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(54) Benævnelse: **Antistoffer, der er rettet mod T-celle-immunglobulin og mucin-protein 3 (TIM-3)**

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DESCRIPTION

Description

BACKGROUND OF THE INVENTION

[0001] 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)).

[0002] 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)).

[0003] 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)).

[0004] 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)).

[0005] WO 2013/006490 discloses antibodies that specifically bind to TIM3.

[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 anti-TIM-3 antibodies.

BRIEF SUMMARY OF THE INVENTION

[0007] In a first aspect, the present invention provides an anti-TIM-3 antibody, or an antigen-binding fragment thereof, comprising an immunoglobulin light chain polypeptide comprising SEQ ID NO: 115 and an immunoglobulin heavy chain polypeptide comprising SEQ ID NO: 34.

[0008] In a second aspect of the invention a pharmaceutical composition comprising the aforementioned anti-TIM-3 antibody, or antigen-binding fragment thereof, and a pharmaceutically acceptable carrier, is provided.

[0009] In addition, the invention provides isolated or purified nucleic acid sequences encoding the foregoing immunoglobulin polypeptides, vectors comprising such nucleic acid sequences, isolated cells comprising such vectors, and a method for producing an anti-TIM-3 antibody comprising the foregoing immunoglobulin polypeptides.

[0010] Further embodiments of the invention are described below or are defined in the subclaims.

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

[0011]

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.

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.

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

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.

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.

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.

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.

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.

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

[0012] The invention provides an anti-TIM-3 antibody, or an antigen-binding fragment thereof, comprising an immunoglobulin heavy chain polypeptide comprising SEQ ID NO: 34 and an immunoglobulin light chain polypeptide comprising SEQ ID NO: 115.

[0013] 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.

[0014] 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)).

[0015] 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.

[0016] 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)).

[0017] 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, Abeam (Cambridge, MA), and R&D Systems, Inc. (Minneapolis, MN).

[0018] 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: Probalistic 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)).

[0019] 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.

[0020] 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).

[0021] 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.

[0022] 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.

[0023] 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).*

[0024] 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 $-NH_2$ can be maintained.

[0025] "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.

[0026] 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.

[0027] 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)).

[0028] 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.

[0029] 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., IC_{50}), 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.

[0030] 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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.

[0031] 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 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.

[0032] In embodiments where the anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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.

[0033] 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 regionthat 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, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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)). 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 etal., 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.

[0040] In one embodiment, 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 antibody, or an antigen-binding fragment thereof, using either protein chemistry or recombinant DNA technology.

[0041] In a preferred embodiment, the anti-TIM-3antibody, or an antigen-binding fragment thereof, 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.

[0042] 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 anti-TIM-3antibody, or an antigen-binding fragment thereof.

[0043] 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).

[0044] 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof. 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)).

[0045] In addition to the nucleic acid sequence encoding the inventive immunoglobulin heavy polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0046] 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 Mal. Biol., 308: 123-144 (2005)).

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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., pcDNA™5/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) FLEXI™ from Promega (Madison, WI).

[0051] 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).

[0052] 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.

[0053] 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.

[0054] 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), DH5α, DH10, MC1061 (ATCC No. 53338), and CC102).

[0055] 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*, *Rhino-sporidium*, *Saccharomyces*, and *Schizosaccharomyces.* Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris.*

[0056] 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 HIS (Invitrogen, Carlsbad, CA).

[0057] 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.

[0058] 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.

[0059] 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.

[0060] The invention provides a pharmaceutical composition comprising the anti-TIM-3 antibody, or an antigen-binding fragment thereof, and a pharmaceutically acceptable (e.g., physiologically acceptable) carrier. 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).

[0061] The invention further provides the anti-TIM-3 antibody, or an antigen-binding fragment thereof, or the pharmaceutical composition for use in treating a disorder in a mammal that is responsive to TIM-3 inhibition or neutralization. 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof of the invention 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof of the invention 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof of the invention 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)).

[0062] Administration of a composition comprising the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0063] 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. 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 is an amount which decreases TIM-3 bioactivity in a human.

[0064] Alternatively, the pharmacologic and/or physiologic effect may be prophylactic, i.e., the effect completely or partially prevents a disease or symptom thereof. 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).

[0065] 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.

[0066] The composition comprising the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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.

[0067] Once administered to a mammal (e.g., a human), the biological activity of the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody. In one embodiment of the invention, the anti-TIM-3 antibody, or an antigen-binding fragment thereof,
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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0068] The stability of the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0069] The stability of the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, comprises a transition mid-point value (T_m) *in vitro* of about 60-100 °C. For example, the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0070] 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 (μM) (e.g., from about 1 picomolar (pM) to about 1 nanomolar (nM), from about 1 nM to about 1 micromolar (μM), or from about 1 μM to about 100 μM). In one embodiment, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0071] The anti-TIM-3 antibody, or an antigen-binding fragment thereof, of the invention may be administered alone or in combination with other drugs. For example, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, of the invention is for use in treating an infectious disease, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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).

[0072] In another embodiment, when the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, is used to treat cancer or an infectious disease, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, can be administered in combination with other agents that inhibit immune checkpoint pathways. For example, the inventive anti-TIM-3 antibody, or an antigen binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 invention for use in 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.

[0073] In addition to therapeutic uses, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, described herein can be used
in diagnostic or research applications. In this respect, the anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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., ^{3}H, ^{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)).

[0074] TIM-3 protein levels can be measured using the inventive anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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.

[0075] The anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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 anti-TIM-3 antibody, or an antigen-binding fragment thereof, 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.

[0076] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0077] This example demonstrates a method of identifying antibodies directed against human TIM-3 from an evolvable library and affinity maturation of the identified antibodies.

[0078] 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.

[0079] 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.

[0080] 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, and331, 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.

[0081] 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)	NonSpecific 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%

[0082] The results of this example confirm a method of affinity maturing monoclonal antibodies directed against TIM-3 identified using an evolvable library.

EXAMPLE 2

[0083] 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.

[0084] 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.

[0085] The results of this example demonstrate that the inventive anti-TIM-3 antibody can inhibit TIM-3 biological activity alone and in combination with antagonists of other negative regulators of the immune system.

EXAMPLE 3

[0086] This example demonstrates that an anti-TIM-3 antibody antagonizes TIM-3 activity in a syngeneic mouse tumor model.

[0087] 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 C57B1/6 mice and grown for 10 days. Mice with tumors measuring 40-90 mm^3 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 mm^3, 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

[0088] 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.

[0089] 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 C57B1/6 mice and grown for 8 days. Mice with tumors measuring 40-80 mm^3 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 and2).

Tumor volumes were measured twice weekly until reaching 2000 mm^3 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

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

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Patentkrav

1. Anti-TIM-3-antistof eller et antigen-bindende fragment deraf, omfattende et immunoglobulin-letkæde-polypeptid omfattende SEQ ID NO: 115 og et immunoglobulin-tungkæde-polypeptid omfattende SEQ ID NO: 34.

2. Antistof eller et antigenbindende fragment deraf ifølge krav 1, yderligere omfattende en konstant IgG1-, IgG2- eller IgG4-region.

3. Antistof eller et antigenbindende fragment deraf ifølge krav 1, yderligere omfattende en konstant IgG4-region.

4. Farmaceutisk sammensætning omfattende antistoffet eller det antigenbindende fragment deraf ifølge et hvilket som helst af kravene 1-3 og en farmaceutisk acceptabel bærer.

5. Antistof eller antigenbindende fragment deraf ifølge et hvilket som helst af kravene 1-3 eller den farmaceutiske sammensætning ifølge krav 4 til anvendelse ved behandling af en cancer i et menneske.

6. Antistof eller antigenbindende fragment deraf ifølge et hvilket som helst af kravene 1-3 eller den farmaceutiske sammensætning ifølge krav 4 til anvendelse ved behandling af en infektionssygdom hos et menneske.

7. Antistof eller antigenbindende fragment deraf ifølge et hvilket som helst af kravene 1-3 eller den farmaceutiske sammensætning ifølge krav 4, til anvendelse til at inducere et immunrespons mod en cancer eller en infektionssygdom eller inducere aktiviteten af en immuneffektorcelle.

8. Antistof eller antigenbindende fragment deraf eller farmaceutisk sammensætning til anvendelse ifølge krav 5 eller krav 7, hvor canceren er melanom, nyrecellecarcinom, lungecancer, blærecancer, brystcancer, livmoderhalscancer, tyktarmscancer, galdeblærecancer, lstrubecancer, levercancer, skjoldbruskkirtelcancer, mavecancer, spytkirtelcancer, prostatacancer, bugspytkirtelcancer eller Merkelcellecancer.

9. Antistof eller antigenbindende fragment deraf til anvendelse ifølge krav 8, hvor canceren er lungecancer.

10. Antistof eller antigenbindende fragment deraf eller farmaceutisk sammensætning til anvendelse ifølge krav 6 eller krav 7, hvor infektionssygdommen er forårsaget af en virus eller en bakterie.

11. Antistof eller antigenbindende fragment deraf eller farmaceutisk sammensætning til anvendelse ifølge krav 10, hvor infektionssygdommen er forårsaget af humant immundefektvirus (HIV), respiratorisk syncytium-virus (RSV), influenzavirus, denguevirus eller hepatitis B virus (HBV).

12. Antistof eller antigenbindende fragment deraf ifølge et hvilket som helst af kravene 1-3, den farmaceutiske sammensætning ifølge krav 4, eller antistoffet eller antigenbindende fragment deraf eller farmaceutisk sammensætning, til anvendelse ifølge et hvilket som helst af kravene 5-11, hvor antistoffet eller det antigenbindende fragment deraf eller det farmaceutiske præparat skal anvendes i kombination med et PD-1-bindende middel.

13. Antistof eller antigenbindende fragment deraf ifølge et hvilket som helst af kravene 1-3, den farmaceutiske sammensætning ifølge krav 4, eller antistoffet eller antigenbindende fragment deraf eller farmaceutisk sammensætning, til anvendelse ifølge et hvilket som helst af kravene 5-11, hvor antistoffet eller det antigenbindende fragment deraf eller det farmaceutiske præparat skal anvendes i kombination med et LAG-3-bindende middel.

14. Isoleret nukleinsyre, der koder for immunoglobulin-letkæde-polypeptidet og immunoglobulin-tungkæde-polypeptidet som defineret i et hvilket som helst af kravene 1-3.

15. Vektor omfattende den isolerede nukleinsyre ifølge krav 14.

16. Isoleret celle omfattende vektoren ifølge krav 15.

17. Isoleret celle omfattende en vektor, der koder immunoglobulin-tungkæde-polypeptidet som defineret i et hvilket som helst af kravene 1-3 og en vektor, der koder for immunoglobulin-letkæde-polypeptidet som defineret i et hvilket som helst af kravene 1-3.

18. Isoleret celle ifølge krav 16 eller krav 17, hvor cellen er en pattedyrscelle.

19. Fremgangsmåde til fremstilling af et anti-TIM-3-antistof, hvilken fremgangsmåde omfatter at udtrykke antistoffet *in vitro* i en værtscelle, der er i stand til at udtrykke antistoffet, og hvor værtscellen omfatter vektoren ifølge krav 15, der koder for antistoffet (*in cis*), eller omfatter en vektor, der koder for antistof- immunoglobulin-tungkæde-polypeptidet som defineret i et hvilket som helst af kravene 1-3 og en vektor, der koder for antistof- immunoglobulin-letkæde-polypeptidet som defineret i et hvilket som helst af kravene 1-3 (*in trans*).

DRAWINGS

Drawing

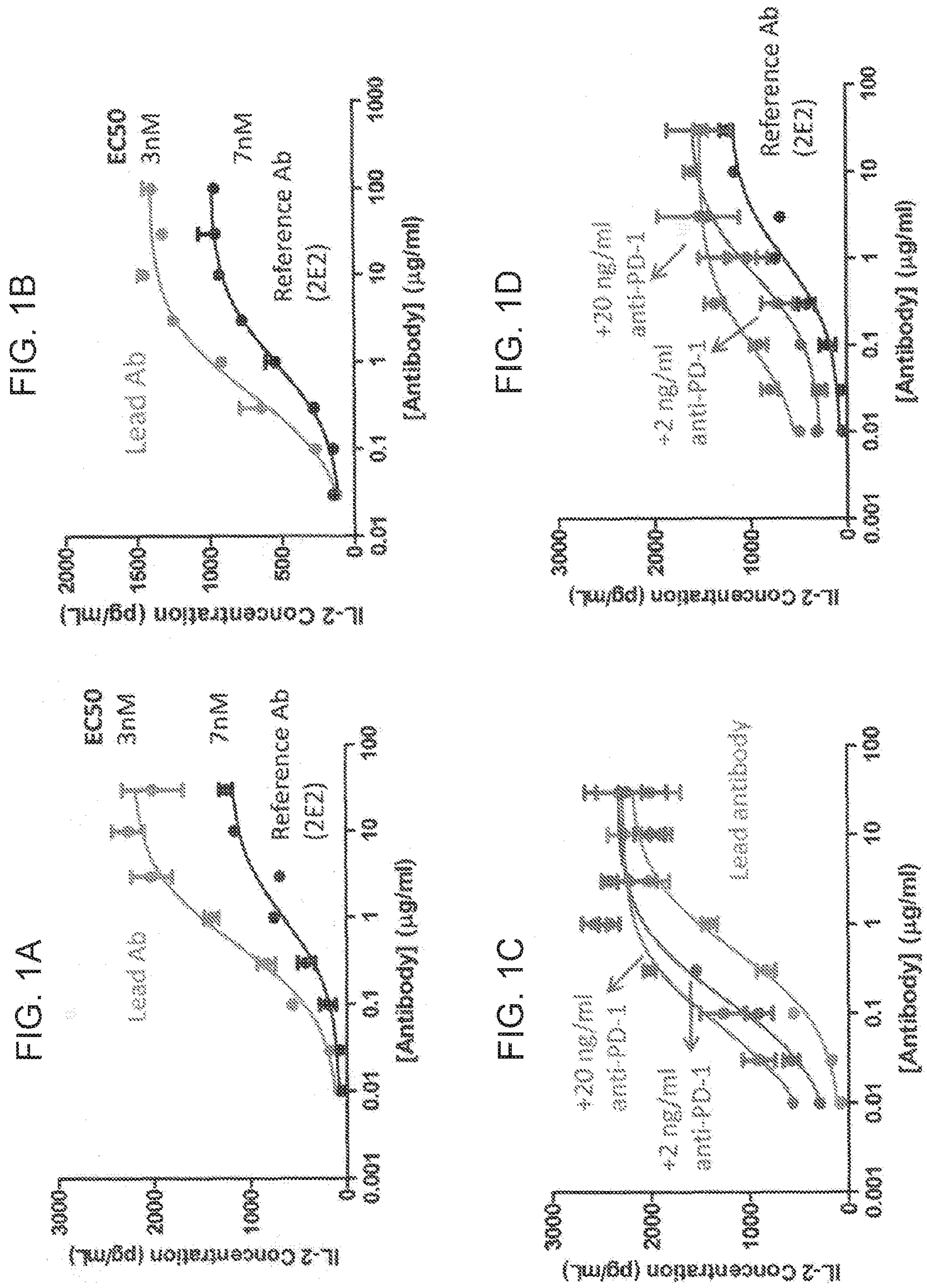


FIG. 2A
Group 1 PBS

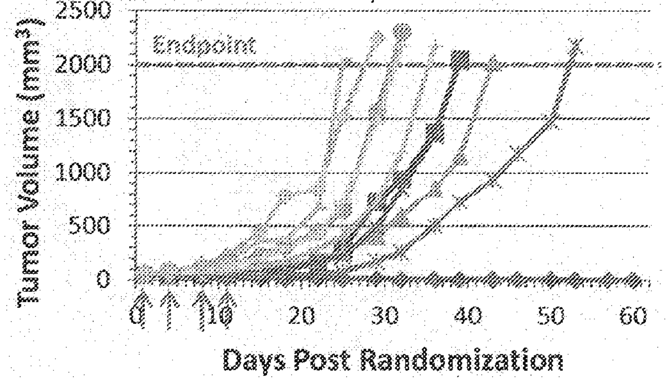


FIG. 2B
Group 2 Anti-TIM-3

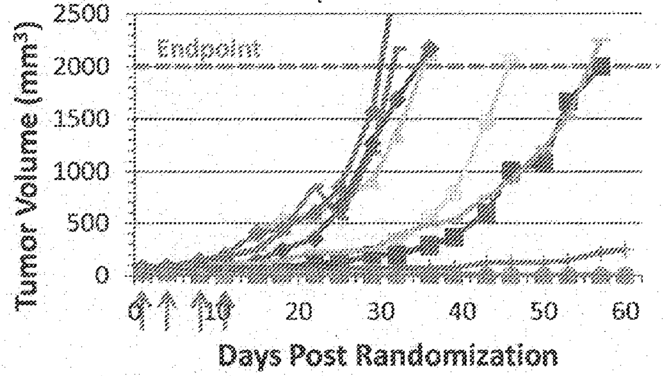


FIG. 2C
Group 3 Anti-PD-1

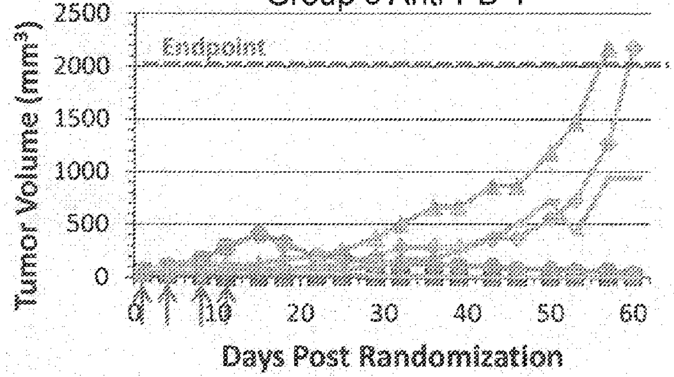
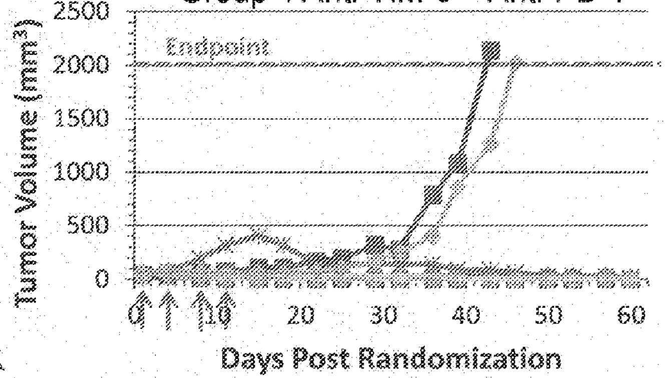
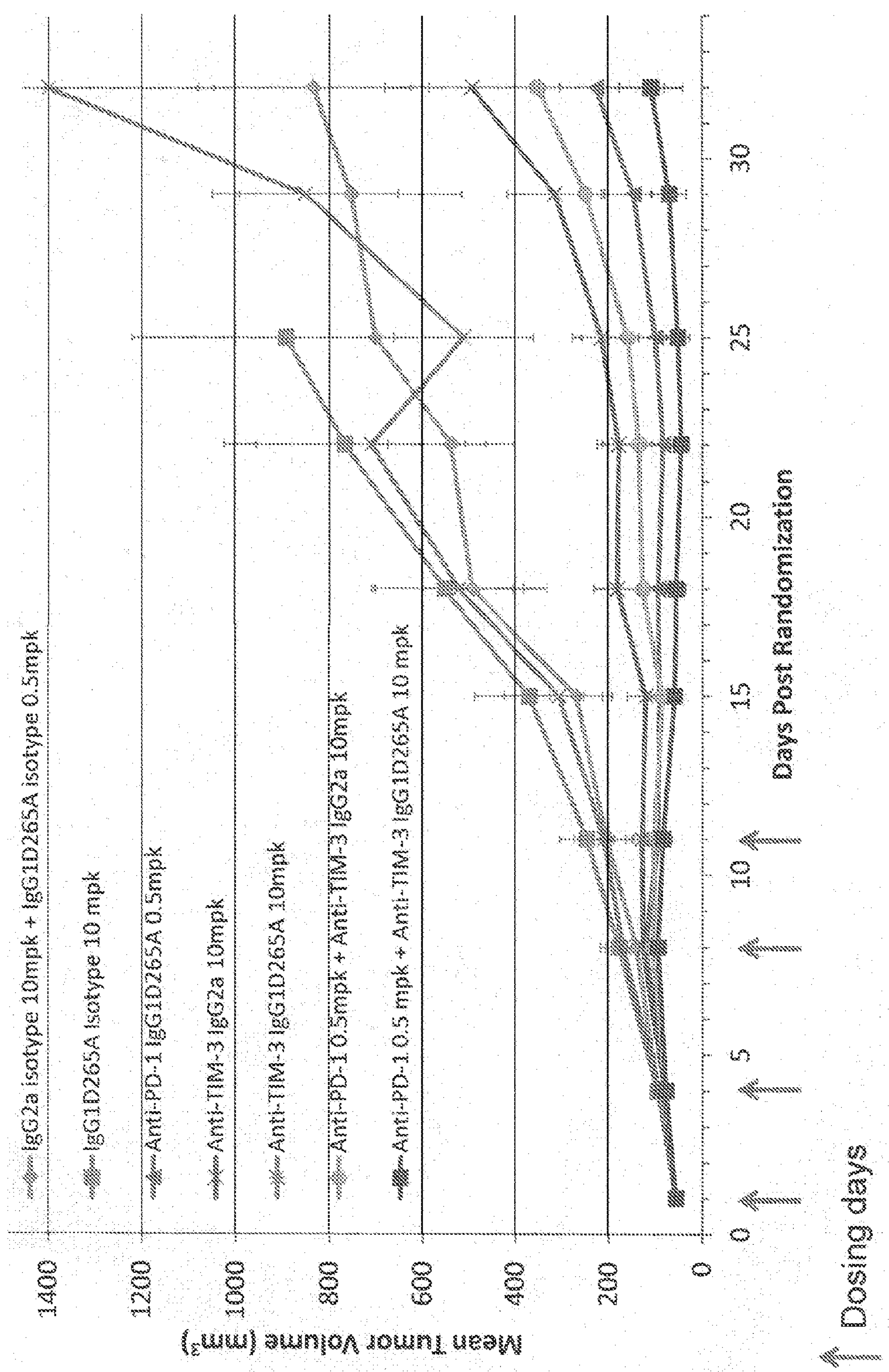


FIG. 2D
Group 4 Anti-TIM-3 + Anti-PD-1



Dosing days

FIG. 3


IgG2a isotype 10mpk + IgG1D265A isotype 0.5mpk
IgG1D265A isotype 10 mpk
Anti-PD-1 IgG1D265A 0.5mpk
Anti-TIM-3 IgG2a 10mpk
Anti-TIM-3 IgG1D265A 10mpk
Anti-PD-1 0.5mpk + Anti-TIM-3 IgG2a 10mpk
Anti-PD-1 0.5 mpk + Anti-TIM-3 IgG1D265A 10 mpk
Mean Tumor Volume (mm³)
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Days Post Randomization
Dosing days

SEKVENSLISTE

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