogeneic PBMCs in a round bottom 96 well plate. Cells were stimulated with increasing concentrations of clone 13 hIgG1 wild type, afucosylated clone 13 hIgG1, or clone 13 IgG1 LALA-PG for five days. Tissue culture supernatants were collected and cytokine production assessed by Luminex multiplex assays as per the manufacturer's instructions.

[00286] As shown in FIG. 22, cultures stimulated with anti-TIGIT antibodies showed increased allogenic effector T cell responses as seen by an increase in IL2 production. Afucosylated clone 13 IgG1 resulted in an amplified antigen specific T-cell response down to 100 pg/ml and this activity was more robust than the response associated with clone 13 IgG1 wild type. The effector null clone 13 IgG1 LALA-PG antibody was inefficient at driving a memory T cell response.

[00287] In addition to investigating activation of T cells in antigen-naïve and memory assays, staphylococcal enterotoxin B peptide was used as a superantigen in combination with clone 13 hIgG1 wild type, clone 13 hIgG1 afucosylated, clone 13 IgG1 DLE, and another anti-TIGIT antibody, H5/L4 IgG1. Afucosylated clone 13 hIgG1 was able to induce activation of T cells, as measured by induction of IL-2, more robustly than clone 13 hIgG1 wild type or H5/L4. FIG. 38A. Clone 13 IgG1 DLE was also able to induce IL-2 production roughly equivalently to both clone 13 hIgG1 wild type and clone 13 hIgG1 afucosylated. Clone 13 IgG1 DLE was found to concomitantly induce production of IL-10, in contrast to the other antibodies. FIG. 38B. This result is consistent with reports that engagement of Fc γ RIIb receptor results in induction of IL-10. IgG1 DLE is expected to engage Fc γ RIIb.

Example 20: Activity of anti-TIGIT antibodies in a murine tumor model.

[00288] Treatment of tumor-bearing (CT26 colon carcinoma) mice with various forms of an anti-TIGIT antibody, either wild type IgG2a, effector null IgG2a LALA-PG or afucosylated

IgG2a resulted in both systemic and tissue specific cytokine induction. To examine potential systemic and tissue-specific effects of treatment with anti-TIGIT antibodies, six Balb/c mice were implanted with CT26 colon cancer cells on the flank and treated with 1 mg/kg anti-TIGIT antibodies clone 13 IgG2a wild type, afucosylated clone 13 IgG2a, clone 13 IgG2a LALA-PG, once every three days for six doses, or no treatment, after tumors reached 100 mm³. 24 hours after the 3rd dose of anti-TIGIT antibody, half of the mice were sacrificed and plasma, spleen, and tumor tissue were collected. Half of each spleen and tumor were lysed in RIPA buffer with mechanical disruption. Plasma and tissue lysates were then analyzed for cytokines using the Milipore 25 pre mix Luminex multiplex kit, which allowed for analysis of 25 different inflammatory cytokines. Tissue cytokine levels were normalized to one another via BCA (bicinchoninic acid assay)-determined protein content.

[00289] As shown in FIG. 23, cytokine analysis did not show drastically increased cytokines in the plasma, which would be indicative of a systemic response, or in the tissues in response to any antibody treatments. Limited cytokines were observed in untreated mice in the plasma and tumor with only modest changes observed following anti-TIGIT treatment indicating that TIGIT treatment did not initiate a global inflammatory response even with the afucosylated antibody.

[00290] In addition to analysis of anti-TIGIT induced cytokine levels, tumor and periphery samples harvested 24 hours after the third dose of anti-TIGIT antibody were analyzed for global changes in the proportion of various T cell subsets by flow cytometry. Tumor and spleen single cell suspensions and PBMCs from the same Balb/c mice as described above for FIG. 23 were stained for various cell surface markers to delineate CD8⁺, CD4⁺ subsets of naïve/memory T cells (CD62L⁺CD44⁻ naïve, CD62L⁻CD44⁻ effector, CD44⁺CD62L⁺ central memory, and CD44⁺CD62L⁻ effector memory), and and Treg (CD25⁺CD127⁻). Samples were run on an Attune Acoustic Focusing Cytometer and analyzed using FlowJo. Gates were set on CD45⁺ live cells.

[00291] As shown in FIG 24, increases in CD8⁺ T cells in the tumor were observed in response to anti-TIGIT antibodies clone 13 mIgG2a wild-type and afucosylated clone 13 mIgG2a antibodies, but not the effector null clone 13 mIgG2a LALA-PG, when compared to untreated animals (FIG. 24 top panel). More modest increases in the percentage of CD8⁺ cells after anti-TIGIT antibody treatment were observed in the PBMCs and spleen. Intra-tumoral changes in naïve, effector, and memory T cells were also noted. Specifically, levels of intra-tumoral naïve CD8⁺ T cells were decreased after treatment with clone 13 mIgG2a wild-type and afucosylated clone 13 mIgG2a, but less so with clone 13 mIgG2a LALA-PG (FIG. 24, lower left panel). An increase in the proportion of CD8⁺CD44⁺CD62L⁻ effector memory cells (FIG. 24,

upper right panel) and CD8⁺CD44⁺CD62L⁺ central memory cells (FIG. 24, upper left hand panel) was observed in the tumor in response to the clone 13 mIgG2a wild-type and afucosylated antibodies, but not in response to the clone 13 mIgG2a LALA-PG antibody. These changes were not noted in the spleen or PBMCs, though some mild changes in these tissues did occur.

[00292] CD4⁺ T cell subsets were also analyzed by flow cytometry for changes induced by anti-TIGIT antibody treatment using a similar strategy as described above for CD8⁺ cells. As shown in FIG. 25 (top left hand panel), treatment with clone 13 mIgG2a wild-type and afucosylated antibodies induced mild decreases in intra-tumoral CD4⁺ cells compared to treatment with clone 13 mIgG2a LALA-PG antibody or untreated animals. This effect was not seen in the PBMCs or spleen. An intra-tumoral decrease in response to clone 13 mIgG2a wild-type and afucosylated antibodies, but not clone 13 LALA-PG antibody, was also observed to a small extent in the CD4⁺ Treg population, suggesting that anti-TIGIT antibody treatment is diminishing the immunosuppressive Treg population in the tumor, but not in the normal tissues. Similar to the results with CD8⁺ T cells, intra-tumoral CD4⁺ naïve T cells were decreased after treatment with clone 13 mIgG2a wild-type and afucosylated antibodies compared to untreated (FIG. 25 lower left panel) and this finding was concurrent with an increase in the proportion of effector memory CD4⁺ T cells in the tumor (FIG. 25, middle right panel). Effector memory CD4⁺ and CD8⁺ T cells are involved in eliciting anti-tumor responses and elevation with TIGIT directed antibodies may contribute to the anti-tumor response. Many of these prominent intra-tumor T cell population changes were not accompanied by large shifts in these populations in the periphery.

[00293] After six doses of anti-TIGIT antibodies, at approximately 20 days after the beginning of treatment, tumors were diminished and almost undetectable. Spleens and plasma were harvested from these mice and cells prepared for flow cytometry as described above. As shown in FIG. 26A-B, analysis of various T cell subsets from spleens of these mice demonstrated no drastic changes in the CD8⁺ or CD4⁺ subsets between the groups.

[00294] Induction of cytokines in the spleen and plasma of mice treated with anti-TIGIT antibodies was assessed using multiplex analysis and the results are shown in FIG. 27. No elevated cytokine production was observed in the spleens of treated animals when compared to untreated animals and generally low levels of cytokines were detected in the plasma, with the exception of the chemokine LIX (murine IL8 homologue) and eotaxin, which were elevated in untreated and certain anti-TIGIT antibody treated animals.

[00295] The generation of antigen-specific memory against the tumor in these animals was evaluated using splenocytes harvested at approximately 20 days after initiation of treatment

with anti-TIGIT antibodies. Splenocytes from mice treated with anti-TIGIT antibodies described above were resuspended in culture media and plated in duplicate in wells of a 96-well plate. Cells were either left unstimulated or restimulated with 1 µg/ml AH1 peptide, which is the dominant target for the CD8⁺ T cell responses against the CT26 colorectal tumor. Forty eight hours later, culture supernatants were collected and analyzed for cytokine production via multiplex analysis.

[00296] As shown in FIG. 28, very little cytokine was produced in the absence of peptide. An interesting exception was the observation of elevated levels of IL5 and IL4 produced by unstimulated splenocytes from the afucosylated clone 13 mIgG2a treated animals (FIG 28, lower panels). However, stimulation of splenocytes with a CT-26 specific peptide resulted in robust IFN γ and TNF α production in clone 13 mIgG2a wild-type and afucosylated antibody treated animals, but not in clone 13 mIgG2a LALA-PG antibody treated animals (FIG. 28, top panels), indicating that clone 13 mIgG2a wild-type and afucosylated antibody treatments are able to stimulate a long-lasting, antigen-specific memory CD8⁺ T cell response. Generation of memory T cell responses are involved in driving long term anti-tumor responses. Increases in IL4 production after peptide restimulation of the clone 13 mIgG2a wild-type and afucosylated antibody treated splenocytes was also observed (FIG.28, bottom right panel). The production of IL4 and IL5 in unstimulated splenocytes in the afucosylated clone 13 mIgG2a group without restimulation may suggest induction of a TH2 response by this antibody.

Example 21: Activity of anti-TIGIT antibodies in additional murine tumor models

[00297] Two murine syngeneic tumor models, EMT6 and E0771 breast carcinomas, were treated with 5 mg/kg anti-TIGIT clone 13 antibody comprising wild type mIgG2a or 0.1 mg/kg, 1 mg/kg, or 5 mg/kg anti-TIGIT clone 13 antibody comprising afucosylated mIgG2a. The dosing schedule was q4dx4 (one dose every four days; four total doses). Tumor length and width were measured and tumor volume was calculated using the formula $\text{Volume (mm}^3\text{)} = 0.5 * \text{Length} * \text{Width}^2$ where length is the longer dimension.

[00298] As shown in FIG. 29A-29B, anti-tumor response in the breast cancer models demonstrated tumor growth delay but minimal curative responses. In the EMT6 model, both clone 13 mIgG2a wild-type and afucosylated antibodies at 5 mg/kg demonstrated good tumor growth delay with an increase in average survival from 30.5 days to 37 and 39 days, respectively (FIG. 29A). Additional dose groups for the clone 13 mIgG2a afucosylated antibody demonstrated some loss of activity at the lower doses, with only 35.2 and 36.8 days average overall survival observed with 1 and 0.1 mg/kg doses, but tumor growth was still delayed compared to untreated controls (FIG. 29A).

[00299] In the E0771 model, a more modest tumor growth delay was observed for both clone 13 mIgG2a wild-type and afucosylated antibodies at 5 mg/kg, with an increase in average survival from 38.6 days to 44 and 42 days, respectively (FIG. 29B). Furthermore, one out of six mice appeared to be cured with the clone 13 mIgG2a afucosylated antibody at the 1 mg/kg and 5 mg/kg doses. The lower dose groups for the clone 13 mIgG2a afucosylated antibody, 1 mg/kg and 0.1 mg/kg, resulted in 39 days average overall survival (FIG. 29B).

[00300] In addition, 1 mg/kg clone 13 mIgG2a wild-type antibody was evaluated in a second CT26 colon cancer model, which was obtained from an external lab (MI Bioresearch). As shown in FIG. 29C, in this CT26 colon cancer model, clone 13 mIgG2a wild-type antibody demonstrated an increase in average overall survival from 30 days to 40 days with treatment but only induced one complete regression (16%). As shown in FIG. 18B, the CT26 colon cancer model that had been maintained at Seattle Genetics showed 50% (3/6) complete regressions with 1 mg/kg clone 13 IgG2a wild-type antibody.

Example 22: RNA-Seq analysis and correlation of in vivo response with molecule signatures

[00301] To evaluate the differences in the responses of the two CT26 colon cancer models, as well as other syngeneic cancer models, RNA-seq analysis was used to identify underlying changes in the transcriptomes between the models. RNA-seq raw reads (fastq format) for representative untreated tumors for each model was available as follows.

A20 (maintained at Seattle Genetics), 2 replicates
CT26 (maintained at Seattle Genetics), 2 replicates
CT26 (obtained from MIBio), 1 replicate
E0771 (obtained from MIBio), 1 replicate
EMT-6 (obtained from MIBio), 1 replicate
MC38 (maintained at Seattle Genetics), 1 replicate

[00302] RNA-seq reads for the samples were processed on a standard pipeline consisting of adapter trimming (cutadapt), alignment to the mouse genome/transcriptome (STAR), and transcript quantification (RSEM). Gene expression values normalized to FPKM (fragments per kilobase per million reads) were used for subsequent analyses. Based on inspection of *in vivo* response curves, models were classified as follows in terms of their overall response to WT TIGIT treatment: complete responders = A20 (Seattle Genetics; FIG. 18A), CT26 (Seattle Genetics; FIG. 18B) and partial responders = CT26 (MIBio; FIG. 29C), EMT-6 (MIBio; FIG. 29A), E0771 (MIBio; FIG. 29B), MC38 (Seattle Genetics; FIG. 18C). Differences between the two CT26 colon cancer models at the transcriptional level were observed, suggesting it may be important to characterize models used for efficacy studies.

[00303] Molecular signatures related to the tumor microenvironment (TME) that may correlate with response were also determined by compiling gene signatures pertaining to the immune system and immune response from two published sources: Mosely et al., “Rational selection of syngeneic preclinical tumor models for immunotherapeutic drug discovery,” *Cancer Immunol Res* 2017;5:29-41; and TCIA, The Cancer Immunome Atlas (tcia.at/home), with signatures taken from Charoentong et al., “Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade,” *Cell Rep* 2017 Jan 3;18(1):248-262. Gene signatures from these sources consist of lists of genes whose expression is high in a particular biological context – in CD8+ T cells, for instance. The TCIA gene signatures are human-derived, so the genes in each of those signatures were mapped to the mouse orthologs, using the R library biomaRt. Approximately 50 gene signatures were considered in total, and each of the eight RNA-seq untreated tumor samples were scored against each of the 50 signatures by summing the sample FPKM values of the genes in the signature.

[00304] To evaluate quantitatively which gene signature scores separated the strong responders from the weak responders, the vector of signature scores across all samples was correlated with a response vector (Pearson correlation, 0 for weak responders, 1 for strong responders). Signatures with particularly high correlation with response included NK cells and activated CD8 T cells (FIG. 30). In both cases, the stronger responders scored higher the immune subsets compared to the weaker responders. Table 4 shows a summary of the correlation between certain immune cell subsets and response of tumor cells to anti-TIGIT antibody treatment.

Table 4:

Gene Signature	Correlation	P value
Gamma delta T cell	0.9262	0.0009
NK cell	0.9200	0.0012
Activated CD8 T cell	0.8535	0.0070
Activated DC	-0.8437	0.0085
Immature B cell	0.7875	0.0203
CD96	0.7569	0.0297
CD8 T cell	0.7569	0.0297
CD56 ^{bright} natural killer cell	0.7319	0.0390
T helper 17 cell	0.7139	0.0467

Activated CD4 T cell	0.7092	0.0488
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[00305] Without intending to be bound by any particular theory, as TIGIT blockade may stimulate both NK-cell and CD8 T-cell-mediated tumor killing, a larger available pool of these immune cell populations in the TME may explain the stronger response in the models scoring higher for those subsets.

[00306] Interestingly, TIGIT expression was not a strong differentiator of response in this evaluation, nor was CD155 expression (FIG. 31). For example, the Seattle Genetics CT26 colon cancer model showed the highest expression of CD155 of all models, while the A20 lymphoma model had the lowest, although both are strong responders.

Example 23: Anti-TIGIT antibody enhances Th1 response

[00307] To assess whether the clone 13 anti-TIGIT antibody aids in driving a naïve antigen-specific T cell response, balb/c mice (5 per group) were vaccinated once with 100 µg of the model antigen ovalbumin (OVA) in the presence of complete Freund's adjuvant (CFA) subcutaneously. At the same time, mice were administered 1 mg/kg clone 13 IgG2a wild-type, clone 13 mIgG2a afucosylated, or clone 13 mIgG2a LALA-PG antibody, and then three more doses three days apart (q3dx4). Fourteen days after vaccination, mice were analyzed for induction of antigen-specific immunity using an anti-OVA IgG1 and IgG2a ELISA. Splenocytes were re-stimulated and assessed for cytokine induction.

[00308] The anti-OVA ELISAs demonstrated that co-administration of either clone 13 IgG2a wild-type or clone 13 mIgG2a LALA-PG antibody while mice were undergoing vaccine-induced antigen-specific priming boosted the levels of IgG1 antibodies generated by ~1.5 fold. Clone 13 mIgG2a afucosylated antibody did not boost antigen-specific IgG1 antibody levels in this experiment (FIG. 32, right panel). These data suggest that TIGIT blockade is able to increase priming of a CD4/Th2 response, but enhanced effector function abrogates this effect. TIGIT blockade also boosted levels of IgG2a antigen-specific antibodies (FIG. 32, right panel), but in this case, the clone 13 mIgG2a afucosylated antibody showed the greatest effect. In sum, an inverse correlation was observed between IgG1 levels and enhanced effector function, while IgG2a levels were directly correlated with enhanced effector function, with the clone 13 mIgG2a afucosylated antibody increasing anti-OVA IgG2a levels by over 9 fold. Clone 13 IgG2a wild-type and clone 13 mIgG2a LALA-PG antibody boosted IgG2a levels also, but only by ~4 fold (FIG. 32, right panel). While TIGIT blockade alone wasn't sufficient to skew this heavily Th2-favored system, which is driven by the mouse strain and adjuvant used, toward Th1 in this experiment, TIGIT blockade was shown to enhance a Th1 response, and the response was correlated with effector function.

[00309] In addition to analyzing antigen-specific antibody production, the induction of antigen-specific T cells was also evaluated. Equivalent numbers of splenocytes from each mouse were re-stimulated *ex vivo* with 1 µg whole protein (OVA) for 72 h followed by T effector cytokine analysis. Production of the activated T cell cytokine IL-2 in response to antigen re-stimulation ("1 µg/ml OVA") was increased in the antigen/adjuvant treated group over untreated animals as expected, and this was further increased in splenocytes from animals treated with effector function enabled (clone 13 IgG2a wild-type) or enhanced (clone 13 mIgG2a afucosylated) anti-TIGIT antibody during priming (FIG. 33, lower right panel). These results are consistent with the ability of TIGIT blockade to enhance generation of a naïve antigen-specific T cell response. Analysis of the Th2 effector cytokines IL-5 and IL-4 demonstrated some additional cytokine induction after antigen re-stimulation over antigen/adjuvant alone from animals treated with anti-TIGIT antibodies, though a less clear correlation between effector function and response was observed (FIG. 33, left panels). For the Th1/CD8 T cell cytokine IFN γ , TIGIT blockade during the antigen priming event more profoundly enhanced the generation of antigen-specific T cells (FIG. 33, upper right panel). The most significant boost was observed in splenocytes from animals treated with the effector enhanced clone 13 mIgG2a afucosylated antibody.

Example 24: Anti-TIGIT antibody induces long-lasting anti-tumor memory CD8 T cells

[00310] Mice that showed a complete response in the CT26 colon cancer syngeneic tumor model following treatment with clone 13 mIgG2a afucosylated or unmodified antibody and four untreated mice were re-challenged with CT26 colon cancer cells. As shown in FIG. 34, the mice that had shown a complete response following 1 mg/kg or 5 mg/kg treatment with clone 13 mIgG2a afucosylated or unmodified antibody appeared to have long-lasting anti-tumor memory CD8 T cells that were capable of rejecting re-challenged tumor cells.

[00311] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that many modifications and variations of this invention can be made without departing from its spirit and scope. The specific embodiments described herein are offered by way of example only and are not meant to be limiting in any way. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Example 25: Anti-TIGIT antibody binding to Fc γ receptors

[00312] *In vivo*, monocytes, macrophages, neutrophils, dendritic cells, and natural killer cells can mediate ADCP, ADCC, and CDC via Fc γ RI, Fc γ RII, and Fc γ RIIIa. To assess cellular Fc γ R binding, CHO cells were transfected with human Fc γ RI, Fc γ IIa, Fc γ IIB, or high affinity

Fc γ RIIIa V/V receptor. Cells were exposed to increasing concentrations of wild-type, afucosylated, LALA-PG, or DLE versions of clone 13 IgG1 anti-TIGIT antibody, or another anti-TIGIT antibody, H5L4. Binding was monitored using a fluorescent-tagged anti-human Fc secondary antibody and flow cytometry.

[00313] As shown in FIG. 39A, all of the antibodies except the IgG1 LALA-PG inactive backbone bound substantially equivalently to Fc γ RI on the surface of CHO cells. The IgG1 afucosylated and IgG1 DLE backbones exhibited increased binding to Fc γ RIIIa on the surface of CHO cells. FIG. 39B. Binding to both Fc γ RIIa and Fc γ RIIb on the surface of CHO cells was highest with the wild-type IgG1 backbone and IgG1 DLE effector enhanced backbone. FIG. 39C and FIG. 39D. The afucosylated IgG1 backbone had lower binding affinity to both Fc γ RIIa and Fc γ RIIb when compared to the IgG1 wild-type backbone, although its binding affinity was greater than the IgG1 LALA-PG backbone. FIG. 39C and FIG. 39D. Reduced binding to the inhibitory Fc γ RIIb receptor may influence immune response mediated by the afucosylated IgG1 backbone.

Example 26: Anti-TIGIT antibody mediated phagocytosis

[00314] The term “antibody-dependent cellular phagocytosis” or “ADCP” refers to the process by which antibody-bound cells are internalized, either in whole or in part, by phagocytic immune cells (e.g., by macrophages, neutrophils and/or dendritic cells) that bind to an Fc region of an antibody.

[00315] Wild-type, afucosylated, and DLE versions of clone 13 IgG1 anti-TIGIT antibody were assayed for antibody-mediated phagocytosis using human monocyte/macrophages and TIGIT-positive Jurkat T cells. Human TIGIT-positive Jurkat T cells labeled with fluorescent red PKH dye were opsonized for 30 minutes with increasing concentrations of anti-TIGIT antibodies. The cells were washed and incubated at a 10:1 ratio with monocyte macrophages for 18 hours. Samples were washed 3 times and subjected to flow cytometry to assess phagocytosis.

[00316] As shown in FIG. 40, the wild type and afucosylated IgG1 clone 13 anti-TIGIT antibodies mediated similar levels of phagocytosis, while the clone 13 IgG1 DLE antibody was less effective at mediating phagocytic activity.

[00317] In addition to ADCC and ADCP, Fc regions of cell-bound antibodies can also activate the complement classical pathway, and elicit CDC. C1q of the complement system binds to the Fc regions of antibodies when they are complexed with antigen. Binding of C1q to cell-bound antibodies can initiate a cascade of events involving the proteolytic activation of C4 and C2 to generate the C3 convertase. Cleavage of C3 to C3b by C3 convertase enables the activation of terminal complement components, including C5b, C6, C7, C8 and C9.

Collectively, these proteins form membrane-attack complex pores on the antibody-coated cells. These pores disrupt the cell membrane integrity, killing the target cell via complement-dependent cytotoxicity or “CDC”.

[00318] Wild-type, afucosylated, LALA-PG, and DLE versions of clone 13 IgG1 anti-TIGIT antibody, as well as H5/L4 were assayed for CDC activity using TIGIT+ Jurkat T cells as target cells. The Jurkat cells were incubated with increasing doses of the antibodies in the presence of human serum (not heat inactivated) for 2 hours at 37°C in media containing SYTOX[®] green, which is excluded from live cells but taken up upon activation of CDC and lysis. After incubation, samples were analyzed on a plate reader, background signal was subtracted, and the % of maximum lysis was calculated by determining the maximum signal from cells killed with a 1% Triton X solution.

[00319] As shown in FIG. 41, none of the anti-TIGIT antibodies tested mediated CDC activity. In contrast, the anti-CD47 antibody hB6H12.3 mediated robust CDC activity in this system. Induction of CDC by antibodies is conformationally dependent, and often depends on the epitope they bind. These results suggest the TIGIT epitope bound by the antibodies do not allow for appropriate C1q binding.

[00320] All publications, patents, patent applications or other documents cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, or other document was individually indicated to be incorporated by reference for all purposes.

Table of Sequences

Name	SEQ ID NO	Sequence
Clone 2 VH Protein	1	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDHYMDWVRQAPGKGLEWVG RTRNKANSYTTTEYAASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCA RGQYYYGSSSRGYYYMDVWGQGTITVTVSS
Clone 2 VH DNA	2	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGG TCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTGACCACTA CATGGACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGG CCGTACTAGAAACAAAGCTAACAGTTACACCACAGAATACGCCGCGTC TGTGAAAGGCAGATTACCATCTCAAGAGATGATTCAAAGAACTCACTG TATCTGCAAATGAACAGCCTGAAAACCGAGGACACGGCGGTGTACTAC TGCGCCAGAGGCCAGTACTACTACGGCAGCAGCAGCAGAGGTTACTAC TACATGGACGTATGGGGCCAGGGAACAACCGTCACCGTCTCCTCA
Clone 2 VH FR1	3	EVQLVESGGGLVQPGGSLRLSCAASG
Clone 2 VH CDR1	4	FTFSDHYMD
Clone 2 VH FR2	5	WVRQAPGKGLEWVG
Clone 2 VH CDR2	6	RTRNKANSYTTTEYAASVKG
Clone 2 VH FR3	7	RFTISRDDSKNSLYLQMNSLKTEDTAVYYC
Clone 2 VH CDR3	8	ARGQYYYGSSSRGYYYMDV
Clone 2 VH FR4	9	WGQGTITVTVSS
Clones 2 and 2C VL Protein	10	EIVLTQSPGTLISLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGA SSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQAVPSPLTFGGGTV EIK
Clone 2 VL DNA	11	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGGTGCATCCAGCAGGGCCACTGGCATCCAGACAGGTTCACTGGC AGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTG AAGATTTTGCAGTGTATTACTGTCAGCAGGCCGTCCCCAGTCTCTCACT TTTGGCGGAGGGACCAAGGTTGAGATCAAA
Clone 2 VL FR1	12	EIVLTQSPGTLISLSPGERATLSC
Clones 2 and 2C VL CDR1	13	RASQSVSSSYLA
Clone 2 VL FR2	14	WYQQKPGQAPRLLIY
Clones 2 and 2C VL CDR2	15	GASSRAT
Clone 2 VL FR3	16	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
Clones 2 and 2CVL CDR3	17	QQAVPSPLT
Clone 2 VL FR4	18	FGGGTKVEIK
Clone 3 VH Protein	19	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDHYMDWVRQAPGKGLEWVG RTRNKANSYTTTEYAASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCA RGQYYYGSSSRGYYYMDVWGQGTITVTVSS
Clone 3 VH DNA	20	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGG TCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTGACCACTA CATGGACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGG CCGTACTAGAAACAAAGCTAACAGTTACACCACAGAATACGCCGCGTC TGTGAAAGGCAGATTACCATCTCAAGAGATGATTCAAAGAACTCACTG TATCTGCAAATGAACAGCCTGAAAACCGAGGACACGGCGGTGTACTAC TGCGCCAGAGGCCAGTACTACTACGGCAGCAGCAGCAGAGGTTACTAC TACATGGACGTATGGGGCCAGGGAACAACCGTCACCGTCTCCTCA
Clone 3 VH FR1	21	EVQLVESGGGLVQPGGSLRLSCAASG
Clone 3 VH CDR1	22	FTFSDHYMD
Clone 3 VH FR2	23	WVRQAPGKGLEWVG
Clone 3 VH CDR2	24	RTRNKANSYTTTEYAASVKG
Clone 3 VH FR3	25	RFTISRDDSKNSLYLQMNSLKTEDTAVYYC
Clone 3 VH CDR3	26	ARGQYYYGSSSRGYYYMDV
Clone 3 VH FR4	27	WGQGTITVTVSS

Clone 3 VL Protein	28	EIVLTQSPGTLSPGERATLSCRASQSVRSSYLAWYQQKPGQAPRLLIYGA SSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQVGPPLTFGGGTKVEIK
Clone 3 VL DNA	29	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGGAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGC AGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTG AAGATTTTGCAGTGTATTACTGTCAGCAGGTCGGACCCCCCTCACTTTT GGCGGAGGGACCAAGGTTGAGATCAAA
Clone 3 VL FR1	30	EIVLTQSPGTLSPGERATLSC
Clone 3 VL CDR1	31	RASQSVRSSYLA
Clone 3 VL FR2	32	WYQQKPGQAPRLLIY
Clone 3 VL CDR2	33	GASSRAT
Clone 3 VL FR3	34	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
Clone 3 VL CDR3	35	QQVGPPLT
Clone 3 VL FR4	36	FGGGTKVEIK
Clone 5 VH Protein	37	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVSA ISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKGP RYQDRAGMDVWGQGTITVTVSS
Clone 5 VH DNA	38	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCTGTGCAGCCTCTGGATTACCTTTAGCACCTATGC CATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTC AGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC AAATGAACAGCCTGAGAGCCGAGGACACGGCGGTGTACTACTGCGCCA AGGGCCCCAGATACCAAGACAGGGCAGGAATGGACGTATGGGGCCAGG GAACAACCTGTCAACCGTCTCTCTCA
Clone 5 VH FR1	39	EVQLLESGGGLVQPGGSLRLSCAASG
Clone 5 VH CDR1	40	FTFSTYAMS
Clone 5 VH FR2	41	WVRQAPGKGLEWVS
Clone 5 VH CDR2	42	AISGSGGSTYYADSVKG
Clone 5 VH FR3	43	RFTISRDN SKNTLYLQMNSLRAEDTAVYYC
Clone 5 VH CDR3	44	AKGPRIYQDRAGMDV
Clone 5 VH FR4	45	WGQGTITVTVSS
Clone 5 VL Protein	46	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAAS SLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQLATPYTFGGGTKV EIK
Clone 5 VL DNA	47	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTT AAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTAT GCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTTCAGTGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGA TTTGTCAACTTACTACTGTACGCAAAAGCCTCGCCACTCCTTACACTTTTG GCGGAGGGACCAAGGTTGAGATCAAA
Clone 5 VL FR1	48	DIQMTQSPSSLSASVGDRVTITC
Clone 5 VL CDR1	49	RASQSISSYLN
Clone 5 VL FR2	50	WYQQKPGKAPKLLIY
Clone 5 VL CDR2	51	AASSLQS
Clone 5 VL FR3	52	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
Clone 5 VL CDR3	53	QQLATPYT
Clone 5 VL FR4	54	FGGGTKVEIK
Clone 13 VH Protein	55	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMG SIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGPSE VGAILGYVWFDWPWGQGLTVTVSS
Clone 13 VH DNA	56	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCC TCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATG CTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGG GAAGCATCATCCCTATCTTTGGTACAGCAAACCTACGCACAGAAAGTTCCA GGGCAGAGTCACGATTACCGCGACGAATCCACGAGCACAGCCTACAT GGAGCTGAGCAGCCTGAGATCTGAGGACACGGCGGTGTACTACTGCGC

		CAGAGGCCCTTCTGAAGTAGGAGCAATACTCGGATATGTATGGTTCGAC CCATGGGGACAGGGTACATTGGTCAACCGTCTCCTCA
Clone 13 VH FR1	57	QVQLVQSGAEVKKPGSSVKVSKASG
Clone 13 VH CDR1	58	GTFSSYAIS
Clone 13 VH FR2	59	WVRQAPGQGLEWMG
Clone 13 VH CDR2	60	SIIPFGTANYAQKFQG
Clone 13 VH FR3	61	RVTITADESTSTAYMELSSLRSEDVAVYYC
Clones 13 and 13A VH CDR3	62	ARGPSEVGAILGYVWFDP
Clone 13 VH FR4	63	WGQGLTLTVSS
Clones 13, 13A, 13B, 13C, and 13D VL Protein	64	DIVMTQSPLSLPVTGPGEASISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLL IYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARRIPITFG GGTKVEIK
Clone 13 VL DNA	65	GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAG AGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAA TGGATACAACCTATTTGGATTGGTACCTGCAGAAGCCAGGGCAGTCTCCA CAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCGGGGTCCCTGACA GGTTCAGTGGCAGTGGATCAGGCACAGATTTTACTGAAAATCAGCA GAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAGGCAAGAC GAATCCCTATCACTTTTGGCGGAGGGACCAAGGTTGAGATCAAA
Clone 13 VL FR1	66	DIVMTQSPLSLPVTGPGEASIS
Clones 13, 13A, 13B, 13C, and 13D VL CDR1	67	RSSQSLLSHNGYNYLD
Clone 13 VL FR2	68	WYLQKPGQSPQLLIY
Clones 13, 13A, 13B, 13C, and 13D VL CDR2	69	LGSNRAS
Clone 13 VL FR3	70	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
Clones 13, 13A, 13B, 13C, and 13D VL CDR3	71	MQARRIPIT
Clone 13 VL FR4	72	FGGGTKVEIK
Clone 14 VH Protein	73	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMG SIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGPSE VGAILGYVWFDPWGQGLTLTVSS
Clone 14 VH DNA	74	CAGGTGCAGCTGGTGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCC TCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATG CTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGG GAAGCATCATCCCTATCTTTGGTACAGCAAACACGCACAGAAGTTCCA GGGAGAGTACAGATTACCGCGGACGAATCCACGAGCACAGCCTACAT GGAGCTGAGCAGCCTGAGATCTGAGGACACGGCGGTGTACTACTGCGC CAGAGGCCCTTCTGAAGTAGGAGCAATACTCGGATATGTATGGTTCGAC CCATGGGGACAGGGTACATTGGTCAACCGTCTCCTCA
Clone 14 VH FR1	75	QVQLVQSGAEVKKPGSSVKVSKASG
Clone 14 VH CDR1	76	GTFSSYAIS
Clone 14 VH FR2	77	WVRQAPGQGLEWMG
Clone 14 VH CDR2	78	SIIPFGTANYAQKFQG
Clone 14 VH FR3	79	RVTITADESTSTAYMELSSLRSEDVAVYYC
Clone 14 VH CDR3	80	ARGPSEVGAILGYVWFDP
Clone 14 VH FR4	81	WGQGLTLTVSS
Clone 14 VL Protein	82	DIVMTQSPLSLPVTGPGEASISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLL IYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAKRLPLTF GGGTKVEIK
Clone 14 VL DNA	83	GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAG AGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAA TGGATACAACCTATTTGGATTGGTACCTGCAGAAGCCAGGGCAGTCTCCA CAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCGGGGTCCCTGACA GGTTCAGTGGCAGTGGATCAGGCACAGATTTTACTGAAAATCAGCA GAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAGGCAAAAC GACTCCCTCTCACTTTTGGCGGAGGGACCAAGGTTGAGATCAAA

Clone 14 VL FR1	84	DIVMTQSPLSLPVTGPGEASISC
Clone 14 VL CDR1	85	RSSQSLHLSNGYNYLD
Clone 14 VL FR2	86	WYLQKPGQSPQLLIY
Clone 14 VL CDR2	87	LGSNRAS
Clone 14 VL FR3	88	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
Clone 14 VL CDR3	89	MQAKRLPLT
Clone 14 VL FR4	90	FGGGTKVEIK
Clone 16 VH Protein	91	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSY AISWVRQAPGQGLEWMG GIIPFGTASYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARQST WHKLYGTDVWGQGTITVTVSS
Clone 16 VH DNA	92	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCC TCGGTGAAGGTCTCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATG CTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGG GAGGGATCATCCCTATCTTTGGTACAGCAAGCTACGCACAGAAGTTCCA GGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACAT GGAGCTGAGCAGCCTGAGATCTGAGGACACGGCGGTGTACTACTGCGC AAGACAGAGCACCTGGCACAAATTGTACGGAACGGACGTATGGGGCCA GGGAACAACCTGTACCGTCTCCTCA
Clone 16 VH FR1	93	QVQLVQSGAEVKKPGSSVKVSCKASG
Clone 16 VH CDR1	94	GTSSY AIS
Clone 16 VH FR2	95	WVRQAPGQGLEWMG
Clone 16 VH CDR2	96	GIIPFGTASYAQKFQG
Clone 16 VH FR3	97	RVTITADESTSTAYMELSSLRSEDTAVYYC
Clone 16 VH CDR3	98	ARQSTWHKLYGTDV
Clone 16 VH FR4	99	WGQGTITVTVSS
Clones 16, 16C, 16D, and 16E VL Protein	100	DIQMTQSPSSVSASVGDRTITCRASQGISSWLAWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGDSLPTFGGGTKV EIK
Clone 16 VL DNA	101	GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTGTCTGGGCGAGTCAGGGTATTAGCAGCTGGTT AGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTAT GCTGCATCCAGTTTGAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGA TTTTGCAACTTATTACTGTCTCAGCAGGGAGACAGTCTCCCTCCTACTTTTG GCGGAGGGACCAAGGTTGAGATCAAA
Clone 16 VL FR1	102	DIQMTQSPSSVSASVGDRTITC
Clones 16, 16C, 16D, and 16E VL CDR1	103	RASQGISSWLA
Clone 16 VL FR2	104	WYQQKPGKAPKLLIY
Clones 16, 16C, 16D, and 16E VL CDR2	105	AASSLQS
Clone 16 VL FR3	106	GVPSRFSGSGSGTDFTLTISLQPEDFATYYC
Clones 16, 16C, 16D, and 16E VL CDR3	107	QQGDSLPT
Clone 16 VL FR4	108	FGGGTKVEIK
Clone 18 VH Protein	109	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMSWVRQAPGQGLEWM GIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARV RYGYADGMDVWGQGTITVTVSS
Clone 18 VH DNA	110	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCC TCAGTGAAGGTTTCTGCAAGGCATCTGGATACACCTTCACCAGCTACT ATATGTCATGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGG GAATAATCAACCCTAGTGGTGGTAGCACAAGCTACGCACAGAAGTTCC AGGGCAGAGTCACCATGACCAGGGACACGTCCACGAGCACAGTCTACA TGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCGGTGTACTACTGCG CCAGAGTGAGGTACGGATACGCAGACGGAATGGACGTATGGGGCCAGG GAACAACCTGTACCGTCTCCTCA
Clone 18 VH FR1	111	QVQLVQSGAEVKKPGASVKVSCKASG
Clone 18 VH CDR1	112	YTFTSYMS

Clone 18 VH FR2	113	WVRQAPGQGLEWMG
Clone 18 VH CDR2	114	IINPSGGSTSYAQKFQG
Clone 18 VH FR3	115	RVTMTRDTSTSTVYMESSLRSEDTAVYYC
Clone 18 VH CDR3	116	ARVRYGYADGMDV
Clone 18 VH FR4	117	WGQGTITVTVSS
Clone 18 VL Protein	118	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYGAS SLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVYHLPFTFGGGTKV EIK
Clone 18 VL DNA	119	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG ACAGAGTCAACATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTT AAATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAGCTCCTGATCTAT GGTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCACTGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGA TTTTGCAACTTACTACTGTGAGCAAGTATACCACCTCCCTTTCACTTTTG GCGGAGGGACCAAGGTTGAGATCAAA
Clone 18 VL FR1	120	DIQMTQSPSSLSASVGDRVTITC
Clone 18 VL CDR1	121	RASQSISSYLN
Clone 18 VL FR2	122	WYQQKPGKAPKLLIY
Clone 18 VL CDR2	123	GASSLQS
Clone 18 VL FR3	124	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
Clone 18 VL CDR3	125	QQVYHLPFT
Clone 18 VL FR4	126	FGGGTKVEIK
Clone 21 VH Protein	127	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSYWGWIRQPPGKGLEWIGSI YYSGSTYYNPSLKSRTVISVDTSKNQFSLKLSVTAADTAVYYCARDPLYQ DAPFDYWGQGTITVTVSS
Clone 21 VH DNA	128	CAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAG ACCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTAGTA GTTACTACTGGGGCTGGATCCGCCAGCCCCAGGGAAGGGGCTGGAGT GGATTGGGAGTATCTATTATAGTGGGAGCACCTACTACAACCCGTCCCT CAAGAGTCGAGTCACCATATCCGTAGACACGTCCAAGAACCAGTTCTCC CTGAAGCTGAGTTCTGTGACCGCCGACACACGGCGGTGTACTACTGCG CCAGAGATCCTTTGTACCAAGACGCTCCCTTCGACTATTGGGGACAGGG TACATTGGTCACCGTCTCCTCA
Clone 21 VH FR1	129	QLQLQESGPGLVKPSETLSLTCTVSG
Clone 21 VH CDR1	130	GSISSSSYWGW
Clone 21 VH FR2	131	WIRQPPGKGLEWIG
Clone 21 VH CDR2	132	SIYYSGSTYYNPSLKS
Clone 21 VH FR3	133	RVTISVDTSKNQFSLKLSVTAADTAVYYC
Clone 21 VH CDR3	134	ARDPLYQDAPFDY
Clone 21 VH FR4	135	WGQGTITVTVSS
Clone 21 VL Protein	136	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDAS NRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRANFPTFGGGTKVEI K
Clone 21 VL DNA	137	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTGCAAGGGCCAGTCAGAGTGTTAGCAGCTACTT AGCCTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTAT GATGCATCCAACAGGGCCACTGGCATCCCAGCCAGGTTCACTGGCAGT GGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAG ATTTGCAGTTTATTACTGTGACAGAGAGCCAACCTCCCTACTTTTGGC GGAGGGACCAAGGTTGAGATCAAA
Clone 21 VL FR1	138	EIVLTQSPATLSLSPGERATLSC
Clone 21 VL CDR1	139	RASQSVSSYLA
Clone 21 VL FR2	140	WYQQKPGQAPRLLIY
Clone 21 VL CDR2	141	DASNRAT
Clone 21 VL FR3	142	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC
Clone 21 VL CDR3	143	QQRANFPT
Clone 21 VL FR4	144	FGGGTKVEIK
Clone 22 VH Protein	145	QVQLQESGPGLVKPSETLSLTCAVSGYISSGYWAWIRQPPGKGLEWIGSI YHSGSTYYNPSLKSRTVISVDTSKNQFSLKLSVTAADTAVYYCARQGYYY GSSGSVDFDLWGRGTLTVTVSS

Clone 22 VH DNA	146	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAG ACCCTGTCCCTCACCTGCGCTGTCTCTGGTTACTCCATCAGCAGTGGTTA CTACTGGGCTTGGATCCGGCAGCCCCAGGGAAGGGGCTGGAGTGGAT TGGGAGTATCTATCATAGTGGGAGCACCTACTACAACCCGTCCCTCAAG AGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGA AGCTGAGTTCTGTGACCGCCGACAGACACGGCGGTGTACTACTGCGCCAG GCAGGGATACTACTACGGCAGCAGCGGCAGTGTAGACTTCGACCTATG GGGGAGAGGTACCTTGGTCACCGTCTCCTCA
Clone 22 VH FR1	147	QVQLQESGPGLVKPSSETLSLTCAVSG
Clone 22 VH CDR1	148	YSISSGYYWA
Clone 22 VH FR2	149	WIRQPPGKGLEWIG
Clone 22 VH CDR2	150	SIYHSGSTYYNPSLKS
Clone 22 VH FR3	151	RVTISVDTSKNQFSLKLSSVTAADTAVYYC
Clone 22 VH CDR3	152	ARQGYYYGSSGSVDFDL
Clone 22 VH FR4	153	WGRGTLVTVSS
Clone 22 VL Protein	154	DIQMTQSPSSVSASVGDRTVITCRASQGISSWLAWYQQKPGKAPKLLIYAA SNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQANSLPPWTFGGGT KVEIK
Clone 22 VL DNA	155	GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTTGTCTGGGCGAGTCAGGGTATTAGCAGCTGGTT AGCCTGGTATCAGCAGAAACCAGGGAAGCCCCTAAGCTCCTGATCTAT GCTGCATCCAATTTGCAAAGTGGGGTCCCATCAAGGTTTCAGCGGAGTGT GATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGA TTTTGCAACTTATTACTGTCAACAGGCAAATAGTCTCCCTCCTTGGACTT TTGGCGGAGGGACCAAGGTTGAGATCAAA
Clone 22 VL FR1	156	DIQMTQSPSSVSASVGDRTVITC
Clone 22 VL CDR1	157	RASQGISSWLA
Clone 22 VL FR2	158	WYQQKPGKAPKLLIY
Clone 22 VL CDR2	159	AASNLQS
Clone 22 VL FR3	160	GVPSRFSGSGSGTDFTLTISLQPEDFATYYC
Clone 22 VL CDR3	161	QQANSLPPWT
Clone 22 VL FR4	162	FGGGTKVEIK
Clone 25 VH Protein	163	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYAISWVRQAPGQGLEWMG WISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR DLSSFWSGDVLGAFDIWGQGTMTVSS
Clone 25 VH DNA	164	CAGGTTCACTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCC TCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATG CCATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGG GATGGATCAGCGCTTACAATGGTAACACAACTATGCACAGAAGCTCC AGGGCAGAGTCACCATGACCACAGACACATCCACGAGCAGAGCTTACA TGGAGCTGAGGAGCCTGAGATCTGACGACACGGCGGTGTACTACTGCG CAAGGGATTTGTCTAGCTTCTGGAGCGGAGACGTGTTAGGAGCCTTCGA CATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCA
Clone 25 VH FR1	165	QVQLVQSGAEVKKPGASVKVSCKASG
Clones 25 and 25A VH CDR1	166	YTFTSYAIS
Clone 25 VH FR2	167	WVRQAPGQGLEWMG
Clones 25 and 25E VH CDR2	168	WISAYNGNTNYAQKLQG
Clone 25 VH FR3	169	RVTMTTDTSTSTAYMELRSLRSDDTAVYYC
Clones 25, 25A, and 25B VH CDR3	170	ARDLSSFWSGDVLGAFDI
Clone 25 VH FR4	171	WGQGTMTVTVSS
Clones 25, 25A, 25B, 25C, 25D, and 25E VL Protein	172	DIQMTQSPSSLSASVGDRTVITCRASQSISSYLNWYQQKPGKAPKLLIYAAS SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSVPPRTFGGGTKVEI K
Clone 25 VL DNA	173	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTTGCCTGGGCAAGTCAGAGCATTAGCAGCTATTT AAATTGGTATCAGCAGAAACCAGGGAAGCCCCTAAGCTCCTGATCTAT GCTGCATCCAGTTTGCAGAAAGTGGGGTCCCATCAAGGTTCAAGTGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGA

		TTTTGCAACTTACTACTGTGTCAGCAAAGCGTCCCCCCCAGGACTTTTGGC GGAGGGACCAAGGTTGAGATCAAA
Clone 25 VL FR1	174	DIQMTQSPSSLSASVGDRVTTC
Clones 25, 25A, 25B, 25C, 25D, and 25E VL CDR1	175	RASQSISSYLN
Clone 25 VL FR2	176	WYQQKPGKAPKLLIY
Clones 25, 25A, 25B, 25C, 25D, and 25E VL CDR2	177	AASSLQS
Clone 25 VL FR3	178	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
Clones 25, 25A, 25B, 25C, 25D, and 25E VL CDR3	179	QQSVPPRT
Clone 25 VL FR4	180	FGGGTKVEIK
Clone 27 VH Protein	181	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAISWVRQAPGQGLEWMG WISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR DLSSFWSGDVLGAFDIWGQGTMTVTVSS
Clone 27 VH DNA	182	CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCC TCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATG CCATCAGCTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGG GATGGATCAGCGCTTACAATGGTAACACAACTATGCACAGAAGCTCC AGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACA TGGAGCTGAGGAGCCTGAGATCTGACGACACGGCGGTGTACTACTGCG CAAGGGATTTGTCTAGCTTCTGGAGCGGAGACGTGTTAGGAGCCTTCGA CATATGGGGTCAGGGTACAATGGTCACCGTCTCCTCA
Clone 27 VH FR1	183	QVQLVQSGAEVKKPGASVKVSCKASG
Clone 27 VH CDR1	184	YTFTSYAIS
Clone 27 VH FR2	185	WVRQAPGQGLEWMG
Clone 27 VH CDR2	186	WISAYNGNTNYAQKLQG
Clone 27 VH FR3	187	RVTMTTDTSTSTAYMELRSLRSDDTAVYYC
Clone 27 VH CDR3	188	ARDLSSFWSGDVLGAFDI
Clone 27 VH FR4	189	WGQGTMTVTVSS
Clone 27 VL Protein	190	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGA STRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQHANHITFGGGTKVE IK
Clone 27 VL DNA	191	GAAATAGTGATGACGCACTCTCCAGCCACCCTGTCTGTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTT AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCTCATCTAT GGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTTCAGTGGCAGTG GGTCTGGGACAGAGTTCACTCTCACCATCAGCAGCCTGCAGTCTGAAGA TTTTGCAGTTTATTACTGTGACGAGCAGCCAATCACATCACTTTTGGCG GAGGGACCAAGGTTGAGATCAAA
Clone 27 VL FR1	192	EIVMTQSPATLSVSPGERATLSC
Clone 27 VL CDR1	193	RASQSVSSNLA
Clone 27 VL FR2	194	WYQQKPGQAPRLLIY
Clone 27 VL CDR2	195	GASTRAT
Clone 27 VL FR3	196	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
Clone 27 VL CDR3	197	QQHANHIT
Clone 27 VL FR4	198	FGGGTKVEIK
Clone 54 VH Protein	199	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHWVRQAPGQGLEWM GIINPSGGSTSYAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCARA SDSYGVGLYYGMDVWGQGTITVTVSS
Clone 54 VH DNA	200	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCC TCAGTGAAGGTTTCTCCTGCAAGGCATCTGGATACACCTTACCAGCTACT ATATGCACTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGG GAATAATCAACCCTAGTGGTGGTAGCACAAGCTACGCACAGAAGTTCC AGGGCAGAGTCACCATGACCATGACGAGGACACGTCCACGAGCACAGTCTACA TGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCGGTGTACTACTGCG CTAGGGCATCTGACTCCTACGGAGTGGGCCTCTACTACGGAATGGACGT ATGGGGCCAGGGAACAACTGTACCGTCTCCTCA

Clone 54 VH FR1	201	QVQLVQSGAEVKKPGASVKVSCKASG
Clone 54 VH CDR1	202	YTFTSYMH
Clone 54 VH FR2	203	WVRQAPGQGLEWMG
Clone 54 VH CDR2	204	IINPSGGSTSYAQKFQG
Clone 54 VH FR3	205	RVTMTTRDTSTSTVYMELSSLRSEDVAVYYC
Clone 54 VH CDR3	206	ARASDSYGVGLYYGMDV
Clone 54 VH FR4	207	WGQGTITVTVSS
Clone 54 VL Protein	208	EIVLTQSPGTLSTSPGERATLSCRASQSVRSYLAWYQQKPGQAPRLLIYGA SSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYYVSPLTFGGGTK VEIK
Clone 54 VL DNA	209	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGGAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGC AGTGGGTCTGGGACAGACTTCACTCTACCATCAGCAGACTGGAGCCTG AAGATTTTGCAGTGTATTACTGTGACGAGTACTACGTCAGTCCTCTCACT TTTGGCGGAGGGACCAAGGTTGAGATCAAA
Clone 54 VL FR1	210	EIVLTQSPGTLSTSPGERATLSC
Clone 54 VL CDR1	211	RASQSVRSYLA
Clone 54 VL FR2	212	WYQQKPGQAPRLLIY
Clone 54 VL CDR2	213	GASSRAT
Clone 54 VL FR3	214	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
Clone 54 VL CDR3	215	QQYYVSPLT
Clone 54 VL FR4	216	FGGGTKVEIK
Human TIGIT cDNA sequence (GenBank Accession No. NM_173799.3)	217	CGTCCTATCTGCAGTCGGCTACTTTTCAGTGGCAGAAAGAGGCCACATCTG CTTCCTGTAGGCCCTCTGGGCAGAAAGCATGCGCTGGTGTCTCCTCTGA TCTGGGCCCCAGGGGCTGAGGCAGGCTCCCCTCGCCTCAGGAATGATGAC AGGCACAATAGAAACAACGGGGAACATTTCTGCAGAGAAAGGTGGCTC TATCATCTTACAATGTACCTCTCCTCCACCACGGCACAAGTGACCCAG GTCAACTGGGAGCAGCAGGACAGCTTCTGGCCATTTGTAATGCTGACT TGGGGTGGCACATCTCCCCATCCTTCAAGGATCGAGTGGCCCCAGGTCC CGGCCTGGGCCTCACCTCCAGTCGCTGACCGTGAACGATACAGGGGA GTACTTCTGCATCTATCACACCTACCCTGATGGGACGTACACTGGGAGA ATCTTCCTGGAGGTCTAGAAAGCTCAGTGGCTGAGCACGGTGCCAGGT TCCAGATTCCATTGCTTGGAGCCATGGCCGCGACGCTGGTGGTCATCTG CACAGCAGTCATCGTGGTGGTCGCGTTGACTAGAAAGAAGAAAGCCCT CAGAATCCATTCTGTGGAAGGTGACCTCAGGAGAAAATCAGCTGGACA GGAGGAATGGAGCCCCAGTGCTCCCTCACCCCCAGGAAGCTGTGTCCA GGCAGAAGCTGCACCTGCTGGGCTCTGTGGAGAGCAGCGGGGAGAGGA CTGTGCCGAGCTGCATGACTACTTCAATGTCTCTGAGTTACAGAAGCCTG GGTAACTGCAGCTTCTTACAGAGACTGGTTAGCAACCAGAGGCATCTT CTGGAAGATACACTTTTGTCTTTGCTATTATAGATGAATATATAAGCAG CTGTACTCTCCATCAGTGTCTGCGTGTGTGTGTGTGTGTGTGTGTGTGT GTGTTTCAGTTGAGTGAATAAATGTCATCCTCTTCTCCATCTTCATTTCT TGGCCTTTTCGTTCTATTCCATTTTGCATTATGGCAGGCCTAGGGTGAGT AACGTGGATCTTGATCATAAATGCAAAATTAATAAATATCTTGACCTGG TTTTAAATCTGGCAGTTTGTAGCAGATCCTATGTCTCTGAGAGACACATT CCTCATAATGGCCAGCATTTTGGGCTACAAGGTTTGTGGTTGATGATG AGGATGGCATGACTGCAGAGCCATCCTCATCTCATTTTTCACGTCATTT TCAGTAACTTTCATCTATTCAAAGGCAGGTTATAAGTAAAGTCTGGTAG CAGCCTCTATGGGGAGATTTGAGAGTGACTAAATCTTGGTATCTGCCCT CAAGAATTACAGTTAAATGGGGAGACAATGTTGTGATGAAAAGGTAT TATAGTAAAGGAGAGAAGGAGACATACACAGGCCTTCAGGAAGAGACGA CAGTTTGGGGTGAGGTAGTTGGCATAGGCTTATCTGTGATGAAGTGGCC TGGGAGCACCAAGGGGATGTTGAGGCTAGTCTGGGAGGAGCAGGAGTT TTGTCTAGGGAACCTGTAGGAAATCTTGGAGCTGAAAGTCCCACAAAG AAGGCCCTGGCACCAAGGGAGTCAGCAAACTTCAGATTTTATTCTCTGG GCAGGCATTTCAAGTTTCTTTTGTGTGACATACTCATCCATTAGACAG CCTGATACAGGCCTGTAGCCTCTTCCGGCCGTGTGTGTGTGGGGAAGCCC CAGGAAACGCACATGCCACACAGGGAGCCAAGTCGTAGCATTTGGGC CTTGATCTACCTTTTTCATCAATACTCTTGAGCCTTTGAAAAAAGA ACGTTTCCCACTAAAAAGAAAATGTGGATTTTAAAAATAGGGACTCTTC

		CTAGGGGAAAAAGGGGGGCTGGGAGTGATAGAGGGTTAAAAAATAA ACACCTTCAAACCTAACTTCTTGAACCTTTTATTCACTCCCTGACGACT TTGTGCTGGGGTTGGGGTAACTGAACCGCTTATTTCTGTTTAATTGCATT CAGGCTGGATCTTAGAAGACTTTTATCCTTCCACCATCTCTCTCAGAGG AATGAGCGGGGAGGTTGGATTTACTGGTGACTGATTTTCTTTCATGGGC CAAGGAACTGAAAGAGAATGTGAAGCAAGGTTGTGTCTTGCATGGT TAAAAATAAAGCATTGTCTGCTTCTTAAGACTTAGACTGGGGTTGACA ATTGTTTTAGCAACAAGACAATTCAACTATTTCTCCTAGGATTTTTATTA TTATTATTTTTTCACTTTTCTACCAAATGGGTACATAGGAAGAATGAAC TGAAATCTGTCCAGAGCTCCAAGTCCTTTGGAAGAAAGATTAGATGAAC GTAAAAATGTTGTGTTTGTGTGGCAGTTTACAGCATTTTCTTGCAAA ATTAGTGCAAACTCTGTTGGAATAGAACACAATTACAAATTGGAAGTG AACTAAAATGTAATGACGAAAAGGGAGTAGTGTTTTGATTGGAGGAG GTGTATATTTCGGCAGAGGTTGGACTGAGAGTTGGGTGTTATTTAACATA ATTATGGTAATTGGGAAACATTTATAAACACTATTGGGATGGTGATAAA ATACAAAAGGGCCTATAGATGTTAGAAATGGGTCAGGTTACTGAAATG GGATTCAATTTGAAAAAAATTTTTTAAATAGAACTCACTGAACTAGAT TCTCCTCTGAGAACCAGAGAAGACCATTTTCATAGTTGGATTCTCTGGAGA CATGCGCTATCCACCACGTAGCCACTTTCCACATGTGGCCATCAACCAC TTAAGATGGGGTTAGTTTAAATCAAGATGTGCTGTTATAATTGGTATAA GCATAAAATCACACTAGATTCTGGAGATTTAA TATGAATAATAAGAATACTATTTTCACTAGTTTGGTATATTGTGTGTCAA AAATGATAATATTTTGGATGTATTGGGTGAAATAAAATATTAACATTAA AAAAAAAAA
Human TIGIT protein (GenBank Accession No. NP_776160.2)	218	MRWCLLLIWAQGLRQAPLASGMMTGTIETTNISAEKGGSIILQCHLSSTT AQVTQVNWEQQDQLLAICNADLGWHISPSFKDRVAPGPGLGLTLQSLTVN DTGEYFCIYHTYPDGTGTGRIFLEVLESSVAEHGARFQIPLLGAMAATLVVI CTAVIVVVALTRKKKALRIHSVEGLRRKSAGQEEWSPSAPSPPGSCVQAE AAPAGLCGEQRGEDCAELHDYFNVLSYRSLGNCSSFFTETG
Cynomolgus monkey TIGIT protein	219	MAFLVAPPMQFVYLLKTL CVFNMVFAKPGFSETVFSHRLSFTVL SAVGYFR WQKRPHLLPV SPLGRSMRWCLFLIWAQGLRQAPLASGMMTGTIETTNIS AKKGGSVILQCHLSSTMAQVTQVNWEQHDHSLAIRNAELGWHIYPAFKD RVAPGPGLGLTLQSLTMNDTGEYFCTYHTYPDGTGTGRIFLEVLESSVAEH SARFQIPLLGAMAMMLVVICIAVIVVVVLARKKSLRIHSVESGLQRKSTG QEEQIPSAPSPPGSCVQAEAAPAGLCGEQQGDDCAELHDYFNVLSYRSLGS CSFFTETG
Mouse TIGIT protein	220	MHGWLLLVWVQGLIQAFLATGATAGTIDTKRNISAEEGGSVILQCHFSSD TAEVTQVDWKQDQLLAISVDLGWHVASVFSRVPVGPGLGLTFQSLTM NDTGEYFCTYHTYPGGIYKGRIFLKVQESSVAQFQTAPLGGTMAAVLGLIC LMVTGVTVLARKKSIRMHSIESGLGRTEAEPQEWNLRLSLSPGSPVQTQTA PAGPCGEQAEDDYADPQEFNVLSYRSLESFIAVSKTG
Clone 2C VH CDR1	221	FTFTDYYMD
Clone 2C VH CDR2	222	RTRNKVNSYYTEY AASVKG
Clone 2C VH CDR3	223	ARGQYYYGSDRRGYYYMDV
Clones 13A, 13C, and 13D VH CDR1	224	GTFLSSAIS
Clone 13A VH CDR2	225	SLIPYFGTANYAQKFQG
Clone 13B VH CDR1	226	GTFSAWAIS
Clones 13B and 13D VH CDR2	227	SIIPYFGKANYAQKFQG
Clone 13B VH CDR3	228	ARGPSEVSGILGYVWFDP
Clone 13C VH CDR2	229	SIPLFGKANYAQKFQG
Clones 13C and 13D VH CDR3	230	ARGPSEVKILGYVWFDP
Clone 16C VH CDR1	231	GTFREY AIS
Clone 16C VH CDR2	232	GIHPIFGTARYAQKFQG

Clones 16D and 16E VH CDR1	233	GTFS DYPIS
Clones 16B, 16D, and 16E VH CDR2	234	GIPIVGGANYAQKFQG
Clone 16C VH CDR3	235	TRQSTWHKLYGTDV
Clone 16D VH CDR3	236	TRQSTWHKLFGTDV
Clone 16E VH CDR3	237	ARQSTWHKVYGTDV
Clone 25A VH CDR2	238	WISAYNGNTKYAQKLQG
Clones 25B, 25C, and 25D VH CDR1	239	YTFTSYPIG
Clones 25B, 25C, and 25D VH CDR2	240	WISSYNGNTNYAQKLQG
Clone 25C VH CDR3	241	ARGASSFWSGDVLGAFDI
Clone 25D VH CDR3	242	ARDLKSFWSGDVLGAFDI
Clone 25E VH CDR1	243	YTFTSYAIA
Clone 25E VH CDR3	244	ARSGSSFWSGDVLGAFDI
Clone 2C VH	245	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYYMDWVRQAPGKGLEWVG RTRNKVNSYYTEYAASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCA RGQYYYGSDRRGYMDVWGQGTITVTVSS
Clone 13A VH	246	QVQLVQSGAEVKKPGSSVKVSCASGGTFLSSAISWVRQAPGQGLEWMGS LIPYFGTANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGPSE VGAILGYVWFDPPWGQGTITVTVSS
Clone 13B VH	247	QVQLVQSGAEVKKPGSSVKVSCASGGTFSAWAISWVRQAPGQGLEWMG SIIPYFGKANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGPS EVSGILGYVWFDPPWGQGTITVTVSS
Clone 13C VH	248	QVQLVQSGAEVKKPGSSVKVSCASGGTFLSSAISWVRQAPGQGLEWMGS IIPYFGKANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGPSE VKGILGYVWFDPPWGQGTITVTVSS
Clone 13D VH	249	QVQLVQSGAEVKKPGSSVKVSCASGGTFLSSAISWVRQAPGQGLEWMGS IIPYFGKANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGPSE VKGILGYVWFDPPWGQGTITVTVSS
Clone 16C VH	250	QVQLVQSGAEVKKPGSSVKVSCASGGTFREY AISWVRQAPGQGLEWMG GIHPYFGTARYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCTRQST WHKLYGTDVWGQGTITVTVSS
Clone 16D VH	251	QVQLVQSGAEVKKPGSSVKVSCASGGTFS DYPISWVRQAPGQGLEWMG GIPIVGGANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCTRQST WHKLFGTDVWGQGTITVTVSS
Clone 16E VH	252	QVQLVQSGAEVKKPGSSVKVSCASGGTFS DYPISWVRQAPGQGLEWMG GIPIVGGANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARQST WHKVYGTDVWGQGTITVTVSS
Clone 25A VH	253	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYAISWVRQAPGQGLEWMG WISAYNGNTKYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR DLSSFWSGDVLGAFDIWGQGTMTVTVSS
Clone 25B VH	254	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYPIGWVRQAPGQGLEWMG WISSYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR DLSSFWSGDVLGAFDIWGQGTMTVTVSS
Clone 25C VH	255	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYPIGWVRQAPGQGLEWMG WISSYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR GASSFWSGDVLGAFDIWGQGTMTVTVSS
Clone 25D VH	256	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYPIGWVRQAPGQGLEWMG WISSYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR DLKSFWSGDVLGAFDIWGQGTMTVTVSS

Clone 25E VH	257	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAIAWVRQAPGQGLEWMG WISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR SGSSFWSGDVLGAFDIWGQGMVTVSS
hTIGIT 68-82 epitope	258	ICNADLGWHISPSFK
Clone 13 heavy chain hIgG1 (and hIgG1 afucosylated) nucleotide sequence; bold indicates nucleotide sequence of variable region (VH)	259	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCA GCTATGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGA GTGGATGGGAAGCATCATCCCTATCTTTGGTACAGCAAACCTACGCA CAGAAGTTCCAGGGCAGAGTCACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCACCG TCTCCTCAGCTAGCACCAAGGGCCCCTCTGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCTGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCTGAACCTGTGACAGTGTCTGGAACCTCAGGAGCCCTGA CCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTACACATGCCACCGT GCCAGCACCTGAACCTCTGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGC AGCCCCGAGAACCACAGGTTTACACCCTGCCCCATCCCGGGATGAGCT GACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTA CAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGTCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG AGCCTCTCCCTGTCTCCGGGCAAA
Clone 13 heavy chain hIgG1 (and hIgG1 afucosylated) amino acid sequence; bold indicates VH	260	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEW MGSIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RGPSEVGAILGYVWFDPWGQGLTVTVSS ASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKLSLSL SPGK
Clone 13 heavy chain hIgG1 LALA- PG nucleotide sequence; bold indicates nucleotide sequence of VH	261	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCA GCTATGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGA GTGGATGGGAAGCATCATCCCTATCTTTGGTACAGCAAACCTACGCA CAGAAGTTCCAGGGCAGAGTCACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCACCG TCTCCTCAGCTAGCACCAAGGGCCCCTCTGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCTGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCTGAACCTGTGACAGTGTCTGGAACCTCAGGAGCCCTGA CCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTACACATGCCACCGT GCCAGCACCTGAAGCTGTGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG

		TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTACCGTCTGCG ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCGGGGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGC AGCCCCGAGAACCACAGGTTTACACCCCTGCCCCCATCCCGGGATGAGCT GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAAACAAC TACAAGACCACGCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTA CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG AGCCTCTCCCTGTCTCCGGGCAAA
Clone 13 heavy chain hIgG1 LALA- PG amino acid sequence; bold indicates VH	262	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEW MGSIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RGPSEVGAILGYVWFDPWGQGLTLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK
Clone 13 heavy chain hIgG4 S228P nucleotide sequence; bold indicates nucleotide sequence of VH	263	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCA GCTATGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGA GTGGATGGGAAGCATCATCCCTATCTTTGGTACAGCAAACCTACGCA CAGAAGTTCCAGGGCAGAGTCACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCACCG TCTCCTCAGCTAGCACCAAGGGCCCATCTGTCTTCCCCCTGGCCCCTTG CTCCAGAAGCACATCTGAGAGCACAGCGGCCCTGGGATGCCTGGTCAA GGACTATTTCCCTGAGCCGGTGACCGTAAGCTGGAACCTCTGGAGCCCTG ACCAGCGCGGTGCACACCTTCCAGCTGTCTTCCAGCTCTTCAGGGCTCT ACTCCCTCAGCAGTGTGGTGACTGTACCCTCCAGCAGCTTGGGCACCAA GACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGA CAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCCCTGCCAGCA CCAGAGTTCTTGGGGGGACCATCTGTCTTCTCTTCCCCCAAAACCCA AGGATACCCTCATGATCTCCCGGACCCCTGAGGTACATGTGTGGTGGT GGACGTGAGCCAGGAGGACCCGAGGTCCAGTTCAACTGGTATGTGGA TGGCGTGGAAGTGCATAATGCTAAGACAAAGCCACGGGAGGAGCAGTT CAACAGCACCTACCGTGTGGTCAGCGTCCTCACAGTGCTGCACCAGGAC TGGCTGAATGGCAAGGAGTACAAATGCAAGGTCTCCAACAAAGGCCTC CCATCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAACCCCGG GAACCACAGGTATACACCTGCCTCCATCCCAAGAAGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCTCTGACA TTGCCGTGGAGTGGGAGAGCAATGGGCAGCCAGAGAACTACAAGA CCACACCTCCCGTGTGGACTCCGATGGCTCCTTCTTCTGTACTCCCGG CTCACAGTGGACAAGAGCAGGTGGCAGGAGGAAATGTCTTCTCTCTGC TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGCAAA
Clone 13 heavy chain hIgG4 S228P amino acid sequence; bold indicates VH	264	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEW MGSIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RGPSEVGAILGYVWFDPWGQGLTLTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGKTYTCNVNHHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREP QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSRLTVDKSRWQEGNVFCFSVMHEALHNHYTQKSLSLSPGK
Clone 13A heavy chain hIgG1 (and hIgG1 afucosylated)	265	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCCTTA GCTCTGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGA

nucleotide sequence; bold indicates nucleotide sequence of VH		GTGGATGGGATCTCTCATCCCTTATTTTGGTACAGCAAACACTACGCA CAGAAGTTCCAGGGCAGAGTCACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCAACCG TCTCCTCAGCTAGCACCAAGGGCCCATCTGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCTGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCTGAACCTGTGACAGTGTCTGGAACCTCAGGAGCCCTGA CCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGT GCCCGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGC AGCCCCGAGAACACAGGTTTACACCCTGCCCCCATCCCGGGATGAGCT GACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCCTCTA CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG AGCCTCTCCCTGTCTCCGGGCAAA
Clone 13A heavy chain hIgG1 (and hIgG1 afucosylated) amino acid sequence; bold indicates VH	266	QVQLVQSGAEVVKPKGSSVKVSCKASGGTFLSSAISWVRQAPGQGLEW MGSLIPYFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDYAVYYC ARGPSEVGAILGYVWFDPWGQGLTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDITLMISRTPETVCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK
Clone 13B heavy chain hIgG1 (and hIgG1 afucosylated) nucleotide sequence; bold indicates nucleotide sequence of VH	267	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCTCTG CCTGGGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG AGTGGATGGGATCCATCATCCCTTATTTTGGTAAGGCAAACTACGC ACAGAAGTTCCAGGGCAGAGTCACCATTACTGCTGATGAATCCACC AGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTG CTGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAAGTGGTATACT GGGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCAAC GTCTCCTCAGCTAGCACCAAGGGCCCATCTGTCTTCCCCCTGGCACCCCT CCTCCAAGAGCACCTCTGGGGGCACAGCTGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCTGAACCTGTGACAGTGTCTGGAACCTCAGGAGCCCTG ACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCT ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA GACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA CAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACC GTGCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCTCCCC CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACAT GCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACT GGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCT GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAACACAGGTTTACACCCTGCCCCCATCCCGGGATGAG CTGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCCT

		CTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGT CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAG AAGAGCCTCTCCCTGTCTCCGGGCAAA
Clone 13B heavy chain hIgG1 (and hIgG1 afucosylated) amino acid sequence; bold indicates VH	268	QVQLVQSGAEVKKPGSSVKV SCKASGGT FSAWAISWVRQAPGQGLEW MGSII PFYFGKANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYC ARGPSEVSGILGYVWFDPWGQG TLTVSS ASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK
Clone 13C heavy chain hIgG1 (and hIgG1 afucosylated) nucleotide sequence; bold indicates nucleotide sequence of VH	269	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCCTTA GCTCTGCTATCAGCTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGA GTGGATGGGAAGTATCATCCCTCTGTTTGTAAGGCAAACTACGCA CAGAAGTTCCAGGGCAGAGTCA CCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAAAGGGTATAC TG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCA CCG TCTCCTCAGCTAGCACCAAGGGCCC ATCTGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCTGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCTGAACCTGTGACAGTGTCTTGAACTCAGGAGCCCTGA CCAGCGGCGTGACACCTTCCCGGCTGTCTTACAGTCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAA ACTCACACATGCCACCGT GCCCAGCACCTGA ACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCA CATGC GTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGG TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCCCAGCCCCATCGAGAAA ACCATCTCCAAAGCCAAAGGGC AGCCCCGAGAACCACAGGTTTACACCCTGCCCCATCCCGGGATGAGCT GACCAAGAACCAGGTCA GCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA TACAAGACCACGCCTCCCGTCTGGACTCCGACGGCTCCTTCTCTCTTA CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG AGCCTCTCCCTGTCTCCGGGCAAA
Clone 13C heavy chain hIgG1 (and hIgG1 afucosylated) amino acid sequence; bold indicates VH	270	QVQLVQSGAEVKKPGSSVKV SCKASGGT FELSSAISWVRQAPGQGLEW MGSII PLFGKANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYC ARGPSEVKGILGYVWFDPWGQG TLTVSS ASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK
Clone 13D heavy chain hIgG1 (and hIgG1 afucosylated) nucleotide sequence; bold indicates nucleotide sequence of VH	271	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCCTTA GCTCTGCTATCAGCTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGA GTGGATGGGATCCATCATCCCTTATTTTGTAAGGCAAACTACGCA CAGAAGTTCCAGGGCAGAGTCA CCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAAAGGGTATAC TG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCA CCG TCTCCTCAGCTAGCACCAAGGGCCC ATCTGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCTGCCCTGGGCTGCCTGGTCAAG

		GACTACTTCCTGAACCTGTGACAGTGTCTTGGAACCTCAGGAGCCCTGA CCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTA CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTACACATGCCACCGT GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGACGTGAGCCACGAAGACCCTGAGGTCAAAGTTCAAATGG TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCCCAGCCCCATCGAGAAAACCATCTCAAAGCCAAAGGGC AGCCCCGAGAACCACAGGTTTACACCCTGCCCCATCCCGGGATGAGCT GACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTA CAGCAAGCTACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG AGCTCTCCCTGTCTCCGGGCAA
Clone 13D heavy chain hIgG1 (and hIgG1 afucosylated) amino acid sequence; bold indicates VH	272	QVQLVQSGAEVKKPGSSVKASGGTFLSSAISWVRQAPGQGLEW MGSII PFYFGKANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC ARGPSEVKGILGYVWFDPWGQGL LVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS LSPGK
Clone 13, 13A, 13B, 13C, and 13D light chain h kappa (and afucosylated) nucleotide sequence; bold indicates nucleotide sequence of VL	273	GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTG GAGAGCCGGCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCA TAGTAATGGATA CAACTATTTGGATTGGTACCTGCAGAAGCCAGG CAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCTCCG GGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTTAC ACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTAC TGCATGCAGGCAAGACGAATCCCTATCACTTTTGGCGGAGGGACCA AGGTTGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCC GCCATCTGATGAGCAGTTGAAATCTGGAAC TGCCTCTGTTGTGTGCCTG CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACACAGACAGGAC AGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAA GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTACCCATCAG GGCTGAGCTCGCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
Clone 13, 13A, 13B, 13C, and 13D light chain h kappa (and afucosylated) amino acid sequence; bold indicates VL	274	DIVMTQSPSLPVT PGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSP QLLIY LGSNRSAGV PDR FSGSGSGT DF TLKISRVE AE DVG VYY CMQAR RIPIT FGGG TK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC
Clone 13 heavy chain mIgG2a (and afucosylated) nucleotide sequence; bold indicates nucleotide sequence of VH	275	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCA GCTATGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTGA GTGGATGGGAAGCATCATCCCTATCTTTGGTACAGCAA ACTACGCA CAGAAGTTCCAGGGCAGAGTACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTCACCG TCTCCTCAGCTAAAACAACAGCCCCATCGGTCTATCCGCTAGCCCCCTGT GTGTGGAGATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAAG GGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGGATCCCTGTC CAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACC CTCAGCAGCTCAGTACTGTAACCTCTAGCACCTGGCCCAGCCAGTCCA

		TCACCTGCAATGTGGCCACCCGGCAAGCAGCACCAAGGTGGACAAGA AAATTGAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATG CCCAGCACCTAACGCTGCTGGTGGACCATCCGTCTTCATCTTCCCTCCAA AGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGT GGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTT GTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAG GATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACC AGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAG ACCTCGGGGCGCCCATCGAGAGAACCATCTCAAACCCAAAGGCTCAG TAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGAC TAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCTGAA GACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTAC AAGAACACTGAACCAGTCTGGACTCTGATGGTTCTTACTTCATGTACA GCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAAGAAATAGTACT CCTGTTCAAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAG CTTCTCCCGGACTCCGGGCAAA
Clone 13 heavy chain mIgG2a (and afucosylated) amino acid sequence; bold indicates VH	276	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAIWVRQAPGQGLEW MGSIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RGPSEVGAILGYVWFDPWGQGLTVTVSSAKTTAPSVYPLAPVCGDITGS SVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSS TWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIF PPKIKDVLMLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHRE DYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRA PQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTE PVLDSGYSFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTP GK
Clone 13 heavy chain mIgG2a LALA-PG nucleotide sequence; bold indicates nucleotide sequence of VH	277	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCA GCTATGCTATCAGCTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGA GTGGATGGGAAGCATCATCCCTATCTTTGGTACAGCAAACCTACGCA CAGAAAGTTCAGGGCAGAGTCACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGCACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTACCCG TCTCCTCAGCTAAAACAACAGCCCCATCGGTCTATCCGCTAGCCCCTGT GTGTGGAGATACAACCTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAG GGTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGGATCCCTGTC CAGTGGTGTGCACACCTTCCCAGCTGTCTGCAGTCTGACCTCTACACC CTCAGCAGCTCAGTGACTCTAACCTCTAGCACCTGGCCACGCCAGTCCA TCACCTGCAATGTGGCCACCCGGCAAGCAGCACCAAGGTGGACAAGA AAATTGAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATG CCCAGCACCTAACGCTGCTGGTGGACCATCCGTCTTCATCTTCCCTCCAA AGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGT GGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTT GTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAG GATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACC AGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAG ACCTCGGGGCGCCCATCGAGAGAACCATCTCAAACCCAAAGGCTCAG TAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGAC TAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCTGAA GACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTAC AAGAACACTGAACCAGTCTGGACTCTGATGGTTCTTACTTCATGTACA GCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAAGAAATAGTACT CCTGTTCAAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAG CTTCTCCCGGACTCCGGGCAAA
Clone 13 heavy chain mIgG2a LALA-PG amino acid sequence; bold indicates VH	278	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAIWVRQAPGQGLEW MGSIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RGPSEVGAILGYVWFDPWGQGLTVTVSSAKTTAPSVYPLAPVCGDITGS SVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSS TWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIF PPKIKDVLMLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHRE DYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRA

		PQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTE PVLDSDSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTP GK
Clone 13 heavy chain mIgG1 nucleotide sequence; bold indicates nucleotide sequence of VH	279	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG TCCTCTGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCA GCTATGCTATCAGCTGGGTGCGACAGGCCCCCTGGACAAGGGCTTGA GTGGATGGGAAGCATCATCCCTATCTTTGGTACAGCAAACTACGCA CAGAAGTTCCAGGGCAGAGTCACCATTACTGCTGATGAATCCACCA GCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACTGC TGTGTACTACTGTGCCAGAGGCCCTTCTGAAGTAGGAGCAATACTG GGATATGTATGGTTTGACCCATGGGGACAGGGTACATTGGTACCCG TCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCGCTAGCCCCCTGG ATCTGCTGCCAACTAACTCCATGGTGACCCTGGGATGCCCTGGTCAAG GGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTGGATCCCTGT CCAGCGGTGTGCACACCTTCCCAGCTGTCTGGAGTCTGACCTCTACAC TCTGAGCAGCTCAGTGACTGTCCCTCCAGCCCTCGGCCAGCGAGACC GTCACCTGCAACGTTGCCACCCGCGCCAGCAGCACCAAGGTGGACAAG AAAATTGTGCCAGGGATTGTGGTTGTAAGCCTTGCATCTGTACAGTCC CAGAAGTATCATCTGTCTTCATCTTCCCCCAAAGCCCAAGGATGTGCT CACCATTACTCTGACTCCTAAGGTACAGTGTGTGTGGTAGACATCAGC AAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGG TGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCACAGCACTT TCCGCTCAGTCAGTGAATTCCCATCATGCACCAGGACTGGCTCAATGG CAAGGAGTTCAAATGCAGGGTCAACAGTGACGCTTTCCTGCCCCCATC GAGAAAACCATCTCCAAAACCAAGGCAGACCGAAGGCTCCACAGGTG TACACCATTCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGT CTGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGT GGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACTCAGCCCA TCATGAACACGAATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCA GAAGAGCAACTGGGAGGCAGGAAATACCTTACCTGCTCTGTGTTACAT GAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTG GCAAA
Clone 13 heavy chain mIgG1 amino acid sequence; bold indicates VH	280	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEW MGSIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCA RGPSEVGAILGYVWFDPWGQGTLVTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLESPLYTLSSSVTVPS SPRPSETVTCNVAPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPK DVLITLTPKVTCTVVDISKDDPEVQFSWVVDVEVHTAQTQPREEQFNST FRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTI PPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMNNTN GSYFVYSKLNQKSNWEAGNTFTCSVLHEGLHNHHTTEKSLSHSPGK
Clone 13 light chain mKappa nucleotide sequence; bold indicates nucleotide sequence of VH	281	GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTG GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCA TAGTAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGGG CAGTCTCCACAGCTCCTGATCTATTTGGGTCTAATCGGGCCTCCG GGGTCCCTGACAGTTTCAGTGGCAGTGGATCAGGCACAGATTTTAC ACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTAC TGCATGCAGGCAAGACGAATCCCTATCACTTTTGGCGGAGGGACCA AGGTTGAGATCAAACGTGCAGATGCGGCGCCAAGTATCCATCTTCC CACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTT CTTGAACAACCTTACCCCAAAGACATCAATGTCAAGTGGAAAGATTGAT GGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGAC AGCAAAGACAGCACCTACAGCATGAGCAGCACCCCTCACGTTGACCAAG GACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAG ACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT
Clone 13 light chain mKappa amino acid sequence; bold indicates VL	282	DIVMTQSPPLSLPVTTPGEPASISCRSSQSLHSNGYNLYLDWYLOKPGQSP QLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAR RIPITFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI NVKWKIDGSRQNGVLNSWTDQDSKSTYSMSSTLTLTKDEYERHNSYTC EATHKTSTSPIVKSFNRENEC

WHAT IS CLAIMED IS:

1. A composition comprising isolated antibodies that bind to human TIGIT (T-cell immunoreceptor with Ig and ITIM domains), wherein the antibodies have a binding affinity (K_D) for human TIGIT of less than 5 nM, and wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.
2. The composition of claim 1, wherein the antibodies have a K_D for human TIGIT of less than 1 nM.
3. The composition of claim 1, wherein the antibodies have a K_D for human TIGIT of less than 100 pM.
4. The composition of any one of claims 1 to 3, wherein the antibodies exhibit cross-reactivity with cynomolgus monkey TIGIT and/or mouse TIGIT.
5. The composition of claim 4, wherein the antibodies exhibit cross-reactivity with both cynomolgus monkey TIGIT and mouse TIGIT.
6. The composition of any one of claims 1 to 5, wherein the antibodies block binding of CD155 to TIGIT.
7. The composition of any one of claims 1 to 5, wherein the antibodies block binding of CD112 to TIGIT.
8. The composition of any one of claims 1 to 5, wherein the antibodies block binding of both CD155 and CD112 to TIGIT.
9. The composition of any one of claims 1 to 8, wherein the antibodies compete for binding to human TIGIT with an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 64.
10. The composition of any one of claims 1 to 9, wherein the antibodies when bound to human TIGIT bind one or both of amino acid positions 81 and 82.

11. The composition of claim 10, wherein the antibodies bind both of amino acid positions 81 and 82.
12. The composition of claim 10 or claim 11, wherein amino acid positions 81 and 82 are Phe81 and Lys82.
13. The composition of any one of claims 1 to 12, wherein the antibodies bind to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82.
14. A composition comprising isolated antibodies that binds to human TIGIT, wherein the antibodies when bound to human TIGIT bind one or both of amino acid positions 81 and 82, and wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.
15. The composition of claim 14, wherein the antibodies bind both of amino acid positions 81 and 82.
16. The composition of claim 14 or claim 15, wherein amino acid positions 81 and 82 are Phe81 and Lys82.
17. A composition comprising isolated antibodies that binds to human TIGIT, wherein the antibodies bind to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82, and wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated.
18. The composition of claim 13 or claim 17, wherein the epitope comprises Phe at position 81.
19. The composition of any one of claims 13, 17, and 18, wherein the epitope comprises Lys or Ser at position 82.
20. The composition of any one of claims 13, and 17 to 19, wherein the epitope comprises Phe at position 81 and Lys or Ser at position 82.
21. The composition of claim 20, wherein the epitope comprises Phe81 and Lys82.

22. The composition of any one of claims 13 and 17 to 21, wherein the epitope is a discontinuous epitope.

23. The composition of any one of claims 13 and 17 to 22, wherein the antibodies bind to an epitope on human TIGIT that further comprises one or more of amino acid positions 51, 52, 53, 54, 55, 73, 74, 75, 76, 77, 79, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93.

24. The composition of claim 23, wherein the epitope further comprises one or more amino acid residues selected from the group consisting of Thr51, Ala52, Gln53, Val54, Thr55, Leu73, Gly74, Trp75, His76, Ile77, Pro79, Asp83, Arg84, Val85, Ala86, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

25. The composition of claim 24, wherein the epitope comprises the amino acid residues Thr51, Ala52, Gln53, Val54, Thr55, Gly74, Trp75, His76, Ile77, Phe81, Lys82, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

26. The composition of claim 24, wherein the epitope comprises the amino acid residues Ala52, Gln53, Leu73, Gly74, Trp75, Pro79, Phe81, Lys82, Asp83, Arg84, Val85, and Ala86.

27. The composition of any one of claims 13 and 17 to 26, wherein the epitope comprises the sequence ICNADLGWHISPSFK (SEQ ID NO: 258).

28. The composition of any one of claims 1 to 27, wherein human TIGIT comprises the sequence of SEQ ID NO: 218.

29. The composition of any one of claims 1 to 28, wherein each of the antibodies comprises one or more of:

(a) a heavy chain CDR1 comprising a sequence selected from SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 comprising a sequence selected from SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186, SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, and SEQ ID NO:295;

(c) a heavy chain CDR3 comprising a sequence selected from SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134, SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, and SEQ ID NO:296;

(d) a light chain CDR1 comprising a sequence selected from SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, and SEQ ID NO:287;

(e) a light chain CDR2 comprising a sequence selected from SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, and SEQ ID NO:288; or

(f) a light chain CDR3 comprising a sequence selected from SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, and SEQ ID NO:215.

30. The composition of claim 29, wherein each of the antibodies comprises:

(a) a heavy chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, and SEQ ID NO:295;

- (c) a heavy chain CDR3 sequence comprising an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:286, SEQ ID NO:228, SEQ ID NO:292, SEQ ID NO:230, and SEQ ID NO:296;
- (d) a light chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:67 and SEQ ID NO:287;
- (e) a light chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:69 and SEQ ID NO:288; and/or
- (f) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

31. The composition of claim 29 or claim 30, wherein each of the antibodies comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 4, 6, 8, 13, 15, and 17, respectively; or
- (b) SEQ ID NOs: 22, 24, 26, 31, 33, and 35, respectively; or
- (c) SEQ ID NOs: 40, 42, 44, 49, 51, and 53, respectively; or
- (d) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (e) SEQ ID NOs: 283, 285, 62, 287, 288, and 71, respectively; or
- (f) SEQ ID NOs: 284, 60, 286, 67, 69, and 71, respectively; or
- (g) SEQ ID NOs: 76, 78, 80, 85, 87, and 89, respectively; or
- (h) SEQ ID NOs: 94, 96, 98, 103, 105, and 107, respectively; or
- (i) SEQ ID NOs: 112, 114, 116, 121, 123, and 125, respectively; or
- (j) SEQ ID NOs: 130, 132, 134, 139, 141, and 143, respectively; or
- (k) SEQ ID NOs: 148, 150, 152, 157, 159, and 161, respectively; or
- (l) SEQ ID NOs: 166, 168, 170, 175, 177, and 179, respectively; or
- (m) SEQ ID NOs: 184, 186, 188, 193, 195, and 197, respectively; or
- (n) SEQ ID NOs: 202, 204, 206, 211, 213, and 215, respectively; or
- (o) SEQ ID NOs: 221, 222, 223, 13, 15, and 17, respectively; or
- (p) SEQ ID NOs: 224, 225, 62, 67, 69, and 71, respectively; or
- (q) SEQ ID NOs: 293, 297, 62, 287, 288, and 71, respectively; or
- (r) SEQ ID NOs: 294, 225, 286, 67, 69, and 71, respectively; or
- (s) SEQ ID NOs: 226, 227, 228, 67, 69, and 71, respectively; or
- (t) SEQ ID NOs: 289, 291, 228, 287, 288, and 71, respectively; or
- (u) SEQ ID NOs: 290, 227, 292, 67, 69, and 71, respectively; or

- (v) SEQ ID NOs: 224, 229, 230, 67, 69, and 71, respectively; or
- (w) SEQ ID NOs: 293, 295, 230, 287, 288, and 71, respectively; or
- (x) SEQ ID NOs: 294, 229, 296, 67, 69, and 71, respectively; or
- (y) SEQ ID NOs: 224, 227, 230, 67, 69, and 71, respectively; or
- (z) SEQ ID NOs: 293, 290, 230, 287, 288, and 71, respectively; or
- (aa) SEQ ID NOs: 294, 290, 230, 67, 69, and 71, respectively; or
- (bb) SEQ ID NOs: 231, 232, 235, 103, 105, and 107, respectively; or
- (cc) SEQ ID NOs: 233, 234, 236, 103, 105, and 107, respectively; or
- (dd) SEQ ID NOs: 233, 234, 237, 103, 105, and 107, respectively; or
- (ee) SEQ ID NOs: 166, 238, 170, 175, 177, and 179, respectively; or
- (ff) SEQ ID NOs: 239, 240, 170, 175, 177, and 179, respectively; or
- (gg) SEQ ID NOs: 239, 240, 241, 175, 177, and 179, respectively; or
- (hh) SEQ ID NOs: 239, 240, 242, 175, 177, and 179, respectively; or
- (ii) SEQ ID NOs: 243, 168, 244, 175, 177, and 179, respectively.

32. The composition of any one of claims 1 to 31, wherein each of the antibodies comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (b) SEQ ID NOs: 283, 285, 62, 287, 288, and 71, respectively; or
- (c) SEQ ID NOs: 284, 60, 286, 67, 69, and 71, respectively.

33. The composition of any one of claims 1 to 32, wherein each of the antibodies comprises:

(a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257; and/or

(b) a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ

ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208.

34. The composition of any one of claims 1 to 33, wherein each of the antibodies comprises:

(a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10; or

(b) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:19 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:28; or

(c) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:37 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:46; or

(d) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:64; or

(e) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:73 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:82; or

(f) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:100; or

(g) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:109 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:118; or

(h) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:127 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:136; or

(i) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:145 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:154; or

(j) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:172; or

(k) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:181 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:190; or

(l) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:199 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:208.

35. The composition of claim 34, wherein each of the antibodies comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:28; or

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:46; or

(d) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID

NO:249 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64; or

(e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:73 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:82; or

(f) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:100; or

(g) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:109 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:118; or

(h) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:127 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:136; or

(i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:154; or

(j) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:172; or

(k) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:190; or

(l) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:199 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:208.

36. The composition of any one of claims 1 to 35, wherein each of the antibodies comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:55, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64.

37. The composition of any one of claims 1 to 36, wherein the antibodies are IgG antibodies.

38. The composition of claim 37, wherein the antibodies are IgG1 antibodies or IgG3 antibodies.

39. The composition of any one of claims 1 to 38, wherein each of the antibodies comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 262, 264, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

40. The composition of claim 39, wherein each of the antibodies comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

41. The composition of claim 39, wherein each of the antibodies comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

42. A composition comprising isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, and wherein each of the antibodies comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

43. A composition comprising isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, and wherein each of the antibodies comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

44. The composition of any one of claims 1 to 43, wherein the antibodies exhibit synergy with an anti-PD-1 antibody or an anti-PD-L1 antibody.

45. The composition of claim 44, wherein synergy is determined using an assay that comprises contacting a co-culture comprising (i) Jurkat effector cells that express PD-1, TIGIT and CD226, wherein the Jurkat effector cells comprise a luciferase reporter gene driven by the IL-2 promoter; and (ii) CHO-K1 artificial antigen presenting cells (aAPCs) expressing a TCR activator, PD-L1 and CD155; with the composition and an anti-PD-1 antibody or an anti-PD-L1 antibody.

46. The composition of any one of claims 1 to 45, wherein regulatory T (Treg) cells are depleted from human PBMCs contacted with the composition.

47. The composition of any one of claims 1 to 46, expression of MCP1, IL-8, and MIP1 α is increased in human PBMCs contacted with the composition.

48. The composition of any one of claims 1 to 47, wherein monocyte/macrophages are activated when contacted with the composition.

49. The composition of any one of claims 1 to 48, wherein CD86 and MHCII are upregulated when CD14⁺ monocyte/macrophages are contacted with the composition.

50. The composition of any one of claims 1 to 49, wherein CD14⁺ monocyte/macrophages contacted with the composition mature into antigen presenting cells.

51. The composition of any one of claims 1 to 50, wherein the memory T cells contacted with the composition show increased IFN γ production in response to antigen.

52. The composition of any one of claims 1 to 51, wherein the memory T cells contacted with the composition demonstrate enhanced response to antigen.

53. The composition of any one of claims 1 to 52, wherein effector memory CD8⁺ T cells and/or effector memory CD4⁺ T cells are increased in tumors contacted with the composition.

54. The composition of any one of claims 1 to 53, wherein the composition enhances a Th1 response in an animal administered the composition.

55. The composition of any one of claims 1 to 54, wherein the antibodies of the composition have lower binding affinity for Fc γ RIIa and/or Fc γ RIIb compared to the same anti-TIGIT antibodies that are not afucosylated.

56. The composition of any one of claims 1 to 55, wherein the composition mediates antibody-dependent cellular phagocytosis (ADCP) of cells that express TIGIT in the presence of monocyte macrophages.

57. The composition of any one of claims 1 to 56, wherein the antibodies are monoclonal.

58. The composition of any one of claims 1 to 57, wherein the antibodies are fully human antibodies.

59. The composition of any one of claims 1 to 58, wherein the antibodies are chimeric antibodies.

60. The composition of any one of claims 1 to 28 and 44 to 59, wherein the antibodies are humanized.

61. The composition of any one of claims 1 to 60, wherein the antibodies are antibody fragments.

62. The composition of claim 61, wherein the antibody fragments are Fab, Fab', F(ab')₂, scFv, or diabodies.

63. The composition of any one of claims 1 to 62, wherein the antibodies are bispecific antibodies.

64. The composition of any one of claims 1 to 63, wherein the antibodies are antibody-drug conjugates.

65. An antibody that binds to human TIGIT (T-cell immunoreceptor with Ig and ITIM domains), wherein the antibody has a binding affinity (K_D) for human TIGIT of less than 5 nM, and wherein the antibody is afucosylated.

66. The antibody of claim 65, wherein the antibody has a K_D for human TIGIT of less than 1 nM.

67. The antibody of claim 66, wherein antibody has a K_D for human TIGIT of less than 100 pM.

68. The antibody of any one of claims 65 to 67, wherein antibody exhibits cross-reactivity with cynomolgus monkey TIGIT and/or mouse TIGIT.

69. The antibody of claim 68, wherein the antibody exhibits cross-reactivity with both cynomolgus monkey TIGIT and mouse TIGIT.

70. The antibody of any one of claims 65 to 69, wherein the antibody blocks binding of CD155 to TIGIT.

71. The antibody of any one of claims 65 to 70, wherein the antibody blocks binding of CD112 to TIGIT.

72. The antibody of any one of claims 65 to 71, wherein the antibody blocks binding of both CD155 and CD112 to TIGIT.

73. The antibody of any one of claims 65 to 72, wherein the antibody competes for binding to human TIGIT with an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 64.

74. The antibody of any one of claims 65 to 73, wherein when bound to human TIGIT binds one or both of amino acid positions 81 and 82.

75. The antibody of claim 74, wherein the antibody binds both of amino acid positions 81 and 82.

76. The antibody of claim 74 or claim 75, wherein amino acid positions 81 and 82 are Phe81 and Lys82.

77. The antibody of any one of claims 65 to 76, wherein the antibody binds to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82.

78. An antibody that binds to human TIGIT, wherein when bound to human TIGIT binds one or both of amino acid positions 81 and 82, and wherein the antibody is afucosylated.

79. The antibody of claim 78, wherein the antibody binds both of amino acid positions 81 and 82.

80. The antibody of claim 78 or claim 79, wherein amino acid positions 81 and 82 are Phe81 and Lys82.

81. An antibody that binds to human TIGIT, wherein the antibody binds to an epitope on human TIGIT that comprises one or both of amino acid positions 81 and 82, and wherein the antibody is afucosylated.

82. The antibody of claim 77 or claim 81, wherein the epitope comprises Phe at position 81.

83. The antibody of any one of claims 77, 81, and 82, wherein the epitope comprises Lys or Ser at position 82.

84. The antibody of any one of claims 77 and 81 to 83, wherein the epitope comprises Phe at position 81 and Lys or Ser at position 82.

85. The antibody of claim 84, wherein the epitope comprises Phe81 and Lys82.

86. The antibody of any one of claims 77 and 81 to 85, wherein the epitope is a discontinuous epitope.

87. The antibody of any one of claims 77 and 81 to 86, wherein the antibody binds to an epitope on human TIGIT that further comprises one or more of amino acid positions 51, 52, 53, 54, 55, 73, 74, 75, 76, 77, 79, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93.

88. The antibody of claim 87, wherein the epitope further comprises one or more amino acid residues selected from the group consisting of Thr51, Ala52, Gln53, Val54, Thr55, Leu73, Gly74, Trp75, His76, Ile77, Pro79, Asp83, Arg84, Val85, Ala86, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

89. The antibody of claim 88, wherein the epitope comprises the amino acid residues Thr51, Ala52, Gln53, Val54, Thr55, Gly74, Trp75, His76, Ile77, Phe81, Lys82, Pro87, Gly88, Pro89, Gly90, Leu91, Gly92, and Leu93.

90. The antibody of claim 88, wherein the epitope comprises the amino acid residues Ala52, Gln53, Leu73, Gly74, Trp75, Pro79, Phe81, Lys82, Asp83, Arg84, Val85, and Ala86.

91. The antibody of any one of claims 77 and 81 to 90, wherein the epitope comprises the sequence ICNADLGWHISPSFK (SEQ ID NO: 258).

92. The antibody of any one of claims 65 to 91, wherein human TIGIT comprises the sequence of SEQ ID NO: 218.

93. The antibody of any one of claims 65 to 92, wherein the antibody comprises one or more of:

(a) a heavy chain CDR1 comprising a sequence selected from SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:221, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:239, SEQ ID NO:243, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 comprising a sequence selected from SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:42, SEQ ID NO:60, SEQ ID NO:78, SEQ ID NO:96, SEQ ID NO:114, SEQ ID NO:132, SEQ ID NO:150, SEQ ID NO:168, SEQ ID NO:186, SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:285, SEQ ID NO:297, SEQ ID NO:291, and SEQ ID NO:295;

(c) a heavy chain CDR3 comprising a sequence selected from SEQ ID NO:8, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:62, SEQ ID NO:80, SEQ ID NO:98, SEQ ID NO:116, SEQ ID NO:134, SEQ ID NO:152, SEQ ID NO:170, SEQ ID NO:188, SEQ ID NO:206, SEQ ID NO:223, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:286, SEQ ID NO:292, and SEQ ID NO:296;

(d) a light chain CDR1 comprising a sequence selected from SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:49, SEQ ID NO:67, SEQ ID NO:85, SEQ ID NO:103, SEQ ID NO:121, SEQ ID NO:139, SEQ ID NO:157, SEQ ID NO:175, SEQ ID NO:193, SEQ ID NO:211, and SEQ ID NO:287;

(e) a light chain CDR2 comprising a sequence selected from SEQ ID NO:15, SEQ ID NO:33, SEQ ID NO:51, SEQ ID NO:69, SEQ ID NO:87, SEQ ID NO:105, SEQ ID NO:123, SEQ ID NO:141, SEQ ID NO:159, SEQ ID NO:177, SEQ ID NO:195, SEQ ID NO:213, and SEQ ID NO:288; or

(f) a light chain CDR3 comprising a sequence selected from SEQ ID NO:17, SEQ ID NO:35, SEQ ID NO:53, SEQ ID NO:71, SEQ ID NO:89, SEQ ID NO:107, SEQ ID NO:125, SEQ ID NO:143, SEQ ID NO:161, SEQ ID NO:179, SEQ ID NO:197, and SEQ ID NO:215.

94. The antibody of claim 93, wherein the antibody comprises:

(a) a heavy chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:58, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:224, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:226, SEQ ID NO:289, and SEQ ID NO:290;

(b) a heavy chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:60, SEQ ID NO:285, SEQ ID NO:225, SEQ ID NO:297, SEQ ID NO:227, SEQ ID NO:291, SEQ ID NO:229, and SEQ ID NO:295;

(c) a heavy chain CDR3 sequence comprising an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:286, SEQ ID NO:228, SEQ ID NO:292, SEQ ID NO:230, and SEQ ID NO:296;

(d) a light chain CDR1 sequence comprising an amino acid sequence selected from SEQ ID NO:67 and SEQ ID NO:287;

(e) a light chain CDR2 sequence comprising an amino acid sequence selected from SEQ ID NO:69 and SEQ ID NO:288; and/or

(f) a light chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO:71.

95. The antibody of claim 94, wherein the antibody comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 4, 6, 8, 13, 15, and 17, respectively; or
- (b) SEQ ID NOs: 22, 24, 26, 31, 33, and 35, respectively; or
- (c) SEQ ID NOs: 40, 42, 44, 49, 51, and 53, respectively; or
- (d) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (e) SEQ ID NOs: 76, 78, 80, 85, 87, and 89, respectively; or
- (f) SEQ ID NOs: 94, 96, 98, 103, 105, and 107, respectively; or
- (g) SEQ ID NOs: 112, 114, 116, 121, 123, and 125, respectively; or
- (h) SEQ ID NOs: 130, 132, 134, 139, 141, and 143, respectively; or
- (i) SEQ ID NOs: 148, 150, 152, 157, 159, and 161, respectively; or

- (j) SEQ ID NOs: 166, 168, 170, 175, 177, and 179, respectively; or
- (k) SEQ ID NOs: 184, 186, 188, 193, 195, and 197, respectively; or
- (l) SEQ ID NOs: 202, 204, 206, 211, 213, and 215, respectively; or
- (m) SEQ ID NOs: 221, 222, 223, 13, 15, and 17, respectively; or
- (n) SEQ ID NOs: 224, 225, 62, 67, 69, and 71, respectively; or
- (o) SEQ ID NOs: 226, 227, 228, 67, 69, and 71, respectively; or
- (p) SEQ ID NOs: 224, 229, 230, 67, 69, and 71, respectively; or
- (q) SEQ ID NOs: 224, 227, 230, 67, 69, and 71, respectively; or
- (r) SEQ ID NOs: 231, 232, 235, 103, 105, and 107, respectively; or
- (s) SEQ ID NOs: 233, 234, 236, 103, 105, and 107, respectively; or
- (t) SEQ ID NOs: 233, 234, 237, 103, 105, and 107, respectively; or
- (u) SEQ ID NOs: 166, 238, 170, 175, 177, and 179, respectively; or
- (v) SEQ ID NOs: 239, 240, 170, 175, 177, and 179, respectively; or
- (w) SEQ ID NOs: 239, 240, 241, 175, 177, and 179, respectively; or
- (x) SEQ ID NOs: 239, 240, 242, 175, 177, and 179, respectively; or
- (y) SEQ ID NOs: 243, 168, 244, 175, 177, and 179, respectively.

96. The antibody of any one of claims 65 to 95, wherein the antibody comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR, and CDR3 comprising the sequences of:

- (a) SEQ ID NOs: 58, 60, 62, 67, 69, and 71, respectively; or
- (b) SEQ ID NOs: 283, 285, 62, 287, 288, and 71, respectively; or
- (c) SEQ ID NOs: 284, 60, 286, 67, 69, and 71, respectively.

97. The antibody of any one of claims 65 to 96, wherein the antibody comprises:

(a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:37, SEQ ID NO:55, SEQ ID NO:73, SEQ ID NO:91, SEQ ID NO:109, SEQ ID NO:127, SEQ ID NO:145, SEQ ID NO:163, SEQ ID NO:181, SEQ ID NO:199, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257; and/or

(b) a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10, SEQ ID NO:28, SEQ ID NO:46, SEQ

ID NO:64, SEQ ID NO:82, SEQ ID NO:100, SEQ ID NO:118, SEQ ID NO:136, SEQ ID NO:154, SEQ ID NO:172, SEQ ID NO:190, or SEQ ID NO:208.

98. The antibody of any one of claims 65 to 97, wherein the antibody comprises:

(a) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:10; or

(b) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:19 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:28; or

(c) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:37 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:46; or

(d) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:64; or

(e) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:73 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:82; or

(f) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:100; or

(g) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:109 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:118; or

(h) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:127 and a light chain variable region

comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:136; or

(i) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:145 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:154; or

(j) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:172; or

(k) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:181 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:190; or

(l) a heavy chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:199 and a light chain variable region comprising an amino acid sequence that has at least 90% sequence identity to SEQ ID NO:208.

99. The antibody of claim 98, wherein the antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:245 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:10; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:28; or

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:46; or

(d) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:55, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, or SEQ ID NO:249 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64; or

(e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:73 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:82; or

(f) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:91, SEQ ID NO:250, SEQ ID NO:251, or SEQ ID NO:252 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:100; or

(g) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:109 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:118; or

(h) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:127 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:136; or

(i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:145 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:154; or

(j) a heavy chain variable region comprising the amino acid sequence of any one of SEQ ID NO:163, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, or SEQ ID NO:257 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:172; or

(k) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:181 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:190; or

(l) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:199 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:208.

100. The antibody of any one of claims 65 to 99, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:55, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:64.

101. The antibody of any one of claims 65 to 100, wherein the antibody is an IgG antibody.

102. The antibody of claim 101, wherein the antibody is an IgG1 antibody or an IgG3 antibody.

103. The antibody of any one of claims 65 to 102, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 262, 264, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

104. The antibody of claim 103, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

105. The antibody of claim 103, wherein the antibody comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

106. An antibody that binds to human TIGIT, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 260, 262, 264, 266, 268, 270, and 272; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

107. An antibody that binds to human TIGIT, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 260; and a light chain comprising the amino acid sequence of SEQ ID NO: 274.

108. An antibody that binds to human TIGIT, wherein the antibody comprises a heavy chain consisting of the amino acid sequence of SEQ ID NOs: 260; and a light chain consisting of the amino acid sequence of SEQ ID NO: 274.

109. The antibody of any one of claims 65 to 108, wherein the antibody exhibits synergy with an anti-PD-1 antibody or an anti-PD-L1 antibody.

110. The antibody of claim 109, wherein synergy is determined using an assay that comprises contacting a co-culture comprising (i) Jurkat effector cells that express PD-1, TIGIT and CD226, wherein the Jurkat effector cells comprise a luciferase reporter gene driven by the IL-2 promoter; and (ii) CHO-K1 artificial antigen presenting cells (aAPCs) expressing a TCR activator, PD-L1 and CD155; with the composition and an anti-PD-1 antibody or an anti-PD-L1 antibody.

111. The antibody of any one of claims 65 to 110, wherein regulatory T (Treg) cells are depleted from human PBMCs contacted with the antibody.

112. The antibody of any one of claims 65 to 111, expression of MCP1, IL-8, and MIP1 α is increased in human PBMCs contacted with the antibody.

113. The antibody of any one of claims 65 to 112, wherein monocyte/macrophages are activated when contacted with the antibody.

114. The antibody of any one of claims 65 to 113, wherein CD86 and MHCII are upregulated when CD14⁺ monocyte/macrophages are contacted with the antibody.

115. The antibody of any one of claims 65 to 114, wherein CD14⁺ monocyte/macrophages contacted with the antibody mature into antigen presenting cells.

116. The antibody of any one of claims 65 to 115, wherein the memory T cells contacted with the antibody show increased IFN γ production in response to antigen.

117. The antibody of any one of claims 65 to 116, wherein the memory T cells contacted with the antibody demonstrate enhanced response to antigen.

118. The antibody of any one of claims 65 to 117, wherein effector memory CD8⁺ T cells and/or effector memory CD4⁺ T cells are increased in tumors contacted with the antibody.

119. The antibody of any one of claims 65 to 118, wherein the antibody enhances a Th1 response in an animal administered the antibody.

120. The antibody of any one of claims 65 to 119, wherein the antibody has lower binding affinity for Fc γ RIIa and/or Fc γ RIIb compared to the same anti-TIGIT antibody that is not afucosylated.

121. The antibody of any one of claims 65 to 120, wherein the composition mediates antibody-dependent cellular phagocytosis (ADCP) of cells that express TIGIT in the presence of monocyte macrophages.

122. The antibody of any one of claims 65 to 121, wherein the antibody is monoclonal.

123. The antibody of any one of claims 65 to 122, wherein the antibody is a fully human antibody.

124. The antibody of any one of claims 65 to 123, wherein the antibody is a chimeric antibody.

125. The antibody of any one of claims 65 to 92 and 109 to 124, wherein the antibody is humanized.

126. The antibody of any one of claims 65 to 125, wherein the antibody is an antibody fragment.

127. The antibody of claim 126, wherein the antibody fragment is a Fab, a Fab', a F(ab')₂, a scFv, or a diabody.

128. The antibody of any one of claims 65 to 127, wherein the antibody is a bispecific antibody.

129. The antibody of any one of claims 65 to 128, wherein the antibody is an antibody-drug conjugate.

130. A pharmaceutical formulation comprising the composition of any one of claims 1 to 64 or the antibody of any one of claims 65 to 129 and a pharmaceutically acceptable carrier.

131. An isolated polynucleotide that encodes (i) the heavy chain of the antibody of any one of claims 106 to 108; (ii) the light chain of the antibody of any one of claims 106 to 108; or (iii) the heavy chain and the light chain of the antibody of any one of claims 106 to 108.

132. The isolated polynucleotide of claim 131, wherein the polynucleotide comprises (i) a nucleotide sequence selected from SEQ ID NOs: 259, 261, 163, 265, 267, 269, and 271; or (ii) a nucleotide sequence of SEQ ID NO: 273; or (iii) a nucleotide sequence selected from SEQ ID NOs: 259, 261, 263, 265, 267, 269, and 271, and a nucleotide sequence of SEQ ID NO: 273.

133. A vector comprising the polynucleotide of claim 131 or claim 132.

134. An isolated host cell comprising the isolated polynucleotide of claim 131 or claim 132, or the vector of claim 133.

135. An isolated host cell that expresses the antibody of any one of claims 106-108.

136. The host cell of claim 134 or claim 135, which is engineered to produce afucosylated antibodies.

137. A method of producing an antibody that binds to human TIGIT, comprising incubating the host cell of any one of claims 134 to 136 under conditions suitable for producing the antibody.

138. The method of claim 137, wherein the host cell is engineered to produce afucosylated antibodies.

139. The method of claim 137, wherein the host cell is cultured in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies.

140. The method of any one of claims 137 to 139, further comprising isolating the antibodies.

141. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by the method of any one of claims 138 to 140.

142. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by the method comprising incubating the host cell of claim 134 or claim 135 under conditions suitable for producing afucosylated antibodies, and isolating the antibodies to form the composition of isolated antibodies.

143. The composition of claim 142, wherein the host cell is engineered to produce afucosylated antibodies.

144. The composition of claim 142, wherein the host cell is cultured in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies.

145. A host cell comprising a polynucleotide comprising a nucleotide sequence encoding the (i) antibodies of the composition of any one of claims 1 to 63, or (ii) the antibody of any one of claims 65 to 128, wherein the host cell is engineered to produce afucosylated antibodies.

146. The host cell of claim 145, wherein the polynucleotide is a vector.

147. A host cell that expresses (i) the antibodies of the composition of any one of claims 1 to 63, or (ii) the antibody of any one of claims 65 to 128, wherein the host cell is engineered to produce afucosylated antibodies.

148. The host cell of any one of claims 145 to 147, wherein the host cell comprises:

(a) the nucleotide sequence of SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, SEQ ID NO:74, SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, SEQ ID NO:182, SEQ ID NO:200, SEQ ID NO:259, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, or SEQ ID NO:271; and/or

(b) the nucleotide sequence of SEQ ID NO:11, SEQ ID NO:29, SEQ ID NO:47, SEQ ID NO:65, SEQ ID NO:83, SEQ ID NO:101, SEQ ID NO:119, SEQ ID NO:137, SEQ ID NO:155, SEQ ID NO:173, SEQ ID NO:191, SEQ ID NO:209, or SEQ ID NO:273.

149. A method of producing afucosylated antibodies that bind TIGIT, comprising culturing the host cell of any one of claims 145 to 148 under conditions suitable for producing the afucosylated antibodies.

150. A method of producing afucosylated antibodies that bind TIGIT, comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies, wherein the host cell comprises a polynucleotide comprising a nucleotide sequence encoding the (i) antibodies of the composition of any one of claims 1 to 63, or (ii) the antibody of any one of claims 65 to 128.

151. The method of claim 150, wherein the polynucleotide is a vector.

152. A method of producing afucosylated antibodies that bind TIGIT, comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for

producing afucosylated antibodies, wherein the host cell expresses (i) the antibodies of the composition of any one of claims 1 to 63, or (ii) the antibody of any one of claims 65 to 128.

153. The method of any one of claims 150 to 152, wherein the host cell comprises:

(a) the nucleotide sequence of SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, SEQ ID NO:74, SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, SEQ ID NO:182, SEQ ID NO:200, SEQ ID NO:259, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, or SEQ ID NO:271; and/or

(b) the nucleotide sequence of SEQ ID NO:11, SEQ ID NO:29, SEQ ID NO:47, SEQ ID NO:65, SEQ ID NO:83, SEQ ID NO:101, SEQ ID NO:119, SEQ ID NO:137, SEQ ID NO:155, SEQ ID NO:173, SEQ ID NO:191, SEQ ID NO:209, or SEQ ID NO:273.

154. The method of any one of claims 150 to 153, wherein the fucose analogue is 2-fluorofucose.

155. The method of any one of claims 149 to 154, further comprising isolating the afucosylated antibodies.

156. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by the method of any one of claims 149 to 155.

157. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by a method comprising incubating the host cell of any one of claims 145 to 147 under conditions suitable for producing afucosylated antibodies, and isolating the antibodies to form the composition of isolated antibodies.

158. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by a method comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated

antibodies, wherein the host cell comprises a polynucleotide comprising a nucleotide sequence encoding the (i) antibodies of the composition of any one of claims 1 to 63, or (ii) the antibody of any one of claims 65 to 128.

159. A composition of isolated antibodies that bind to human TIGIT, wherein at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the antibodies in the composition are afucosylated, wherein the antibodies are produced by a method comprising culturing a host cell in the presence of a fucose analogue under conditions suitable for producing afucosylated antibodies, wherein the host cell expresses (i) the antibodies of the composition of any one of claims 1 to 63, or (ii) the antibody of any one of claims 65 to 128.

160. A kit comprising:
the composition of any one of claims 1 to 64, the antibody of any one of claims 65 to 129, or the pharmaceutical composition of claim 130; and
an additional therapeutic agent.

161. The kit of claim 160, wherein the additional therapeutic is an anti-cancer agent.

162. The kit of claim 160 or claim 161, wherein the additional therapeutic agent is an antibody.

163. The kit of any one of claims 160 to 162, wherein the additional therapeutic agent is an antagonist or an inhibitor of a T cell coinhibitor; an agonist of a T cell coactivator; an immune stimulatory cytokine; or SGN-2FF.

164. The kit of any one of claims 160 to 163, wherein the additional therapeutic agent binds a protein selected from CD25, PD-1, PD-L1, Tim3, Lag3, CTLA4, 41BB, OX40, CD3, CD40, CD47M, GM-CSF, CSF1R, TLR, STING, RIGI, TAM receptor kinase, NKG2A, NKG2D, GD2, HER2, EGFR, PDGFR α , SLAMF7, VEGF, CTLA-4, CD20, cCLB8, KIR, and CD52.

165. The kit of claim any one of claims 160 to 164, wherein the additional therapeutic agent is selected from an anti-CD25 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-Tim3 antibody, anti-Lag3 antibody, anti-CTLA4 antibody, anti-41BB

antibody, anti-OX40 antibody, anti-CD3 antibody, anti-CD40 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-STING antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-HER2 antibody, an anti-EGFR antibody, an anti-PDGFR- α -antibody, an anti-SLAMF7 antibody, an anti-VEGF antibody, an anti-CTLA-4 antibody, an anti-CD20 antibody, an anti-cCLB8 antibody, an anti-KIR antibody, and an anti-CD52 antibody.

166. The kit of any one of claims 160 to 165, wherein the additional therapeutic agent comprises a cytokine selected from IL-15, IL-21, IL-2, GM-CSF, M-CSF, G-CSF, IL-1, IL-3, IL-12, and IFN γ .

167. The kit of any one of claims 160 to 165, wherein the additional therapeutic agent is selected from SEA-CD40, avelumab, durvalumab, nivolumab, pembrolizumab, pidilizumab, atezolizumab, Hul4.18K322A, Hu3F8, dinituximab, trastuzumab, cetuximab, olaratumab, necitumumab, elotuzumab, ramucirumab, pertuzumab, ipilimumab, bevacizumab, rituximab, obinutuzumab, siltuximab, ofatumumab, lirilumab, and alemtuzumab.

168. The kit of claim 160 or claim 161, wherein the additional therapeutic agent is selected from an alkylating agent (*e.g.*, cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, or temozolomide), an anthracycline (*e.g.*, doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (*e.g.*, paclitaxel or docetaxel), a histone deacetylase inhibitor (*e.g.*, vorinostat or romidepsin), an inhibitor of topoisomerase (*e.g.*, irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (*e.g.*, bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (*e.g.*, azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (*e.g.*, actinomycin or bleomycin), a platinum-based agent (*e.g.*, cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (*e.g.*, vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, or docetaxel), galardin, thalidomide, lenalidomide, and pomalidomide.

169. A method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of the composition of any one of claims 1 to 64, the antibody of any one of claims 65 to 129, or the pharmaceutical composition of claim 130.

170. The method of claim 169, wherein the cancer is a cancer that is enriched for expression of CD112 or CD155.

171. The method of claim 169 or claim 170, wherein the cancer is a cancer that is enriched for T cells or natural killer (NK) cells that express TIGIT.

172. The method of any one of claims 169 to 171, wherein the cancer is bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, gastric cancer, pancreatic cancer, colorectal cancer, colon cancer, kidney cancer, clear cell renal carcinoma, head and neck cancer, lung cancer, lung adenocarcinoma, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, melanoma, neoplasm of the central nervous system, mesothelioma, lymphoma, leukemia, chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, Hodgkin lymphoma, myeloma, or sarcoma.

173. The method of claim 172, wherein the cancer is lymphoma, leukemia, chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, or Hodgkin lymphoma.

174. The method of any one of claims 169 to 173, further comprising administering to the subject a therapeutically effective amount of an additional therapeutic agent.

175. The method of claim 174, wherein the additional therapeutic is an anti-cancer agent.

176. The method of claim 174 or claim 175, wherein the additional therapeutic agent is an antibody.

177. The method of any one of claims 174 to 176, wherein the additional therapeutic agent is an antagonist or an inhibitor of a T cell coinhibitor; an agonist of a T cell coactivator; or an immune stimulatory cytokine; or SGN-2FF.

178. The method of any one of claims 174 to 177, wherein the additional therapeutic agent binds a protein selected from CD25, PD-1, PD-L1, Tim3, Lag3, CTLA4, 41BB, OX40, CD3, CD40, CD47M, GM-CSF, CSF1R, TLR, STING, RIGI, TAM receptor kinase, NKG2A, NKG2D, GD2, HER2, EGFR, PDGFR α , SLAMF7, VEGF, CTLA-4, CD20, cCLB8, KIR, and CD52.

179. The method of claim 178, wherein the additional therapeutic agent is selected from an anti-CD25 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-Tim3 antibody, anti-Lag3 antibody, anti-CTLA4 antibody, anti-41BB antibody, anti-OX40 antibody, anti-CD3 antibody, anti-CD40 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-STING antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-HER2 antibody, an anti-EGFR antibody, an anti-PDGFR- α -antibody, an anti-SLAMF7 antibody, an anti-VEGF antibody, an anti-CTLA-4 antibody, an anti-CD20 antibody, an anti-cCLB8 antibody, an anti-KIR antibody, and an anti-CD52 antibody.

180. The method of claim 174 or claim 175, wherein the additional therapeutic agent comprises a cytokine selected from IL-15, IL-21, IL-2, GM-CSF, M-CSF, G-CSF, IL-1, IL-3, IL-12, and IFN γ .

181. The method of any one of claims 174 to 179, wherein the additional therapeutic agent is selected from SEA-CD40, avelumab, durvalumab, nivolumab, pembrolizumab, pidilizumab, atezolizumab, Hul4.18K322A, Hu3F8, dinituximab, trastuzumab, cetuximab, olaratumab, necitumumab, elotuzumab, ramucirumab, pertuzumab, ipilimumab, bevacizumab, rituximab, obinutuzumab, siltuximab, ofatumumab, lirilumab, and alemtuzumab.

182. The method of claim 174 or claim 175, wherein the additional therapeutic agent is selected from an alkylating agent (*e.g.*, cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, or temozolomide), an anthracycline (*e.g.*, doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (*e.g.*, paclitaxel or docetaxel), a histone deacetylase inhibitor (*e.g.*, vorinostat or romidepsin), an inhibitor of topoisomerase (*e.g.*, irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (*e.g.*, bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (*e.g.*,

azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (*e.g.*, actinomycin or bleomycin), a platinum-based agent (*e.g.*, cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (*e.g.*, vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, or docetaxel), galardin, thalidomide, lenalidomide, and pomalidomide.

183. The method of any one of claims 174 to 182, wherein the composition, the antibody, or the pharmaceutical composition is administered concurrently with the additional therapeutic agent.

184. The method of any one of claims 174 to 183, wherein the composition, the antibody, or the pharmaceutical composition is administered sequentially to the additional therapeutic agent.