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(54) Titre : ANTICORPS DIRIGES CONTRE L'IMMUNOGLOBULINE DE CELLULE T ET PROTEINE 3 DE MUCINE (TIM-3)
(54) Title: ANTIBODIES DIRECTED AGAINST T CELL IMMUNOGLOBULIN AND MUCIN PROTEIN 3 (TIM-3)

(57) **Abrégé/Abstract:**

The invention relates to an isolated immunoglobulin heavy chain polypeptide and an isolated immunoglobulin light chain polypeptide that bind to a protein encoded by the T Cell Immunoglobulin and Mucin Protein-3 (HM-3). The invention provides a TIM-3-binding agent that comprises the aforementioned immunoglobulin heavy chain polypeptide and immunoglobulin light chain polypeptide. The invention also provides related vectors, compositions, and methods of using the TIM-3-binding agent to treat a disorder or disease that is responsive to TIM-3 inhibition, such as cancer, an infectious disease, or an autoimmune disease.



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ANTIBODIES DIRECTED AGAINST T CELL IMMUNOGLOBULIN AND MUCIN
PROTEIN 3 (TIM-3)

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0001] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 369,646 Byte ASCII (Text) file named "723697_ST25.TXT," created on March 31, 2016.

BACKGROUND OF THE INVENTION

[0002] The protein T Cell Immunoglobulin and Mucin Domain-3 (TIM-3), also known as Hepatitis A Virus Cellular Receptor 2 (HAVCR2), is a Th1-specific cell surface protein that regulates macrophage activation and enhances the severity of experimental autoimmune encephalomyelitis in mice. TIM-3 is highly expressed on the surface of multiple immune cell types, including, for example, Th1 IFN- γ + cells, Th17 cells, natural killer (NK) cells, monocytes, and tumor-associated dendritic cells (DCs) (see, e.g., Clayton et al., *J. Immunol.*, 192(2): 782-791 (2014); Jones et al., *J. Exp. Med.*, 205: 2763-2779 (2008); Monney et al., *Nature*, 415: 536-541 (2002); Hastings et al., *Eur. J. Immunol.*, 39: 2492-2501 (2009); Seki et al., *Clin. Immunol.*, 127: 78-88 (2008); Ju et al., *B. J. Hepatol.*, 52: 322-329 (2010); Anderson et al., *Science*, 318: 1141-1143 (2007); Baitsch et al., *PLoS ONE*, 7: e30852 (2012); Ndhlovu et al., *Blood*, 119: 3734-3743 (2012). TIM-3 also is highly expressed on “exhausted” or impaired CD8+ T-cells in a variety of chronic viral infections (e.g., HIV, HCV, and HBV) and in certain cancers (see, e.g., McMahan et al., *J. Clin. Invest.*, 120(12): 4546-4557 (2010); Jin et al., *Proc Natl Acad Sci USA*, 107(33): 14733-14738 (2010); Golden-Mason et al., *J. Virol.*, 83(18): 9122-9130 (2009); Jones et al., *supra*; Fourcade et al., *J. Exp. Med.*, 207(10): 2175-2186 (2010); Sakuishi et al., *J. Exp. Med.*, 207(10): 2187-2194 (2010); Zhou et al., *Blood*, 117(17): 4501-4510 (2011); Ngiow et al., *Cancer Res.*, 71(10): 3540-3551 (2011)).

[0003] Putative ligands for TIM-3 include phosphatidylserine (Nakayama et al., *Blood*, 113: 3821-3830 (2009)), galectin-9 (Zhu et al., *Nat. Immunol.*, 6: 1245-1252 (2005)), high-mobility group protein 1 (HMGB1) (Chiba et al., *Nature Immunology*, 13: 832-842 (2012)), and

carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1) (Huang et al., *Nature*, 517(7534): 386-90 (2015)).

[0004] TIM-3 functions to regulate various aspects of the immune response. The interaction of TIM-3 and galectin-9 (Gal-9) induces cell death and *in vivo* blockade of this interaction exacerbates autoimmunity and abrogates tolerance in experimental models, strongly suggesting that TIM-3 is a negative regulatory molecule. In contrast to its effect on T-cells, the TIM-3-Gal-9 interaction exhibits antimicrobial effects by promoting macrophage clearance of intracellular pathogens (see, e.g., Sakuishi et al., *Trends in Immunology*, 32(8): 345-349 (2011)). *In vivo*, suppression of TIM-3 has been shown to enhance the pathological severity of experimental autoimmune encephalomyelitis (Monney et al., *supra*; and Anderson, A. C. and Anderson, D. E., *Curr. Opin. Immunol.*, 18: 665-669 (2006)). Studies also suggest that dysregulation of the TIM-3-galectin-9 pathway could play a role in chronic autoimmune diseases, such as multiple sclerosis (Anderson and Anderson, *supra*). TIM-3 promotes clearance of apoptotic cells by binding phosphatidyl serine through its unique binding cleft (see, e.g., DeKruyff et al., *J. Immunol.*, 184(4):1918-1930 (2010)).

[0005] Inhibition of TIM-3 activity, such as through use of monoclonal antibodies, is currently under investigation as an immunotherapy for tumors based on preclinical studies (see, e.g., Ngiow et al., *Cancer Res.*, 71(21): 1-5 (2011); Guo et al., *Journal of Translational Medicine*, 11: 215 (2013); and Ngiow et al., *Cancer Res.*, 71(21): 6567-6571 (2011)).

[0006] There is a need for additional antagonists of TIM-3 (e.g., an antibody) that binds TIM-3 with high affinity and effectively neutralizes TIM-3 activity. The invention provides such TIM-3-binding agents.

BRIEF SUMMARY OF THE INVENTION

[0007] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Xaa1 Ala Xaa2 Ser Gly Phe Xaa3 Xaa4 Xaa5 Thr Phe Ser Xaa6 Tyr Xaa7 Met Xaa8 Trp Val Arg Gln Ala Xaa9 Gly Lys Gly Leu Xaa10 Trp Val Ser Xaa11 Ile Ser Xaa12 Gly Gly Xaa13 Tyr Thr Tyr Tyr Gln Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Xaa14 Glu Asp Thr

Ala Val Tyr Tyr Cys Xaa15 Ser Xaa16 Xaa17 Xaa18 Xaa19 Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala (SEQ ID NO: 1), wherein (a) Xaa1 is deleted (i.e., absent) or is alanine (Ala), (b) Xaa2 is alanine (Ala), proline (Pro), aspartic acid (Asp), glycine (Gly), threonine (Thr), or valine (Val), (c) the subsequence Xaa3 Xaa4 Xaa5 is deleted or is Thr-Phe-Ile, (d) Xaa6 is serine (Ser), asparagine (Asn), arginine (Arg), or threonine (Thr), (e) Xaa7 is aspartic acid (Asp) or alanine (Ala), (f) Xaa8 is serine (Ser) or threonine (Thr), (g) Xaa9 is proline (Pro) or leucine (Leu), (h) Xaa10 is aspartic acid (Asp) or glutamic acid (Glu), (i) Xaa11 is threonine (Thr) or alanine (Ala), (j) Xaa12 is glycine (Gly) or serine (Ser), (k) Xaa13 is serine (Ser), threonine (Thr), aspartic acid (Asp), glycine (Gly), asparagine (Asn), or lysine (Lys), (l) Xaa14 is alanine (Ala) or valine (Val), (m) Xaa15 is alanine (Ala) or threonine (Thr), and (n) the subsequence Xaa16 Xaa17 Xaa18 Xaa19 is deleted or is Pro-Tyr-Tyr-Ala.

[0008] The invention provides an isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Ile Gln Met Thr Xaa1 Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Xaa2 Ser Gln Ser Ile Xaa3 Xaa4 Tyr Leu Asn Trp Tyr Xaa5 Gln Lys Xaa6 Xaa7 Lys Ala Pro Lys Leu Leu Xaa8 Tyr Xaa9 Ala Ser Xaa10 Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Xaa11 Tyr Tyr Cys Gln Gln Xaa12 Xaa13 Xaa14 Xaa15 Pro Xaa16 Thr Phe Gly Xaa17 Gly Thr Lys Xaa18 Glu Ile Lys Arg (SEQ ID NO: 45), wherein (a) Xaa1 is glutamine (Gln) or histidine (His), (b) Xaa2 is alanine (Ala) or threonine (Thr), (c) Xaa3 is serine (Ser), arginine (Arg), asparagine (Asn), or threonine (Thr), (d) Xaa4 is serine (Ser), arginine (Arg), aspartic acid (Asp), threonine (Thr), or glycine (Gly), (e) Xaa5 is glutamine (Gln) or histidine (His), (f) Xaa6 is proline (Pro) or alanine (Ala), (g) Xaa7 is glycine (Gly), lysine (Lys), or arginine (Arg), (h) Xaa8 is isoleucine (Ile) or methionine (Met), (i) Xaa9 is alanine (Ala), glycine (Gly), aspartic acid (Asp), threonine (Thr), serine (Ser), valine (Val), or isoleucine (Ile), (j) Xaa10 is serine (Ser) or threonine (Thr), (k) Xaa11 is valine (Val), methionine (Met), or alanine (Ala) (l) Xaa12 is serine (Ser) or arginine (Arg), (m) Xaa13 is tyrosine (Tyr), histidine (His), phenylalanine (Phe), aspartic acid (Asp), serine (Ser), or asparagine (Asn), (n) Xaa14 is serine (Ser) or asparagine (Asn), (o) Xaa15 is threonine (Thr), serine (Ser), alanine (Ala), or proline (Pro), (p) Xaa16 is leucine (Leu) or histidine (His), (q) Xaa17 is glycine (Gly), arginine (Arg), or glutamic acid (Glu), and (r) Xaa18 is valine (Val) or leucine (Leu).

[0009] The invention also provides an isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Xaa1 Leu Xaa2 Xaa3 Xaa4 Xaa5 Ser Gly Gly Xaa6 Leu Xaa7 Gln Pro Gly Gly Ser Leu Arg Leu Xaa8 Cys Xaa9 Ala Ser Gly Phe Thr Phe Xaa10 Xaa11 Ser Tyr Xaa12 Met Xaa13 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Xaa14 Ile Ser Gly Ser Gly Gly Xaa15 Thr Tyr Tyr Xaa16 Asp Ser Val Lys Gly Xaa17 Phe Thr Ile Ser Xaa18 Asp Asn Ser Xaa19 Asn Thr Xaa20 Tyr Leu Gln Met Asn Xaa21 Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Xaa22 Lys Lys Tyr Tyr Xaa23 Xaa24 Pro Ala Asp Tyr Trp Xaa25 Gln Gly Thr Leu Val Thr Val Ser Ser Gly (SEQ ID NO: 126), wherein (a) Xaa1 is leucine (Leu), valine (Val), or methionine (Met), (b) the subsequence Xaa2 Xaa3 Xaa4 is deleted or is Glu-Ser-Leu, (c) Xaa5 is deleted or is glutamic acid (Glu), (d) Xaa6 is glycine (Gly) or aspartic acid (Asp), (e) Xaa7 is valine (Val) or isoleucine (Ile), (f) Xaa8 is serine (Ser) or tyrosine (Tyr), (g) Xaa9 is alanine (Ala) or valine (Val), (h) Xaa10 is serine (Ser), asparagine (Asn), arginine (Arg), threonine (Thr), aspartic acid (Asp), or glycine (Gly), (i) Xaa11 is deleted or is glycine (Gly), (j) Xaa12 is alanine (Ala) or threonine (Thr), (k) Xaa13 is serine (Ser) or asparagine (Asn), (l) Xaa14 is alanine (Ala), glycine (Gly), valine (Val), serine (Ser), phenylalanine (Phe), isoleucine (Ile), threonine (Thr), or aspartic acid (Asp), (m) Xaa15 is serine (Ser) or asparagine (Asn), (n) Xaa16 is alanine (Ala), valine (Val), or asparagine (Asn), (o) Xaa17 is arginine (Arg) or glutamine (Gln), (p) Xaa18 is arginine (Arg) or lysine (Lys), (q) Xaa19 is lysine (Lys) or asparagine (Asn), (r) Xaa20 is leucine (Leu), valine (Val), threonine (Thr), methionine (Met), or proline (Pro), (s) Xaa21 is serine (Ser) or asparagine (Asn), (t) Xaa22 is alanine (Ala) or glycine (Gly), (u) Xaa23 is glycine (Gly), valine (Val), aspartic acid (Asp), alanine (Ala), threonine (Thr), or asparagine (Asn), (v) Xaa24 is glycine (Gly), serine (Ser), valine (Val), aspartic acid (Asp), asparagine (Asn), or threonine (Thr), and (w) Xaa25 is glycine (Gly) or aspartic acid (Asp).

[0010] The invention also provides an isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Asn Lys Asn Tyr Leu Xaa1 Trp Tyr Xaa2 Xaa3 Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Xaa4 Gly Val Pro Asp Arg Phe Xaa5 Gly Ser Xaa6 Ser Gly Thr Asp Phe Thr Leu Xaa7 Ile Xaa8 Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Xaa9 Gln Tyr Tyr Xaa10

Ser Pro Xaa11 Thr Phe Gly Gly Gly Thr Lys Ile Glu Xaa12 Lys (SEQ ID NO: 260), wherein (a) Xaa1 is alanine (Ala) or threonine (Thr), (b) Xaa2 is glutamine (Gln) or histidine (His), (c) Xaa3 is glutamine (Gln) or histidine (His), (d) Xaa4 is serine (Ser), tyrosine (Tyr), aspartic acid (Asp), glycine (Gly), threonine (Thr), asparagine (Asn), lysine (Lys), glutamic acid (Glu), leucine (Leu), proline (Pro), or valine (Val), (e) Xaa5 is serine (Ser) or asparagine (Asn), (f) Xaa6 is glycine (Gly), glutamic acid (Glu), alanine (Ala), aspartic acid (Asp), asparagine (Asn), serine (Ser), threonine (Thr), or valine (Val), (g) Xaa7 is threonine (Thr) or isoleucine (Ile), (h) Xaa8 is serine (Ser) or isoleucine (Ile), (i) Xaa9 is glutamine (Gln) or histidine (His), (j) Xaa10 is serine (Ser), asparagine (Asn), arginine (Arg), glycine (Gly), or threonine (Thr), (k) Xaa11 is leucine (Leu) or isoleucine (Ile), and (l) Xaa12 is leucine (Leu) or valine (Val).

[0011] In addition, the invention provides isolated or purified nucleic acid sequences encoding the foregoing immunoglobulin polypeptides, vectors comprising such nucleic acid sequences, TIM-3-binding agents comprising the foregoing immunoglobulin polypeptides, nucleic acid sequences encoding such TIM-3-binding agents, vectors comprising such nucleic acid sequences, isolated cells comprising such vectors, compositions comprising such TIM-3-binding agents or such vectors with a pharmaceutically acceptable carrier, and methods of treating cancer, infectious diseases, or autoimmune diseases in mammals by administering effective amounts of such compositions to mammals.

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

[0012] Figure 1A is a graph which depicts experimental results demonstrating secretion of IL-2 induced by a lead anti-TIM-3 antibody in a mixed lymphocyte reaction as described in Example 2.

[0013] Figure 1B is a graph which depicts experimental results demonstrating secretion of IL-2 induced by a lead anti-TIM-3 antibody in activated CD4⁺ T cells as described in Example 2.

[0014] Figure 1C is a graph which depicts experimental results demonstrating secretion of IL-2 induced by a lead anti-TIM-3 antibody in combination with an anti-PD-1 antibody in a mixed lymphocyte reaction as described in Example 2.

[0015] Figure 1D is a graph which depicts experimental results demonstrating secretion of IL-2 induced by a lead anti-TIM-3 antibody in combination with an anti-PD-1 antibody in activated CD4⁺ T cells as described in Example 2.

[0016] Figure 2A is a graph which depicts experimental results demonstrating the effect of PBS treatment on tumor volume in the MC38 syngeneic tumor model as described in Example 3. Arrows denote dosing days.

[0017] Figure 2B is a graph which depicts experimental results demonstrating the effect of anti-TIM-3 antibody treatment on tumor volume in the MC38 syngeneic tumor model as described in Example 3. Arrows denote dosing days.

[0018] Figure 2C is a graph which depicts experimental results demonstrating the effect of anti-PD-1 antibody treatment on tumor volume in the MC38 syngeneic tumor model as described in Example 3. Arrows denote dosing days.

[0019] Figure 2D is a graph which depicts experimental results demonstrating the effect of anti-TIM-3 antibody in combination with anti-PD-1 antibody treatment on tumor volume in the MC38 syngeneic tumor model as described in Example 3. Arrows denote dosing days.

[0020] Figure 3 is a graph depicting experimental results demonstrating the effects of surrogate anti-TIM-3 and anti-PD-1-antibodies of different isotypes on tumor volume in the MC38 syngeneic tumor model as described in Example 4. Arrows denote dosing days.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The invention provides an isolated immunoglobulin heavy chain polypeptide and/or an isolated immunoglobulin light chain polypeptide, or a fragment (e.g., antigen-binding fragment) thereof. The term “immunoglobulin” or “antibody,” as used herein, refers to a protein that is found in blood or other bodily fluids of vertebrates, which is used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. The polypeptide is “isolated” in that it is removed from its natural environment. In a preferred embodiment, an immunoglobulin or antibody is a protein that comprises at least one complementarity determining region (CDR). The CDRs form the “hypervariable region” of an antibody, which is responsible for antigen binding (discussed further below). A whole immunoglobulin typically consists of four polypeptides: two identical copies of a heavy (H) chain polypeptide and two

identical copies of a light (L) chain polypeptide. Each of the heavy chains contains one N-terminal variable (V_H) region and three C-terminal constant (C_{H1} , C_{H2} , and C_{H3}) regions, and each light chain contains one N-terminal variable (V_L) region and one C-terminal constant (C_L) region. The light chains of antibodies can be assigned to one of two distinct types, either kappa (κ) or lambda (λ), based upon the amino acid sequences of their constant domains. In a typical immunoglobulin, each light chain is linked to a heavy chain by disulphide bonds, and the two heavy chains are linked to each other by disulphide bonds. The light chain variable region is aligned with the variable region of the heavy chain, and the light chain constant region is aligned with the first constant region of the heavy chain. The remaining constant regions of the heavy chains are aligned with each other.

[0022] The variable regions of each pair of light and heavy chains form the antigen binding site of an antibody. The V_H and V_L regions have the same general structure, with each region comprising four framework (FW or FR) regions. The term “framework region,” as used herein, refers to the relatively conserved amino acid sequences within the variable region which are located between the hypervariable or complementary determining regions (CDRs). There are four framework regions in each variable domain, which are designated FR1, FR2, FR3, and FR4. The framework regions form the β sheets that provide the structural framework of the variable region (see, e.g., C.A. Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001)).

[0023] The framework regions are connected by three complementarity determining regions (CDRs). As discussed above, the three CDRs, known as CDR1, CDR2, and CDR3, form the “hypervariable region” of an antibody, which is responsible for antigen binding. The CDRs form loops connecting, and in some cases comprising part of, the beta-sheet structure formed by the framework regions. While the constant regions of the light and heavy chains are not directly involved in binding of the antibody to an antigen, the constant regions can influence the orientation of the variable regions. The constant regions also exhibit various effector functions, such as participation in antibody-dependent complement-mediated lysis or antibody-dependent cellular toxicity via interactions with effector molecules and cells.

[0024] The isolated immunoglobulin heavy chain polypeptide and the isolated immunoglobulin light chain polypeptide of the invention desirably bind to the T Cell

Immunoglobulin and Mucin Protein 3 (TIM-3). TIM-3 is a 60 kDa type 1 transmembrane protein comprised of three domains: an N-terminal Ig variable (IgV)-like domain, a central Ser/Thr-rich mucin domain, and a transmembrane domain with a short intracellular tail (see, e.g., Kane, L.P., *Journal of Immunology*, 184(6): 2743-2749 (2010)). TIM-3 was initially identified on terminally differentiated Th1 cells, and negatively regulates the T-cell response by inducing T-cell apoptosis (see, e.g., Hastings et al., *Eur. J. Immunol.*, 39(9): 2492-2501 (2009)). TIM-3 also is expressed on activated Th17 and Tc1 cells, and dysregulation of Tim-3 expression on CD4⁺ T-cells and CD8⁺ T-cells is associated with several autoimmune diseases, viral infections, and cancer (see, e.g., Liberal et al., *Hepatology*, 56(2): 677-686 (2012); Wu et al., *Eur. J. Immunol.*, 42(5): 1180-1191 (2012); Anderson, A.C., *Curr. Opin. Immunol.*, 24(2): 213-216 (2012); and Han et al., *Frontiers in Immunology*, 4: 449 (2013)).

[0025] The inventive isolated immunoglobulin heavy chain polypeptide and the inventive isolated immunoglobulin light chain polypeptide can form an agent that binds to TIM-3 and another antigen, resulting in a “dual reactive” binding agent (e.g., a dual reactive antibody). For example, the agent can bind to TIM-3 and to another negative regulator of the immune system such as, for example, programmed death 1 (PD-1) and/or the Lymphocyte Activation Gene 3 protein (LAG-3).

[0026] Certain other antibodies which bind to TIM-3, and components thereof, are known in the art (see, e.g., U.S. Patent 8,101,176; U.S. Patent 8,552,156; and U.S. Patent 8,841,418). Anti-TIM-3 antibodies also are commercially available from sources such as, for example, Abcam (Cambridge, MA), and R&D Systems, Inc. (Minneapolis, MN).

[0027] The invention provides an immunoglobulin heavy chain polypeptide that comprises, consists of, or consists essentially of the amino acid sequence Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Xaa1 Ala Xaa2 Ser Gly Phe Xaa3 Xaa4 Xaa5 Thr Phe Ser Xaa6 Tyr Xaa7 Met Xaa8 Trp Val Arg Gln Ala Xaa9 Gly Lys Gly Leu Xaa10 Trp Val Ser Xaa11 Ile Ser Xaa12 Gly Gly Xaa13 Tyr Thr Tyr Tyr Gln Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Xaa14 Glu Asp Thr Ala Val Tyr Tyr Cys Xaa15 Ser Xaa16 Xaa17 Xaa18 Xaa19 Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala (SEQ ID NO: 1), wherein (a) Xaa1 is deleted or is alanine (Ala), (b) Xaa2 is alanine (Ala), proline (Pro), aspartic acid (Asp), glycine (Gly),

threonine (Thr), or valine (Val), (c) the subsequence Xaa3 Xaa4 Xaa5 is deleted or is Thr-Phe-Ile, (d) Xaa6 is serine (Ser), asparagine (Asn), arginine (Arg), or threonine (Thr), (e) Xaa7 is aspartic acid (Asp) or alanine (Ala), (f) Xaa8 is serine (Ser) or threonine (Thr), (g) Xaa9 is proline (Pro) or leucine (Leu), (h) Xaa10 is aspartic acid (Asp) or glutamic acid (Glu), (i) Xaa11 is threonine (Thr) or alanine (Ala), (j) Xaa12 is glycine (Gly) or serine (Ser), (k) Xaa13 is serine (Ser), threonine (Thr), aspartic acid (Asp), glycine (Gly), asparagine (Asn), or lysine (Lys), (l) Xaa14 is alanine (Ala) or valine (Val), (m) Xaa15 is alanine (Ala) or threonine (Thr), and (n) the subsequence Xaa16 Xaa17 Xaa18 Xaa19 is deleted or is Pro-Tyr-Tyr-Ala.

[0028] The inventive heavy chain polypeptide can comprise, consist of, or consist essentially of the amino acid sequence of SEQ ID NO: 1 with any one of the aforementioned amino acid substitutions and amino acid deletions in any suitable combination. In one embodiment, the immunoglobulin heavy chain polypeptide comprises, consists of, or consists essentially of an amino acid sequence of any one of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, and SEQ ID NO: 44.

[0029] The invention also provides an immunoglobulin heavy chain polypeptide that comprises, consists of, or consists essentially of the amino acid sequence Glu Val Gln Xaa1 Leu Xaa2 Xaa3 Xaa4 Xaa5 Ser Gly Gly Xaa6 Leu Xaa7 Gln Pro Gly Gly Ser Leu Arg Leu Xaa8 Cys Xaa9 Ala Ser Gly Phe Thr Phe Xaa10 Xaa11 Ser Tyr Xaa12 Met Xaa13 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Xaa14 Ile Ser Gly Ser Gly Gly Xaa15 Thr Tyr Tyr Xaa16 Asp Ser Val Lys Gly Xaa17 Phe Thr Ile Ser Xaa18 Asp Asn Ser Xaa19 Asn Thr Xaa20 Tyr Leu Gln Met Asn Xaa21 Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Xaa22 Lys Lys Tyr Tyr Xaa23 Xaa24 Pro Ala Asp Tyr Trp Xaa25 Gln Gly Thr Leu Val Thr Val Ser Ser Gly (SEQ ID NO: 126), wherein (a) Xaa1 is leucine (Leu), valine (Val), or methionine (Met), (b) the subsequence

Xaa2 Xaa3 Xaa4 is deleted or is Glu-Ser-Leu, (c) Xaa5 is deleted or is glutamic acid (Glu), (d) Xaa6 is glycine (Gly) or aspartic acid (Asp), (d) Xaa7 is valine (Val) or isoleucine (Ile), (e) Xaa8 is serine (Ser) or tyrosine (Tyr), (f) Xaa9 is alanine (Ala) or valine (Val), (g) Xaa10 is serine (Ser), asparagine (Asn), arginine (Arg), threonine (Thr), aspartic acid (Asp), or glycine (Gly), (h) Xaa11 is deleted or is glycine (Gly), (i) Xaa12 is alanine (Ala) or threonine (Thr), (j) Xaa13 is serine (Ser) or asparagine (Asn), (k) Xaa14 is alanine (Ala), glycine (Gly), valine (Val), serine (Ser), phenylalanine (Phe), isoleucine (Ile), threonine (Thr), or aspartic acid (Asp) (l) Xaa15 is serine (Ser) or asparagine (Asn), (m) Xaa16 is alanine (Ala), valine (Val), or asparagine (Asn), (n) Xaa17 is arginine (Arg) or glutamine (Gln), (o) Xaa18 is arginine (Arg) or lysine (Lys), (p) Xaa19 is lysine (Lys) or asparagine (Asn), (q) Xaa20 is leucine (Leu), valine (Val), threonine (Thr), methionine (Met), or proline (Pro), (r) Xaa21 is serine (Ser) or asparagine (Asn), (s) Xaa22 is alanine (Ala) or glycine (Gly), (t) Xaa23 is glycine (Gly), valine (Val), aspartic acid (Asp), alanine (Ala), threonine (Thr), or asparagine (Asn), (u) Xaa24 is glycine (Gly), serine (Ser), valine (Val), aspartic acid (Asp), asparagine (Asn), or threonine (Thr), and (v) Xaa25 is glycine (Gly) or aspartic acid (Asp).

[0030] The inventive heavy chain polypeptide can comprise, consist of, or consist essentially of the amino acid sequence of SEQ ID NO: 126 with one of the aforementioned amino acid substitutions and amino acid deletions in any suitable combination. In one embodiment, the immunoglobulin heavy chain polypeptide comprises, consists of, or consists essentially of an amino acid sequence of any one of SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO:

182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, 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, SEQ ID NO: 257, SEQ ID NO: 258, and SEQ ID NO: 259.

[0031] When the inventive immunoglobulin heavy chain polypeptide consists essentially of an amino acid sequence of any one of SEQ ID NO: 1-SEQ ID NO: 44 or SEQ ID NO: 126- SEQ ID NO: 259, additional components can be included in the polypeptide that do not materially affect the polypeptide, e.g., by influencing affinity of the inventive heavy chain polypeptide to TIM-3. Examples of such components include, for example, protein moieties such as biotin that facilitate purification or isolation, passenger mutations, sequences free of problematic sites including free cysteines, additional glycosylation sites, and high-likelihood deamidation or isomerization sites.

[0032] When the inventive immunoglobulin heavy chain polypeptide consists of an amino acid sequence of any one of SEQ ID NO: 1-SEQ ID NO: 44 or SEQ ID NO: 126-SEQ ID NO: 259, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin heavy chain polypeptide).

[0033] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises an amino acid sequence that is at least 90% identical (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%, at least 99%, or

100% identical) to any one of SEQ ID NO: 1-SEQ ID NO: 44 or SEQ ID NO: 126-SEQ ID NO: 259. Nucleic acid or amino acid sequence “identity,” as described herein, can be determined by comparing a nucleic acid or amino acid sequence of interest to a reference nucleic acid or amino acid sequence. The percent identity is the number of nucleotides or amino acid residues that are the same (i.e., that are identical) as between the sequence of interest and the reference sequence divided by the length of the longest sequence (i.e., the length of either the sequence of interest or the reference sequence, whichever is longer). A number of mathematical algorithms for obtaining the optimal alignment and calculating identity between two or more sequences are known and incorporated into a number of available software programs. Examples of such programs include CLUSTAL-W, T-Coffee, and ALIGN (for alignment of nucleic acid and amino acid sequences), BLAST programs (e.g., BLAST 2.1, BL2SEQ, and later versions thereof) and FASTA programs (e.g., FASTA3x, FASTM, and SSEARCH) (for sequence alignment and sequence similarity searches). Sequence alignment algorithms also are disclosed in, for example, Altschul et al., *J. Molecular Biol.*, 215(3): 403-410 (1990), Beigert et al., *Proc. Natl. Acad. Sci. USA*, 106(10): 3770-3775 (2009), Durbin et al., eds., *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, UK (2009), Soding, *Bioinformatics*, 21(7): 951-960 (2005), Altschul et al., *Nucleic Acids Res.*, 25(17): 3389-3402 (1997), and Gusfield, *Algorithms on Strings, Trees and Sequences*, Cambridge University Press, Cambridge UK (1997)).

[0034] In another embodiment, the invention provides an immunoglobulin light chain polypeptide that comprises, consists of, or consists essentially of the amino acid sequence Asp Ile Gln Met Thr Xaa1 Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Xaa2 Ser Gln Ser Ile Xaa3 Xaa4 Tyr Leu Asn Trp Tyr Xaa5 Gln Lys Xaa6 Xaa7 Lys Ala Pro Lys Leu Leu Xaa8 Tyr Xaa9 Ala Ser Xaa10 Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Xaa11 Tyr Tyr Cys Gln Gln Xaa12 Xaa13 Xaa14 Xaa15 Pro Xaa16 Thr Phe Gly Xaa17 Gly Thr Lys Xaa18 Glu Ile Lys Arg (SEQ ID NO: 45), wherein (a) Xaa1 is glutamine (Gln) or histidine (His), (b) Xaa2 is alanine (Ala) or threonine (Thr), (c) Xaa3 is serine (Ser), arginine (Arg), asparagine (Asn), or threonine (Thr), (d) Xaa4 is serine (Ser), arginine (Arg), aspartic acid (Asp), threonine (Thr), or glycine (Gly), (e) Xaa5 is glutamine (Gln) or histidine (His), (f) Xaa6 is proline (Pro) or alanine

(Ala), (g) Xaa7 is glycine (Gly), lysine (Lys), or arginine (Arg), (h) Xaa8 is isoleucine (Ile) or methionine (Met), (i) Xaa9 is alanine (Ala), glycine (Gly), aspartic acid (Asp), threonine (Thr), serine (Ser), valine (Val), or isoleucine (Ile), (j) Xaa10 is serine (Ser) or threonine (Thr), (k) Xaa11 is valine (Val), methionine (Met), or alanine (Ala) (l) Xaa12 is serine (ser) or arginine (Arg), (m) Xaa13 is tyrosine (Tyr), histidine (His), phenylalanine (Phe), aspartic acid (Asp), serine (Ser), or asparagine (Asn), (n) Xaa14 is serine (Ser) or asparagine (Asn), (o) Xaa15 is threonine (Thr), serine (Ser), alanine (Ala), or proline (Pro), (p) Xaa16 is leucine (Leu) or histidine (His), (q) Xaa17 is glycine (Gly), arginine (Arg), or glutamic acid (Glu), and (r) Xaa18 is valine (Val) or leucine (Leu).

[0035] In one embodiment, the isolated immunoglobulin light chain polypeptide comprises, consists of, or consists essentially of an amino acid sequence of any one of SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, and SEQ ID NO: 125.

[0036] The invention also provides an immunoglobulin light chain polypeptide that comprises, consists of, or consists essentially of the amino acid sequence Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Asn Lys Asn Tyr Leu Xaa1 Trp Tyr Xaa2 Xaa3 Lys Pro Gly Gln Pro

Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Xaa4 Gly Val Pro Asp Arg Phe Xaa5 Gly Ser Xaa6 Ser Gly Thr Asp Phe Thr Leu Xaa7 Ile Xaa8 Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Xaa Gln Tyr Tyr Xaa10 Ser Pro Xaa11 Thr Phe Gly Gly Gly Thr Lys Ile Glu Xaa12 Lys (SEQ ID NO: 260), wherein (a) Xaa1 is alanine (Ala) or threonine (Thr), (b) Xaa2 is glutamine (Gln) or histidine (His), (c) Xaa3 is glutamine (Gln) or histidine (His), (d) Xaa4 is serine (Ser), tyrosine (Tyr), aspartic acid (Asp), glycine (Gly), threonine (Thr), asparagine (Asn), lysine (Lys), glutamic acid (Glu), leucine (Leu), proline (Pro), or valine (Val), (e) Xaa5 is serine (Ser) or asparagine (Asn), (f) Xaa6 is glycine (Gly), glutamic acid (Glu), alanine (Ala), aspartic acid (Asp), asparagine (Asn), serine (Ser), threonine (Thr), or valine (Val), (g) Xaa7 is threonine (Thr) or isoleucine (Ile), (h) Xaa8 is serine (Ser) or isoleucine (Ile), (i) Xaa9 is glutamine (Gln) or histidine (His), (j) Xaa10 is serine (Ser), asparagine (Asn), arginine (Arg), glycine (Gly), or threonine (Thr), (k) Xaa11 is leucine (Leu) or isoleucine (Ile), and (l) Xaa12 is leucine (Leu) or valine (Val).

[0037] In one embodiment, the isolated immunoglobulin light chain polypeptide comprises, consists of, or consists essentially of an amino acid sequence of any one of SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, SEQ ID NO: 266, SEQ ID NO: 267, SEQ ID NO: 268, SEQ ID NO: 269, SEQ ID NO: 270, SEQ ID NO: 271, SEQ ID NO: 272, SEQ ID NO: 273, SEQ ID NO: 274, SEQ ID NO: 275, SEQ ID NO: 276, SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, SEQ ID NO: 281, SEQ ID NO: 282, SEQ ID NO: 283, SEQ ID NO: 284, SEQ ID NO: 285, SEQ ID NO: 286, SEQ ID NO: 287, SEQ ID NO: 288, SEQ ID NO: 289, SEQ ID NO: 290, SEQ ID NO: 291, SEQ ID NO: 292, SEQ ID NO: 293, SEQ ID NO: 294, SEQ ID NO: 295, SEQ ID NO: 296, SEQ ID NO: 297, SEQ ID NO: 298, SEQ ID NO: 299, SEQ ID NO: 300, SEQ ID NO: 301, SEQ ID NO: 302, SEQ ID NO: 303, SEQ ID NO: 304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID NO: 309, SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 314, SEQ ID NO: 315, SEQ ID NO: 316, SEQ ID NO: 317, SEQ ID NO: 318, SEQ ID NO: 319, SEQ ID NO: 320, SEQ ID NO: 321, SEQ ID NO: 322, SEQ ID NO: 323, SEQ ID NO: 324, SEQ ID NO: 325, SEQ ID NO: 326, SEQ ID NO: 327, and SEQ ID NO: 328.

[0038] When the inventive immunoglobulin light chain polypeptide consists essentially of an amino acid sequence of any one of SEQ ID NO: 45-SEQ ID NO: 125 or SEQ ID NO: 260-SEQ

ID NO: 328, additional components can be included in the polypeptide that do not materially affect the polypeptide, such as those described herein. When the inventive immunoglobulin light chain polypeptide consists of an amino acid sequence of any one of SEQ ID NO: 45-SEQ ID NO: 125 or SEQ ID NO: 260-SEQ ID NO: 328, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin light chain polypeptide).

[0039] The invention provides an isolated immunoglobulin light chain polypeptide which comprises an amino acid sequence that is at least 90% identical (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%, at least 99%, or 100% identical) to any one of SEQ ID NO: 45-SEQ ID NO: 125 and SEQ ID NO: 260-SEQ ID NO: 328. Nucleic acid or amino acid sequence “identity” can be determined using the methods described herein.

[0040] One or more amino acids of the aforementioned immunoglobulin heavy chain polypeptides and/or light chain polypeptides can be replaced or substituted with a different amino acid. An amino acid “replacement” or “substitution” refers to the replacement of one amino acid at a given position or residue by another amino acid at the same position or residue within a polypeptide sequence.

[0041] Amino acids are broadly grouped as “aromatic” or “aliphatic.” An aromatic amino acid includes an aromatic ring. Examples of “aromatic” amino acids include histidine (H or His), phenylalanine (F or Phe), tyrosine (Y or Tyr), and tryptophan (W or Trp). Non-aromatic amino acids are broadly grouped as “aliphatic.” Examples of “aliphatic” amino acids include glycine (G or Gly), alanine (A or Ala), valine (V or Val), leucine (L or Leu), isoleucine (I or Ile), methionine (M or Met), serine (S or Ser), threonine (T or Thr), cysteine (C or Cys), proline (P or Pro), glutamic acid (E or Glu), aspartic acid (D or Asp), asparagine (N or Asn), glutamine (Q or Gln), lysine (K or Lys), and arginine (R or Arg).

[0042] Aliphatic amino acids may be sub-divided into four sub-groups. The “large aliphatic non-polar sub-group” consists of valine, leucine, and isoleucine. The “aliphatic slightly-polar sub-group” consists of methionine, serine, threonine, and cysteine. The “aliphatic polar/charged sub-group” consists of glutamic acid, aspartic acid, asparagine, glutamine, lysine, and arginine. The “small-residue sub-group” consists of glycine and alanine. The group of charged/polar

amino acids may be sub-divided into three sub-groups: the “positively-charged sub-group” consisting of lysine and arginine, the “negatively-charged sub-group” consisting of glutamic acid and aspartic acid, and the “polar sub-group” consisting of asparagine and glutamine.

[0043] Aromatic amino acids may be sub-divided into two sub-groups: the “nitrogen ring sub-group” consisting of histidine and tryptophan and the “phenyl sub-group” consisting of phenylalanine and tyrosine.

[0044] The amino acid replacement or substitution can be conservative, semi-conservative, or non-conservative. The phrase “conservative amino acid substitution” or “conservative mutation” refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz and Schirmer, *Principles of Protein Structure*, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz and Schirmer, *supra*).

[0045] Examples of conservative amino acid substitutions include substitutions of amino acids within the sub-groups described above, for example, lysine for arginine and vice versa such that a positive charge may be maintained, glutamic acid for aspartic acid and vice versa such that a negative charge may be maintained, serine for threonine such that a free -OH can be maintained, and glutamine for asparagine such that a free -NH₂ can be maintained.

[0046] “Semi-conservative mutations” include amino acid substitutions of amino acids within the same groups listed above, but not within the same sub-group. For example, the substitution of aspartic acid for asparagine, or asparagine for lysine, involves amino acids within the same group, but different sub-groups. “Non-conservative mutations” involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc.

[0047] In addition, one or more amino acids can be inserted into the aforementioned immunoglobulin heavy chain polypeptides and/or light chain polypeptides. Any number of any suitable amino acids can be inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide. In this respect, at least one amino acid (e.g., 2

or more, 5 or more, or 10 or more amino acids), but not more than 20 amino acids (e.g., 18 or less, 15 or less, or 12 or less amino acids), can be inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide. In some embodiments, 1-10 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) are inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide. In this respect, the amino acid(s) can be inserted into any one of the aforementioned immunoglobulin heavy chain polypeptides and/or light chain polypeptides in any suitable location. For instance, the amino acid(s) can be inserted into a CDR (e.g., CDR1, CDR2, or CDR3) of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide.

[0048] The inventive isolated immunoglobulin heavy chain polypeptide and light chain polypeptides are not limited to polypeptides comprising the specific amino acid sequences described herein. The immunoglobulin heavy chain polypeptide or light chain polypeptide can be any heavy chain polypeptide or light chain polypeptide that competes with the immunoglobulin heavy chain polypeptide or light chain polypeptide of the above-described sequences for binding to TIM-3. For example, the immunoglobulin heavy chain polypeptide or light chain polypeptide can be any heavy chain polypeptide or light chain polypeptide that binds to the same epitope of TIM-3 recognized by the heavy and light chain polypeptides described herein. Antibody competition can be assayed using routine peptide competition assays which utilize ELISA, Western blot, or immunohistochemistry methods (see, e.g., U.S. Patents 4,828,981 and 8,568,992; and Braitbard et al., *Proteome Sci.*, 4: 12 (2006)).

[0049] The invention provides a TIM-3-binding agent comprising, consisting essentially of, or consisting of one or more of the inventive isolated amino acid sequences described herein. By “TIM-3-binding agent” is meant a molecule, preferably a proteinaceous molecule, which binds specifically to the TIM-3 protein. Preferably, the TIM-3-binding agent is an antibody or a fragment (e.g., antigen-binding fragment) thereof. The TIM-3-binding agent of the invention comprises, consists essentially of, or consists of the inventive immunoglobulin heavy chain polypeptide and/or the inventive immunoglobulin light chain polypeptide. In one embodiment, the TIM-3-binding agent comprises, consists essentially of, or consists of the inventive immunoglobulin heavy chain polypeptide or the inventive immunoglobulin light chain polypeptide. In another embodiment, the TIM-3-binding agent comprises, consists essentially

of, or consists of the inventive immunoglobulin heavy chain polypeptide and the inventive immunoglobulin light chain polypeptide.

[0050] Any amino acid residue of the inventive immunoglobulin heavy chain polypeptide and/or the inventive immunoglobulin light chain polypeptide can be replaced, in any combination, with a different amino acid residue, or can be deleted or inserted, so long as the biological activity of the TIM-3-binding agent is not materially diminished (e.g., enhanced or improved) as a result of the amino acid replacements, insertions, and/or deletions. The “biological activity” of an TIM-3-binding agent refers to, for example, binding affinity for a particular TIM-3 epitope, neutralization or inhibition of TIM-3 binding to its receptor(s), neutralization or inhibition of TIM-3 activity *in vivo* (e.g., IC₅₀), pharmacokinetics, and cross-reactivity (e.g., with non-human homologs or orthologs of the TIM-3 protein, or with other proteins or tissues). Other biological properties or characteristics of an antigen-binding agent recognized in the art include, for example, avidity, selectivity, solubility, folding, immunotoxicity, expression, and formulation. The aforementioned properties or characteristics can be observed, measured, and/or assessed using standard techniques including, but not limited to, ELISA, competitive ELISA, surface plasmon resonance analysis (BIAcore™), or KINEXA™, *in vitro* or *in vivo* neutralization assays, receptor-ligand binding assays, cytokine or growth factor production and/or secretion assays, and signal transduction and immunohistochemistry assays.

[0051] The terms “inhibit” or “neutralize,” as used herein with respect to the activity of a TIM-3-binding agent, refer to the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, alter, eliminate, stop, or reverse the progression or severity of, for example, the biological activity of TIM-3, or a disease or condition associated with TIM-3. The TIM-3-binding agent of the invention preferably inhibits or neutralizes the activity of TIM-3 by at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 100%, or a range defined by any two of the foregoing values.

[0052] The TIM-3-binding agent of the invention can be a whole antibody, as described herein, or an antibody fragment. The terms “fragment of an antibody,” “antibody fragment,” “functional fragment of an antibody,” and the like are used interchangeably herein to mean one or more fragments of an antibody that retain the ability to specifically bind to an antigen (see,

generally, Holliger et al., *Nat. Biotech.*, 23(9): 1126-1129 (2005)). The TIM-3-binding agent can contain any TIM-3-binding antibody fragment. The antibody fragment desirably comprises, for example, one or more CDRs, the variable region (or portions thereof), the constant region (or portions thereof), or combinations thereof. Examples of antibody fragments include, but are not limited to, (i) a Fab fragment, which is a monovalent fragment consisting of the V_L , V_H , C_L , and CH_1 domains, (ii) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, (iii) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a Fab' fragment, which results from breaking the disulfide bridge of an $F(ab')_2$ fragment using mild reducing conditions, (v) a disulfide-stabilized Fv fragment (dsFv), and (vi) a domain antibody (dAb), which is an antibody single variable region domain (VH or VL) polypeptide that specifically binds antigen.

[0053] In embodiments where the TIM-3-binding agent comprises a fragment of the immunoglobulin heavy chain or light chain polypeptide, the fragment can be of any size so long as the fragment binds to, and preferably inhibits the activity of, TIM-3. In this respect, a fragment of the immunoglobulin heavy chain polypeptide desirably comprises between about 5 and 18 (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or a range defined by any two of the foregoing values) amino acids. Similarly, a fragment of the immunoglobulin light chain polypeptide desirably comprises between about 5 and 18 (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or a range defined by any two of the foregoing values) amino acids.

[0054] When the TIM-3-binding agent is an antibody or antibody fragment, the antibody or antibody fragment desirably comprises a heavy chain constant region (F_c) of any suitable class. Preferably, the antibody or antibody fragment comprises a heavy chain constant region that is based upon wild-type IgG1, IgG2, or IgG4 antibodies, or variants thereof. It will be appreciated that each antibody class, or isotype, engages a distinct set of effector mechanisms for disposing of or neutralizing antigen once recognized. As such, in some embodiments, when the TIM-3-binding agent is an antibody or antibody fragment, it can exhibit one or more effector functions, such as participation in antibody-dependent complement-mediated lysis or antibody-dependent cellular toxicity via interactions with effector molecules and cells (e.g., activation of the complement system).

[0055] The TIM-3-binding agent also can be a single chain antibody fragment. Examples of single chain antibody fragments include, but are not limited to, (i) a single chain Fv (scFv), which is a monovalent molecule consisting of the two domains of the Fv fragment (i.e., V_L and V_H) joined by a synthetic linker which enables the two domains to be synthesized as a single polypeptide chain (see, e.g., Bird et al., *Science*, 242: 423-426 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA*, 85: 5879-5883 (1988); and Osbourn et al., *Nat. Biotechnol.*, 16: 778 (1998)) and (ii) a diabody, which is a dimer of polypeptide chains, wherein each polypeptide chain comprises a V_H connected to a V_L by a peptide linker that is too short to allow pairing between the V_H and V_L on the same polypeptide chain, thereby driving the pairing between the complementary domains on different V_H-V_L polypeptide chains to generate a dimeric molecule having two functional antigen binding sites. Antibody fragments are known in the art and are described in more detail in, e.g., U.S. Patent Application Publication 2009/0093024 A1.

[0056] The TIM-3-binding agent also can be an intrabody or fragment thereof. An intrabody is an antibody which is expressed and which functions intracellularly. Intrabodies typically lack disulfide bonds and are capable of modulating the expression or activity of target genes through their specific binding activity. Intrabodies include single domain fragments such as isolated V_H and V_L domains and scFvs. An intrabody can include sub-cellular trafficking signals attached to the N or C terminus of the intrabody to allow expression at high concentrations in the sub-cellular compartments where a target protein is located. Upon interaction with a target gene, an intrabody modulates target protein function and/or achieves phenotypic/functional knockout by mechanisms such as accelerating target protein degradation and sequestering the target protein in a non-physiological sub-cellular compartment. Other mechanisms of intrabody-mediated gene inactivation can depend on the epitope to which the intrabody is directed, such as binding to the catalytic site on a target protein or to epitopes that are involved in protein-protein, protein-DNA, or protein-RNA interactions.

[0057] The TIM-3-binding agent also can be an antibody conjugate. In this respect, the TIM-3-binding agent can be a conjugate of (1) an antibody, an alternative scaffold, or fragments thereof, and (2) a protein or non-protein moiety comprising the TIM-3-binding agent. For example, the TIM-3-binding agent can be all or part of an antibody conjugated to a peptide, a fluorescent molecule, or a chemotherapeutic agent.

[0058] The TIM-3-binding agent can be, or can be obtained from, a human antibody, a non-human antibody, or a chimeric antibody. By “chimeric” is meant an antibody or fragment thereof comprising both human and non-human regions. Preferably, the TIM-3-binding agent is a humanized antibody. A “humanized” antibody is a monoclonal antibody comprising a human antibody scaffold and at least one CDR obtained or derived from a non-human antibody. Non-human antibodies include antibodies isolated from any non-human animal, such as, for example, a rodent (e.g., a mouse or rat). A humanized antibody can comprise, one, two, or three CDRs obtained or derived from a non-human antibody. In one embodiment of the invention, CDRH3 of the inventive TIM-3-binding agent is obtained or derived from a mouse monoclonal antibody, while the remaining variable regions and constant region of the inventive TIM-3-binding agent are obtained or derived from a human monoclonal antibody.

[0059] A human antibody, a non-human antibody, a chimeric antibody, or a humanized antibody can be obtained by any means, including via *in vitro* sources (e.g., a hybridoma or a cell line producing an antibody recombinantly) and *in vivo* sources (e.g., rodents). Methods for generating antibodies are known in the art and are described in, for example, Köhler and Milstein, *Eur. J. Immunol.*, 5: 511-519 (1976); Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988); and Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001)). In certain embodiments, a human antibody or a chimeric antibody can be generated using a transgenic animal (e.g., a mouse) wherein one or more endogenous immunoglobulin genes are replaced with one or more human immunoglobulin genes. Examples of transgenic mice wherein endogenous antibody genes are effectively replaced with human antibody genes include, but are not limited to, the Medarex HUMAB-MOUSE™, the Kirin TC MOUSE™, and the Kyowa Kirin KM-MOUSE™ (see, e.g., Lonberg, *Nat. Biotechnol.*, 23(9): 1117-25 (2005), and Lonberg, *Handb. Exp. Pharmacol.*, 181: 69-97 (2008)). A humanized antibody can be generated using any suitable method known in the art (see, e.g., An, Z. (ed.), *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley & Sons, Inc., Hoboken, New Jersey (2009)), including, e.g., grafting of non-human CDRs onto a human antibody scaffold (see, e.g., Kashmiri et al., *Methods*, 36(1): 25-34 (2005); and Hou et al., *J. Biochem.*, 144(1): 115-120 (2008)). In one embodiment, a humanized antibody can be

produced using the methods described in, e.g., U.S. Patent Application Publication 2011/0287485 A1.

[0060] In one embodiment, a CDR (e.g., CDR1, CDR2, or CDR3) or a variable region of the immunoglobulin heavy chain polypeptide and/or the immunoglobulin light chain polypeptide described herein can be transplanted (i.e., grafted) into another molecule, such as an antibody or non-antibody polypeptide, using either protein chemistry or recombinant DNA technology. In this regard, the invention provides a TIM-3-binding agent comprising at least one CDR of an immunoglobulin heavy chain and/or light chain polypeptide as described herein. The TIM-3-binding agent can comprise one, two, or three CDRs of an immunoglobulin heavy chain and/or light chain variable region as described herein.

[0061] In a preferred embodiment, the TIM-3-binding agent binds an epitope of TIM-3 which blocks the binding of TIM-3 to any of its putative ligands (e.g., phosphatidylserine, galectin-9, high-mobility group protein 1 (HMGB1), and carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1)) and inhibits TIM-3-mediated signaling. The invention also provides an isolated or purified epitope of TIM-3 which blocks the binding of TIM-3 to any of its putative ligands in an indirect or allosteric manner.

[0062] The invention also provides one or more isolated or purified nucleic acid sequences that encode the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, and the inventive TIM-3-binding agent.

[0063] The term “nucleic acid sequence” is intended to encompass a polymer of DNA or RNA, i.e., a polynucleotide, which can be single-stranded or double-stranded and which can contain non-natural or altered nucleotides. The terms “nucleic acid” and “polynucleotide” as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecule, and thus include double- and single-stranded DNA, and double- and single-stranded RNA. The terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated and/or capped polynucleotides. Nucleic acids are typically linked via phosphate bonds to form nucleic acid sequences or polynucleotides, though many other linkages are known in the art (e.g., phosphorothioates, boranophosphates, and the like).

[0064] The invention further provides a vector comprising one or more nucleic acid sequences encoding the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive TIM-3-binding agent. The vector can be, for example, a plasmid, episome, cosmid, viral vector (e.g., retroviral or adenoviral), or phage. Suitable vectors and methods of vector preparation are well known in the art (see, e.g., Sambrook et al., *Molecular Cloning, a Laboratory Manual*, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994)).

[0065] In addition to the nucleic acid sequence encoding the inventive immunoglobulin heavy polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive TIM-3-binding agent, the vector preferably comprises expression control sequences, such as promoters, enhancers, polyadenylation signals, transcription terminators, signal peptides (e.g., the osteonectin signal peptide), internal ribosome entry sites (IRES), and the like, that provide for the expression of the coding sequence in a host cell. Exemplary expression control sequences are known in the art and described in, for example, Goeddel, *Gene Expression Technology: Methods in Enzymology*, Vol. 185, Academic Press, San Diego, Calif. (1990).

[0066] A large number of promoters, including constitutive, inducible, and repressible promoters, from a variety of different sources are well known in the art. Representative sources of promoters include for example, virus, mammal, insect, plant, yeast, and bacteria, and suitable promoters from these sources are readily available, or can be made synthetically, based on sequences publicly available, for example, from depositories such as the ATCC as well as other commercial or individual sources. Promoters can be unidirectional (i.e., initiate transcription in one direction) or bi-directional (i.e., initiate transcription in either a 3' or 5' direction). Non-limiting examples of promoters include, for example, the T7 bacterial expression system, pBAD (araA) bacterial expression system, the cytomegalovirus (CMV) promoter, the SV40 promoter, the RSV promoter. Inducible promoters include, for example, the Tet system (U.S. Patents 5,464,758 and 5,814,618), the Ecdysone inducible system (No et al., *Proc. Natl. Acad. Sci.*, 93: 3346-3351 (1996)), the T-REX™ system (Invitrogen, Carlsbad, CA), LACSWITCH™ system (Stratagene, San Diego, CA), and the Cre-ERT tamoxifen inducible recombinase system (Indra

et al., *Nuc. Acid. Res.*, 27: 4324-4327 (1999); *Nuc. Acid. Res.*, 28: e99 (2000); U.S. Patent 7,112,715; and Kramer & Fussenegger, *Methods Mol. Biol.*, 308: 123-144 (2005)).

[0067] The term “enhancer” as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences.

[0068] The vector also can comprise a “selectable marker gene.” The term “selectable marker gene,” as used herein, refers to a nucleic acid sequence that allow cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, e.g., International Patent Application Publications WO 1992/008796 and WO 1994/028143; Wigler et al., *Proc. Natl. Acad. Sci. USA*, 77: 3567-3570 (1980); O'Hare et al., *Proc. Natl. Acad. Sci. USA*, 78: 1527-1531 (1981); Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, 78: 2072-2076 (1981); Colberre-Garapin et al., *J. Mol. Biol.*, 150: 1-14 (1981); Santerre et al., *Gene*, 30: 147-156 (1984); Kent et al., *Science*, 237: 901-903 (1987); Wigler et al., *Cell*, 11: 223-232 (1977); Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, 48: 2026-2034 (1962); Lowy et al., *Cell*, 22: 817-823 (1980); and U.S. Patents 5,122,464 and 5,770,359.

[0069] In some embodiments, the vector is an “episomal expression vector” or “episome,” which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, e.g., Conese et al., *Gene Therapy*, 11: 1735-1742 (2004)). Representative commercially available episomal expression vectors include, but are not limited to, episomal plasmids that utilize Epstein Barr Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, CA) and pBK-CMV

from Stratagene (La Jolla, CA) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin of replication in lieu of EBNA1 and oriP.

[0070] Other suitable vectors include integrating expression vectors, which may randomly integrate into the host cell's DNA, or may include a recombination site to enable the specific recombination between the expression vector and the host cell's chromosome. Such integrating expression vectors may utilize the endogenous expression control sequences of the host cell's chromosomes to effect expression of the desired protein. Examples of vectors that integrate in a site specific manner include, for example, components of the flp-in system from Invitrogen (Carlsbad, CA) (e.g., pcDNATM5/FRT), or the cre-lox system, such as can be found in the pExchange-6 Core Vectors from Stratagene (La Jolla, CA). Examples of vectors that randomly integrate into host cell chromosomes include, for example, pcDNA3.1 (when introduced in the absence of T-antigen) from Life Technologies (Carlsbad, CA), UCOE from Millipore (Billerica, MA), and pCI or pFN10A (ACT) FLEXITM from Promega (Madison, WI).

[0071] Viral vectors also can be used. Representative commercially available viral expression vectors include, but are not limited to, the adenovirus-based Per.C6 system available from Crucell, Inc. (Leiden, The Netherlands), the lentiviral-based pLP1 from Invitrogen (Carlsbad, CA), and the retroviral vectors pFB-ERV plus pCFB-EGSH from Stratagene (La Jolla, CA).

[0072] Nucleic acid sequences encoding the inventive amino acid sequences can be provided to a cell on the same vector (i.e., in *cis*). A unidirectional promoter can be used to control expression of each nucleic acid sequence. In another embodiment, a combination of bidirectional and unidirectional promoters can be used to control expression of multiple nucleic acid sequences. Nucleic acid sequences encoding the inventive amino acid sequences alternatively can be provided to the population of cells on separate vectors (i.e., in *trans*). Each of the nucleic acid sequences in each of the separate vectors can comprise the same or different expression control sequences. The separate vectors can be provided to cells simultaneously or sequentially.

[0073] The vector(s) comprising the nucleic acid(s) encoding the inventive amino acid sequences can be introduced into a host cell that is capable of expressing the polypeptides encoded thereby, including any suitable prokaryotic or eukaryotic cell. As such, the invention

provides an isolated cell comprising the inventive vector. Preferred host cells are those that can be easily and reliably grown, have reasonably fast growth rates, have well characterized expression systems, and can be transformed or transfected easily and efficiently.

[0074] Examples of suitable prokaryotic cells include, but are not limited to, cells from the genera *Bacillus* (such as *Bacillus subtilis* and *Bacillus brevis*), *Escherichia* (such as *E. coli*), *Pseudomonas*, *Streptomyces*, *Salmonella*, and *Erwinia*. Particularly useful prokaryotic cells include the various strains of *Escherichia coli* (e.g., K12, HB101 (ATCC No. 33694), DH5 α , DH10, MC1061 (ATCC No. 53338), and CC102).

[0075] Preferably, the vector is introduced into a eukaryotic cell. Suitable eukaryotic cells are known in the art and include, for example, yeast cells, insect cells, and mammalian cells. Examples of suitable yeast cells include those from the genera *Kluyveromyces*, *Pichia*, *Rhinosporidium*, *Saccharomyces*, and *Schizosaccharomyces*. Preferred yeast cells include, for example, *Saccharomyces cerevisiae* and *Pichia pastoris*.

[0076] Suitable insect cells are described in, for example, Kitts et al., *Biotechniques*, 14: 810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, 4: 564-572 (1993); and Lucklow et al., *J. Virol.*, 67: 4566-4579 (1993). Preferred insect cells include Sf-9 and HI5 (Invitrogen, Carlsbad, CA).

[0077] Preferably, mammalian cells are utilized in the invention. A number of suitable mammalian host cells are known in the art, and many are available from the American Type Culture Collection (ATCC, Manassas, VA). Examples of suitable mammalian cells include, but are not limited to, Chinese hamster ovary cells (CHO) (ATCC No. CCL61), CHO DHFR-cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA*, 97: 4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), and 3T3 cells (ATCC No. CCL92). Other suitable mammalian cell lines are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), as well as the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Other suitable mammalian cell lines include, but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, and BHK or HaK hamster cell lines, all of which are available from the ATCC. Methods for

selecting suitable mammalian host cells and methods for transformation, culture, amplification, screening, and purification of cells are known in the art.

[0078] In one embodiment, the mammalian cell is a human cell. For example, the mammalian cell can be a human lymphoid or lymphoid derived cell line, such as a cell line of pre-B lymphocyte origin. Examples of human lymphoid cells lines include, without limitation, RAMOS (CRL-1596), Daudi (CCL-213), EB-3 (CCL-85), DT40 (CRL-2111), 18-81 (Jack et al., *Proc. Natl. Acad. Sci. USA*, 85: 1581-1585 (1988)), Raji cells (CCL-86), PER.C6 cells (Crucell Holland B.V., Leiden, The Netherlands), and derivatives thereof.

[0079] A nucleic acid sequence encoding the inventive amino acid sequence may be introduced into a cell by “transfection,” “transformation,” or “transduction.” “Transfection,” “transformation,” or “transduction,” as used herein, refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or chemical methods. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, e.g., Murray E.J. (ed.), *Methods in Molecular Biology*, Vol. 7, *Gene Transfer and Expression Protocols*, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.*, 7: 2031-2034 (1987)). Phage or viral vectors can be introduced into host cells, after growth of infectious particles in suitable packaging cells, many of which are commercially available.

[0080] The invention provides a composition comprising an effective amount of the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive TIM-3-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence. Preferably, the composition is a pharmaceutically acceptable (e.g., physiologically acceptable) composition, which comprises a carrier, preferably a pharmaceutically acceptable (e.g., physiologically acceptable) carrier, and the inventive amino acid sequences, antigen-binding agent, or vector. Any suitable carrier can be used within the context of the invention, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the composition may be administered and the particular method used to

administer the composition. The composition optionally can be sterile. The composition can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. The compositions can be generated in accordance with conventional techniques described in, e.g., Remington: *The Science and Practice of Pharmacy, 21st Edition*, Lippincott Williams & Wilkins, Philadelphia, PA (2001).

[0081] The invention further provides a method of treating a disorder in a mammal that is responsive to TIM-3 inhibition or neutralization. The method comprises administering the aforementioned composition to a mammal having a disorder that is responsive to TIM-3 inhibition or neutralization, whereupon the disorder is treated in the mammal. A disorder that is “responsive to TIM-3 inhibition” or “responsive to TIM-3 neutralization” refers to any disease or disorder in which a decrease in TIM-3 levels or activity has a therapeutic benefit in mammals, preferably humans, or the improper expression (e.g., overexpression) or increased activity of TIM-3 causes or contributes to the pathological effects of the disease or disorder. Disorders that are responsive to TIM-3 inhibition include, for example, cancer, infectious diseases, and autoimmune diseases. The inventive method can be used to treat any type of cancer known in the art, such as, for example, melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, leukemia, lymphoma, or Merkel cell carcinoma (see, e.g., Bhatia et al., *Curr. Oncol. Rep.*, 13(6): 488-497 (2011)). The inventive method can be used to treat any type of infectious disease (i.e., a disease or disorder caused by a bacterium, a virus, a fungus, or a parasite). Examples of infectious diseases that can be treated by the inventive method include, but are not limited to, diseases caused by a human immunodeficiency virus (HIV), a respiratory syncytial virus (RSV), an influenza virus, a dengue virus, a hepatitis B virus (HBV, or a hepatitis C virus (HCV)). The inventive method can be used to treat any type of autoimmune disease (i.e., as disease or disorder caused by immune system overactivity in which the body attacks and damages its own tissues), such as those described in, for example, MacKay I.R. and Rose N.R., eds., *The Autoimmune Diseases, Fifth Edition*, Academic Press, Waltham, MA (2014). Examples of autoimmune diseases that can be treated by the inventive method include, but are not limited to, multiple

sclerosis, type 1 diabetes mellitus, rheumatoid arthritis, scleroderma, Crohn's disease, psoriasis, systemic lupus erythematosus (SLE), and ulcerative colitis.

[0082] Administration of a composition comprising the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive TIM-3-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence induces an immune response against a cancer or infectious disease in a mammal. An "immune response" can entail, for example, antibody production and/or the activation of immune effector cells (e.g., T-cells).

[0083] As used herein, the terms "treatment," "treating," and the like refer to obtaining a desired pharmacologic and/or physiologic effect. Preferably, the effect is therapeutic, i.e., the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. To this end, the inventive method comprises administering a "therapeutically effective amount" of the TIM-3-binding agent. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the TIM-3-binding agent to elicit a desired response in the individual. For example, a therapeutically effective amount of a TIM-3-binding agent of the invention is an amount which decreases TIM-3 bioactivity in a human.

[0084] Alternatively, the pharmacologic and/or physiologic effect may be prophylactic, i.e., the effect completely or partially prevents a disease or symptom thereof. In this respect, the inventive method comprises administering a "prophylactically effective amount" of the TIM-3-binding agent. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (e.g., prevention of disease onset).

[0085] A typical dose can be, for example, in the range of 1 pg/kg to 20 mg/kg of animal or human body weight; however, doses below or above this exemplary range are within the scope of the invention. The daily parenteral dose can be about 0.00001 µg/kg to about 20 mg/kg of total body weight (e.g., about 0.001 µg/kg, about 0.1 µg/kg, about 1 µg/kg, about 5 µg/kg, about 10 µg/kg, about 100 µg/kg, about 500 µg/kg, about 1 mg/kg, about 5 mg/kg, about 10 mg/kg, or a range defined by any two of the foregoing values), preferably from about 0.1 µg/kg

to about 10 mg/kg of total body weight (e.g., about 0.5 µg/kg, about 1 µg/kg, about 50 µg/kg, about 150 µg/kg, about 300 µg/kg, about 750 µg/kg, about 1.5 mg/kg, about 5 mg/kg, or a range defined by any two of the foregoing values), more preferably from about 1 µg/kg to 5 mg/kg of total body weight (e.g., about 3 µg/kg, about 15 µg/kg, about 75 µg/kg, about 300 µg/kg, about 900 µg/kg, about 2 mg/kg, about 4 mg/kg, or a range defined by any two of the foregoing values), and even more preferably from about 0.5 to 15 mg/kg body weight per day (e.g., about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 6 mg/kg, about 9 mg/kg, about 11 mg/kg, about 13 mg/kg, or a range defined by any two of the foregoing values). Therapeutic or prophylactic efficacy can be monitored by periodic assessment of treated patients. For repeated administrations over several days or longer, depending on the condition, the treatment can be repeated until a desired suppression of disease symptoms occurs, or alternatively, the treatment can be continued for the lifetime of the patient. However, other dosage regimens may be useful and are within the scope of the invention. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0086] The composition comprising an effective amount of the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive TIM-3-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence can be administered to a mammal using standard administration techniques, including oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. The composition preferably is suitable for parenteral administration. The term “parenteral,” as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. More preferably, the composition is administered to a mammal using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0087] Once administered to a mammal (e.g., a human), the biological activity of the inventive TIM-3-binding agent can be measured by any suitable method known in the art. For example, the biological activity can be assessed by determining the stability of a particular TIM-3-binding agent. In one embodiment of the invention, the TIM-3-binding agent (e.g., an

antibody) has an *in vivo* half life between about 30 minutes and 45 days (e.g., about 30 minutes, about 45 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 10 hours, about 12 hours, about 1 day, about 5 days, about 10 days, about 15 days, about 25 days, about 35 days, about 40 days, about 45 days, or a range defined by any two of the foregoing values). In another embodiment, the TIM-3-binding agent has an *in vivo* half life between about 2 hours and 20 days (e.g., about 5 hours, about 10 hours, about 15 hours, about 20 hours, about 2 days, about 3 days, about 7 days, about 12 days, about 14 days, about 17 days, about 19 days, or a range defined by any two of the foregoing values). In another embodiment, the TIM-3-binding agent has an *in vivo* half life between about 10 days and about 40 days (e.g., about 10 days, about 13 days, about 16 days, about 18 days, about 20 days, about 23 days, about 26 days, about 29 days, about 30 days, about 33 days, about 37 days, about 38 days, about 39 days, about 40 days, or a range defined by any two of the foregoing values).

[0088] The stability of the inventive TIM-3 binding agent can be measured using any other suitable assay known in the art, such as, for example, measuring serum half-life, differential scanning calorimetry (DSC), thermal shift assays, and pulse-chase assays. Other methods of measuring protein stability *in vivo* and *in vitro* that can be used in the context of the invention are described in, for example, *Protein Stability and Folding*, B.A. Shirley (ed.), Human Press, Totowa, New Jersey (1995); *Protein Structure, Stability, and Interactions (Methods in Molecular Biology)*, Shiver J.W. (ed.), Humana Press, New York, NY (2010); and Ignatova, *Microb. Cell Fact.*, 4: 23 (2005).

[0089] The stability of the inventive TIM-3-binding agent can be measured in terms of the transition mid-point value (T_m), which is the temperature where 50% of the amino acid sequence is in its native confirmation, and the other 50% is denatured. In general, the higher the T_m , the more stable the protein. In one embodiment of the invention, the inventive TIM-3 binding agent comprises a transition mid-point value (T_m) *in vitro* of about 60-100 °C. For example, the inventive TIM-3 binding agent can comprise a T_m *in vitro* of about 65-80 °C (e.g., 66 °C, 68 °C, 70 °C, 71 °C, 75 °C, or 79 °C), about 80-90 °C (e.g., about 81 °C, 85 °C, or 89 °C), or about 90-100 °C (e.g., about 91 °C, about 95 °C, or about 99 °C).

[0090] The biological activity of a particular TIM-3-binding agent also can be assessed by determining its binding affinity to TIM-3 or an epitope thereof. The term “affinity” refers to the

equilibrium constant for the reversible binding of two agents and is expressed as the dissociation constant (K_D). Affinity of a binding agent to a ligand, such as affinity of an antibody for an epitope, can be, for example, from about 1 picomolar (pM) to about 100 micromolar (μ M) (e.g., from about 1 picomolar (pM) to about 1 nanomolar (nM), from about 1 nM to about 1 micromolar (μ M), or from about 1 μ M to about 100 μ M). In one embodiment, the TIM-3-binding agent can bind to an TIM-3 protein with a K_D less than or equal to 1 nanomolar (e.g., 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.05 nM, 0.025 nM, 0.01 nM, 0.001 nM, or a range defined by any two of the foregoing values). In another embodiment, the TIM-3-binding agent can bind to TIM-3 with a K_D less than or equal to 200 pM (e.g., 190 pM, 175 pM, 150 pM, 125 pM, 110 pM, 100 pM, 90 pM, 80 pM, 75 pM, 60 pM, 50 pM, 40 pM, 30 pM, 25 pM, 20 pM, 15 pM, 10 pM, 5 pM, 1 pM, or a range defined by any two of the foregoing values). Immunoglobulin affinity for an antigen or epitope of interest can be measured using any art-recognized assay. Such methods include, for example, fluorescence activated cell sorting (FACS), separable beads (e.g., magnetic beads), surface plasmon resonance (SPR), solution phase competition (KINEXA™), antigen panning, competitive binding assays, and/or ELISA (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY, 2001).

[0091] The TIM-3-binding agent of the invention may be administered alone or in combination with other drugs. For example, the TIM-3-binding agent can be administered in combination with other agents for the treatment or prevention of the diseases disclosed herein, such as agents that are cytotoxic to cancer cells, modulate the immunogenicity of cancer cells, or promote immune responses to cancer cells. In this respect, for example, the TIM-3-binding agent can be used in combination with at least one other anticancer agent including, for example, any chemotherapeutic agent known in the art, ionization radiation, small molecule anticancer agents, cancer vaccines, biological therapies (e.g., other monoclonal antibodies, cancer-killing viruses, gene therapy, and adoptive T-cell transfer), and/or surgery. When the inventive method treats an infectious disease, the TIM-3-binding agent can be administered in combination with at least one anti-bacterial agent or at least one anti-viral agent. In this respect, the anti-bacterial agent can be any suitable antibiotic known in the art. The anti-viral agent can be any vaccine of any suitable type that specifically targets a particular virus (e.g., live-attenuated vaccines, subunit

vaccines, recombinant vector vaccines, and small molecule anti-viral therapies (e.g., viral replication inhibitors and nucleoside analogs). When the inventive method treats an autoimmune disease, the TIM-3-binding agent can be used in combination with an anti-inflammatory agent including, for example, corticosteroids (e.g., prednisone and fluticasone) and non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin, ibuprofen, and naproxen).

[0092] In another embodiment, when the inventive TIM-3 binding agent is used to treat cancer or an infectious disease, the TIM-3 binding agent can be administered in combination with other agents that inhibit immune checkpoint pathways. For example, the inventive TIM-3 binding agent can be administered in combination with agents that inhibit or antagonize the programmed death 1 protein (PD-1), lymphocyte activation gene-3 protein (LAG-3), and/or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways. Combination treatments that simultaneously target two or more of these immune checkpoint pathways have demonstrated improved and potentially synergistic antitumor activity (see, e.g., Sakuishi et al., *J. Exp. Med.*, 207: 2187-2194 (2010); Ngiow et al., *Cancer Res.*, 71: 3540-3551 (2011); and Woo et al., *Cancer Res.*, 72: 917-927 (2012)). In one embodiment, the inventive TIM-3 binding agent is administered in combination with an antibody that binds to LAG-3 and/or an antibody that binds to PD-1. In this respect, the inventive method of treating a disorder that is responsive to TIM-3 inhibition (e.g., cancer or an infectious disease) in a mammal can further comprise administering to the mammal a composition comprising (i) an antibody that binds to a TIM-3 protein and (ii) a pharmaceutically acceptable carrier or a composition comprising (i) an antibody that binds to a PD-1 protein and (ii) a pharmaceutically acceptable carrier.

[0093] In addition to therapeutic uses, the TIM-3-binding agent described herein can be used in diagnostic or research applications. In this respect, the TIM-3-binding agent can be used in a method to diagnose a disorder or disease in which the improper expression (e.g., overexpression) or increased activity of TIM-3 causes or contributes to the pathological effects of the disease or disorder. In a similar manner, the TIM-3-binding agent can be used in an assay to monitor TIM-3 protein levels in a subject being tested for a disease or disorder that is responsive to TIM-3 inhibition. Research applications include, for example, methods that utilize the TIM-3-binding agent and a label to detect a TIM-3 protein in a sample, e.g., in a human body fluid or in a cell or tissue extract. The TIM-3-binding agent can be used with or without modification, such as

covalent or non-covalent labeling with a detectable moiety. For example, the detectable moiety can be a radioisotope (e.g., ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I), a fluorescent or chemiluminescent compound (e.g., fluorescein isothiocyanate, rhodamine, or luciferin), an enzyme (e.g., alkaline phosphatase, beta-galactosidase, or horseradish peroxidase), or prosthetic groups. Any method known in the art for separately conjugating an antigen-binding agent (e.g., an antibody) to a detectable moiety may be employed in the context of the invention (see, e.g., Hunter et al., *Nature*, 194: 495-496 (1962); David et al., *Biochemistry*, 13: 1014-1021 (1974); Pain et al., *J. Immunol. Meth.*, 40: 219-230 (1981); and Nygren, *J. Histochem. and Cytochem.*, 30: 407-412 (1982)).

[0094] TIM-3 protein levels can be measured using the inventive TIM-3-binding agent by any suitable method known in the art. Such methods include, for example, radioimmunoassay (RIA), and FACS. Normal or standard expression values of TIM-3 can be established using any suitable technique, e.g., by combining a sample comprising, or suspected of comprising, TIM-3 with a TIM-3-specific antibody under conditions suitable to form an antigen-antibody complex. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials (see, e.g., Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987)). The amount of TIM-3 polypeptide expressed in a sample is then compared with a standard value.

[0095] The TIM-3-binding agent can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing a diagnostic assay. If the TIM-3-binding agent is labeled with an enzyme, the kit desirably includes substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides a detectable chromophore or fluorophore). In addition, other additives may be included in the kit, such as stabilizers, buffers (e.g., a blocking buffer or lysis buffer), and the like. The relative amounts of the various reagents can be varied to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. The reagents may be provided as dry powders (typically lyophilized), including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

[0096] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0097] This example demonstrates a method of identifying antibodies directed against human TIM-3 from an evolvable library and affinity maturation of the identified antibodies.

[0098] An IgG evolvable library, based on germline sequence V-gene segments joined to human donor-derived recombined (D)J regions, was constructed as described in Bowers et al. *Proc. Natl. Acad. Sci. USA*, 108(51): 20455-20460 (2011). IgG heavy chain (HC) and light chain (LC) were cloned into separate episomal vectors (Horlick et al., *Gene*, 243(1-2): 187-194 (2000)), with each vector encoding a distinct antibiotic selectable marker. Cells expressing surface-displayed, fully-human antibodies that bind to human TIM-3 were identified from a screening campaign of the evolvable library using magnetic beads coated with huTIM-3 extracellular domain. A panel of antibodies was isolated that bound specifically to TIM-3.

[0099] Stable cell lines co-expressing the HC and LC of each antibody identified in using the evolvable library described above were transfected with activation induced cytidine deaminase (AID) to initiate *in vitro* SHM. AID was also transfected directly into the original mixed population of cells expanded from the library screen. In all cases, cell populations were stained for both IgG expression and binding to antigen, collected by flow cytometry as a bulk population, and then expanded for sequence analysis by next generation sequencing (NGS). This process was repeated iteratively to accumulate SHM-derived mutations in the variable regions of both the heavy and light chains, and their derivatives, for each strategy. Maturation of the initial library hit antibodies was demonstrated by binding studies using BIACORE™, and binding to TIM-3 presented on the surface of a CHO cell line.

[00100] Matured antibodies were characterized to meet stringent requirements for therapeutic antibody development, including assessment of “developability” criteria as well as functional potency across assays. Developability criteria included thermal stability, expression level, absence of problematic sequence motifs (e.g., variable-region N-linked glycosylation sites, free cysteines, high-likelihood sites for deamidation, isomerization, etc.). In addition, high affinity binding to cynomolgus monkey TIM-3 was selected for to facilitate preclinical studies. Lead

and back-up antibodies with potent antagonistic activity were identified that met all criteria for further development. The lead antibody contained a heavy chain immunoglobulin polypeptide comprising SEQ ID NO: 34 and a light chain immunoglobulin polypeptide comprising SEQ ID NO: 115, and was designated APE5137. The APE5137 heavy chain CDR1, CDR2, and CDR3 comprised SEQ ID NOs: 329, 330, and 331, respectively. The APE5137 light chain CDR1, CDR2, and CDR3 comprised SEQ ID NOs: 332, 333, and 334, respectively. The back-up antibody contained a heavy chain immunoglobulin polypeptide comprising SEQ ID NO: 238 and a light chain immunoglobulin polypeptide comprising SEQ ID NO: 327, and was designated APE5121. The APE5121 heavy chain CDR1, CDR2, and CDR3 comprised SEQ ID NOs: 335, 336, and 337, respectively. The APE5121 light chain CDR1, CDR2, and CDR3 comprised SEQ ID NOs: 338, 339, and 340, respectively.

[00101] The characteristics of the lead and back-up anti-TIM-3 antibodies are described in Table 1.

Table 1

	Heavy Chain SEQ ID NO:	Light Chain SEQ ID NO:	BIAcore™ KD		T _m (Thermofluor Analysis)	Non-Specific Binding	Purity (Size Exclusion Chromatography)
			Human TIM-3	Cyno TIM-3			
Lead Antibody	34	115	50pM	190pM	72 °C	None detectable	>97%
Back-Up Antibody	238	327	<50pM	1.5nM	71 °C	None detectable	>97%

[00102] The results of this example confirm a method of affinity maturing monoclonal antibodies directed against TIM-3 identified using an evolvable library.

EXAMPLE 2

[00103] This example demonstrates that an inventive anti-TIM-3 monoclonal antibody can inhibit TIM-3 signaling and enhance T-cell activation *in vitro* alone, and in combination with an anti-PD-1 antibody.

[00104] The functional antagonist activity of antibodies exhibiting improved TIM-3 binding properties (described in Example 1) was tested in a human CD4⁺ T-cell mixed lymphocyte

reaction (MLR) assay in which activation of CD4⁺ T-cells in the presence of anti-TIM-3 antibodies is assessed by measuring IL-2 secretion. The anti-TIM-3 antibodies were tested alone or in combination with 2 ng/mL or 20 ng/mL of an antagonistic anti-PD-1 antibody. Specifically, isolated peripheral blood monocytes from a human donor were differentiated into dendritic cells (DCs) and then mixed with CD4⁺ T-cells isolated from a second donor. IL-2 levels were measured after 48 hours. Antagonism of TIM-3 alone, and in combination with antagonism of PD-1, was expected to result in increased T-cell activation as measured by increased IL-2 production. The anti-TIM-3 antibody increased IL-2 secretion both alone and in combination with the anti-PD1 antibody at 48 hours in the MLR assay, with the anti-TIM-3 antibody exhibiting increased activity in combination with the anti-PD-1 antibody, as shown in Figures 1A-1D.

[00105] The results of this example demonstrate that the inventive TIM-3 binding agent can inhibit TIM-3 biological activity alone and in combination with antagonists of other negative regulators of the immune system.

EXAMPLE 3

[0100] This example demonstrates that an anti-TIM-3 antibody antagonizes TIM-3 activity in a syngeneic mouse tumor model.

[0101] Surrogate rat antibodies recognizing mouse PD-1 (RMP1-14) and mouse TIM-3 (RMT3-23) were purchased from Bio X Cell (West Lebanon, NH) and tested in a MC38 syngeneic tumor model alone and in combination. Specifically, MC38 colon adenocarcinoma cells (1×10^6 s.c.) were implanted into C57Bl/6 mice and grown for 10 days. Mice with tumors measuring 40-90 mm³ were randomized (day of randomization designated day 1) to four groups of 10 animals/group and dosed with each antibody at 10 mg/kg on days 1, 4, 8 and 11. Mice injected with PBS served as a control. Tumor volumes were measured twice weekly until reaching 2000 mm³, which was designated as the endpoint at which time mice were sacrificed. The results of this experiment are shown in Figures 2A-2D, and demonstrate that the combination of surrogate anti-PD-1 and anti-TIM-3 antibodies can inhibit tumor growth in a mouse model, suggesting that dual blockade of immune checkpoint pathways could lead to increased clinical efficacy.

EXAMPLE 4

[0102] This example demonstrates certain effects of antibody isotype on anti-tumor activity of an anti-TIM-3 antibody alone or in combination with an anti-PD-1 antibody in a syngeneic mouse tumor model.

[0103] Surrogate rat/mouse chimeric antibodies recognizing mouse PD-1 and mouse TIM-3 of mouse IgG1(D265A) and mouse IgG2a isotypes were constructed from the rat antibodies tested in Example 3. These antibodies were tested in a MC38 syngeneic tumor model alone and in combination with anti-PD-1 antibody of the mouse IgG1(D265A) isotype. Specifically, MC38 colon adenocarcinoma cells (1×10^6 s.c.) were implanted into C57Bl/6 mice and grown for 8 days. Mice with tumors measuring 40-80 mm³ were randomized (day of randomization designated day 1) to seven groups of 10 animals/group and dosed with each antibody or antibody combination on days 1, 4, 8 and 11 as set forth in Table 2. Mice injected with isotype-matched control antibodies not recognizing any mouse antigens served as controls (Groups 1 and 2). Tumor volumes were measured twice weekly until reaching 2000 mm³, which was designated as the endpoint at which time mice were sacrificed.

Table 2

Group	Treatment	Dose
1	Isotype IgG2a + Isotype IgG1(D265A)	10 mg/kg, 0.5 mg/kg
2	Isotype IgG1 (D265A)	10 mg/kg
3	Anti-mPD-1 IgG1(D265A)	0.5 mg/kg
4	Anti-mTIM-3 IgG2a	10 mg/kg
5	Anti-mTIM-3 IgG1(D265A)	10 mg/kg
6	Anti-mPD-1 IgG1(D265A) + Anti-mTIM-3 IgG2a	0.5 mg/kg, 10 mg/kg
7	Anti-mPD-1 IgG1(D265A)+ Anti-mTIM-3 IgG1(D265A)	0.5 mg/kg, 10 mg/kg

[0104] Interim results for this experiment are shown in Figure 3, which demonstrates that a single agent anti-mouse TIM-3 antibody with effector function (i.e., IgG2a) has increased anti-tumor activity as compared with an anti-mouse TIM-3 antibody with minimal effector function (i.e., IgG1(D265A)). In addition, an anti-mouse TIM-3 antibody with minimal effector function (i.e., IgG1(D265A)) in combination with a regimen of an anti-mouse PD-1 IgG1(D265A) antibody exhibited slightly increased anti-tumor activity compared with an anti-mouse PD-1 IgG1(D265A) antibody alone. An anti-mouse TIM-3 antibody with full effector function

(IgG2a) in combination with an anti-mouse PD-1 IgG1(D265A) antibody exhibited similar anti-tumor activity as an anti-mouse PD-1 IgG1(D265A) antibody alone.

[0105] The results of this example demonstrate that anti-mouse TIM-3 and anti-mouse PD-1 antibodies of different isotypes, and moreover with different levels of effector function, alone and in combination, can inhibit tumor growth in a mouse model. The data furthermore demonstrate that, in some embodiments, antibodies (or fragments thereof) with only minimal effector function, administered alone or in combination with other antibodies (or fragments thereof, which may or may not display significant effector function), can provide effective therapy.

[0106] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0107] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No

language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0108] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. An isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Xaa1 Ala Xaa2 Ser Gly Phe Xaa3 Xaa4 Xaa5 Thr Phe Ser Xaa6 Tyr Xaa7 Met Xaa8 Trp Val Arg Gln Ala Xaa9 Gly Lys Gly Leu Xaa10 Trp Val Ser Xaa11 Ile Ser Xaa12 Gly Gly Xaa13 Tyr Thr Tyr Tyr Gln Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Xaa14 Glu Asp Thr Ala Val Tyr Tyr Cys Xaa15 Ser Xaa16 Xaa17 Xaa18 Xaa19 Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala (SEQ ID NO: 1), wherein

- (a) Xaa1 is deleted or is alanine (Ala),
- (b) Xaa2 is alanine (Ala), proline (Pro), aspartic acid (Asp), glycine (Gly), threonine (Thr), or valine (Val),
- (c) the subsequence Xaa3 Xaa4 Xaa5 is deleted or is Thr-Phe-Ile,
- (d) Xaa6 is serine (Ser), asparagine (Asn), arginine (Arg), or threonine (Thr),
- (e) Xaa7 is aspartic acid (Asp) or alanine (Ala),
- (f) Xaa8 is serine (Ser) or threonine (Thr),
- (g) Xaa9 is proline (Pro) or leucine (Leu),
- (h) Xaa10 is aspartic acid (Asp) or glutamic acid (Glu),
- (i) Xaa11 is threonine (Thr) or alanine (Ala),
- (j) Xaa12 is glycine (Gly) or serine (Ser),
- (k) Xaa13 is serine (Ser), threonine (Thr), aspartic acid (Asp), glycine (Gly), asparagine (Asn), or lysine (Lys),
- (l) Xaa14 is alanine (Ala) or valine (Val),
- (m) Xaa15 is alanine (Ala) or threonine (Thr), and
- (n) the subsequence Xaa16 Xaa17 Xaa18 Xaa19 is deleted or is Pro-Tyr-Tyr-Ala.

2. The isolated immunoglobulin heavy chain polypeptide of claim 1, wherein the subsequence Xaa3 Xaa4 Xaa5 is deleted.

3. The isolated immunoglobulin heavy chain polypeptide of claim 2, which comprises the amino acid sequence of any one of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5-SEQ ID NO: 8, SEQ ID NO: 10-SEQ ID NO: 12, SEQ ID NO: 14-SEQ ID NO: 27, or SEQ ID NO: 29-SEQ ID NO: 44

4. The isolated immunoglobulin heavy chain polypeptide of claim 1, wherein the subsequence Xaa3 Xaa4 Xaa5 is Thr-Phe-Ile.

5. The isolated immunoglobulin heavy chain polypeptide of claim 4, which comprises the amino acid sequence of any one of SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 13, and SEQ ID NO: 28.

6. The isolated immunoglobulin heavy chain polypeptide of claim 1, wherein the subsequence Xaa16 Xaa17 Xaa18 Xaa19 is deleted.

7. The isolated immunoglobulin heavy chain polypeptide of claim 6, which comprises the amino acid sequence of any one of SEQ ID NO: 3- SEQ ID NO: 44.

8. The isolated immunoglobulin heavy chain polypeptide of claim 1, wherein the subsequence Xaa16 Xaa17 Xaa18 Xaa19 is Pro-Tyr-Tyr-Ala.

9. The isolated immunoglobulin heavy chain polypeptide of claim 8, which comprises the amino acid sequence of SEQ ID NO: 2.

10. An isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Ile Gln Met Thr Xaa1 Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Xaa2 Ser Gln Ser Ile Xaa3 Xaa4 Tyr Leu Asn Trp Tyr Xaa5 Gln Lys Xaa6 Xaa7 Lys Ala Pro Lys Leu Leu Xaa8 Tyr Xaa9 Ala Ser Xaa10 Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Xaa11 Tyr Tyr Cys Gln Gln Xaa12 Xaa13 Xaa14 Xaa15 Pro Xaa16 Thr Phe Gly Xaa17 Gly Thr Lys Xaa18 Glu Ile Lys Arg (SEQ ID NO: 45), wherein

- (a) Xaa1 is glutamine (Gln) or histidine (His),
- (b) Xaa2 is alanine (Ala) or threonine (Thr),
- (c) Xaa3 is serine (Ser), arginine (Arg), asparagine (Asn), or threonine (Thr),
- (d) Xaa4 is serine (Ser), arginine (Arg), aspartic acid (Asp), threonine (Thr), or glycine (Gly),
- (e) Xaa5 is glutamine (Gln) or histidine (His),
- (f) Xaa6 is proline (Pro) or alanine (Ala),
- (g) Xaa7 is glycine (Gly), lysine (Lys), or arginine (Arg),
- (h) Xaa8 is isoleucine (Ile) or methionine (Met),
- (i) Xaa9 is alanine (Ala), glycine (Gly), aspartic acid (Asp), threonine (Thr), serine (Ser), valine (Val), or isoleucine (Ile),
- (j) Xaa10 is serine (Ser) or threonine (Thr),
- (k) Xaa11 is valine (Val), methionine (Met), or alanine (Ala)
- (l) Xaa12 is serine (Ser) or arginine (Arg),
- (m) Xaa13 is tyrosine (Tyr), histidine (His), phenylalanine (Phe), aspartic acid (Asp), serine (Ser), or asparagine (Asn),
- (n) Xaa14 is serine (Ser) or asparagine (Asn),
- (o) Xaa15 is threonine (Thr), serine (Ser), alanine (Ala), or proline (Pro),
- (p) Xaa16 is leucine (Leu) or histidine (His),
- (q) Xaa17 is glycine (Gly), arginine (Arg), or glutamic acid (Glu), and
- (r) Xaa18 is valine (Val) or leucine (Leu).

11. The isolated immunoglobulin light chain polypeptide of claim 10, which comprises the amino acid sequence of any one of SEQ ID NO: 46 – SEQ ID NO: 125.

12. An isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Xaa1 Leu Xaa2 Xaa3 Xaa4 Xaa5 Ser Gly Gly Xaa6 Leu Xaa7 Gln Pro Gly Gly Ser Leu Arg Leu Xaa8 Cys Xaa9 Ala Ser Gly Phe Thr Phe Xaa10 Xaa11 Ser Tyr Xaa12 Met Xaa13 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Xaa14 Ile Ser Gly Ser Gly Gly Xaa15 Thr Tyr Tyr Xaa16 Asp Ser Val Lys Gly Xaa17 Phe Thr Ile Ser Xaa18 Asp

Asn Ser Xaa19 Asn Thr Xaa20 Tyr Leu Gln Met Asn Xaa21 Leu Arg Ala Glu Asp Thr Ala Val
Tyr Tyr Cys Xaa22 Lys Lys Tyr Tyr Xaa23 Xaa24 Pro Ala Asp Tyr Trp Xaa25 Gln Gly Thr Leu
Val Thr Val Ser Ser Gly (SEQ ID NO: 126), wherein

- (a) Xaa1 is leucine (Leu), valine (Val), or methionine (Met),
- (b) the subsequence Xaa2 Xaa3 Xaa4 is deleted or is Glu-Ser-Leu,
- (c) Xaa5 is deleted or is glutamic acid (Glu),
- (d) Xaa6 is glycine (Gly) or aspartic acid (Asp),
- (d) Xaa7 is valine (Val) or isoleucine (Ile),
- (e) Xaa8 is serine (Ser) or tyrosine (Tyr),
- (f) Xaa9 is alanine (Ala) or valine (Val),
- (g) Xaa10 is serine (Ser), asparagine (Asn), arginine (Arg), threonine (Thr),
aspartic acid (Asp), or glycine (Gly),
- (h) Xaa11 is deleted or is glycine (Gly),
- (i) Xaa12 is alanine (Ala) or threonine (Thr),
- (j) Xaa13 is serine (Ser) or asparagine (Asn),
- (k) Xaa14 is alanine (Ala), glycine (Gly), valine (Val), serine (Ser),
phenylalanine (Phe), isoleucine (Ile), threonine (Thr), or aspartic acid (Asp)
- (l) Xaa15 is serine (Ser) or asparagine (Asn),
- (m) Xaa16 is alanine (Ala), valine (Val), or asparagine (Asn),
- (n) Xaa17 is arginine (Arg) or glutamine (Gln),
- (o) Xaa18 is arginine (Arg) or lysine (Lys),
- (p) Xaa19 is lysine (Lys) or asparagine (Asn),
- (q) Xaa20 is leucine (Leu), valine (Val), threonine (Thr), methionine (Met), or
proline (Pro),
- (r) Xaa21 is serine (Ser) or asparagine (Asn),
- (s) Xaa22 is alanine (Ala) or glycine (Gly),
- (t) Xaa23 is glycine (Gly), valine (Val), aspartic acid (Asp), alanine (Ala),
threonine (Thr), or asparagine (Asn),
- (u) Xaa24 is glycine (Gly), serine (Ser), valine (Val), aspartic acid (Asp),
asparagine (Asn), or threonine (Thr), and

(v) Xaa25 is glycine (Gly) or aspartic acid (Asp).

13. The isolated immunoglobulin heavy chain polypeptide of claim 12, wherein the subsequence Xaa2 Xaa3 Xaa4 is deleted.

14. The isolated immunoglobulin heavy chain polypeptide of claim 13, which comprises the amino acid sequence of any one of SEQ ID NO: 127-SEQ ID NO: 227, SEQ ID NO: 229-SEQ ID NO: 231, SEQ ID NO: 233-SEQ ID NO: 235, SEQ ID NO: 237-SEQ ID NO: 243, or SEQ ID NO: 245-SEQ ID NO: 259.

15. The isolated immunoglobulin heavy chain polypeptide of claim 12, wherein the subsequence Xaa2 Xaa3 Xaa4 is Glu-Ser-Leu.

16. The isolated immunoglobulin heavy chain polypeptide of claim 15 which comprises the amino acid sequence of SEQ ID NO: 228, SEQ ID NO: 232, SEQ ID NO: 236, or SEQ ID NO: 244.

17. The isolated immunoglobulin heavy chain polypeptide of claim 12, wherein Xaa5 is deleted.

18. The isolated immunoglobulin heavy chain polypeptide of claim 17, which comprises the amino acid sequence of any one of SEQ ID NO: 222, SEQ ID NO: 231, SEQ ID NO: 235, or SEQ ID NO: 243.

19. The isolated immunoglobulin heavy chain polypeptide of claim 12, wherein Xaa5 is glutamic acid (Glu).

20. The isolated immunoglobulin heavy chain polypeptide of claim 19, which comprises the amino acid sequence of any one of SEQ ID NO: 126-SEQ ID NO: 221, SEQ ID NO: 223-SEQ ID NO: 230, SEQ ID NO: 232-SEQ ID NO: 234, SEQ ID NO: 236-SEQ ID NO: 242, or SEQ ID NO: 244-SEQ ID NO: 259.

21. An isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala

Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Asn Lys Asn Tyr Leu Xaa1 Trp Tyr Xaa2 Xaa3 Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Xaa4 Gly Val Pro Asp Arg Phe Xaa5 Gly Ser Xaa6 Ser Gly Thr Asp Phe Thr Leu Xaa7 Ile Xaa8 Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Xaa Gln Tyr Tyr Xaa10 Ser Pro Xaa11 Thr Phe Gly Gly Gly Thr Lys Ile Glu Xaa12 Lys (SEQ ID NO: 260), wherein

- (a) Xaa1 is alanine (Ala) or threonine (Thr),
- (b) Xaa2 is glutamine (Gln) or histidine (His),
- (c) Xaa3 is glutamine (Gln) or histidine (His),
- (d) Xaa4 is serine (Ser), tyrosine (Tyr), aspartic acid (Asp), glycine (Gly), threonine (Thr), asparagine (Asn), lysine (Lys), glutamic acid (Glu), leucine (Leu), proline (Pro), or valine (Val),
- (e) Xaa5 is serine (Ser) or asparagine (Asn),
- (f) Xaa6 is glycine (Gly), glutamic acid (Glu), alanine (Ala), aspartic acid (Asp), asparagine (Asn), serine (Ser), threonine (Thr), or valine (Val),
- (g) Xaa7 is threonine (Thr) or isoleucine (Ile),
- (h) Xaa8 is serine (Ser) or isoleucine (Ile),
- (i) Xaa9 is glutamine (Gln) or histidine (His),
- (j) Xaa10 is serine (Ser), asparagine (Asn), arginine (Arg), glycine (Gly), or threonine (Thr),
- (k) Xaa11 is leucine (Leu) or isoleucine (Ile), and
- (l) Xaa12 is leucine (Leu) or valine (Val).

22. The isolated immunoglobulin light chain polypeptide of claim 21, which comprises the amino acids sequence of any one of SEQ ID NO: 261-SEQ ID NO: 328.

23. An isolated nucleic acid sequence encoding the immunoglobulin heavy chain polypeptide of any one of claims 1-9 or 12-20.

24. An isolated nucleic acid sequence encoding the immunoglobulin light chain polypeptide of any one of claims 10, 11, 21, or 22.

25. A vector comprising the isolated nucleic acid sequence of claim 23 or claim 24.

26. A T Cell Immunoglobulin and Mucin Protein 3 (TIM-3)-binding agent comprising the immunoglobulin heavy chain polypeptide of any one of claims 1-9 or 12-20 and/or the immunoglobulin light chain polypeptide of any one of claims 10, 11, 21, or 22.
27. The TIM-3-binding agent of claim 26, which comprises the immunoglobulin heavy chain polypeptide of any one of claims 1-9 or 12-20 and the immunoglobulin light chain polypeptide of any one of claims 10, 11, 21, or 22.
28. The TIM-3-binding agent of claim 26, which comprises the immunoglobulin heavy chain polypeptide of any one of claims 1-9 or 12-20 or the immunoglobulin light chain polypeptide of any one of claims 10, 11, 21, or 22.
29. The TIM-3-binding agent of any one of claims 26-28, which is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.
30. The TIM-3-binding agent of claim 29, which is a F(ab')₂ fragment, a Fab' fragment, a Fab fragment, a Fv fragment, a scFv fragment, a dsFv fragment, a dAb fragment, or a single chain binding polypeptide.
31. An isolated nucleic acid sequence encoding the TIM-3-binding agent of any one of claims 26-30.
32. A vector comprising the isolated nucleic acid sequence of claim 31.
33. An isolated cell comprising the vector of claim 32.
34. A composition comprising (a) the TIM-3-binding agent of any one of claims 26-30 or the vector of claim 32 and (b) a pharmaceutically acceptable carrier.
35. A method of treating a disorder in a mammal that is responsive to TIM-3 inhibition, which method comprises administering an effective amount of the composition of claim 34 to a mammal having a disorder that is responsive to TIM-3 inhibition, whereupon the disorder is treated in the mammal.
36. The method of claim 35, wherein the disorder is cancer.

37. The method of claim 36, wherein the cancer is melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, or Merkel cell carcinoma.

38. The method of claim 35, wherein the disorder is an infectious disease.

39. The method of claim 38, wherein the infectious disease is caused by a virus or a bacterium.

40. The method of claim 39, wherein the virus is human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), influenza virus, dengue virus, or hepatitis B virus (HBV).

41. The method of claim 35, wherein the disorder is an autoimmune disease.

42. The method of claim 41, wherein the autoimmune disease is multiple sclerosis, type 1 diabetes mellitus, rheumatoid arthritis, scleroderma, Crohn's disease, psoriasis, systemic lupus erythematosus (SLE), or ulcerative colitis.

43. The method of any one of claims 35-42, wherein the half-life of the TIM-3-binding agent in the mammal is between 30 minutes and 45 days.

44. The method of any one of claims 35-43, wherein the TIM-3-binding agent binds to TIM-3 with a K_D between about 1 picomolar (pM) and about 100 micromolar (μ M).

45. The method of any one of claims 35-44, further comprising administering a PD-1-binding agent to the mammal.

46. The method of claim 45, wherein the PD-1-binding agent is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

47. The method of any one of claims 35-46, further comprising administering a LAG-3-binding agent to the mammal.

48. The method of claim 47, wherein the LAG-3-binding agent is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

49. An isolated immunoglobulin heavy chain polypeptide which comprises a complementarity determining region 1 (CDR1) consisting of the amino acid sequence of SEQ ID NO: 329, a CDR2 consisting of the amino acid sequence of SEQ ID NO: 330, and/or a CDR3 consisting of the amino acid sequence of SEQ ID NO: 331.

50. An isolated immunoglobulin light chain polypeptide which comprises a CDR1 consisting of the amino acid sequence of SEQ ID NO: 332, a CDR2 consisting of the amino acid sequence of SEQ ID NO: 333, and/or a CDR3 consisting of the amino acid sequence of SEQ ID NO: 334.

51. An isolated immunoglobulin heavy chain polypeptide which comprises a CDR1 consisting of the amino acid sequence of SEQ ID NO: 335, a CDR2 consisting of the amino acid sequence of SEQ ID NO: 336, and/or a CDR3 consisting of the amino acid sequence of SEQ ID NO: 337.

52. An isolated immunoglobulin light chain polypeptide which comprises a CDR1 consisting of the amino acid sequence of SEQ ID NO: 338, a CDR2 consisting of the amino acid sequence of SEQ ID NO: 339, and/or a CDR3 consisting of the amino acid sequence of SEQ ID NO: 340.

53. A TIM-3-binding agent comprising the immunoglobulin heavy chain polypeptide of claim 49 or 51 and/or the immunoglobulin light chain polypeptide of claim 50 or 52.

54. The TIM-3-binding agent of claim 53, which comprises the immunoglobulin heavy chain polypeptide of claim 49 or 51 and the immunoglobulin light chain polypeptide of claim 50 or 52.

55. The TIM-3 binding agent of claim 53, which comprises the immunoglobulin heavy chain polypeptide of claim 49 and the immunoglobulin light chain polypeptide of claim

50, or the immunoglobulin heavy chain polypeptide of claim 51 and the immunoglobulin light chain polypeptide of claim 52.

56. The TIM-3-binding agent of any one of claims 53-55, which is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

57. The TIM-3-binding agent of claim 56, which is a F(ab')₂ fragment, a Fab' fragment, a Fab fragment, a Fv fragment, a scFv fragment, a dsFv fragment, a dAb fragment, or a single chain binding polypeptide.

FIG. 1A

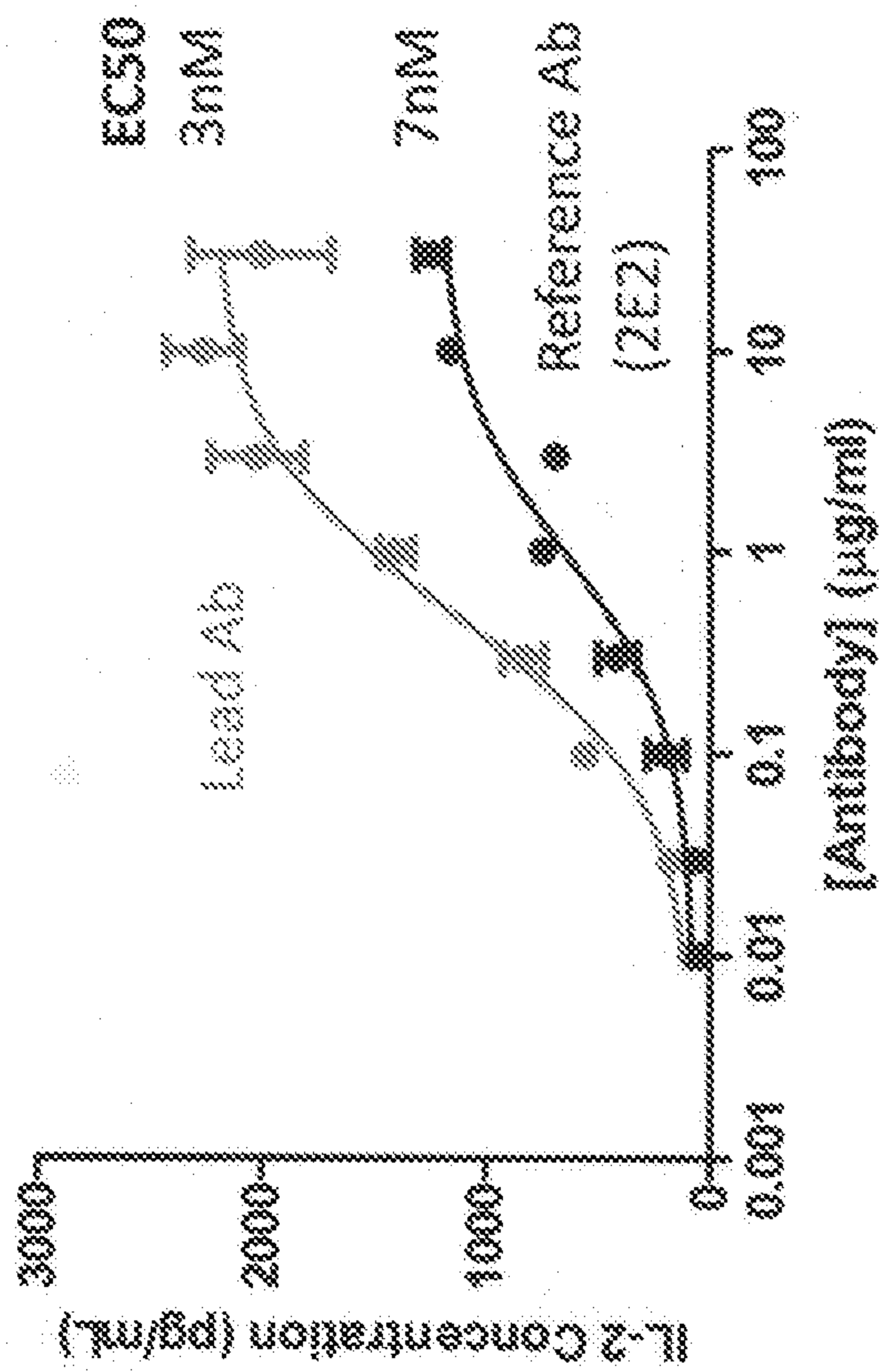


FIG. 1B

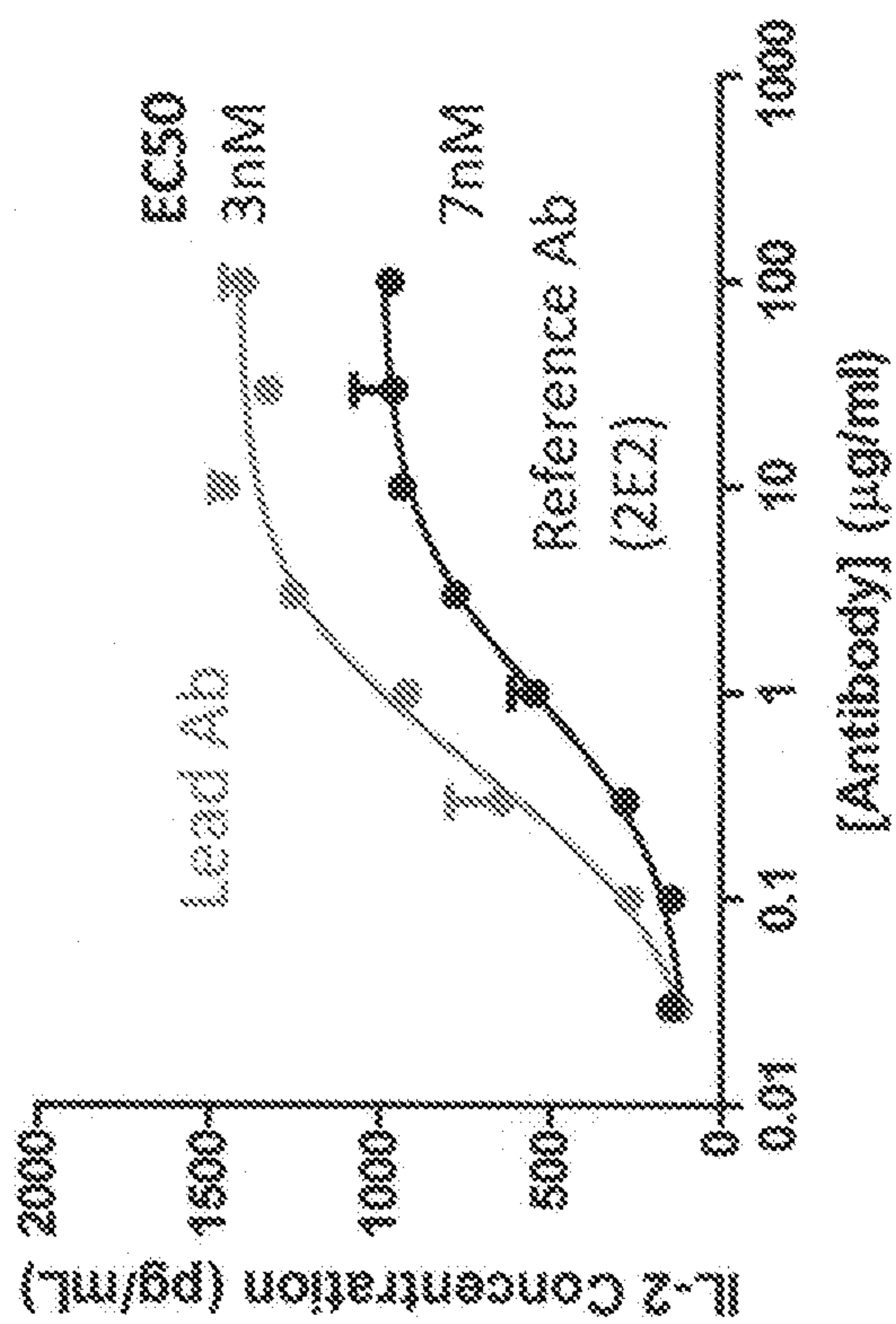


FIG. 1C

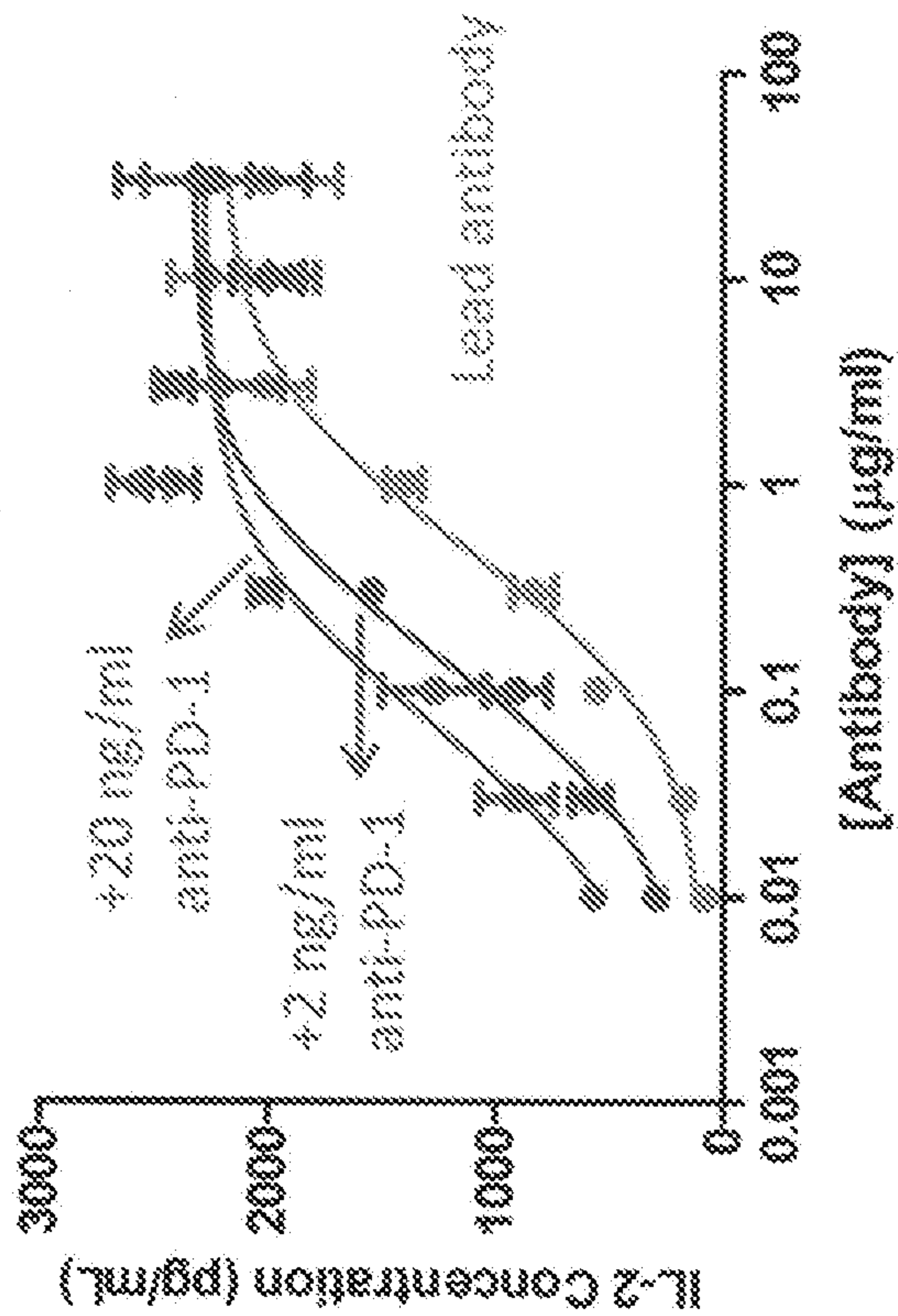
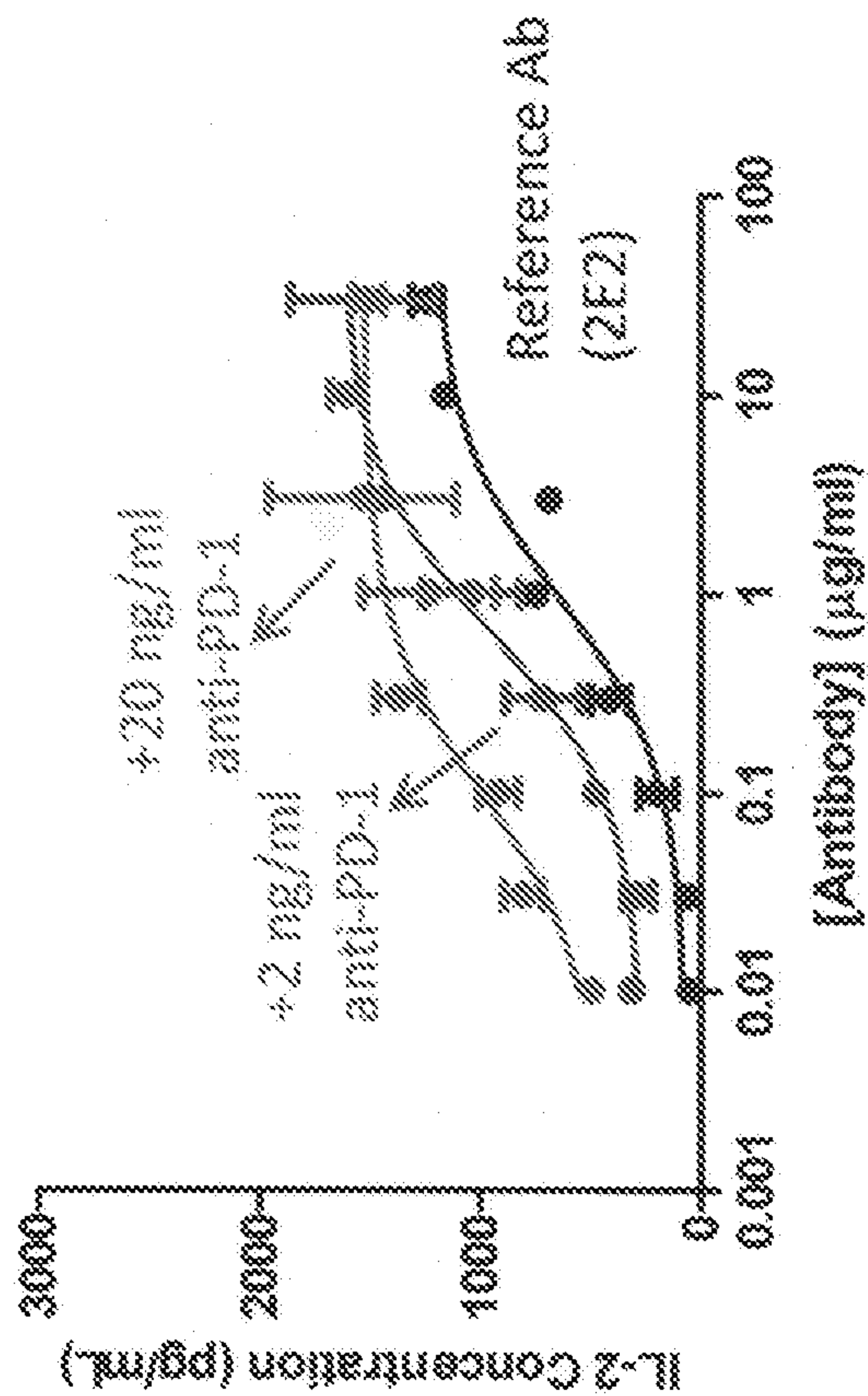
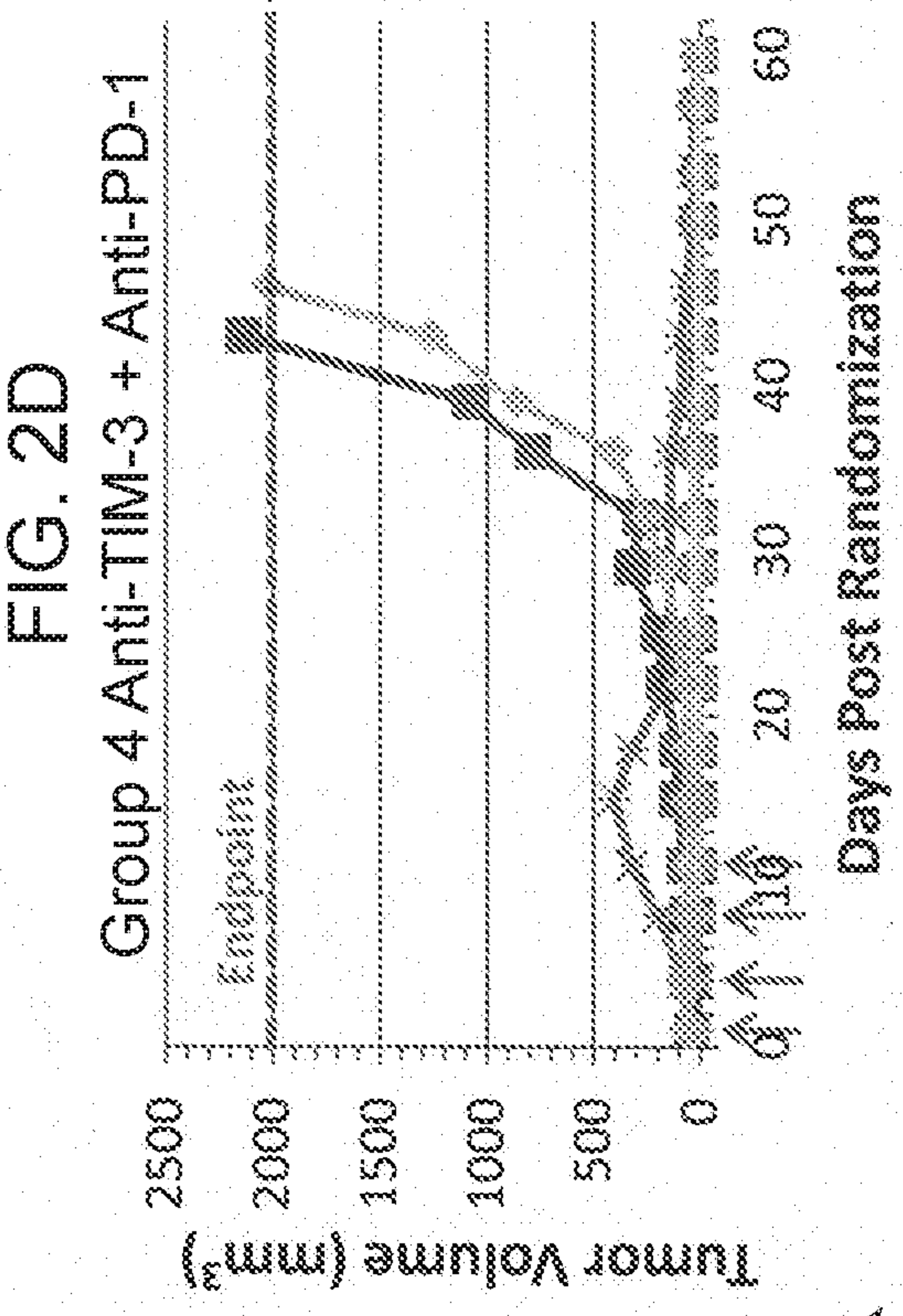
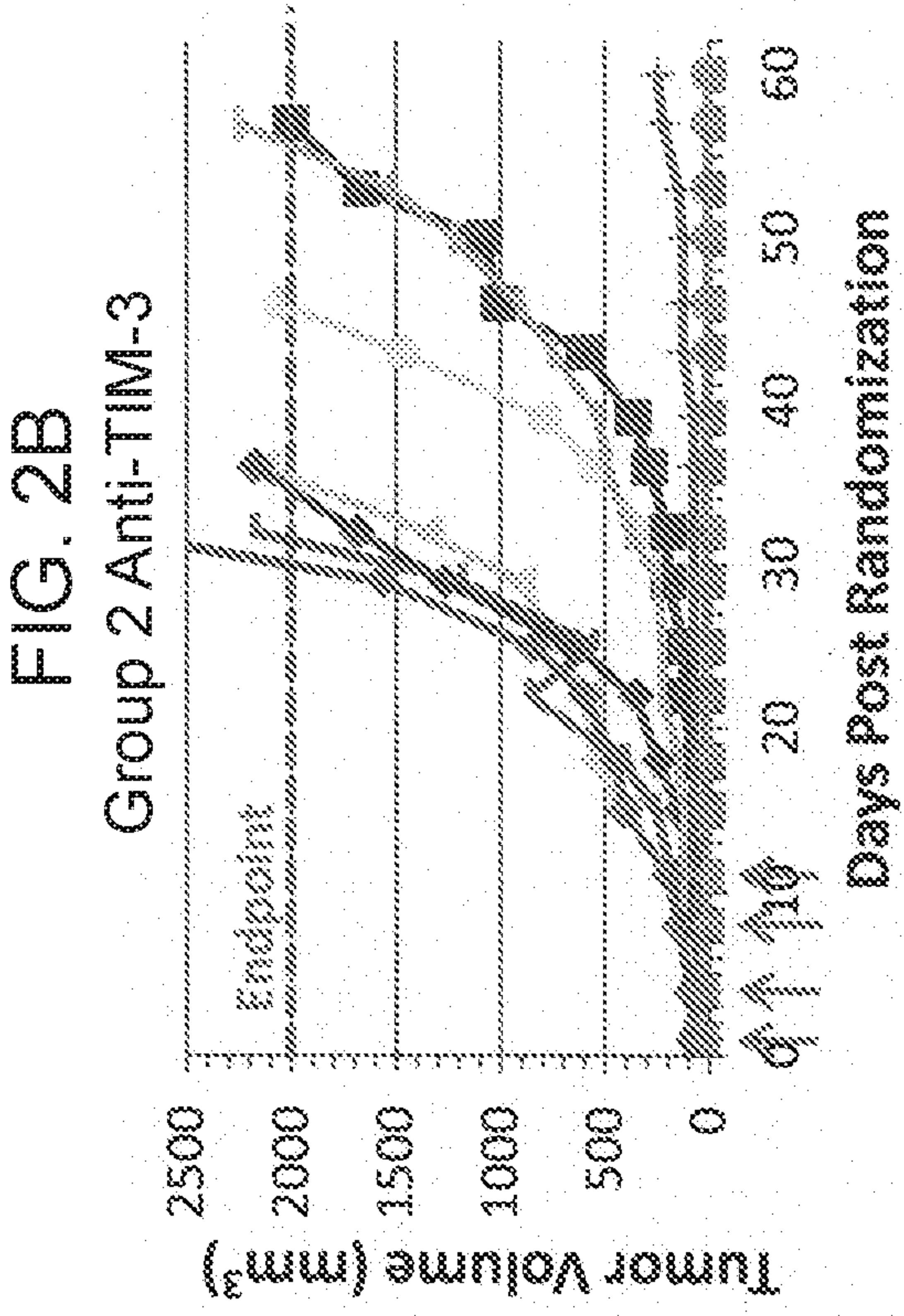
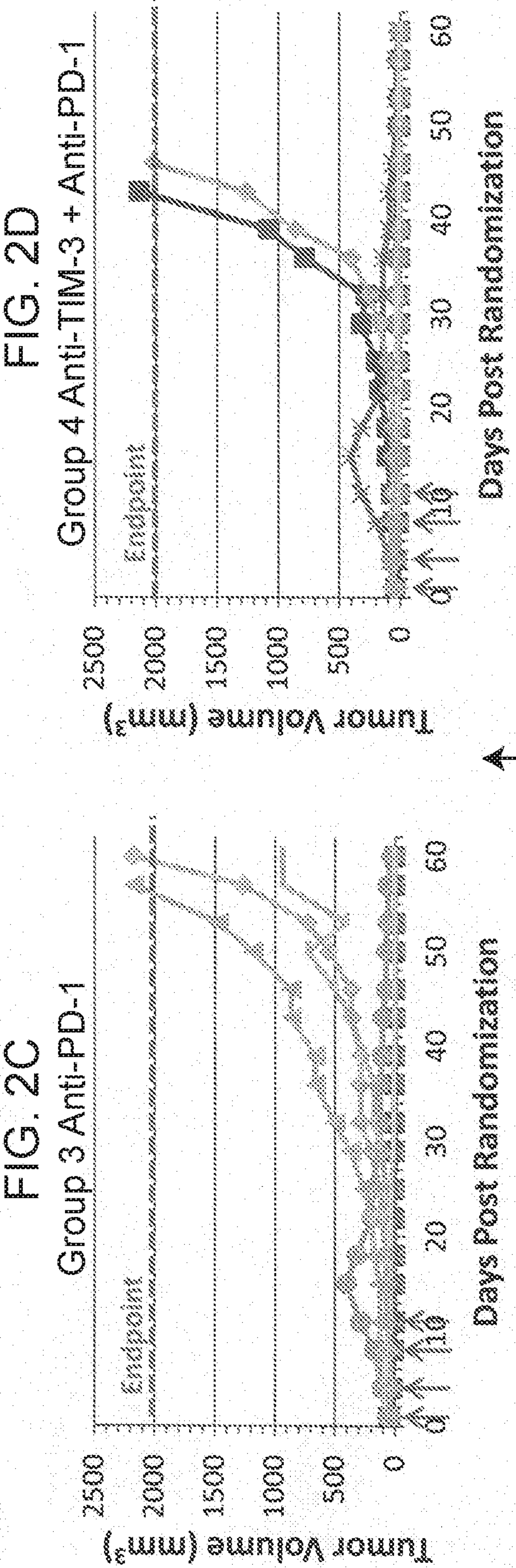
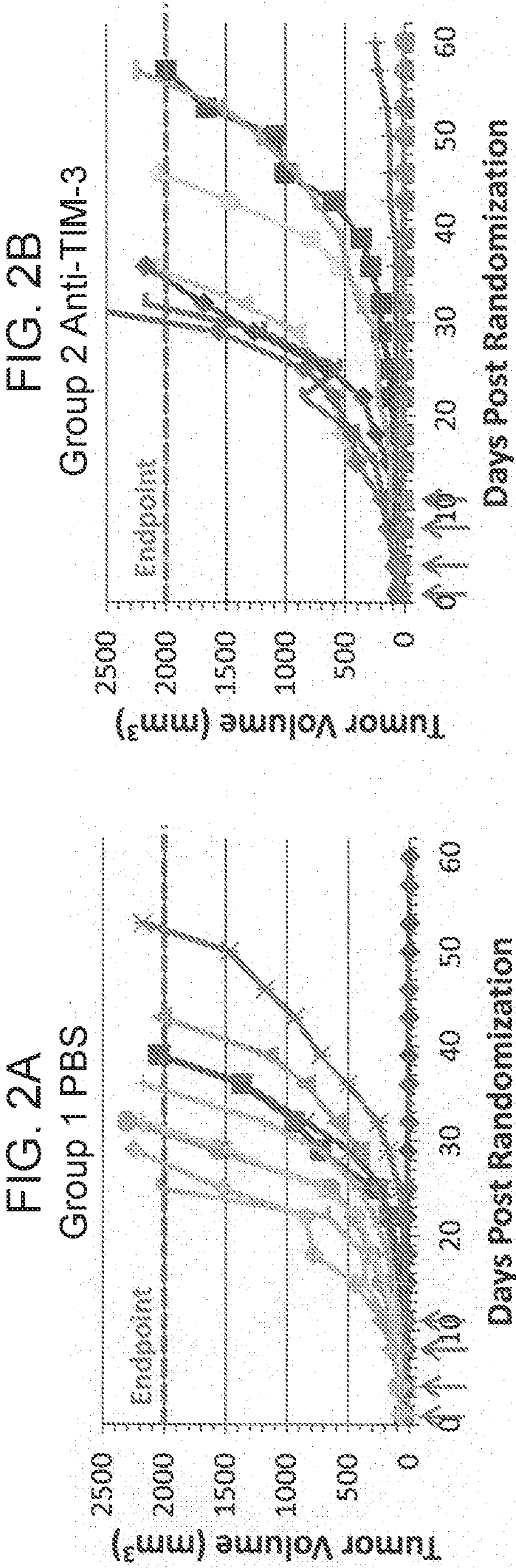


FIG. 1D





↑
Dosing days

FIG. 3

