HUMAN ANTI-CD27 ANTIBODIES, METHODS, AND USES

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to human antibodies to the CD27 protein and their uses and, more particularly, human antibodies to human CD27 protein and their use in treating inflammatory disorders.

Related Art

CD27 is a type I transmembrane protein and member of the TNF receptor superfamily (TNFSF27) expressed as a surface antigen on a majority of T cells, natural killer cells and antibody secreting plasma and memory B-cells. CD70 is a cytokine, also called tumor necrosis factor ligand superfamily member 7 (TNFSF7), and the cognate ligand for CD27. TNFSF ligand-receptor interactions are able to regulate T-dependent B-cell differentiation (Jacquot S. 2000 Immunol Res. 21(1):23-30) and induce apoptotic cell death in different cells.

CD27:CD70 ligation results in activation of canonical and non-canonical NF-kβ signaling pathways that in turn stimulates B- and T-cell proliferation, plasma cell differentiation and subsequent antibody secretion (Yamamoto, H. 1998 J Immunol. 161(9): 4753-9). CD27 co-stimulation with OX40, 4-1BB also promotes the survival of activated T cells (Croft, M. 2003 Cytokine Growth Factor Rev. 14(3-4): 265-73), thereby regulating a number of effector and memory T cells and controls T cell function directly by promoting production of cytokines, such as IL-4 and IFNgamma, or modulating T-cell responses to the actions of other cytokines, such as IL2 and IL-12.

Studies in both humans and animals suggest an important role of the CD27:CD70 pathway in various immune-related diseases, including systemic lupus erythematosus (SLE) (Doerner T Lupus 2004 13(5):283-9), rheumatoid arthritis (Tak, PP et al. 1996 clin Immunol Immunopathol 80(2): 129-38) and multiple sclerosis (Hintzen RQ et al.1991 J Neuroimmunol 35(1-3):211-7). On the other hand, CD70 has been reported to be expressed to varying degrees on malignant B cells and the

CD70:CD27 complex is able to mediate an antitumor response by activating antitumor immunity and reducing tumor growth (Borst J, Hendriks J and Xiao Y. 2005. Curr Opin Immunol. 17(3):275-81). CD27 may also control the accumulation of CD4+ and CD8+ T-cells at sites of infection (Hendricks et al. 2000 Nature Immunol 1, 433 - 440).

CD70 is not expressed on normal non-hematopoietic cells. CD70 expression appears to be temporally restricted to antigen-activated T- and B cells and its expression is down-regulated when antigenic stimulation ceases. Evidence from animal models suggests that CD70 may contribute to immunological disorders such as, e.g., rheumatoid arthritis (Brugnoni et al., 1997 Immunol. Lett. 55:99-104), psoriatic arthritis (Brugnoni et al., 1997, Immunol. Lett. 55:99-104), and lupus (Oelke et al., 2004, Arthritis Rheum. 50:1850-60). In addition to its potential role in inflammatory responses, CD70 is also expressed on a variety of transformed cells including lymphoma B cells, Hodgkin's and Reed-Sternberg cells, malignant cells of neural origin, and a number of carcinomas.

Agonist CD27 binding antibodies described in W02008/051424 (Univ. South Hampton) are noted as useful for promoting T-cell immunity and such antibodies have a binding epitope which causes them to be unaffected (not inhibited) by CD70.

While studies in rodents involving alteration of CD27 and/or CD70 have demonstrated potentially important roles of this receptor ligand interaction, there is a need to provide human antibodies specific for human CD27 and other CD27:CD70 interaction blocking agents that can exert a clinically useful cytotoxic, cytostatic, or immunomodulatory effect on CD27-expressing cells, particularly without exerting undesirable agonist effects on CD27-expressing cells in the absence of CD70. Such compounds may be useful therapeutic agents in modulating the development of neoplastic cells or immune disorders that are mediated by CD27-expressing cells.

Summary of the Invention

The present invention provides human CD27 binding, monoclonal antibodies capable of blocking activities associated with CD27-CD70 interaction on cells, tissues, or organs in a host subject. Amino acid sequences of exemplary CD27 binding monoclonal antibodies are provided which are encoded by nucleic acids for expression

in a host cell. In addition, the CD27 monoclonal antibodies of the invention define at least three non-overlapping epitopes on the extracellular domain of CD27 which when engaged by an antibody of the invention, are prevented from CD70-type ligand ligation driven signaling and downstream biological activity.

Another aspect of the invention is an isolated anti-CD27 antibody reactive with a CD27 protein epitope defined by residues between positions 21-191 of the CD27 protein.

Another aspect of the invention is an isolated antibody having a heavy chain variable region sequence selected from the sequences shown in SEQ ID NOs: 76, 78, 80, 102-126, 128-136, and 145-147, and a light chain variable region sequence selected from the sequences shown in SEQ ID NOs: 77, 79, 81-101, 127, 137-144, and 148, including variants of those sequences, e.g., conservative substitutions.

A further aspect of the invention is an isolated antibody having heavy and light chain CDR sequences selected from the sequences shown in SEQ ID NOs: 1-75 and 151-158, including variants of those sequences, e.g., conservative substitutions.

Another aspect of the invention is an isolated polynucleotide encoding an antibody of the invention.

In another aspect, the invention relates to an antibody which binds to a common epitope defined by the region on the protein to which antibodies C2177 and/or C2186 or human antibodies generated therefrom described in Tables 30-39 bind or which compete for binding to the CD27 protein with antibodies C2177 and/or C2186 or human antibodies generated therefrom described in Tables 30-39. In another embodiment, the invention relates to an antibody which binds to an epitope of the extracellular domain of CD27 defined by the region on the protein to which antibody C2191 bind or human antibodies generated therefrom described in Tables 30-39 and competes for binding to the CD27 protein with antibody C2191 or human antibodies generated therefrom described in Tables 30-39. In another embodiment, invention relates to an antibody which binds to an epitope of the extracellular domain of CD27 defined by the region on the protein to which antibody C2192 binds or human antibodies generated therefrom described in Tables 30-39 and competes for binding to

the CD27 protein with antibody C2192 or human antibodies generated therefrom described in Tables 30-39. In another aspect, the invention comprises an antibody or fragment thereof derived from one or more of antibodies C2177, C2186, C2191, and C2192 or human antibodies generated therefrom described in Tables 30-39 having other functional binding characteristics exhibited by one or more of antibodies C2177, C2186, C2191, and C2192, or human antibodies generated therefrom described in Tables 30-39, such as inhibiting the binding of CD27 to CD70 positive cells.

Thus, one aspect of the invention relates to an engineered antibody comprising an engineered (e.g., humanized or human adapted) heavy chain and light chain, wherein:

(1) the engineered heavy chain variable region comprises or is derived from one or more complementarity determining regions (CDRS) from the mouse antibodies C2177, C2191, C2192 and C2186 heavy chain and a framework from a human acceptor antibody heavy chain, optionally having one or more human framework residue substitutions, and

(2) the engineered light chain variable region comprises one or more complementarity determining regions from the mouse antibodies C2177, C2191, C2192 and C2186 light chain and a framework from a human acceptor antibody light chain optionally having one or more human framework residue substitutions; and

(3) the engineered antibody specifically binds to human CD27 and interferes with its interaction with CD70.

In a further embodiment, the engineered antibody may be composed of one or more CDRs that are further engineered with one or more substitutions or deletions, for example, those that are 90%, 95%, 98% or 99.5% identical to one or more CDRs of antibodies C2177, C2191, C2192 and/or C2186.

Another embodiment relates to the treatment or prevention of pathological conditions associated with CD27 bioactivity by administering a therapeutically or prophylactically effective amount of an antibody of the present invention, portion thereof or a mixture of antibodies of the present invention or portions thereof to a subject in need of such treatment.

In a further embodiment, the invention comprises antigen epitopes as a component of a vaccine. The polypeptides or polynucleotides encoding the polypeptide epitopes described above comprising subfragments or three-dimensional analogs of some or all of SEQ ID NO: 1 residues 21-191, or conservative changes thereof, are recognized by the antibodies of the invention. The polypeptides and polynucleotides are useful for actively immunizing a host to elicit production of antibodies against CD27 capable of the combating or preventing pathological conditions associated with CD27 bioactivity.

The invention also relates to methods of generating, purifying, formulating, and packaging an antibody of the invention for use in the treatment or prevention of pathological conditions associated with CD27 bioactivity by administering a therapeutically or prophylactically effective amount of an antibody or portion thereof.

Brief Description Of The Drawings

Fig. 1 is a graph showing the effects of the C2177 antibody on T-cell proliferation.

Fig. 2 shows the crystal structure of CD27:C2177:C2191 ternary complex. N- and C-termini of CD27 fragment are labeled. Heavy chains of Fabs are darker than their light chains.

Fig. 3 is a diagram of CD27 protein–C2177 antibody contacts with CD27 protein residues (epitope) in circles and C2177 antibody residues (paratope) in boxes.

Fig. 4 is a diagram of CD27 protein–C2191 antibody contacts with CD27 protein residues (epitope) are in circles and C2191 antibody residues (paratope) in boxes.

Detailed Description

Abbreviations

CDR – complementarity determining region; CFSE - carboxyfluorescein diacetate, succinimidyl ester; ECD – extracellular domain; FR – framework; H - heavy chain; GvHD graft-versus-host disease; L – light chain; IFN – interferon (g, gamma); Ig – immunoglobulin; Mab - monoclonal antibody; MMP - matrix metalloproteinase; PBMC – peripheral blood mononuclear cells; VL – Variable light chain; VH – Variable heavy chain

Definitions

As used herein, an "antibody" includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus, the antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to, at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein, which can be incorporated into an antibody of the present invention. The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or a specified fragment or portion thereof, including single chain and single domain antibodies and fragments thereof. Functional fragments include antigen-binding fragments to a preselected target. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH, domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH, domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment,

VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (I 988) Science 242:423-426, and Huston et al. (1988) Proc. Natl. Acad Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Conversely, libraries of scFv constructs can be used to screen for antigen binding capability and then, using conventional techniques, spliced to other DNA encoding human germline gene sequences. One example of such a library is the “HuCAL: Human Combinatorial Antibody Library” (Knappik, A. et al. J Mol Biol (2000) 296(1):57-86).

The term “CDR” refers to the complementarity determining region or hypervariable region amino acid residues of an antibody that participate in or are responsible for antigen-binding. The hypervariable region or CDRs of the human IgG subtype of antibody comprise amino acid residues from residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat et al. (1991 Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) and/or those residues from a hypervariable loop (i.e., residues 26-32 (L1) , 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) or the current H2 Chothia definition of 52-57, and 96-101 (H3) in the heavy chain variable domain as described by (Chothia et al., J. Mol. Biol. 196: 901-917 (1987)).

Framework or FR1-4 residues are those variable domain residues other than and bracketing the hypervariable regions. More recently, a universal numbering system has been developed and widely adopted, international ImMunoGeneTics information system® (IMGT) (LaFranc, et al. 2005. Nucl Acids Res. 33:D593–D597).

Herein, the CDRs are referred to in terms of both the amino acid sequence and the location within the light or heavy chain by sequential numbering. As the "location" of the CDRs within the structure of the immunoglobulin variable domain is conserved between species and present in structures called loops, by using numbering systems that align variable domain sequences according to structural features, CDR and framework residues and are readily identified. This information is used in grafting and replacement of CDR residues from immunoglobulins of one species into an acceptor framework from, typically, a human antibody.

The term “CD27” refers to the human TNF receptor superfamily (TNFSF27), the product of the human gene 939 (CD27 gene), also called human CD27L receptor, MGC20393, S152, T14, T-cell activation antigen CD27 and include all of the variants, isoforms and species homologs of CD27. The expressed human CD27 (NCBI Accession No. NP_001233) is a polypeptide of 260 amino acids in length having a 20 amino acid secretion signal at the N-terminus. Accordingly, the antibodies of the invention may, in certain cases, cross-react with CD27 from species other than human. In other cases, the antibodies may be completely specific for human CD27 and not exhibit species or other types of cross-reactivity. By CD27 biological activities is meant, any downstream activities resulting from CD27 receptor binding and/or activation as a result of activation of CD27 by one or more ligands, especially CD70 polypeptides (TNFSF7, NP_ 001243), or other ligands, such as SIVA. CD27 transduces signals that lead to the activation of NF-κ--β and MAPK8/JNK. Adaptor proteins TRAF2 and TRAF5 have been shown to mediate the signaling process of this receptor. CD27 biologic activities may also result from the binding of certain truncated forms of CD27 or fragments of CD27 to ligands which themselves exhibit biologic activities, for example, a polypeptide which comprises from about residues 21-191 of the full-length protein can bind to CD70. The CD27 antigen cytoplasmic tail, residues 213-260, binds to the N-terminus of the SIVA protein (also known as the apoptosis-inducing factor: CD27BP; SIVA1, Siva-1, NP_006418 (175 aa)); and Siva-2, SIVA2, NP_068355 (110 aa).

The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of

molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

"Humanization" (also called Reshaping or CDR-grafting) or "engineering" includes established techniques for reducing the immunogenicity of monoclonal antibodies (mAbs) from xenogeneic sources (commonly rodent) and for improving affinity or the effector functions (ADCC, complement activation, C1q binding). The engineered mAb can be produced using the techniques of molecular biology, using phage displayed randomized sequences, or synthesized de novo. For example, in order to construct a humanized antibody with incorporated CDR regions from a nonhuman species, the design might include variations, such as conservative amino acid substitutions in residues of the CDRs, and back substitution of residues from the nonhuman mAb into the human framework regions (backmutations). The positions can be discerned or identified by sequence comparison methods, consensus sequence analysis, or structural analysis of the variable regions' 3D structure. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way or by simple sequence alignment algorithms (e.g., Clustal W), FR (framework) residues can be selected from known antibody sequences, found in such publicly accessible databases as VBASE or Kabat, and the consensus sequences optimized so that the desired antibody characteristic, such as affinity for the target antigen(s), is achieved. As the datasets of known parameters for antibody structures increases, so does the sophistication and refinement of these techniques. Another approach to humanization is to modify only surface residues of the rodent sequence with the most common residues found in human mAbs and has been termed "resurfacing" or "veneering." A large number of both human and non-human Ig sequences are now known and freely available and used by those skilled in the art, e.g., the database and tools developed by

LeFranc et al found under the name IMGT; websites curated by the U.S. National Center for Biologics (NCBI); Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983) now also greatly expanded and available online, each entirely incorporated herein by reference. Humanization or engineering of antibodies of the present invention can be performed using any method known or those developed using human immunoglobulin sequence information. Such methods are taught in, for example, Winter U.S. Pat No. 6982361 and Bowdish et al. WO03/025019, the contents of which are incorporated herein by reference.

As used herein, K_D refers to the dissociation constant, specifically, the antibody K_D for a predetermined antigen, and is a measure of affinity of the antibody for a specific target. High affinity antibodies have a K_D of 10^{-8} M or less, more preferably 10^{-9} M or less and even more preferably 10^{-10} M or less, for a predetermined antigen. The reciprocal of K_D is K_A, the association constant. The term "k_{dis}" or "k_2," or "k_d" as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The "K_D" is the ratio of the rate of dissociation (k_2), also called the "off-rate (k_{off})" to the rate of association rate (k_1) or "on-rate (k_{on})." Thus, K_D equals k_2/k_1 or k_{off} / k_{on} and is expressed as a molar concentration (M). It follows that the smaller the K_D, the stronger the binding. Thus, a K_D of 10^{-6} M (or 1 microM) indicates weak binding compared to 10^{-9} M (or 1nM). These values may be calculated using surface plasmon resonance and/or the Kinexa method as known in the art.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. The term also includes "recombinant antibody" and "recombinant monoclonal antibody" as all antibodies are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal or a hybridoma prepared by the fusion of antibody secreting animal cells and an fusion partner, (b) antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human or other species antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of

immunoglobulin gene sequences to other DNA sequences. An "isolated antibody," as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities. An isolated antibody that specifically binds to an epitope, isoform or variant of human CD27 may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., CD27 species homologs). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. In one embodiment of the invention, a combination of "isolated" monoclonal antibodies having different specificities are combined in a well defined composition.

As used herein, "specific binding," “immunospecific binding” and “binds immunospecifically” refers to antibody binding to a predetermined antigen. Typically, the antibody binds with a dissociation constant (K_D) of 10^{-7} M or less, and binds to the predetermined antigen with a K_D that is at least twofold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein, or any other specified polypeptide) other than the predetermined antigen. The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen." As used herein “highly specific” binding means that the relative K_D of the antibody for the specific target epitope is at least 10-fold less than the K_D for binding that antibody to other ligands.

As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG) that is encoded by heavy chain constant region genes. Some antibody classes further encompass subclasses which are also encoded by the heavy chain constant regions and further decorated by oligosaccharides at specific residues within the constant region domains (e.g. IgG1, IgG2, IgG3 and IgG4) which further impart biological functions to the antibody. For example, in human antibody isotypes IgG1, IgG3 and to a lesser extent, IgG2 display effector functions as do murine IgG2a antibodies.

By “effector” functions or “effector positive” is meant that the antibody comprises domains distinct from the antigen specific binding domains capable of interacting with receptors or other blood components such as complement, leading to, for example, the recruitment of macrophages and events leading to destruction of cells

bound by the antigen binding domains of the antibody. Antibodies have several effector functions mediated by binding of effector molecules. For example, binding of the C1 component of complement to antibodies activates the complement system. Activation of complement is important in the opsonisation and lysis of cell pathogens. The activation of complement stimulates the inflammatory response and may also be involved in autoimmune hypersensitivity. Further, antibodies bind to cells via the Fc region, with a Fc receptor site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (eta receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody- coated particles, clearance of immune complexes, lysis of antibody- coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production.

1. Composition of an Antibody of the Invention

A CD27-neutralizing antibody of the invention is an antibody that inhibits, blocks, or interferes with at least one CD27 activity or CD70 binding, in vitro, in situ and/or in vivo and does not promote, stimulate, induce, or agonize CD27 activity or ligand binding nor does antibody binding mimic the downstream effects of CD27-ligand ligation, in particular CD70 interaction with CD27, such as signal transduction in a host cell. A suitable CD27-neutralizing antibody, specified portion, or variant can also, optionally, affect at least one CD27 activity or function, such as but not limited to, RNA, DNA or protein synthesis, protein release, T-cell activation, B-cell proliferation or differentiation, antibody secretion, CD27 receptor signaling, CD27 cleavage, CD27-ligand binding, CD27 or CD70 induction, synthesis or secretion.

In relation to the CD27:CD70 co-stimulatory pathway blocking activity of the CD27-neutralizing antibodies of the present invention, the treatment of autoimmune disorders with elevated T- or B-cell effector functions may be beneficial.

The present invention is based upon the discovery of anti-human CD27 monoclonal antibodies capable of inhibiting CD27 activation by CD70 and incapable of CD27 self-activation in the absence of CD70 stimulus. Hybridomas and transfectomas capable of secreting such an antibody were generated. An NF-kβ reporter gene assay was used to identify several candidate antibodies capable of inhibiting CD70-mediated NF-kβ reporter activation of CD27 expressing host cells. Second, the antibodies were characterized as being unable to induce dose-dependent agonistic activity when incubated with CD27 coupled luciferase reporter transfected cells in the absence of CD70 stimulus. Third, it was demonstrated that the antibodies dose-dependently inhibit CD70-dependent human naïve CD4 T-cell proliferation. Fourth, the CD27-neutralizing antibodies generated are capable of reducing CD70-mediated stimulation of plasma cell generation from human primary B-cells in a dose dependent manner. Fifth, no significant dose-dependent agonistic activity was observed in primary T- or B-cells with tested anti-CD27 antibodies.

The antibodies of the invention can interfere with CD27:CD70 ligation, inhibit both T-cell effector functions and B-cell differentiation to plasma cells in cell culture and thus may be beneficial for treatment of immune-mediated diseases including, but not limited to, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease, Crohn's Disease, chronic obstructive pulmonary disease or other syndrome, pathology, disease or disorder related to the aberrant functions or activation of CD27-expressing cell populations. The CD27-binding antibodies described herein recognize at least three distinct regions on the extracellular domain of human CD27, indicating the additional discovery of multiple sites on CD27 suitable for the targeting of antibodies or other compounds with similar function blocking capabilities. Thus, expression and purification of the antibody binding domains provided herein as amino acid sequences further provides a tool which can be the means for selection of novel molecules exhibiting CD27-neutralizing activity.

In one embodiment, the anti-human CD27 antibody, has a binding region comprising a light chain variable (VL) or heavy chain variable (VH) region having the amino acid sequence as shown in SEQ ID NO: 76-144 and which antibody or binding portion thereof immunospecifically binds CD27. In another embodiment of the

invention, the antibody or antigen binding portion thereof, binds to CD27 protein and, in addition, the antibodies possesses specified functional properties of antibodies of the invention, such as:

binding to immobilized human CD27;

inhibition of human soluble CD27 binding to cells expressing CD70;

inhibition of human CD70 mediated CD27 signaling measured by NF-kappaB reporter gene assay at an IC50 of less than 0.5 ug/ml;

inhibition of CD70-mediated proliferation of naïve T-cells;

inhibition of CD70 mediated plasma blast formation from primary human B-cells;

inhibition of human CD70-mediated soluble mediator release from T and B primary cells or cell lines;

binding to human CD27 with K_d of less than 100 nM (10^{-7} M);

minimal activation of CD27 signaling in the absence of CD70 stimulus; and

binding to an epitope on the human CD27 extracellular domain to which the Mabs having one or more of the variable region sequences of SEQ ID NOS: 76-144 bind and competes for binding with the Mabs identified having one or more of the variable region sequences of SEQ ID NOS: 76-144.

Since it is well known in the art that antibody heavy and light chains CDR domains play a particularly important role in the binding specificity/affinity of an antibody for an antigen, the recombinant antibodies of the invention disclosed herein preferably comprise one or more of the heavy and light chain CDRs of SEQ ID NOS: 1-75. Such antibodies can be prepared by chemically joining together the various portions (e.g., CDRs, framework) of the antibody using conventional techniques, by preparing and expressing a (i.e., one or more) nucleic acid molecule that encodes the antibody using conventional techniques of recombinant DNA technology or by using any other suitable method.

In one embodiment, the human antibodies of the invention have the sequence of one or more of the heavy and light chain CDRs of SEQ ID NOS: 1-75. In addition to

these CDR sequences, the ordinarily skilled artisan will appreciate that some deviation from the exact CDR sequences may be possible or desirable while still retaining the ability of the antibody to bind CD27 (e.g., conservative substitutions). Accordingly, in another embodiment, the human antibody may be composed of one or more CDRs that are, for example, 90%, 95%, 98% or 99.5% identical to the CDRs listed in SEQ ID NOs: 1-75.

In another embodiment, the epitope bound by the antibodies of the invention, comprising as few as five to all of residues 21-191 of CD27 protein or a nucleic acid coding sequence therefor, can be used to immunize a subject in order to produce the antibodies of the invention directly in the host for the purpose of treating, preventing, or ameliorating disease or symptoms of disease associated with the production of CD27.

2. Generation of CD27-neutralizing Antibodies

A CD27-neutralizing antibody exhibiting the desired bioactivity spectrum as exemplified herein by the disclosed and described antibodies, can be generated by a variety of techniques, including the standard somatic cell hybridization technique (hybridoma method) of Kohler and Milstein (1975) Nature 256:495. In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as described herein to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

A CD27-neutralizing antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human anti-CD27 antibody can be isolated from such animals and immortalized using suitable methods, such as the methods described herein. Alternatively, the antibody coding sequences may be cloned, introduced into a suitable vector, and used to transfect a host cell for expression

and isolation of the antibody by methods taught herein and those known in the art.

The use of transgenic mice carrying human immunoglobulin (Ig) loci in their germline configuration provides for the isolation of high affinity fully human monoclonal antibodies directed against a variety of targets including human self antigens for which the normal human immune system is tolerant (Lonberg, N. et al., US5569825, US6300129 and 1994, Nature 368:856-9; Green, L. et al., 1994, Nature Genet. 7:13-21; Green, L. & Jakobovits, 1998, Exp. Med. 188:483-95; Lonberg, N and Huszar, D., 1995, Int. Rev. Immunol. 13:65-93; Kucherlapati, et al. US6713610; Bruggemann, M. et al., 1991, Eur. J. Immunol. 21:1323- 1326; Fishwild, D. et al., 1996, Nat. Biotechnol. 14:845-851; Mendez, M. et al., 1997, Nat. Genet. 15:146-156; Green, L., 1999, J. Immunol. Methods 231:11-23; Yang, X. et al., 1999, Cancer Res. 59:1236-1243; Brüggemann, M. and Taussig, M J., Curr. Opin. Biotechnol. 8:455-458, 1997; Tomizuka et al. WO02043478). The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes. In addition, companies, such as Abgenix, Inc. (Freemont, Calif.) and Medarex (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology as described above.

In another embodiment, the human antibody is selected from a phage library, where that phage comprises human immunoglobulin genes and the library expresses human antibody binding domains as, for example, single chain antibodies (scFv), as Fabs, or some other construct exhibiting paired or unpaired antibody variable regions (Vaughan et lo al. Nature Biotechnology 14:309-314 (1996): Sheets et al. PITAS (USA) 95:6157-6162 (1998)); Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al. J. Mol. Biol., 222:581 (1991)). Human monoclonal antibodies of the invention can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such phage display methods for isolating human antibodies are established in the art. See for example: U.S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner et al.; U.S. Patent Nos. 5,427,908 and 5, 580,717 to Dower et al.; U.S. Patent Nos. 5,969,108 and 6,172,197 to McCafferty et al.; and U.S. Patent Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to

Griffiths et al.

Preparation of immunogenic antigens, and monoclonal antibody production can be performed using any suitable technique, such as recombinant protein production. The immunogenic antigens can be administered to an animal in the form of purified protein, or protein mixtures including whole cells or cell or tissue extracts, or the antigen can be formed de novo in the animal's body from nucleic acids encoding said antigen or a portion thereof.

The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art. DNA encoding the monoclonal antibodies is readily isolated and sequenced using methods known in the art (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Where a hybridoma is produced, such cells can serve as a source of such DNA. Alternatively, using display techniques wherein the coding sequence and the translation product are linked, such as phage or ribosomal display libraries, the selection of the binder and the nucleic acid is simplified. After phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria.

Humanized Antibodies

The invention further provides humanized (engineered or human adapted) immunoglobulins (or antibodies) which bind human CD27. The humanized forms of immunoglobulins have variable framework region(s) substantially from a human immunoglobulin (termed an acceptor immunoglobulin) and CDRs substantially from a non-human Mab which specifically binds CD27. The constant region(s), if present, are also substantially from a human immunoglobulin. The humanized antibodies exhibit K_D for CD27 of at least about 10^{-6} M (1 microM), about 10^{-7} M (100 nM), or less. The binding affinity of the humanized antibodies may be greater or less than that of the mouse antibody from which they were derived. To affect a change in affinity, e.g.,

improve affinity, of the humanized antibody for CD27 substitutions in either the CDR residues or the human residues may be made.

The substitution of mouse CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework adopts the same or similar conformation to the mouse variable framework from which the CDRs originated. This is achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies, be derived from human germline immunoglobulin sequences, or can be consensus sequences of several human antibody and/or germline sequences.

Suitable human antibody sequences are identified by computer comparisons of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies. The comparison is performed separately for heavy and light chains but the principles are similar for each.

In one example, the amino acid sequence of a CD27-neutralizing mAb is used to query a human antibody database compiled from public antibody sequence databases. The heavy chain variable regions disclosed or described herein can be used to find the human variable region with the highest sequence identity. The variable region of the light chain disclosed or described herein can, similarly, be used to find the human variable region with the highest sequence identity. A DNA construct in which the regions coding for the CDRs of one of the heavy chain variable regions from the murine Mab donor are transferred into the selected human heavy chain variable sequence, replacing the CDRs of the human variable region is prepared for each murine variable region.

The unnatural juxtaposition of murine CDR regions with human variable framework region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, lead to loss of binding affinity.

As noted supra, the humanized antibodies of the invention comprise variable framework region(s) substantially from a human immunoglobulin and CDRs substantially from a mouse immunoglobulin (e.g., C2177, C2186, C2191, or C2192 mouse antibodies). Having identified the CDRs of mouse antibodies and appropriate human acceptor immunoglobulin sequences, the next step is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a HAMA response in humans. Amino acids are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences can be done by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids. With regard to the empirical method, it has been found to be particularly convenient to create a library of variant sequences that can be screened for the desired activity, binding affinity or specificity. One format for creation of such a library of variants is a phage display vector. Alternatively, variants can be generated using other methods for varigation of a nucleic acid sequence encoding the targeted residues within the variable domain.

Another method of determining whether further substitutions are required, and the selection of amino acid residues for substitution, can be accomplished using computer modeling. Computer hardware and software for producing three-dimensional images of immunoglobulin molecules are widely available. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by

energy minimization and by verifying that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

Usually the CDR regions in humanized antibodies are substantially identical, and more usually, identical to the corresponding CDR regions in the mouse antibody from which they were derived. Although not usually desirable, it is sometimes possible to make one or more conservative amino acid substitutions of CDR residues without appreciably affecting the binding affinity of the resulting humanized immunoglobulin. Occasionally, substitutions of CDR regions can enhance binding affinity.

Other than for the specific amino acid substitutions discussed above, the framework regions of humanized immunoglobulins are usually substantially identical, and, more usually, identical to the framework regions of the human antibodies from which they were derived. Of course, many of the amino acids in the framework region make little or no direct contribution to the specificity or affinity of an antibody. Thus, many individual conservative substitutions of framework residues can be tolerated without appreciable change of the specificity or affinity of the resulting humanized immunoglobulin.

Because of the degeneracy of the code, a variety of nucleic acid sequences will encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by de nova solid-phase DNA synthesis or by PCR mutagenesis of an earlier prepared variant of the desired polynucleotide. All nucleic acids encoding the antibodies described in this application are expressly included in the invention.

The variable segments of humanized antibodies produced as described supra are typically linked to at least a portion of a human immunoglobulin constant region. The antibody will contain both light chain and heavy chain constant regions. The heavy chain constant region usually includes CH1, hinge, CH2, CH3, and, sometimes, CH4 domains.

The humanized antibodies may comprise any type of constant domains from any class of antibody, including IgM, IgG, IgD, IgA and IgE, and any subclass (isotype), including IgG1, IgG2, IgG3 and IgG4. When it is desired that the humanized

antibody exhibit cytotoxic activity, the constant domain is usually a complement-fixing constant domain and the class is typically IgG_1. When such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype.

Nucleic acids encoding humanized light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Such control sequences include a signal sequence, a promoter, an enhancer, and a transcription termination sequence (see Queen et al., Proc. Natl. Acad. Sci. USA 86, 10029 (1989); WO 90/07861; Co et al., J. Immunol. 148, 1149 (1992), which are incorporated herein by reference in their entirety for all purposes).

Efficacy of a therapeutic protein can be limited by unwanted immune reactions. Non-human monoclonal antibodies can have substantial stretches of linear amino acid sequences and local structural conformations that can elicit immune response in humans. The first attempt to reduce immunogenicity of non-human antibodies was the construction of human-murine antibody chimeras, which was then followed by methods for humanization of those chimeras in the late 1980's (review in Almagro and Fransson, *Front Biosci* **13**: 1619-1633, 2008).

One of the most often used humanization approaches is the so-called "Complementarity-Determining Regions (CDR) grafting" wherein murine CDR's are grafted into human antibody Framework Regions (FR's). Nevertheless, application of this method more often than not results in a substantial loss of binding to antigen and thus a reduction in potency of the antibody-based drug. Hence, it is highly valuable to use sound design principles for creating antibody molecules that elicit minimal immunogenic reactions while retaining the binding and biophysical profiles of the parent non-human molecule when injected into humans.

The humanization of 2177 and 2191, two mouse monoclonal antibodies (mAb) with binding specificity to CD27 is described. The frameworks (FR) of these

antibodies were replaced by human germline gene FRs using the first step of the Janssen proprietary humanization technology called Human Framework Adaption (HFA) disclosed in the patent application Raghunathan, G., US20090118127 A1 and further exemplified in Fransson et al (J Mol Biol 398:214-231, 2010). This technology enables a set of mAbs specific for CD27 with superior binding and inhibition properties to those measured for the parental mouse antibodies 2177 and 2191.

3. Methods of Using an Anti-CD27 Antibody

As described in detail below, the present invention demonstrates that four isolated monoclonal antibodies (C2177, C2186, C2191, and C2192) bind three non-overlapping epitopes on CD27 and display in vitro and/or in vivo CD27 inhibiting activities. Significantly, the reactivity of the MAbs includes the ability to dose-dependently block CD27 interaction with CD70, reduce CD27 signaling in the presence of CD70, reduce IL-4 and IFNg production by T-cells, and inhibit CD70-dependent human naïve CD4+ T-cell proliferation, CD70-dependent B-cell proliferation and plasma cell generation. Moreover, isolated antibodies do not significantly induce CD27 activation in the absence of CD70 stimulus.

Given the properties of the monoclonal antibodies as described in the present invention, the antibodies or antigen binding fragments thereof are suitable both as therapeutic and prophylactic agents for treating or preventing CD27-associated conditions in humans and animals.

In general, use will comprise administering a therapeutically or prophylactically effective amount of one or more monoclonal antibodies or antigen binding fragments of the present invention, or an antibody or molecule selected to have similar spectra of binding and biologic activity, to a susceptible subject or one exhibiting a condition in which CD27 activity is known to have pathological sequelae, such as immunological disorders or tumor growth and metastasis. Any active form of the antibody can be administered, including Fab and F(ab')2 fragments.

Preferably, the antibodies used are compatible with the recipient species such that the immune response to the MAbs does not result in an unacceptably short circulating half-life or induce an immune response to the MAbs in the subject. The

MAbs administered may exhibit some secondary functions, such as binding to Fc receptors of the subject and activation of ADCC mechanisms, in order to deplete the target cell population using cytolytic or cytotoxic mechanisms or they may be engineered to by limited or devoid of these secondary effector functions in order to preserve the target cell population.

Treatment of individuals may comprise the administration of a therapeutically effective amount of the antibodies of the present invention. The antibodies can be provided in a kit as described below. The antibodies can be used or administered as a mixture, for example, in equal amounts, or individually, provided in sequence, or administered all at once. In providing a patient with antibodies, or fragments thereof, capable of binding to CD27, or an antibody capable of protecting against CD27 in a recipient patient, the dosage of administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc.

In a similar approach, another therapeutic use of the monoclonal antibodies of the present invention is the active immunization of a patient using an anti-idiotypic antibody raised against one of the present monoclonal antibodies. Immunization with an anti-idiotype which mimics the structure of the epitope could elicit an active anti-CD27 response (Linthicum, D. S. and Farid, N. R., Anti-idiotypes, Receptors, and Molecular Mimicry (1988), pp 1-5 and 285-300).

Likewise, active immunization can be induced by administering one or more antigenic and/or immunogenic epitopes as a component of a vaccine. Vaccination could be performed orally or parenterally in amounts sufficient to enable the recipient to generate protective antibodies against this biologically functional region, prophylactically or therapeutically. The host can be actively immunized with the antigenic/immunogenic peptide in pure form, a fragment of the peptide, or a modified form of the peptide. One or more amino acids, not corresponding to the original protein sequence can be added to the amino or carboxyl terminus of the original peptide, or truncated form of peptide. Such extra amino acids are useful for coupling the peptide to another peptide, to a large carrier protein, or to a support. Amino acids

that are useful for these purposes include: tyrosine, lysine, glutamic acid, aspartic acid, cysteine and derivatives thereof. Alternative protein modification techniques may be used, e.g., NH2-acetylation or COOH-terminal amidation, to provide additional means for coupling or fusing the peptide to another protein or peptide molecule or to a support.

The antibodies capable of protecting against unwanted CD27 bioactivity are intended to be provided to recipient subjects in an amount sufficient to effect a reduction, resolution, or amelioration in the CD27-related symptom or pathology. An amount is said to be sufficient or a "therapeutically effective amount" to "effect" the reduction of symptoms if the dosage, route of administration, etc. of the agent are sufficient to influence such a response. Responses to antibody administration can be measured by analysis of subject's affected tissues, organs, or cells as by imaging techniques or by ex vivo analysis of tissue samples. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

Therapeutic Applications

The CD27-neutralizing antibodies of the present invention, antigen binding fragments, or specified variants thereof can be used to measure or cause effects in an cell, tissue, organ or animal (including mammals and humans), to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, a condition mediated, affected or modulated by CD27 or cells expressing CD27. Thus, the present invention provides a method for modulating or treating at least one CD27 related disease, in a cell, tissue, organ, animal, or patient, as known in the art or as described herein, using at least one CD27 antibody of the present invention. Particular indications are discussed below.

Immune Related Disease

The present invention also provides a method for modulating or treating an immune related inflammatory disease, in a cell, tissue, organ, animal, or patient including, but not limited to rheumatoid arthritis, juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondilitis,

gastric ulcer, seronegative arthropathies, osteoarthritis, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosis, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener's granulomatosis, sarcoidosis, orchitis/vasectomy reversal procedures, allergic/atopic diseases, asthma, allergic rhinitis, eczema, allergic contact dermatitis, allergic conjunctivitis, hypersensitivity pneumonitis, transplants, organ transplant rejection, graft-versus-host disease, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, trauma/hemorrhage, burns, ionizing radiation exposure, acute pancreatitis, adult respiratory distress syndrome, rheumatoid arthritis, alcohol-induced hepatitis, chronic inflammatory pathologies, sarcoidosis, Crohn's pathology, sickle cell anemia, diabetes, nephrosis, atopic diseases, hypersensitity reactions, allergic rhinitis, hay fever, perennial rhinitis, conjunctivitis, endometriosis, asthma, urticaria, systemic anaphalaxis, dermatitis, pernicious anemia, hemolytic disesease, thrombocytopenia, graft rejection of any organ or tissue, kidney translplant rejection, heart transplant rejection, liver transplant rejection, pancreas transplant rejection, lung transplant rejection, bone marrow transplant (BMT) rejection, skin allograft rejection, cartilage transplant rejection, bone graft rejection, small bowel transplant rejection, fetal thymus implant rejection, parathyroid transplant rejection, xenograft rejection of any organ or tissue, allograft rejection, anti-receptor hypersensitivity reactions, Graves disease, Raynoud’s disease, type B insulin-resistant diabetes, asthma, myasthenia gravis, antibody-meditated cytotoxicity, type III hypersensitivity reactions, systemic lupus erythematosus, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), antiphospholipid syndrome, pemphigus, scleroderma, mixed connective tissue disease, idiopathic Addison’s disease, diabetes mellitus, chronic active hepatitis, primary billiary cirrhosis, vitiligo, vasculitis, post-MI cardiotomy syndrome, type IV hypersensitivity , contact dermatitis, hypersensitivity pneumonitis, allograft rejection, granulomas due to intracellular organisms, drug sensitivity, metabolic/idiopathic, Wilson's disease, hemachromatosis, alpha-1-antitrypsin deficiency, diabetic retinopathy, hashimoto's thyroiditis,

osteoporosis, hypothalamic-pituitary-adrenal axis evaluation, primary biliary cirrhosis, thyroiditis, encephalomyelitis, cachexia, cystic fibrosis, neonatal chronic lung disease, chronic obstructive pulmonary disease (COPD), familial hematophagocytic lymphohistiocytosis, dermatologic conditions, psoriasis, alopecia, nephrotic syndrome, nephritis, glomerular nephritis, acute renal failure, hemodialysis, uremia, toxicity, preeclampsia, OKT3 therapy, anti-CD3 therapy, cytokine therapy, chemotherapy, radiation therapy (e.g., including but not limited to asthenia, anemia, cachexia, and the like), chronic salicylate intoxication, and the like.

Pulmonary Disease

The present invention also provides a method for modulating or treating a pulmonary or pleural disease in a cell, tissue, organ, animal or patient, including, but not limited to, modulating the immune-response to associated or ancillary cells or cellular processes involving CD27 in, for example, pneumonia; lung abscess; occupational lung diseases caused be agents in the form or dusts, gases, or mists; asthma, bronchiolitis fibrosa obliterans, respiratory failure, hypersensitivity diseases of the lungs including hypersensitivity pneumonitis (extrinsic allergic alveolitis), allergic bronchopulmonary aspergillosis, and drug reactions; adult respiratory distress syndrome (ARDS), Goodpasture’s Syndrome, chronic obstructive airway disorders (COPD), idiopathic interstitial lung diseases such as idiopathic pulmonary fibrosis, sarcoidosis, desquamative interstitial pneumonia, acute interstitial pneumonia, respiratory bronchiolitis-associated interstitial lung disease, idiopathic bronchiolitis obliterans with organizing pneumonia, lymphocytic interstitial pneumonitis, Langerhans' cell granulomatosis, idiopathic pulmonary hemosiderosis; acute bronchitis, pulmonary alveolar proteinosis, bronchiectasis, pleural disorders, atelectasis, cystic fibrosis, and tumors of the lung, and pulmonary embolism.

Malignant Disease

The present invention also provides a method for modulating or treating a malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, modulating the immune-response to associated or ancillary cells or cellular processes involving CD27 in, at least one of: leukemia, acute leukemia, acute

lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodyplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignamt lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, solid tumors as primary disease or as metastatic disease, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, renal cell carcinoma, lung cancer including mesothelioma, breast cancer, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, adenocarcinomas, squamous cell carcinomas, sarcomas, malignant melanoma, particularly metastatic melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

Cardiovascular Disease

The present invention also provides a method for modulating or treating a cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, modulating the immune-response to associated or ancillary cells or cellular processes involving CD27 in, at least one of myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, arteriosclerosis, atherosclerosis, restenosis, diabetic atheriosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), post perfusion syndrome, cardiopulmonary bypass inflammation response, chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrythmias, ventricular fibrillation, His bundle arrythmias, atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aordic and peripheral aneuryisms, aortic dissection, inflammation of the aorta, occulsion of the abdominal aorta and its branches, peripheral vascular disorders, occulsive arterial disorders, peripheral atherlosclerotic disease, thromboangitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease,

acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphederma, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like.

Neurologic Disease

The present invention also provides a method for modulating or treating at neurologic disease in a cell, tissue, organ, animal or patient, including, but not limited to, modulating the immune-response to associated or ancillary cells or cellular processes involving CD27 in: neurodegenerative diseases, multiple sclerosis, migraine headache, AIDS dementia complex, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders' such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders, such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranucleo Palsy; structural lesions of the cerebellum; spinocerebellar degenerations, such as spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); systemic disorders (Refsum's disease, abetalipoprotemia, ataxia, telangiectasia, and mitochondrial multi.system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; and disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome; diffuse Lewy body disease; senile dementia related to Lewy body development; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerrorden-Spatz disease; and Dementia pugilistica, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one TNF antibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

Other Therapeutic Uses of CD27-neutralizing Antibodies

In addition to the above described conditions and diseases, the present invention also provides a method for modulating or treating fibrotic conditions of various etiologies by modulating the immune-response to associated or ancillary cells or cellular processes involving CD27 in, for example: liver fibrosis (including but not limited to alcohol-induced cirrhosis, viral-induced cirrhosis, autoimmune-induced hepatitis); lung fibrosis (including but not limited to scleroderma, idiopathic pulmonary fibrosis); kidney fibrosis (including but not limited to scleroderma, diabetic nephritis, glomerular pehpritis, lupus nephritis); dermal fibrosis (including but not limited to scleroderma, hypertrophic and keloid scarring, burns); myelofibrosis; neurofibromatosis; fibroma; intestinal fibrosis; and fibrotic adhesions resulting from surgical procedures.

The present invention also provides a method for modulating or treating or ameliorating the symptoms of an infectious disease in a cell, tissue, organ, animal or patient, by modulating the immune-response to associated or ancillary cells or cellular processes involving CD27 in, for example: acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections, HIV infection/HIV neuropathy, meningitis, hepatitis (A,B or C, or the like), septic arthritis, peritonitis, pneumonia, epiglottitis, E. coli, hemolytic uremic syndrome, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, mycobacterium tuberculosis, mycobacterium avium intracellulare, pneumocystis carinii pneumonia, pelvic inflammatory disease, orchitis or epidydimitis, legionella, lyme disease, influenza a, Epstein-Barr virus, vital-associated hemaphagocytic syndrome, vital encephalitis/aseptic meningitis, and the like.

The contents of all cited references (including literature references, issued patents, published patent applications, and co-pending patent applications) throughout this application are hereby expressly incorporated by reference.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of

the invention and are not intended to be limiting thereof.

4. Pharmaceutical formulations

The invention provides for stable formulations of an CD27-neutralizing antibody, which is preferably an aqueous phosphate buffered saline or mixed salt solution, as well as preserved solutions and formulations as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one CD27-neutralizing antibody in a pharmaceutically acceptable formulation. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5.

In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the above-described compounds together with a suitable amount of carrier vehicle. Additional pharmaceutical methods may be employed to control the duration of action. Controlled release preparations may be achieved through the use of polymers to complex or absorb the compounds. Another possible method to control the duration of action by controlled release preparations is to incorporate the compounds of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacylate)-microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington *supra* (2006).

5. Administration of a CD27-neutralizing Antibody

At least one CD27-neutralizing antibody in either the stable or preserved

formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including intravenous (I.V.), intramusclular (I.M.); subcutaneously (S.C.); transdermal, pulmonary, transmucosal, using an formulation in an implant, osmotic pump, cartridge, micropump, or other means appreciated by the skilled artisan, as well-known in the art.

In one method of administering a CD27-neutralizing antibody, the drug substance is given intravenously from a previously installed catheter equipped with an infusion bag. The CD27-neutralizing antibody is supplied in 20-ml single-use vials, such as those supplied by ImmunoGen, Inc. (Cambridge, MA). Each vial contains protein at a concentration of from 0.05 to about 2.0 mg/ml in a buffered solution (pH 6.5 ± 0.5) comprised essentially of monobasic potassium phosphate (0.57 mg/ml), monobasic sodium phosphate monohydrate (0.20 mg/ml), dibasic sodium phosphate (0.555 mg/ml), and sodium chloride (8.16 mg/ml) in purified water, USP. The drug product is prefiltered twice upon instilling the dose volume into the infusion bag by passing it through a low protein-binding 5-µ filter and is administered to patients through an inline 0.22 µm filter within 8 h of preparation. After infusion, the i.v. line should be flushed with fluid to ensure delivery of the full drug dose.

In general, if administering a systemic dose of the antibody, it is desirable to provide the recipient with a dosage of antibody which is in the range of from about 1 ng/kg-100 ng/kg, 100 ng/kg-500 ng/kg, 500 ng/kg-1 ug/kg, 1 ug/kg-100 ug/kg, 100 ug/kg-500 ug/kg, 500 ug/kg-1 mg/kg, 1 mg/kg-50 mg/kg, 50 mg/kg-100 mg/kg, 100 mg/kg-500 mg/kg (body weight of recipient), although a lower or higher dosage may be administered. Dosages as low as about 1.0 mg/kg may be expected to show some efficacy. Preferably, about 5 mg/kg is an acceptable dosage, although dosage levels up to about 50 mg/kg are also preferred especially for therapeutic use. Alternatively, administration of a specific amount of the antibody may be given which is not based upon the weight of the patient such as an amount in the range of 1 ug – 100 ug, 1 mg – 100 mg, or 1 gm – 100 gm. For example, site specific administration may be to body compartment or cavity such as intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic,

intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means.

The treatment may be given in a single dose schedule, or preferably a multiple dose schedule in which a primary course of treatment may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. Examples of suitable treatment schedules include: (i) 0, 1 month and 6 months, (ii) 0, 7 days and 1 month, (iii) 0 and 1 month, (iv) 0 and 6 months, or other schedules sufficient to elicit the desired responses expected to reduce disease symptoms, or reduce severity of disease.

6. Articles of Manufacture Comprising a CD27-neutralizing Antibody

The invention includes an article of manufacture containing materials useful for the treatment of the disorders described above comprising a CD27-neutralizing antibody, a container and a label or package insert on or associated with the container. The article of manufacture preferably contains at least one vial comprising a solution of at least one CD27-neutralizing antibody with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of time. The invention may comprise an article of manufacture, comprising packaging material, a first vial comprising lyophilized CD27-neutralizing antibody, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a practitioner or patient how to reconstitute the CD27-neutralizing antibody in the aqueous diluent to form a solution.

Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper, optionally, capable of being pierced by a hypodermic injection needle).

At least one active agent in the composition is a CD27-neutralizing antibody. The label or package insert indicates that the composition is used for treating the indication of choice, such as SLE. The package insert herein may indicate that the antibody or composition is used to treat a condition that does not respond, or respond poorly, to treatment with the standard of care as outlined herein for specific diseases and diagnoses. In other embodiments, the package insert may indicate that the antibody, antibody-conjugate or composition can be used also to treat a disease characterized by the need to modulate the immune-response of cellular processes involving CD27.

Yet another aspect of the present invention is a kit for detecting CD27 in a biological sample. The kit includes a container holding one or more antibodies which binds an epitope of CD27 and instructions for using the antibody for the purpose of binding to CD27 to form an immunological complex and detecting the formation of the immunological complex such that the presence or absence of the immunological complex correlates with presence or absence of CD27 in the sample. Examples of containers include multiwell plates which allow simultaneous detection of CD27 in multiple samples.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples.

EXAMPLE 1: CD27 REAGENTS AND METHODS

In order to generate and test CD27-binding monoclonal antibodies, protein constructs were generated which represent the full length of human CD70 and human CD27 and the extracellular domain (ECD) of human CD27.

Human CD27 (SEQ ID NO: 149) is a type 1 transmembrane protein comprised of a signal peptide (from residues 1 to 20), extracellular (ECD, from residues 21 to 191), transmembrane (TM, from residues 191 to 212) and intracellular (ICD, from residues 213 to 260) domains. Human CD70 (SEQ ID NO: 2) is a type 2 transmembrane polypeptide of 193 amino acids in length comprised of, from the N-terminus, an intracellular domain (ICD, from residues 1 to 17), transmembrane (TM,

from residues 18 to 38) and extracellular domain (ECD, from residues 39 to 193). The complete CD70 coding sequence was clonally expressed on the surface of HEK 293 cells.

For mAb ELISA and Proteon-based direct binding assays, amino acids 1-121 of the CD27 ECD were transiently expressed in HEK293 cells with a C-terminal His6-tag peptide and purified by metal ion chromatography. For phage panning and ELISA assays, amino acids 1-173 of the ECD with a C-terminal His6-tag were HEK expressed and purified by metal ion chromatography followed by size exclusion chromatography on Superdex 75. Both of these CD27 proteins were biotinylated using NHS-ester chemistry targeting amine residues on the protein. For crystallization, amino acids 1-101 with a C-terminal His6-tag were expressed in a baculovirus system and purified by metal ion chromatography by Proteose, Inc. For mouse immunization, CD27-Fc protein was purchased from R&D systems. For some studies, the complete CD27 coding sequence was clonally expressed on the surface of HEK 293 cells.

Human CD27 and human CD70 cDNA clones were ordered from Open Biosystems. Standard molecular biology techniques were used to generate expression constructs. Briefly, the open reading frames of the CD27 and CD70 genes were PCR amplified and cloned into the mammalian expression vectors via restriction endonuclease digestion and ligation, or via ligase independent cloning (LIC). Full length CD27 and CD70 genes were cloned into the expression vector and were clonally expressed on the surface of mammalian cells. The extracellular domain of CD27 was cloned into mammalian expression vectors and transiently expressed in HEK293 cells with a hexa-his tail.

Example 2: GENERATION of CD27-NEUTRALIZING ANTIBODIES

Murine anti-human CD27 antibodies were generated by the hybridoma method of Kohler and Milstein (1975). Ten 12-14 week old C3H/HeJ mice were obtained from Charles River Laboratories. The mice were immunized subcutaneously (SQ) at the base of tail (BOT) with 50 microgm Hu CD27 Fc (R&D Systems) in combination with $0.33x10^5$ units each of murine interferon-alpha and -beta (Biosource) in a final volume of 100 microL on day 1. On days 2 and 3, the mice were injected SQ BOT

with the interferons (same doses as on day 1). The mice were boosted with 50 microgm Hu CD27-Fc in combination with 50 microgm anti-murine CD40 agonist Mab (R&D Systems, MAB440) given SQ BOT in PBS on day 14; four days prior to splenic harvest for fusion.

For titer assessment, a capture phase EIA was performed. Briefly, plates (Nunc-Maxisorp) were coated with 0.1 microgram goat anti-ms Fc (Jackson Immunotech) in bicarbonate buffer overnight at 4^{o} C. After blocking and washing steps, dilutions of sera were added and plates were incubated for 30 minutes at RT. Following washing steps, the plates were incubated for 30 minutes at RT with 0.25 microgm/mL of biotinylated Hu CD27-ECD in blocking buffer and probed with HRP labeled Streptavidin (Jackson Immunotech) diluted 1:40,000 in 0.4% BSA/PBS of for 30 minutes at RT. Plates were washed as described above; then OPD (Sigma fast tabs) substrate solution was added, incubation for 10 minutes at room temperature, the color substrate development stopped by the addition of 4N sulfuric acid at 25 microL/well, and the absorbance measured at 490nm.

A cell bank of the non-secreting BALB/c mouse myeloma fusion partner, FO was purchased from ATCC (# CRL-1646). One frozen vial of FO cells was thawed and resuspended in DMEM with Glutamax™ (modified) medium (Invitrogen) supplemented with 10% (v/v) FBS (Hyclone). The cells were expanded, cryopreserved and deemed sterile and free of mycoplasma by Charles River Laboratories. The C1833A (Centocor) cell line was also used in this fusion. This cell line was derived in-house by knocking down expression of the CHOP gene in the FO cell line so it requires growth under selection with geneticin. Cells were treated as FO's above with the exception of growing in DMEM with Glutamax™ (modified) medium supplemented with 10% (v/v) FBS (Hyclone) and 500 ug/mL of geneticin (Gibco). Both the FO and C1833A cell lines were subjected to cell synchronization prior to fusion. Briefly, 1.5-2 x108 cells were seeded into 180 mL of DMEM with Glutamax™ (modified) medium supplemented with 0.25% (v/v) FBS (Hyclone) and incubated at 37ºC for 13 hours. An additional 20 mL of FBS was added for a final FBS concentration of 10% and incubated for an additional 13 hours at 37ºC prior to use. C1833A cells were

constantly under geneticin selection throughout cell synchronization process. The myeloma cells were washed in PBS, counted, and viability determined (>78%) via Guava Viacount software prior to fusion.

On the day of fusion, the animals were euthanized by CO_2 asphyxiation. The spleens were removed aseptically and immersed in 10 mL of cold phosphate-buffered saline (PBS) containing antibiotics (PSA) (Sigma).

A single cell suspension of splenocytes was prepared and subjected to RBC lysis using RBC lysis buffer (Sigma). Washed cells were labeled for magnetic sorting as per the manufacturer's instructions, using anti-murine Thy1.2, anti-murine/human CD11b and anti-murine IgM magnetic beads (Miltenyi Biotec # 130-049-101, 130-149-601 and 130-047-301 respectively) and then sorted using the AutoMacs Pro instrument by running the Deplete program. Both the unlabeled (plasmablast B cell enriched) and labeled cell fractions were collected then counted via the Guava PCA. Positively labeled cells were discarded. Unlabeled cells were divided in half for fusion to both FO and C1833A fusion partners. Fusions were carried out at a 1:1 ratio of murine myeloma cells to viable spleen cells according to the method of De St. Groth (J Immunological Methods. 35:1-21. 1980). Briefly, spleen and myeloma cells were mixed together, pelleted and washed once in 50 mL of PBS. The pellet was resuspended with 1 mL of polyethylene glycol (PEG) solution (2 g PEG molecular weight 4000, 2 mL DMEM, and 0.4 mL DMSO) at 37°C over 30 seconds. The cell/fusion mixture was then immersed in a 37°C water bath for approximately 60 seconds with gentle agitation. The fusion reaction was stopped by slowly adding 37°C DMEM over 1 minute. The fused cells were allowed to rest or 5 minutes at room temperature and then centrifuged at 150 x g for 5 minutes. Cells were then resuspended in HAT medium [DMEM with Glutamax™ (modified), supplemented with 20% FBS, 5% Origen, 25 microg/mL gentamicin (Sigma) and HAT (100 microM hypoxanthine, 0.4 microM aminopterin, and 16 microM thymidine (Sigma), and seeded in 96-well flat bottom polystyrene tissue culture plates (Corning # 3997) or methylcellulose medium (StemCell Technologies, MediumD cat# 03804) containing ~2.25 µg/mL of AF488 human CD27 (Janssen Research & Development, LLC). Plates were incubated in a humidified 37°C incubator with 7% CO_2 for 7-10 days. Single colonies were

selected from methylcellulose plates for screening utilizing the ClonepixFL or under a white light microscope.

EXAMPLE 3: Bioactivity of Recombinant Mabs

The ability of the binding domains from the murine antibodies to bind CD27 and to block certain bioactivities of CD27 was analyzed using various in vitro assays as described below.

A solid phase EIA was used to screen the hybridoma supernatants for antibodies capable of binding human CD27. Plates (Nunc-Maxisorp #446612) were coated overnight with 4 µg/mL Fab goat anti-huFc (Jackson #109-006-098) in Bicarbonate buffer O/N at 4^{o} C. Without washing, the wells were blocked with 200 microL of 0.4% (w/v) bovine serum albumin (BSA) in PBS for 1 hr at RT. After washing with 0.15 M saline containing 0.02% (w/v) Tween 20, 50 microl of huCD27-Fc in 0.4% BSA/PBS was added to the plates for 1 hr at RT. After washing again, 50 microl of undiluted hybridoma supernatants were incubated on coated plates for 30 minutes at RT. Plates were washed three times and then incubated with 50 microL of goat anti-murine Fc HRP (Jackson # 115-036-071) diluted 1:10,000 for 30 minutes at RT. Plates were again washed and developed as described above for titer assessment. For assessment relative binding capacity of hybridoma Mabs similar assay was performed using Maxisorp 384 well plates (NUNC 464718) with serially diluted hybridoma supernatants (normalized to a starting concentration of 5 microg/mL. This assay identified 386 positive hybridomas.

All 386 CD27 specific hybridomas were screened for the ability to inhibit binding of huCD27 to huCD70 using biochemical binding assays with IM-9 cells, a B-lymphoblastoid cell line found to endogenously expressing human CD70. Maxisorp plates (VWR # 62409-314) were coated with recombinant human CD27/Fc (R&D Systems, Cat# 382-CD) at 250 nanogram (ng)/mL and incubated overnight at 4˚C. The next day plates were blocked with blocking buffer (Pierce, Cat#37543) and then washed with wash buffer I, that contains PBS without Ca++ or Mg++, 0.01% Tween-20. Controls (mouse MAB to hCD27, R&D Systems, Cat# MAB382; Mouse IgG1 isotype control, R&D Systems, Cat# MAB002; mouse IgG2a isotype control, R&D

Systems, Cat# MAB003) were included on each plate. 50 uL/well of hybridoma samples or controls were mixed with 50 μL/well of harvested IM-9 cells, human B-lymphoblastoid cell line (ATCC, CCL-159) and were incubated for 1 hour at RT without shaking. At the end of incubation, plates were washed with wash buffer II, to remove all unbound cells, and then lysed with 50 uL/well of Cell Titer Glo reagent (Promega, Cat# G7571). After 10 minutes incubation with shaking, plates were read on Envision (PerkinElmer, 2102 Multilabel reader). The luminescent signal generated is proportional to the amount of ATP present and directly correlated to the number of live cells present in the well captured by CD27 binding. Based on the results of the biochemical binding assay, about 50% of CD27 specific clones were neutralizing.

To eliminate redundancy among the neutralizing clones, competition binding assays were performed to bin the antibodies into competition groups. In this assay, hybridoma supernatants were assessed individually as both capture and detection reagents with each of the positive hybridomas in the panel. Antibodies forming effective capture/detection reagents with each other likely recognize spatially-separated epitopes on the CD27 protein, thus allowing both antibodies to bind to the target protein at the same time. Groups of clones exhibiting similar patterns of activity across the entire panel likely bind to similar epitopes. Selecting clones from different groups therefore provided antibodies recognizing different epitopes. Briefly, 384 well Nunc Maxisorp plates (464718) were coated with goat anti-mouse Fc (JIR115-005-071) in coating buffer overnight at 4ºC. Plates were then blocked with 0.4% BSA in PBS for 30 minutes at room temperature. At this step and all subsequent steps plates are washed with PBS, 0.02% Tween-20. Each well of a row (one row per supernatant) received 20 uL of supernatant (neat supernatant was used for the initial screen but for rescreening the subclones, supes were normalized to 2 ug/ml of mAb) was along with controls (mouse anti- huCD27, R&D Systems, Cat# MAB382; mouse isotype control Cat#555439, Becton-Dickenson) then incubated for 30 minutes at RT. After washing, 25 uL of unlabeled Hu CD27–ECD-His-tag was prepared in PBS plus 10% mouse sera (Bioreclamation mouse serum CD-1 lot#MSEBREC.18565) at 0.3 (or 0.8 for concentration normalized) microg/ml was added to all wells, followed by 30 minutes incubation at room temperature then washed. Each supernatant was added down a

single column and incubated for 30 minutes at RT with 25 uL of a mixture prepared as follows: (- pre-incubate supernatants with goat anti-mouse Fc HRP (Jackson 115-036-008), by mixing 150 uL of 1:1000 goat anti-mouse Fc HRP with each 1000 uL of supernatant (for the primary screen) or 90 uL of 1:2000 per 600 uL of supernatant adjusted to 2 ug/mL: (for rescreening subclones). After 30 minutes incubation at room temperature, add 200 microL of 100% normal mouse sera per mL and incubate an additional 30 minutes at RT. Plates were washed then incubated for 15 minutes at RT with 100 uL/well of citrate-phosphate substrate solution (0.1 M citric acid and 0.2 M sodium phosphate, 0.01% H2O2, and 1 mg/mL OPD). Substrate development was stopped by addition of 25 uL of 4N sulfuric acid and the absorbance measured at 490nm using an automated plate reader. This binning assay identified three groups that recognize non-overlapping binding sites on huCD27 antigen. Selected antibodies from all three groups were scaled up for antibody production, purification and further testing in functional assays.

Inhibition of cell signaling

Binding of CD70 to CD27 induces signaling that leads to downstream activation of the transcription factor, NF-kβ. A NF-kβ reporter assay was established for further antibody characterization. The assay was run in two modes: (1) to assess antibody antagonism by neutralization of CD70 induced CD27 activation and (2) to assess antibody agonism by activation of CD27 signaling without CD70 ligation. HEK-293F cells were transfected with a total of 36 ng of DNA containing both human CD27 and luciferase constructs, under control of the NF-kβ promoter. HEK-293F transfectants were plated $5x10^4$ cells per well in 40 uL Freestyle media (Gibco) in 96-well plates. Dilutions of CD27-neutralizing hybridoma mAbs were added to the assay plate in Freestyle media (Gibco) for a final concentration of 50 ug/mL with 1:3 dilutions and plates were incubated at 37°C (95% O_2/5% CO_2) for one hour. To test for ability of hybridoma mAbs to neutralize CD70:CD27 signaling, terminally irradiated (4000 rads) HEK-293E CD70 episomal cells were added at 20% of the number of CD27 transfectant cells to the assay plate. To test for agonist activity of hybridoma mAbs, addition of CD70 episomal cells was omitted. Assay plates were incubated overnight at 37°C (95% O_2/5% CO_2) and developed using the Steady-Glo® Luciferase

Assay System (Promega) according to the instructions of the manufacturer. Four CD27-neutralizing hybridoma mAbs, C2177, C2186, C2191, and C2192, that dose-dependently blocked the CD70-mediated CD27 signaling without causing significant dose-dependent agonistic activation of the CD27 receptor in the absence of CD70 stimulus were selected for further characterization. The IC_{50}s for blocking IM-9 cell binding to CD27 and CD70-mediated signaling in NF-kβ reporter gene assay are summarized for these four antibodies in Table 1. Agonism activity in the NF-kβ reporter gene assay is shown as fold increase in CD27 signaling relative to an irrelevant isotype control antibody (mouse IgG1 to rat EMP protein) in the absence of CD70 stimulus at the maximum tested concentration of antibody.

Affinity for CD27

The K_Ds of antibodies C2177, C2186, C2191, and C2192 for monomeric soluble CD27 at 25 ºC were measured by Biacore and are reported in Table 1. Assays were carried out on a BIACORE 3000 (BIAcore, Inc.) surface plasmon resonance (SPR) instrument. The samples were prepared in Dulbecco's phosphate buffered saline pH 7.4 containing 0.005% surfactant (polysorbate 20). Goat anti-mouse Fc specific antibody (Jackson Immunoresearch laboratories Prod # 115-005-071) was covalently attached to carboxymethyl dextran coated gold surfaces (CM-5 Chip, Biacore). Prior to immobilization the chip was pretreated with 50 mM NaOH, 100 mM HCl and 0.1% sodium dodecyl sulfate with injection of deionized water in between the pre-treatments. The antibodies were diluted with 10 mM sodium acetate buffer pH 4.5 and coupled to the carboxymethylated dextran surface of the chip using the manufacturer instructions for amine-coupling chemistry. The remaining reactive groups on the surface were deactivated using ethanolamine-HCl. The mAb were captured on the sensor surface via Fc domain. The associations of human CD27 ECD injected at increasing concentrations (0.6-150 nM, 4-fold dilution series) were monitored for three minutes and the dissociations for ten minutes. Regeneration of capture surfaces to baseline was optimized using two 3 second pulses of 100 mM phosphoric acid. Data were processed using the Scrubber software, version 1.1g (BioLogic Software). Double reference subtraction of the data was performed to correct for buffer contribution to the signal and instrument noise. The kinetic analysis of the processed data was carried out using

the Biaevaluation 4.0.1 software (GE Healthcare Bio-Sciences, Uppsala, Sweden). Binding profiles were described by a 1:1 binding model indicating a monovalent binding of CD27.

Table 1.

			IM-9		
		Biacore	**Binding[1]**	**NF-kβ Reporter**	
		K_D	IC_{50}	IC_{50}[2]	Agonism[3]
mAb	Isotype	nM	ug/ml	ug/ml	at 50 ug/ml
C2177	mIgG1	3.07	0.063	0.040	1.850
C2186	mIgG1	2.55	0.059	0.105	2.547
C2191	mIgG1	2.62	0.059	0.080	3.053
C2192	mIgG2a	0.21	0.054	0.232	5.394

[1] IC_{50} for mAb inhibition of IM-9 cells binding to immobilized CD27

[2] IC_{50} for mAb inhibition in the reporter gene assay

[3] Agonist activity of mAbs at 50 ug/ml in the absence of CD70, measured as fold-increase in reporter gene signal relative to isotype control antibodies.

Inhibition of cell proliferation

The proliferation of T-cells sub-optimally activated in culture with anti-CD3 plus anti-CD28 antibodies is enhanced by CD70 ligation of CD27 expressed on the T-cells. The four murine neutralizing antibodies were assessed for their ability to inhibit T-cell proliferation in the presence of CD70 and to induce proliferation in the absence CD70. Frozen CD4+ T cells were purchased from AllCells, LLC. Cells were thawed and placed into IMDM medium containing 10% FBS, 1% l-glutamine, and 1% Penicillin-Streptomycin. Prior to plating cells, anti-CD3 (OKT3) antibody was coated onto a U-bottom plate at 1 ug/mL in PBS overnight at 4^{o}C. Cells were counted, brought to a concentration of $1x10^6$ cells/mL, and plated at $1x10^5$ cells/well. Soluble anti-CD28 was added as a secondary activation signal at 1 ug/mL per well. Irradiated (6000 rads) HEK cells transfected with either human CD70 or vector alone (mock)

were added to appropriate wells at $2x10^4$ cells/well (20%). Cells were stimulated for 3 days, 0.9 uCi thymidine [methyl-3H] was added to all sample wells and the cells were incubated for 18-24 hours. On the fourth day of stimulation, cells were harvested onto a filter plate using the PE Filtermate Harvester. The plate was allowed to dry and 30 uL of MicroScintTM-20 was added to all sample wells. The plate was read on a PE TopCount NXT, and data collected was as CPM. Antibody C2177 shows dose-dependent inhibition of CD70 mediated T-cell proliferation and very weak intrinsic agonistic activity in the absence of CD70 (Figure 1). Similar results were observed for the C2186, C2191 and C2192 antibodies. The IC_{50} and maximal % inhibition for these antibodies are reported in Table 2. None of the antibodies showed consistent stimulation of proliferation in the absence CD70 ligation, indicating a lack of intrinsic agonist activity.

In addition, the C2177, C2186, C2191 and C2192 antibodies showed dose-dependent inhibition of CD70-mediated T-cell proliferation as measured in a CSFE assay with no effect on proliferation in the absence of CD70 stimulus. Frozen CD3+ T cells were purchased from AllCells, LLC. Cells were thawed and placed into IMDM medium containing 10% FBS, 1% L-glutamine, and 1% Penicillin-Streptomycin. Cells were pre-labeled with 2.5 mM CFSE (Invitrogen), quenched with FBS and washed with T cell media. CFSE is a dye that passively diffuses into cells and become highly fluorescent upon binding with intracellular amines. Upon cell division each daughter cell will contain half of the CFSE label of the parental cell, thus cell proliferation may be monitored by tracking numbers of cells with different CFSE intensity. The cells were brought to a concentration of 1×10^6 cells/mL, and plated at $1x10^5$ cells/well. Prior to plating cells, anti-CD3 (OKT3) antibody was coated onto a U-bottom plate at 0.5 ug/mL in PBS overnight at 4^o C. Soluble anti-CD28 was added as a secondary activation signal at 0.1 ug/mL per well. Irradiated (6000 rads) HEK cells transfected with either human CD70 or vector alone (mock) were added to appropriate wells at $2x10^4$ cells/well (20%). Cells were stimulated for 4 days and analyzed by FACS analysis to count divided cells containing different intensity levels of CFSE label.

Inhibition of plasma blast differentiation

The CD27-neutralizing hybridoma mAbs were also tested in a plasma blast differentiation assay with primary human B-cells. CD19+ human B lymphocytes that had been negatively selected from peripheral blood of normal donors (obtained from AllCells) were cultured for 6 days in the presence of either 1 ug/mL anti-CD40 antibody (clone MAB89, Abcam) and 100 ng/mL Interleukin 21 (Invitrogen) or 1 ug/mL soluble human recombinant CD40 ligand and 2 ug/mL 'enhancer for ligands' (both Alexis Biochemicals) and 100 ng/ml Interleukin 21 in 96-well plates at 10^5 B cell per well. CD27-neutralizing hybridoma or isotype control antibodies were added in the presence or absence of $2x10^4$ irradiated (6000 rads) CD70-expressing HEK 293 cells or MOCK-transfected HEK 293 cells. CD27-neutralizing hybridoma mAbs and matching isotype controls were used at 25, 2.5, and 0.25 ug/mL. On day 6, cell samples were analyzed by flow cytometry and fractions of plasma blasts were identified as forward scattering/high, IgDminus, CD38bright, CD20low. The effect of CD27-neutralizing mab was calculated as plasma blast frequency in B-cell cultures containing CD70 expressing cells and hybridoma mabs normalized to plasma blast frequency in corresponding B-cell cultures containing mock-transfected cells. The percent inhibition by the C2177, C2186, C2191 or C2192 mAbs at 2.5 ug/mL is shown in Table 2. Agonistic activity in the absence of CD70 stimulus was not observed for any of these mAbs.

Table 2.

MAb	T-Cell Proliferation - CD4+ Cells		Plasma blast differentiation
	IC_{50} ug/ml	% Inhibition at highest concentration (30 ug/ml) (Mean ± SEM)	% Inhibition at 2.5 ug/ml (Mean ± SEM)
C2177	0.245	75.4±4.1 (n=6, 2 donors)	78 ±20 (n = 4)
C2186	0.775	65.3±4.4 (n=6, 2 donors)	93 ± 27 (n = 4)
C2191	0.3	58.3±6.0 (n=6, 2 donors)	105 ± 9 (n = 4)
C2192	0.445	75.7±5.7 (n=6, 2 donors)	74 ± 0 (n = 2)

EXAMPLE 4: EPITOPE MAPPING AND GROUPING

To more carefully evaluate the initial binning, competition assays were carried out with some of the purified neutralizing mAbs and with the CD27-neutralizing antibody, MAB 382 (R&D Systems). Briefly, 5 µl (10 µg/mL) of CD27-Fc chimeric protein (R&D Sysytems, Cat# 382-CD) was coated on a MSD HighBind plate (Meso Scale Discovery, Gaithersburg, MD) per well for 2 hr at RT. 5% MSD Blocker A buffer (Meso Scale Discovery, Gaithersburg, MD) was added to each well and incubated for 2 hr at RT. Plates were washed three times with 0.1 M HEPES buffer, pH 7.4, followed by the addition of a mixture of 10 nM labeled CD27 antibody with different concentrations of a competitor antibody (1 nM to 2 uM). Antibodies were labeled with MSD Sulfo-Tag™ NHS-ester, an amine-reactive N-hydroxysuccinimide ester which couples to primary amine groups of proteins to form a stable amide bond. After a 2-hour incubation with gentle shaking at RT, plates were washed 3 times with 0.1M HEPES buffer (pH 7.4). MSD Read Buffer T was diluted with distilled water (4-fold) and dispensed at a volume of 150 µL/well. The plates were analyzed using a SECTOR Imager 6000 which detects electrochemiluminescence through Sulfo-Tag labels that emit light upon electrochemical stimulation initiated at the electrode surfaces

of MSD microplates.

The competition studies defined three competition groups for the antibodies summarized in Table 3, confirming the initial binning assays. C2179, C2192 and MAB382 constitute one group; C2177, C2182, C2186 and C2193 are a second group; and C2191 constitutes a separate group.

Table 3.

Competitor	**Labeled Antibody**				
	C2179	C2177	C2182	C2186	C2191
C2177	-	+	+	+	-
C2179	+	-	-	-	-
C2182	-	+/-	+	+	-
C2186	-	+	+	+	-
C2191	-	-	-	-	+
C2192	+	-	+	+	-
C2193	-	+/-	+	+	-
MAB382	+	-	-	-	-

EXAMPLE 5: EPITOPE AND PARATOPE IDENTIFICATION BY X-RAY CRYSTALLOGRAPHY

The detailed epitopes and paratopes of antibodies C2177 and C2191 were determined by co-crystallization of their corresponding Fabs with CD27 ECD fragment (residues 1-101) as a trimeric complex and structure determination by X-ray crystallography. The His-tagged chimeric versions (mouse variable domain, human constant domain) of C2177 Fab and C2191 Fab were expressed in HEK293 cells and purified using affinity and size exclusion chromatography. The His-tagged ECD fragment (residues 1-101) of human CD27 was further purified by anion exchange

chromatography. The ternary complex CD27:C2177 Fab:C2191 Fab was prepared by mixing CD27 with the excess of Fabs at a molar ratio 1:1.25:1.25. The complex was incubated for 2 h at 4°C, separated from the uncomplexed species using size-exclusion chromatography, and concentrated to 12 mg/mL in 20 mM Tris pH 8.5, 250 mM NaCl. Crystallization of the complex was carried out by the vapor-diffusion method in sitting drops at 20˚C. The crystals of the complex were obtained from 24% PEG 3350, 0.2 M ammonium chloride, 0.1 M Tris buffer, pH 8.5. For X-ray data collection, one crystal was soaked for a few seconds in a cryo-protectant solution containing crystallization solution supplemented with 20% glycerol, and flash frozen in the stream of nitrogen at 100 K. Diffraction data were collected at the Rigaku MicroMaxTM-007HF X-ray generator equipped with a Saturn 944 CCD detector and an X-stream 2000 cryocooling system (Rigaku) over a 240° crystal rotation with 2-min exposures per 0.25°-image and were processed with the program XDS (Kabsch W. 2010. Acta Crystallogr. D66:125-132). The crystals belong to the monoclinic space group P21 with unit cell parameters: a = 141.1 Å, b = 53.0 Å, c = 143.4 Å, α = 90°, β = 112.2°, γ = 90°.

The crystal structure of the ternary complex was determined at 3.5 Å resolution and refined to the crystallographic R-factor of 26%. The Fabs of C2177 and C2191 bind CD27 at spatially distinct non-overlapping epitopes (Fig. 2). C2177 Fab binds the N-terminal (distal from the cell surface) portion of CD27. The epitope covers 700 Å2 and includes 9 residues: K5, S6, P8, H11, W13, G16, K17, H36, R37 (Fig. 3). The paratope is defined as antibody residues in contact (within 4 Å) with the antigen. The C2177 paratope includes 5 residues from VL (Y31, Y36, Y53, N57, N96) and 9 residues from VH (S31, W33, Y52, D55, D57, Y101, Y102, D104, Y105) (Fig. 3). All 6 CDRs are involved in antigen recognition. H36 and R37 are the central residues of the epitope. They stack against Y31 of VL and Y102 of VH; H36 also forms a salt bridge to D104 of VH.

C2191 Fab binds CD27 at the 'side' surface (Fig. 2) and covers 800 Å2 of the surface. The epitope includes 10 residues: F28, D43, P44, I46, P47, G48, V49, H60, S63, H66. The C2191 paratope includes 7 residues from VL (Y34, F36, Y53, L54, R96, L98, W100) and 8 residues from VH (S31, Y32, Y50, N57, Y59, R100, G101, N102) (Fig. 4). The antibody-antigen interactions are dominated by the hydrophobic

interactions between residues 44-49 of CD27 and a hydrophobic patch at the VL CDRs.

The different location of the C2177 and C2191 epitopes suggests different mechanisms of action of these antibodies. C2191 probably directly competes with CD70 ECD for the overlapping epitopes on the 'side' surface of CD27. C2177 antibody, on the contrary, does not compete for the same epitope but rather prevents the approach of the cells bearing CD27 and CD70. This observation is supported by the fact that C2191 prevents binding of soluble CD70 ECD to CD27 whereas C2177 does not.

EXAMPLE 6: ANTIBODY MODULATION OF HUMAN LYMPHOCYTE RESPONSE

An immune deficient mouse model, NOD/SCID-IL2Rγ^{null} (NSG) mice, was developed to study aspects of the human immune system control by T-cell responses (Markus G Manz & James P Di Santo Renaissance for mouse models of human hematopoiesis and immunobiology *Nature Immunology* 10, 1039 - 1042 (2009)). Adoptive transfer of human PBMCs into immune-compromised (NSG) mice was employed to evaluate the effects of an anti-CD27 antibody on human cell engraftment and/or proliferation. The model allows the evaluation of the effects of targeting human CD27 on antibody production and T-cell mediated responses.

Antibodies C2177 and C2191 were administered at the time of cell transfer and then twice a week for 3 weeks. On day 21, the mice were sacrificed, cells were purified from blood and spleen and subsequently characterized by flow cytometry. CTLA4-Ig (Orencia, BMS) was included as a positive control for immune suppression. Human cell engraftment/expansion was measured by evaluating the presence of human $CD45^+$ cells in the blood and spleen samples.

The mice were closely monitored and the time of sacrifice was determined based on XGVH symptoms in accordance with animal welfare guidelines. The experimental readouts used to evaluate the effects of anti-CD27 treatment included: body weight (twice weekly), observable signs of XGVH (twice weekly), such as posture, activity level, grooming, skin lesions (in particular, around the eyes and ears) using 1-5 score system, absolute count of human cell subsets and activation status using

flow cytometric analysis of (1) human PBMC injected, (2) mouse PB (once/week), and (3) spleen and bone marrow; determination of total human Ig, IgM and IgG in serum, spleen and BM using an ELISA, and, upon sacrifice, histology or immunohistochemistry to determine the levels of human infiltration in target organs, such as liver, kidney, lung and spleen.

The treatment groups were as follows:

1. PBMC (20 to 40 million cells per mouse, i.p.)
2. PBMC + CTLA4-Ig (10 mg/kg)
3. PBMC + Isotype control antibody, 2x/week for 3 weeks
4. PBMC + anti-CD27 antibody 2x/week for 3 weeks

Mice dosed with 10 mg/kg anti-CD27 mAbs, C2177 and C2191, (hybridoma antibodies chimerized on a human IgG_4 (ala/ala, ser->pro) scaffold) had statistically significant fewer human $CD45^+$ cells when compared to PMBC alone or isotype control in PMBCs isolated from the blood or spleen samples.

EXAMPLE 7: HUMAN FRAMEWORK ADAPTATION OF THE C2177 AND C2191 MABS

The antigen-binding site and the regions used to transfer the antigen specificity from antibodies C2177 and C2191 into the human FR's were reclassified as outlined in Raghunathan G. US20090118127 A1, 2009. In brief, the antigen-binding regions have been defined using various terms (review in Almagro and Fransson, *Front Biosci* **13**: 1619-1633, 2008). The term "Complementarity Determining Regions (CDRs)" is based on sequence variability (Wu and Kabat, J. Exp. Med. 132:211-250, 1970). There are six CDRs; three for V_H (H-CDR1, H-CDR2, H-CDR3), and three for V_L (L-CDR1, L-CDR2, L-CDR3) (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). "Hypervariable regions," "HVR's," or "HVL's" refers to the regions of an antibody variable domain which are variable in structure as defined by Chothia and Lesk (Chothia and Lesk, Mol. Biol. 196:901-917, 1987). There are six HVR's, three for VH (H1, H2, and H3) and three for VL (L1, L2, and L3).

In the HFA method, the regions targeted for transferring the specificity of the non-human antibody into the human FRs (HFRs) are the CDRs as defined by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National

Institutes of Health, Bethesda, Md., 1991) except in the region corresponding to the CDR-1 of V_H. For this region a combination of CDR and HVL (extended CDR-1 of V_H) are transferred from the non-human antibody into the human FRs (as provided in Tables 30, 31, 34, 35). In addition, variants with a shorter transferred CDR-H2 (called Kabat-7 [Raghunathan G. US20090118127 A1, 2009]) are generated and tested.

Human FR selection.

Human FRs, defined as the regions in the V regions not comprised in the antigen-binding site, were selected from the repertoire of functional human germilne IGHV, IGKV, IGKJ and IGHJ genes. The repertoire of human germline gene sequences was obtained by searching IMGT database (Kaas, et al., Nucl. Acids. Res. 32, D208-D210, 2004; Lefranc M.-P et al., Nucl. Acids Res., 33, D593-D597, 2005) and compiling all "01" alleles as of Oct 01, 2007. From this compilation, redundant genes (100% identical at amino acid level) and those with unpaired cysteine residues were removed from the compilation.

Initial selection of human sequences for HFR was based on sequence similarity of the human IGHV germline genes to the entire length of the mouse V_H region including FR-1 to 3 as well as H-CDR-1 and H-CDR-2. In the next stage, the selected human sequences were rank ordered using a score that takes into account both the length of the CDRs and sequence similarities between CDRs of mouse and human sequences. A standard mutation matrix, such as the BLOSUM 62 substitution matrix (Henikoff and Henikoff, Proc Natl Acad Sci U S A. 89, 10915-9, 1992) was used for scoring alignments of the CDRs of mouse and human sequences and a large penalty was applied if there was an insertion and/or deletion in the CDR loops. FR-4 was selected based on sequence similarity of the IGHJ germline genes (Kaas, et al., Nucl. Acids. Res. 32, D208-D210, 2004; Lefranc M.-P et al., Nucl. Acids Res., 33, D593-D597, 2005) with mouse antibodies C2177 and C2191 sequences. A similar procedure was used to choose human FRs for V_L. IGVK, germline genes were used for selecting FRs 1-3 and L-CDR 1-3. IGJK germline genes were used for selecting FR-4.

In addition to sequence criteria, a 3D homology model for the Fv fragments was constructed using Modeler (Sali and Blundell. J. Mol. Biol. 234: 779 ,1993) in the program suite from Accelrys, Inc. The models were utilized for analysis of the HFR variants, including CDR characterization and assessment of developability liabilities. Additional

considerations for selection of HFR variants were to minimize the number of exposed methionine and tryptophan residues, eliminate potential N-glycosylation sites and to favor human germlines with the highest expression profile *in silico* (de Wildt, J. Mol. Biol. 185: 895, 1999).

For path 1 framework adaptation and optimization of C2177, six V_H and four V_L HFR variants were included in the library. The V_H and V_L HFR variants were paired in a combinatorial manner to yield 24 HFR variant pairs plus 10 controls pairing all HFR variants with the counterpart V region of C2177 plus the parent C2177 itself to give a total of 35 combinations. Similarly for C2191, five V_H and four V_L HFR variants were paired in a combinatorial manner to yield 20 HFR plus 9 controls combining all HFR V variants paired with the counterpart V region of C2191 plus the parent C2191 parent itself for a total of 30 variants. DNA encoding the selected variable domains was recombined using standard methods to assemble complete MAbs with human IgG1 and kappa constant regions. The resulting reference chimeric antibody of C2177, designated M40, is comprised of variable regions H7 and L18. The corresponding chimeric antibody of C2191, designated M41, is comprised of variable regions H10 and L20. The mAbs were transiently expressed in 48-well plates in HEK 293E cells. Supernatant fluid from the cultures was tested for expression and binding activity 96 hours following transfection. The expression level of secreted mAb was evaluated using Octet technology to measure the rate of antibody binding to Protein A biosensors. The expression level was quantified by comparison to standard samples of known antibody concentration. An 8-point standard curve consisting of a 1:2 serial dilution of antibody of the identical isotype, was assembled, starting at 100 ug/ml. Biosensors were hydrated for 10 minutes in spent medium, and the binding rate of standards and unknown samples was measured for 2 minutes. Data was analyzed using the 5 parameter weighted dose-response equation and the initial slope binding rate algorithm. Samples with expression >1 ug/ml were diluted to 1 ug/ml with spent medium and screened using a single point ELISA. For this ELISA, 96 well black maxisorp plates were coated with 50uL of 3ug/ml goat anti human IgG FC diluted in carbonate-bicarbonate buffer, pH 9.4 at 4C overnight and then washed three times with wash buffer (PBS with 0.05% Tween-20), blocked with 300 µl StartingBlock (Thermo Scientific) solution for 1 hour, then washed as before. Samples or standards were diluted to 100 ng/ml in spent medium, and 50 ul was added to the assay plate

at room temperature for 1 hour with shaking. The plates were washed thrice and 50 ul per well of human CD27 ECD with His Tag was added at 60 ng/ml diluted in Assay Buffer (PBS with 1% FBS and 0.05% Tween-20) and incubated for 1 hour at room temperature. After washing, 50 ul per well of Qiagen peroxidase conjugated penta-his at 1:2000 dilution in assay buffer was added and incubated 1 hour at room temperature with shaking. The BM ChemiLum Substrate (BM Chemilum, POD, Roche) was mixed per manufacturer's instructions, and 50 ul was added to the plates after a final wash. After 10 minutes the plates are read on Perkin Elmer Envision Reader.

The results of screening the C2177 combinatorial library showed that all V-regions bind to CD27 with varying strengths. Several HFR variants gave a higher binding signal than the parent C2177 while others showed binding that was comparable or lower than the parent. All VLs bound antigen at detectable levels and did not influence binding HFR variants expressed at acceptable but lower levels than parent. Twenty-four of the C2177 HFR antibodies (VH, VL combinations) showed CD27 binding and expression >1 ug/ml.

The results of screening the C2191 combinatorial library showed that all except one VH bound to CD27 with varying signals. With the exception of pairing with this VH, all VLs showed binding to CD27. Several HFR variants gave a higher binding signal than the parent C2177, while others showed binding that was comparable or lower than the parent. Seventeen C2191 HFR antibodies (VH, VL combinations) demonstrated CD27 binding and expression >1 ug/ml.

Based on relative binding affinity for CD27 measured by ELISA, fifteen C2177 and eleven C2191 variants were chosen for pilot-scale expression and purification. Pilot-scale expression was done transiently in CHO-S cells at a volume of 750 ml. The harvested supernatants were purified via Protein A chromatography and the purified proteins were assessed for their affinity and functional activity.

The affinities of the HFR C2177 human MAb variants were measured by Surface Plasmon Resonance (SPR) using a ProteOn XPR36 protein interaction array system (BioRad). The rates of CD27 association and dissociation were measured for each variant. The biosensor surface was prepared by covalently coupling Goat anti-Human IgG (Fc) antibodies to the surface of a GLC chip (BioRad) using the manufacturer instructions for

amine-coupling chemistry. Approximately 5,000 RU (response units) of antibody were immobilized. The kinetic experiments were performed at 25 ºC in running buffer (PBS, 0.01% P20, 0.01% BSA). 1:3 serial dilutions of human CD27 ECD from, starting at 300 nM were prepared in running buffer. About 350 RU of mAb were captured on each channel of the sensor chip. An isotype-matched antibody control was immobilized in channel 6 and used as a reference surface. Capture of mAb was followed by three minutes injection (association phase) of antigen at 30 uL/min, followed by 10 minutes of buffer flow (dissociation phase). The chip surface was regenerated by injection of 0.85% phosphoric acid at 100 uL/min. Data was processed on the instrument software. Double reference subtraction of the data was performed by subtracting the curves generated by buffer injection from the reference-subtracted curves for analyte injections. Kinetic analysis of the data was performed using 1:1 Langmuir binding model with global fit. The result for each mAb was reported in the format of K_a (On-rate), K_d (Off-rate), K_D (Equilibrium dissociation constant), and percent activity. The affinities of the C2177 HFR variants were similar to the parent M40 mAb, showing less than a threefold change in K_D for all variants. Similarly, the affinities of the HFR C2191 human mAb variants showed less than a two-fold difference from the parent M41 mAb.

The bioactivity of the HFR variants was measured by their inhibition of CD70-mediated induction of NFkB in a Luciferase reporter assay. HEK cells were transfected with an NFkB inducible luciferase expression vector pGL4-32-NFkB-Luc2 (Promega), and CD27 expression plasmid or empty vector and incubated overnight in Freestyle expression medium (Gibco, #12338). The next day cells were plated in 96-well culture plates in 40 uL, and 50,000 cells per well. Then, 40 uL antibodies or controls were added to cells using a serial dilution of 1:3, starting at 30 ug/ml final in-well concentration, and incubated for 1 to 2 hours. During this incubation, CD70 episomal cells are prepared for stimulation. Briefly, adherent cells were resuspended using standard cell culture techniques and incubated for 1 hour with Mitomycin C at 25 ug/mL to stop cell expansion. After incubation, CD70+ cells were washed in medium, diluted, and 40 uL was added at 10,000 cells per well. The plates were incubated overnight. The next day Steady Glo reagent (Promega) was prepared per the manufacturer's instructions and 120 uL was added per well. Plates were incubated at room temperature for 20 minutes while shaking. Luminescence was measured on a Perkin Elmer

Envision Reader. The IC_{50}s of the C2177 HFR variants were similar to each other and to M40 parental MAb, ranging from 0.11 nM to 0.21 nM. The IC_{50}s of the C2191 HFR variants also were similar to each other and to the M41 parent, varying from 0.13 nM to 1.39 nM.

Consideration of affinity, bioactivity and biophysical properties led to the selection of the C2177 variant M69, comprised of the variable regions H28 (SEQ ID NO: 111) and L35 (SEQ ID NO: 82), and the C2191 variant M91, comprised of the variable regions H31 (SEQ ID NO: 131) and L42 (SEQ ID NO: 140), for affinity maturation. A summary of K_Ds, purification yield, binding to CD27 ECD for the cell culture supernatants ("ELISA"), and inhibition (IC_{50}) of CD27 mediated NFκβ response by CD70 for the M40 parent and its M69 HFR variant and for the M41 parent and its M91 variant are shown in Table 4.

Table 4.

Protein ID	VH	VL	Proteon KD (nM)	Yield (mg)	ELISA signal	NFkB IC50 (nM)
C2177 parent M40	H7	L18	0.96	n.d.	1.00	0.14
M69	H28	L35	0.77	10.08	1.09	0.11
C2191 parent M41	H10	L20	10.3	n.d.	1.00	0.30
M91	H31	L42	7.9	5.64	1.09	0.28

EXAMPLE 8: OPTIMIZATION OF C2177 HFR MAB M69

M69 has an affinity around 1 nM to human CD27 ECD and contains the same CDRs as C2177, and the HFA parent CD27M40. Optimization of M69 involved multiple libraries to increase affinity and remove PTM sites introduced or identified in the process.

As described in Example 9, a parallel phage display library approach for HFR and optimization of C2177 identified diversity in the proline at position 52a of CDR-H2. This position was not randomized in the library design. The co-structure of the C2177 and C2191 Fabs with CD27 (Example 5) indicates that P52a is not directly involved in antigen binding. Nevertheless, mutation at this position could alter the CDR-H2 loop conformation and enable

more optimal interactions with CD27 by surrounding residues D27. Thus, a library was designed to randomly diversify P52a and its neighboring residues Y52, G53 and D54 using NNK mutagenesis (library C27H28L2). Also in the path 2 optimization, a Y32 to F mutation in CDR-L1 showed improved binding. Thus, a second library was designed with random diversity in Y32 together with diversity in residues Y30a, D30d, A50, which lie in the same structural plane as Y32 (library C27L35L2).

In addition, the complete CDR-H3 and CDR-L1 loops were evaluated using libraries of limited diversity. Tables 5 and 6 show the design of these libraries.

Table 5. Limited diversity affinity maturation design for CDR-H3 (C27H28L3)

VH Parent amino acid and position	**Diversity**
Ser95	A,S
Asp96	A,D
Tyr97	A,D,S,Y
Tyr98	A,D,S,Y
Gly99	A,G
Asp100	A, D
Tyr100a	A,D,S,Y
Gly100b	A,G
Phe100c	A,F,S,V
Ala101	A,G
Tyr102	A,D,S,Y

Table 6. Limited diversity affinity maturation design for CDR-L1 (C27L35L3)

VH Parent amino acid and position	**Diversity**
Lys24	A,K,E,T
Ala25	A,G

Ser26	A,S
Gln27	A,Q,E,P
Ser28	A,S
Val29	A,V
Asp30	A,D
Tyr30a	A,D,S
Ala30b	A,G
Gly30c	A,G
Asp30d	A,D
Ser31	A,S
Tyr32	A,D,S
Met33	A,M,T,V
Asn34	A,N,D,T

Fab libraries were constructed in a pIX phage Fab display system as described in WO2009/085462, Shi et al, J Mol Biol 397: 385-396 (2010), and Tornetta et al. J Immunol Methods 360: 39-46 (2010) with minor modifications to restriction enzyme sites. These libraries were panned against biotinylated CD27-ECD according to panning schemes known in the art, such as described in WO2009/085462 and in Shi et al, J Mol Biol 397: 385-396 (2010), directed to increasing affinity by selecting for a slower off-rate or faster on-rate. Phage was produced by helper phage infection. Binders were retrieved by addition of beads to form a bead/antigen/phage complex. After the final wash, phage was rescued by infection of exponentially growing TG-1 Escherichia coli cells. Phage was again produced and subjected for additional rounds of panning.

For follow-up screening, DNA was prepared from glycerol stocks of phage panning rounds and the pIX gene was excised by NheI/SpeI digestion. After ligation, the DNA was transformed into TG-1 cells and grown on LB/Agar plates overnight. The next day, colonies were picked, grown overnight, and the cultures used for (i) colony PCR and sequencing of the V- regions, and (ii) induction of Fab production. For Fab production, the overnight culture was diluted 10-100 fold in new media and grown for 5-6 hours at 37 degrees C. Fab production was induced by the addition of fresh media containing IPTG and the cultures were

grown overnight at 30 degrees C. The following day, the cultures were spun down and the supernatants, containing the soluble Fab proteins, were used for Fab ELISA. For the ELISA, the soluble Fab proteins were captured onto plates by a polyclonal anti-Fd(CH1) antibody. After washing and blocking, biotinylated human CD27 ECD was added at 0.2 nM concentration. This concentration enables ranking of the Fab variants, defined as percent binding of the parent, in which the parent Fab, present as a control in all plates, is defined as 100% binding. The biotinylated CD27 ECD was detected by HRP-conjugated streptavidin and chemiluminescence read in a plate reader. At this concentration of CD27, ranking of the Fab variants, normalized to the parent Fab, is possible. By this criterion, 10 heavy and 6 light chains binding human CD27 at 100% or higher relative to M69 Fab were selected.

From the CDR-H2 library (C27H28L2), the parental Y was predominantly selected at position 52 indicating preference for this residue. At position 52a, P was replaced with A, S, V, and G residues among the Fabs with the best binding activity. At position 53, the parental G was selected along with R and N. At position 54, only the parental D was recovered. Nine clones from this library (Table 7) were subcloned into IgG vectors for expression and characterization as mAbs.

Table 7. Nine VH clones selected from full diversity library C27H28L2

Peptide ID	**Y52**	**P52a**	**G53**	**D54**
H237	F	V	R	D
H238	Y	V	G	D
H239	Y	A	G	D
H240	Y	A	R	D
H241	Y	G	R	D
H242	Y	A	N	D
H243	Y	G	G	D
H244	Y	S	G	D
H245	Y	S	R	D

For the CDR-H3 library (C27H28L3), the only diversity recovered was S95A and A101G. One clone from this library containing both mutations (Table 8) was subcloned into the IgG vectors for expression and characterization as a mAb.

Table 8. Single VH clone selected from C27H28L3

Peptide ID	**S95**	**D96**	**Y97**	**Y98**	**G99**	**D100**	**Y100a**	**G100b**	**F100c**	**A101**	**Y102**
H236	A	D	Y	Y	G	D	Y	G	F	G	Y

For the four position L-CDR1 library (C27L35L2), position 30a showed enrichment of the parental Y and W. At position 30d, residues S, H, and E were enriched along with the parental D. At position 32, the parental Y was replaced with F and W. At position 50, T was preferred over the parental A. In general, the best clones had more hydrophobic side chains compared to parent. Five clones from this library (Table 9) were subcloned into IgG vectors for expression and characterization as mAbs.

For the complete CDR-L1 library with limited diversity (C27L35L3), only one sequence was recovered, with the only difference from parent being Y32 changed to F, similar to the four position VL library above. This complete CDR-L1 library did not include an F in position 32, and thus the recovered clone was likely a contaminant from the four position VL library. This clone (L255) was subcloned into the IgG vectors for expression and characterization as a mAb (Table 9).

Table 9. Six VL clones selected from C27L35L1 and C27L35L2

Peptide ID	**Y30a**	**D30d**	**Y32**	**A50**
L255	Y	D	F	A
L256	Y	D	W	V
L257	Y	D	W	T
L258	Y	S	F	T

L260	W	H	W	T
L261	Y	S	F	E

The 6 variant light chains were paired with the 10 variant heavy chains to give 60 combinations that were expressed HEK293E cells. Supernatants were screened for expression level, binding to human CD27 ECD as measured by ELISA, and affinity as measured on a ProteOn instrument. The expression level of all variants was sufficient for screening purposes. Affinity was increased up to 40-fold for some variants. Two mAbs M596 and M600, were selected for further mutagenesis to remove potential sites of post-translational modification. The VH and VL chain combinations for these mAbs are given in Table 10. The antibodies differ by only two residues in their light chains.

Table 10. Heavy and light chain pairing of selected C2177 affinity matured leads

Antibody ID	Light Chain Peptide ID	CDR-L1 (SEQ ID NO)	CDR-L2 (SEQ ID NO)	Heavy Chain Peptide ID	CDR-H2 (SEQ ID NO)
M596	L257	KASQSVDYAGDS**W**MN (26)	**T**ASNLES (39)	H239	RIY**A**GDGDTN (residues 1-10 of 15)
M600	L255	KASQSVDYAGDS**F**MN (25)	AASNLES (37)	H239	RIY**A**GDGDTN (residues 1-10 of 15)

M596 differs from the parent molecule, M69, at three positions: P52aA in CDR-H2, Y32W in CDR-L1, and A50T in CDR-L2. M600 differs from M69 at two positions: P52aA mutation in CDR-H2 and Y32F in CDR-L1.

Three shared potential post-translational modification sites were identified in M596 and M600. There is a potential N-linked glycosylation site at position N58 in CDR-H2 and two potential isomerization sites in CDR-H2 and CDR-L1 encoded by “DG” and “DS,” respectively. In addition, M596 contains a non-germline tryptophan residue in CDR-L1 that

could be susceptible to oxidation.

To remove the glycosylation risk, three individual single substitutions were created at N58 and one at S60 (Table 11). The constructs were expressed in HEK293E cells and supernatants were evaluated for affinity to CD27 using the ProteOn instrument. All of the variants had affinities close to those of the parents which were 25 pM and 49 pM for M596 and M600, respectively. Variants M680 and M678, both derived from M600, were selected for evaluation of further substitutions to eliminate the isomerization sites. Variants M680 and M678 have A at positions 60 and 58, respectively, and have the additional advantage of lacking the tryptophan in CDR-L1 that was present in the M596 parent.

Table 11.

VH Parent amino acid and position	**Diversity**
Asn58	N, A, R, T
Ser60	S, A

To evaluate the impact of mutating the potential isomerization sites in M678 and M680, a small library was designed to remove both sites in parallel. Each mutation was substituted individually into CDR-H2 of the heavy chains or CDR-L1 of the common light chain and then paired in a combinatorial library. The diversity of this library is shown in Table 12.

Table 12

VH Parent amino acid and position	**Diversity**
Asp54	D, E
Gly55	G, A
VL Parent amino acid and position	**Diversity**
Asp34	D, E

These mAbs were expressed and affinity was evaluated as for the glycosylation site variants. The D34E mutation in the CDR-L1 potential isomerization site led to a consistent two-fold increase in affinity and therefore this site was successfully removed. The D54E mutation in the CDR-H2 potential isomerization site lowered the affinity more than tenfold. However, the G55A mutation did not significantly affect the affinity. Variants M703 and M706 retain the affinity of the M600 parent and have a reduced risk of impact on function from PTM. Table 13 shows the selected variants from each stage of the PTM-risk assessment, their heavy and light chain pairing, affinity, and sequence modifications in the CDRs. The mutation selected to remove the potential glycosylation site is underlined. The mutations to remove the two potential isomerization sites are bolded and double underlined.

Table 13.

mAb ID	VH	VL	KD (pM)	CDR-H2 (SEQ ID NO)	CDR-L1 (SEQ ID NO)
M596	H239	L257	25	RIYAGDGDTNYSPSFQG (165)	KASQSVDYAGDSWMN (26)
M600	H239	L255	49	RIYAGDGDTNYSPSFQG (165)	KASQSVDYAGDSFMN (25)
M678	H259	L255	53	RIYAGDGDT<u>A</u>YSPSFQG (166)	KASQSVDYAGDSFMN (25)
M680	H260	L255	30	RIYAGDGDTNY<u>A</u>PSFQG (167)	KASQSVDYAGDSFMN (25)
M703	H270	L267	28	RIYAGD<u>**A**</u>DT<u>A</u>YSPSFQG (168)	KASQSVDYAG<u>**E**</u>SFMN (29)

M706	H272	L267	13	RIYAGD**A**DTNY**A**PSFQG (169)	KASQSVDYAG**E**SFMN (29)

EXAMPLE 9: COMBINDED HFR AND OPTIMIZATION OF C2177 MAB

In this approach, a limited set of HFR variants were evaluated in a Fab format for expression, pIX display and binding and the best candidates were then advanced into optimization. CDRs from C2177 were human framework adapted into two heavy chains VH5-51 (SEQ ID NO: 102 H24) and VH1-46 (SEQ ID NO: 106 H25) and two light chains Vk4-1 and Vk012 (SEQ ID NO: 90 L36). These HFA variable domains were paired together in a 2x2 matrix as Fabs with human CH1 and Ck constant regions in the Fab pIX phage display vector. The VH1-46/Vk012 variant (M55, H25/L36) showed binding to CD27 and good display characteristics and was selected for construction of affinity maturation libraries.

The Fab libraries for pIX phage display were constructed as described above for Example 8. Based on the experimental co-structure of CD27 with C2191 and C2177 (Example 5), diversity libraries were designed in CDR residues in and around the antibody paratope. The emphasis on variation was in CDRs L1, L3, and H2. A total of 4-6 residues within an individual CDR were diversified with an NNK codon, encoding for all 20 amino acids. The size of each library was estimated to $\leq 6 \times 10^7$ variants, which can be covered using standard library restriction endonuclease cloning techniques. Table 14 shows the residues that were subjected to full diversification in the different CDR libraries.

Table 14: Affinity maturation library design for C2177

VH CDR	**Parent amino acid and position**
CDR-H2	Y52
	G53
	D54
	D56
	N58
CDR-H3	Y97
	Y98
	D100

	Y100a
VL CDR	**Parent amino acid and position**
CDR-L1	Y30a
	A30b
	G30c
	D30d
	Y32
CDR-L3	Q90
	N92
	E93
	D94
	Y96

Fab libraries displayed on phage coat protein IX were panned against biotinylated hCD27ECD/Fc. Phage was produced by helper phage infection of a plasmid library of the variants. Binders were retrieved by addition of streptavidin-coated magnetic beads to form a bead/antigen/phage complex. After the final wash, phage was rescued by infection of exponentially growing MC1061F' Escherichia coli cells. Phage was again produced and subjected for additional rounds of panning. Soluble Fab from selected clones was produced and evaluated for binding activity as described about for Path 1. Hits were obtained only from the CDR-L1 and CDR-L2 libraries. Twenty-one clones from these two libraries demonstrated binding greater than that of the parent HFR Fab. Clones containing C or M in the diversified sequences were discarded. Ten Fabs were converted for expression in a IgG4SPAAa/kappa background for further characterization. The IgG4PAA heavy chain is human IgG4 containing a serine to proline substitution in the hinge region (Angal et al., Mol Immunol 30: 105 (1993) and alanine substitutions at two positions in CH2 (ML Alegre et al, Transplantation; 57: 1537-43 (1994)). The mAbs were produced in HEK293E cells as replicas of the Fabs and as a matrix of heavy and light chain combinations. Affinity was measured on a ProteOn instrument using the culture supernatants (Table 15). Mutation of P52a to Q (M158) or S (M157) in CDR-H2 decreased the K_D 6-fold compared to that of the parental mAb (M159). The Y36F mutation in CDR-L1 (M149) decreased K_D 4-fold and the

addition of G33H and D34E mutations (M155) led to a 6-fold decrease in K_D. Combination of the P52S mutation with eitherY36F (M160) or Y36F plus G33H and D34E decreased the K_D 20-fold to 100 pM. The combinations of substitutions in M158, M160 and M166 were selected for further characterization.

Table 15: Initial panel of mAbs derived from Fab maturation libraries

Protein DNA ID	**H&L**	**CDR-H2 (SEQ ID NO)**	**CDR-L1 (SEQ ID NO)**	**K_D (nM)**
M149	H25,L219	RIYPGDGDTNYNGKFKG (3)	KASQSVDYAGDS**F**MN (25)	0.54
M150	H25,L218	RIYPGDGDTNYNGKFKG (3)	KASQSVDY**F**GDS**L**MN (32)	4.04
M151	H25,L224	RIYPGDGDTNYNGKFKG (3)	KASQSVDY**YNS**S**F**MN (36)	1.07
M152	H25,L223	RIYPGDGDTNYNGKFKG (3)	KASQSVDY**WS**DS**F**MN (35)	1.54
M153	H25,L222	RIYPGDGDTNYNGKFKG (3)	KASQSVDY**V**G**T**S**F**MN (34)	1.41
M154	H25,L221	RIYPGDGDTNYNGKFKG (3)	KASQSVDY**FRT**S**F**MN (33)	1.56
M155	H25,L217	RIYPGDGDTNYNGKFKG (3)	KASQSVDYA**HE**S**F**MN (31)	0.37
M156	H25,L216	RIYPGDGDTNYNGKFKG (3)	KASQSVDY**FSE**S**F**MN (170)	0.71
M157	H197,L220	RIY**Q**GDGDTNYNGKFKG (22)	KASQSVDYAGDSYMN (24)	0.39
M158	H196,L220	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDYAGDSYMN (24)	0.36
M159	H25,L220	RIYPGDGDTNYNGKFKG (3)	KASQSVDYAGDSYMN (24)	2.23

M160	H196,L219	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDYAGDS**F**MN (25)	0.12
M161	H196,L218	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDY**F**GDS**L**MN (32)	1.34
M162	H196,L224	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDY**YNS**S**F**MN (36)	0.43
M163	H196,L223	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDY**WS**DS**F**MN (35)	0.30
M164	H196,L222	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDY**V**G**T**S**F**MN (34)	0.27
M165	H196,L221	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDY**FRT**S**F**MN (33)	0.18
M166	H196,L217	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDYA**HE**S**F**MN (31)	0.10
M167	H196,L216	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDY**FSE**S**F**MN (170)	0.91

M158, M160 and M166 proteins were produced in 750mL cultures of HEK293 cells, purified, and analyzed for binding kinetics to CD27-His on Biacore. The K_D values were about 1 log higher than those measured by ProteOn with crude supernatants but showed the same values relative to each other (Table 16).

Table 16

mAb ID	k_{on} **($M^{-1}s^{-1}$)**	k_{off} **(s^{-1})**	**K_D (pM)**
M158	(1.5±0.03)E+06	(6.6±1.7)E-04	439±114
M160	(1.6±0.15)E+06	(1.8±0.01)E-04	117±11
M166	(1.62±0.04)E+06	(2.03±0.16)E-04	126±11

When re-evaluating the original HFA combinations, the VH5-51 adapted VH showed a 2-fold lower K_D than the VH1-46 scaffold. The P52aS mutation in H-CDR2 was

introduced into the VH5-51 VH creating H221. H221 was expressed with the L220, L219 and L217 light chains from the HFA parental mAb (M159) and the affinity improved variants M160 and M166, respectively, to generate mAbs M171, M169 and M170. BIAcore kinetic measurements on the purified mAbs showed a two-fold improvement in K_D in comparison to the corresponding VH1-46 variants (compare Tables 16 and 17).

Table 17

mAb ID	**H/L Peptide ID**	**K_{on} Ave ($M^{-1}s^{-1}$)**	**K_{off} Ave (s^{-1})**	**K_D Ave (pM)**
M169	H221,L219	(1.48± 0.13)E+06	1.02 ± 0.07)E-04	69 ± 8
M170	H221,L217	1.66E+06	1.16E-04	70
M171	H221,L220	1.53E+06	3.58E-04	234

Sequence analysis of M160, M169 and M170 identified a potential isomerization site at D54-G55 and a potential deamidation site at N61-G62 in CDR-H2 of H196 and H221. Additionally, a potential isomerization site was identified at D34 within CDR-L1 of L219. Mutations were introduced to remove these sites and evaluated for their impact on activity (Table 18). Purified mAbs were analyzed for affinity to CD27-His on ProteOn. The mutations either had no effect or a positive effect on K_D. For example, both M668 and M671 had almost 2 fold lower K_Ds than their parental mAbs, M160 and CM169, respectively.

Table 18: mAb variants with PTM sequences mutated

Parent mAb	**mAb ID**	**H/L Peptide ID**	CDR-H2 (SEQ ID NO)	CDR-L1 (SEQ ID NO)	**k_{on} Ave ($M^{-1}s^{-1}$)**	**k_{off} Ave (s^{-1})**	**K_D Ave (pM)**
M160	M160	H196,L219	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDYAGDS**F**MN (25)	2.07E+06	2.23E-04	108
M166	M166	H196,L217	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDYA**HE**S**F**MN (31)	1.84E+06	2.34E-04	127
M169	M169	H221,L219	RIY**S**GDGDTNYNGKFKG (19)	KASQSVDYAGDS**F**MN (25)	2.36E+06	1.39E-04	59
M160	M668	H255,L266	RIYSGD**A**DTNYA**Q**KFKG (20)	KASQSVDYAG**E**SFMN (29)	2.14E+06	1.29E-04	60
M160	M669	H256,L266	RIYSGD**A**DTNYN**Q**KFKG (21)	KASQSVDYAG**E**SFMN (29)	2.49E+06	1.45E-04	58

M169	M670	H257,L266	RIYSGD**A**DTNY**AQ**KFKG (20)	KASQSVDYAG**E**SFMN (29)	1.73E+06	1.13E-04	65
M169	M671	H258,L266	RIYSGD**A**DTNYN**Q**KFKG (21)	KASQSVDYAG**E**SFMN (29)	2.52E+06	9.73E-05	39
M166	M672	H255,L217	RIYSGD**A**DTNY**AQ**KFKG (20)	KASQSVDYAGESFMN (29)	1.83E+06	2.64E-04	144
M166	M673	H256,L217	RIYSGD**A**DTNYN**Q**KFKG (21)	KASQSVDYAGESFMN (29)	1.89E+06	1.89E-04	100

EXAMPLE 10: OPTIMIZATION OF C2191 HFR MAB M91

The methods applied for the optimization of the M91 (H31/L42) were as described in Example 8, except as noted. An alanine/germline scan of the CDRs of C2191 was carried out in a Fab format to evaluate positions important for interaction with CD27, using the C2191 parent VH and VL regions in a Fab format with human Ch1 and Ck constant regions. The libraries replaced the residues in the CDRs with alanine or the residue present in the corresponding germline sequence. Some positions in the CDRs were excluded as they had low or no solvent exposure based on modeling and subsequently on the determined structure (Example 5). Putative somatic mutations were back-mutated to mouse germline amino acids to assess their contribution to antibody affinity. Briefly, the mouse V regions were cloned into the Fab pIX display vector and the binding of the parent to biotinylated human CD27-ECD protein was verified by ELISA. Single mutations (according to the library design) were introduced by site-directed mutagenesis, performed essentially as described by Stratagene (La Jolla, CA, USA). Sequence confirmed mutants were cherry-picked into new plates and grown together with parental Fab and negative control Fabs. The final single amino-acid-substitution variants were generated in *E. coli*, and then screened for expression and CD27 binding by ELISA. The expression and binding signals for the parent clones were averaged and set to 1.0 and the signals of the mutants were normalized relative to the parent. Two forms of antigen were used in the ELISAs: CD27 ECD(1-173 residues) and CD27 ECD-Fc chimera (R&D Systems).

The results of this scan coupled with co-crystal structure provided the basis for design of affinity maturation libraries. For the heavy chain; the positions selected for variation were T33 in H-CDR1 and Y50, S52, S52a, N56 and Y58 in CDR-H2 (Table 23). T33 is not a contact residue but a T33A mutation improved binding. Positions S52 and S52a are not

contact residues but the substitutions in the scan showed some increased binding. The tyrosines at positions 50 and 58 are both contact sites and substitutions at these sites were selected in the parallel optimization path described in Example 11. Position N56 was not evaluated in the alanine/germline scan but it is a contact site and adjacent to T33, S52, and S52a in the crystal structure. For the light chain, the positions selected for variation were T30a, S30b, G30c and Y30d in CDR-L1 and L50 and N53 in CDR-L2 (Table 23). None of these residues contact antigen directly but are adjacent to residues that are in contact which the scan showed had substantial negative impact on binding. A L50A mutation had a moderate effect on binding and, in the crystal structure, is the only residue in CDR-L2 likely in contact with antigen. In addition, N53 was selected for limited diversification. Two parallel libraries were constructed, one with Y30d mutated to W, and another with Y30d kept as Y, since W could make the paratope more hydrophobic and thus less developable. Tables 19 and 20 below show the VH and VL affinity maturation library designs for M91.

Table 19.

2191	HC_CDR1	HC_CDR2				
Antigen contact?	No	Yes	No	No	Yes	Yes
Position in HC (SEQ ID NO: 131)	T33	Y50	S52	S52a	N56	Y58
Position in HC (SEQ ID NO: 131)	T33	Y50	S52	S53	N57	Y59
Sequence Diversity	All 20 amino acids	Y	All 20 amino acids	All 20 amino acids	All 20 amino acids	Y
		A				I
		W				L
		H				W

Table 20.

2191	LC_CDR1				LC_CDR2	
Antigen contact	No	No	No	Yes	Yes	No
Position in LC (SEQ ID NO: 140)	T30a	S30b	G30c	Y30d	L50	N53
Position in LC (SEQ ID NO: 140)	T31	S32	G33	Y34	L54	N57
Sequence Diversity	All 20 amino acids	All 20 amino acids	G	Y*	All 20 amino acids	N
			R	W*		K
						R

* Two separate libraries containing either Y or W at this position were created

Fab libraries displayed on phage coat protein IX were panned against biotinylated CD27-ECD. A total of 12 heavy and 12 light chain variants were selected that bound to CD27 equally or better than the parental chimeric Fab of C2191. The variants were converted to IgG1/kappa antibodies, produced in HEK293E cells as 144 combinations, and culture supernatants were evaluated for binding by ProteOn. Significant increases (>100-fold) in affinity were observed for some variants. Of the 144 VH and VL pairings, 8 were selected for further characterization (Table 21). These mAbs were classified into three sub-groups: Group 1 variants have the same heavy chain (H227, SEQ ID NO: 133) paired with four different light chains, while Group 2 and Group 3 each have one light chain paired with two different heavy chains. The four selected light chains varied at all four positions diversified in CDR-L1 (RASKSVS$X_1X_2X_3X_4$SFMH) (SEQ ID NO: 158); where X_1 is A, E, H, or L; X_2 is D, G, V, or W; X_3 is G or R; and X_4 is W or Y). They also varied in both positions diversified in CDR-L2 (X_1ASX_2LES) (SEQ ID NO:171); where X_1 is L or V; and where X_2 is K, N, or R). CDR-L3 was unaltered from the L42 sequence (SEQ ID NO: 140) and is QHSRELPWT.

Table 21. Pairing of heavy and light chain sequences of selected 2191 affinity matured leads

Antibody ID	Light Chain Peptide ID	CDR-L1 (SEQ ID NO:)	CDR-L2 (SEQ ID NO:)	Heavy Chain Peptide ID	CDR-H1 (SEQ ID NO:)	CDR-H2 (SEQ ID NO:)
M427	C27L244	RASKSVS**AW**GYSFMH (60)	**V**AS**R**LES (68)	C27H227	GFTFSSY**G**MS (44)	YI**DE**GGG**Q**T**I**YPDSVKG (47)
M429	C27L245	RASKSVS**HVRW**SFMH (61)	LAS**K**LES (69)	C27H227	GFTFSSY**G**MS (44)	YI**DE**GGG**Q**T**I**YPDSVKG (47)
M488	C27L249	RASKSVS**EGRW**SFMH (62)	**V**AS**R**LES (68)	C27H227	GFTFSSY**G**MS (44)	YI**DE**GGG**Q**T**I**YPDSVKG (47)
M489	C27L250	RASKSVS**LDRW**SFMH (63)	LASNLES (67)	C27H227	GFTFSSY**G**MS (44)	YI**DE**GGG**Q**T**I**YPDSVKG (47)
M492	C27L249	RASKSVS**EGRW**SFMH (62)	**V**AS**R**LES (68)	C27H228	GFTFSSY**S**MS (45)	YI**DA**GGG**F**T**I**YPDSVKG (48)
M493	C27L250	RASKSVS**LDRW**SFMH (63)	LASNLES (67)	C27H228	GFTFSSY**S**MS (45)	YI**DA**GGG**F**T**I**YPDSVKG (48)
M501	C27L250	RASKSVS**LDRW**SFMH (63)	LASNLES (67)	C27H231	GFTFSSY**S**MS (45)	**H**I**DA**GGG**R**T**W**YPDSVKG (49)
M526	C27L249	RASKSVS**EGRW**SFMH (62)	**V**AS**R**LES (68)	C27H222	GFTFSSY**G**MS (44)	YI**DR**GGG**V**T**I**YPDSVKG (50)

These eight variants were produced by transient expression in HEK293E cells in a volume of 750 ml. The harvested supernatants were purified via Protein A chromatography, and each variant was analyzed by SDS-PAGE and SE-HPLC to determine purity of the sample and percentage of monomer in the purified sample. All of the variants were greater than 90% pure and greater than 90% monomeric. To evaluate association properties of the antibodies, retention factors (k') were determined by performing cross-interaction chromatography for each purified variant (Jacobs SA, Wu SJ, Feng Y, Bethea D & O'Neil KT (2010) Cross-interaction chromatography: a rapid method to identify highly soluble monoclonal antibody candidates. *Pharm Res* **27**, 65-71). In this method, sample antibodies were passed through a column coupled with human IgG and evaluated for retention relative to control antibodies. Briefly, 50 mg of human IgG (Sigma Aldrich) were coupled to a 1mL NHS-Sepharose column (GE Healthcare) following the manufacturer's instructions. Uncoupled IgG was removed by washing with 0.1M Tris, pH 8, 0.5M NaCl and unreacted NHS groups were blocked with the same buffer. The coupling efficiency was determined by

measuring the protein concentration remaining in the unreacted coupling buffer and washes using Pierce's Coomassie Plus Assay Kit (Thermo Pierce) and subtracting from the amount of protein before immobilization. A control column was also prepared using the same protocol but without conjugation of IgG to the resin. The control column was run first on a Dionex UltiMate 3000 HPLC after being equilibrated with PBS, pH 7at a flow rate of 0.1mL/min. 20µL of the stock protein solution was injected first to ensure non-specific binding sites were blocked followed by 20 µL of 10% acetone to check the integrity of the column. Samples to be analyzed were diluted to 0.1mg/mL in PBS, pH 7. 20 uL of each sample was injected onto each column and allowed to run at 0.1mL/min for 30min. Retention times were recorded and the retention factor (k') was calculated for each variant. The k' value was calculated as the difference in the retention times on the IgG and blank columns. All of the variants were purified to greater than 90% purity based on SDS-PAGE and SE-HPLC. All k' values were calculated to be less than 0.3, indicative of good solution properties (Table 22).

Table 22. Batch analysis of purified affinity matured variants

Antibody ID	HC	LC	Conc. (mg/ml)	Total Protein	% Monomer	Gel	k'
M427	H227	L244	1.42	15.63	100	ok	0.02
M429	H227	L245	0.97	10.21	100	ok	0.07
M488	H227	L249	2.00	27.06	98.9	ok	0.07
M489	H227	L250	1.59	23.03	100	ok	0.17
M492	H228	L249	2.07	27.96	97.4	ok	0.25
M493	H228	L250	0.58	8.12	100	ok	0.24
M501	H231	L250	2.01	26.08	100	ok	0.28
M526	H222	L249	0.90	10.74	100	ok	0.10

The eight variant mAbs and the HFR parent were evaluated for their affinity to CD27 ECD by BIAcore and their IC_{50} in the κβ-reporter assay. Kinetic constants and affinity were measured by BIAcore. Table 23 summarizes the data collected on these variants. The expression and ELISA signal for binding to CD27 as measured from the initial small culture supernatants are also included in this table.

Table 23: C2191 AM library subset data summary

Protein ID	Expression (ug/ml	ELISA signal	NFκβ IC_{50} (pM)	k_a	k_d	K_D (pM)
M41	n.d.	n.d.	45	6.20E+05	8.44E-03	13650
M427	14.6	0.93	19	7.18E+05	3.00E-05	41.7
M429	16.1	0.86	42	6.62E+05	2.02E-05	30.4
M488	20.7	0.92	23	1.01E+06	3.96E-05	39.4
M489	24.2	0.96	41	9.06E+05	2.18E-05	24.1
M492	20.1	0.77	15	1.03E+06	1.47E-04	142.0
M493	24.5	0.85	14	7.23E+05	1.05E-04	145.0
M501	30.9	0.77	4	8.31E+05	6.16E-05	74.2
M526	14.3	0.69	6	1.00E+06	1.71E-04	171.0

EXAMPLE 11: COMBINDED HFR AND OPTIMIZATION OF C2191 MAB

In this approach, a limited set of HFR variants were evaluated in a Fab format for expression, pIX display and binding and the best candidates were then advanced into optimization. CDRs from C2191 were human framework adapted into two heavy chains (VH3-23 and VH3-11) and two light chains (Vk4-1 and Vk012). These HFA variable domains were paired together in a 2x2 matrix as Fabs with human CH1 and Ck constant regions in the Fab pIX phage display vector. The VH3-23/Vk012 variant (H39 (SEQ ID NO: 145)) and L40 (SEQ ID NO: 137) showed binding to CD27 and good display characteristics and was selected for construction of affinity maturation libraries. This Fab is referred to as "parent."

For selection of antibodies with improved affinity, multiple residues in all CDRs except CDR-H3 of H39 were fully diversified using NNK degenerate codons (Table 24). Each CDR library was constructed separately and subjected to phage panning for selection of affinity matured variants.

Table 24: Affinity maturation library design for C2191 variants

VH CDR	**Parent amino acid and position**
CDR-H2	Y50
	S52
	S53
	N56
	Y58
CDR-H3	H95
	R96
	G97
	N98
	P99
VL CDR	**Parent amino acid and position**
CDR-L1	T30a
	S30b
	G30c
	Y30d

	F32
CDR-L2	L50
	A51
	S52
	N53
	L54
	E55
	S56
CDR-L3	H90
	R92
	E93
	L94
	Y96

The C2191 libraries with diversity in CDR-H2, CDR-L1 or CDR-L2 yielded 50 unique Fabs with improved binding to human CD27 relative to the parental HFR Fab as measured in the single point ELISA. These clones were further ranked in a multi-point ELISA and seventeen clones were selected for conversion to human IgG4alaala/kappa for further characterization (Table 25).

Table 25: Affinity matured Fabs selected for conversion to IgG

Fab ID	**HC Peptide ID**	**LC Peptide ID**	**CDR-H2 (SEQ ID NO:)**	**CDR-L1 (SEQ ID NO:)**	**CDR-L2 (SEQ ID NO:)**
Parent	H39	L40	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F116	H39	L59	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	VGNRLED (70)
F119	H39	L62	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	VGDRRQE (71)
F178	H145	L40	YISGGGGQTLYPDSVKG (54)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F18	H40	L40	AIDHGGGRTYYPDSVKG (51)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F19	H41	L40	AIDHGGGRTWYPDSVKG (52)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F243	H39	L124	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	VGSRMAF (72)
F250	H39	L131	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	VGDRANW (73)

F256	H39	L137	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	VGSRLDY (74)
F279	H39	L160	YISSGGGNTYYPDSVKG (46)	RASKSVSYVRWSFMH (64)	LASNLES (67)
F291	H39	L172	YISSGGGNTYYPDSVKG (46)	RASKSVSHIRWSFMH (65)	LASNLES (67)
F292	H39	L173	YISSGGGNTYYPDSVKG (46)	RASKSVSHVRWSFMH (61)	LASNLES (67)
F295	H39	L176	YISSGGGNTYYPDSVKG (46)	RASKSVSTSGYSFMH (59)	VADRVEV (173)
F297	H190	L40	TIDRGGGSTWYPDSVKG (55)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F298	H191	L40	AIDGGGGATYYPDSVKG (56)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F299	H192	L40	VIDHGGGSTHYPDSVKG (57)	RASKSVSTSGYSFMH (59)	LASNLES (67)
F302	H39	L179	YISSGGGNTYYPDSVKG (46)	RASKSVSLIRWSFMH (172)	LASNLES (67)
F57	H79	L40	AIDHGGGQTLYPDSVKG (53)	RASKSVSTSGYSFMH (59)	LASNLES (67)

IgG1/k mAbs were constructed as replicas of the Fabs and as a matrix of heavy and light chain variable regions (Table 26) and tested for affinity and solution properties. The mAb form of the parent Fab is denoted M131. M141 and M408 were selected for further characterization.

Table 26: mAbs derived from Fab affinity maturation

Fab ID	**mAb ID**	**H & L Peptide ID**
Parent	M131	H39, L40
F18	M132	H40, L40
F57	M133	H79, L40
F298	M134	H191, L40
F299	M135	H192, L40
F292	M136	H39, L173
F279	M137	H39, L160
F256	M138	H39, L137
Combination	M139	C27H40, L173
Combination	M140	C27H40, L160

Combination	M141	C27H79, L173
Combination	M142	C27H79, L160
Combination	M143	C27H191, L160
Combination	M144	C27H191, L173
Combination	M145	C27H192, L173
Combination	M146	H192, L160
Combination	M408	H192, L137

EXAMPLE 12: CHARACTERIZATION OF AFFINITY MATURED MABS

Selected affinity matured mAbs derived from the parental C2177 and C2191 hybridoma antibodies were codon optimized, introduced into a different vector for dual-expression of heavy and light chains, expressed in CHO-GS cell culture, and purified for further characterization. The IDs of these antibodies in relation to the matured variants described in the Examples above are shown in Table 27.

Table 27.

Parent	DNA ID	Single-gene DNA ID	LC Peptide ID	HC Peptide ID
2191	429	696	27L245	27H227
2191	492	695	27L249	27H228
2191	488	694	27L249	27H227
2191	141	707	27H79	27L173
2191	408	708	27H192	27L137
2177	703	709	27H270	27L267
2177	706	710	27H272	27L267
2177	671	711	27H258	27L266
2177	668	713	27H255	27L266

Summary data for these mAbs is shown below for the K_D analysis by Biacore and the IC_{50} measured in a NF-κβ reporter gene assay (Tables 28 and 29). For this NF- κβ reporter assay, HEK-293F cells were transfected with a total of 36 ng of DNA containing both human CD27 and luciferase constructs, under control of the NF-κβ promoter. HEK-293F transfectants were plated $5x10^4$ cells per well in 40 μL Freestyle media (Gibco) in 96-well

plates. Dilutions of anti-CD27 hybridomas mAbs were added to the assay plate in Freestyle media for a final concentration of 50 μg/mL with 1:3 dilutions and plates were incubated at 37°C (5% CO_2) for one hour. To test for ability of mAbs to neutralize CD70:CD27 signaling, terminally irradiated (4000 rads) HEK-293E CD70 episomal cells were added at 20% of the number of CD27 transfectant cells to the assay plate. To test for agonist activity of hybridoma mAbs, addition of CD70 episomal cells was omitted. Assay plates were incubated overnight at 37°C (5% CO_2) and developed using the Steady-Glo® Luciferase Assay System (Promega) according to the instructions of the manufacturer.

Table 28. Characterization of matured variants derived from C2191

mAb	**k_a (1/Ms)**	**k_d (1/s)**	**K_D (pM)**	**NF-κβ Assay IC_{50} (pM)**
C2191 parent chimera (M41)	4.35E+05	0.0103	23678	2300
M694	4.80E+05	7.60E-05	105	420
M695	5.10E+05	1.70E-04	202	250
M696	3.70E+05	2.1E-05	57	330
M707	5.85E+05	1.24E-04	213	260
M708	6.430E+05	2.25E-04	350	260

Table 29. Characterization of matured variants derived from C2177

mAb	k_a (1/Ms)	k_d (1/s)	K_D (pM)	NF- κβ Assay IC_{50} (pM)
C2177 parent chimera (M40)	1.06E+06	1.32E-03	1240	466
M709	2.30E+06	5.89E-05	26	272
M710	2.55E+06	4.88E-05	19	320
M711	1.97E+05	5.82E-05	30	258
M713	2.06E+05	1.16E-05	56	296

CDR and V Region Sequences

Table 30. 2177 path 1

VH		**CDR-H1 (SEQ ID NO:)**	**CDR-H2 (SEQ ID NO:)**	**CDR-H3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
H7	M40 parent	SSWMN (1)	RIYPGDGDTNYNGKFKG (3)	SDYYGDYGFAY (23)
		Extended CDR-H1	**Kabat-7 (+2 HFR residues to show PMT mutations)**	**Kabat**
H28	HFR(M69)	GYAFSSSWMN (2)	RIYPGDGDTNYS (4)	SDYYGDYGFAY (23)
H236		GYAFSSSWMN(2)	RIYPGDGDTNYS	**A**DYYGDYGF**G**Y (162)
H237		GYAFSSSWMN(2)	RI**FVR**DGDTNYS (5)	SDYYGDYGFAY (23)
H238		GYAFSSSWMN(2)	RIY**V**GDGDTNYS (6)	SDYYGDYGFAY (23)
H239	M596, M600	GYAFSSSWMN(2)	RIY**A**GDGDTNYS (7)	SDYYGDYGFAY (23)
H240		GYAFSSSWMN(2)	RIY**AR**DGDTNYS (8)	SDYYGDYGFAY (23)
H241		GYAFSSSWMN(2)	RIY**GR**DGDTNYS (9)	SDYYGDYGFAY (23)

H242		GYAFSSSWMN(2)	RIY**AN**DGDTNYS (10)	SDYYGDYGFAY (23)
H243		GYAFSSSWMN(2)	RIY**G**GDGDTNYS (11)	SDYYGDYGFAY (23)
H244		GYAFSSSWMN(2)	RIY**S**GDGDTNYS (12)	SDYYGDYGFAY (23)
H245		GYAFSSSWMN(2)	RIY**SR**DGDTNYS (13)	SDYYGDYGFAY (23)
H259	M678	GYAFSSSWMN(2)	RIY**A**GDGDT**A**YS (14)	SDYYGDYGFAY (23)
H260	M680	GYAFSSSWMN(2)	RIY**A**GDGDTNY**A (15)**	SDYYGDYGFAY (23)
H270	M703=M709	GYAFSSSWMN(2)	RIY**A**GD**A**DT**A**YS (16)	SDYYGDYGFAY (23)
H272	M706=M710	GYAFSSSWMN(2)	RIY**A**GD**A**DTNY**A (17)**	SDYYGDYGFAY (23)
			$RIX_1X_2X_3DX_4DTX_5YX_6$ (151)	

For SEQ ID NO:151, X_1 is F or Y; X_2 is A, G, S, OR V; X_3 is G, N, or R; X_4 is A or G; X_5 is A or N; and X_6 is A or S

Table 31. 2177 path 2

VH		**CDR-H1 (SEQ ID NO:)**	**CDR-H2 (SEQ ID NO:)**	**CDR-H3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
H7	M40 parent	SSWMN (1)	RIYPGDGDTNYNGKFKG (3)	SDYYGDYGFAY (23)
		Extended CDR-H1	**Kabat-7** (the rest of CDR is the same in mouse and HFR)	**Kabat**
H24	HFR(M50)	GYAFSSSWMN (2)	RIYPGDGDTNYNGKFKG (18)	SDYYGDYGFAY (23)
H221	M169, M170, M171	GYAFSSSWMN (2)	RIY**S**GDGDTNYNGKFKG (19)	SDYYGDYGFAY (23)
H257	M670	GYAFSSSWMN (2)	RIY**S**GD**A**DTNY**AQ**KFKG (20)	SDYYGDYGFAY (23)
H258	M671=M711	GYAFSSSWMN (2)	RIY**S**GD**A**DTNYN**Q**KFKG (21)	SDYYGDYGFAY (23)
H25	HFR (M55); M149-156; M159	GYAFSSSWMN (2)	RIYPGDGDTNYNGKFKG (18)	SDYYGDYGFAY (23)
H196	M158; M160-167	GYAFSSSWMN (2)	RIY**S**GDGDTNYNGKFKG (19)	SDYYGDYGFAY (23)
H255	M668=M713; M672;	GYAFSSSWMN (2)	RIY**S**GD**A**DTNY**AQ**KFKG (20)	SDYYGDYGFAY (23)
H256	M669; M673	GYAFSSSWMN (2)	RIY**S**GD**A**DTNYN**Q**KFKG (21)	SDYYGDYGFAY (23)
H197	M157	GYAFSSSWMN (2)	RIY**Q**GDGDTNYNGKFKG (22)	SDYYGDYGFAY (23)
			$RIYX_1GDX_2DTNYX_3X_4KFKG$ (152)	

For SEQ ID NO:152, X_1 is P, Q, or S; X_2 is A or G; X_3 is A or N; X_4 is G or Q.

Table 32. **2177 path 1**

VL		**CDR-L1 (SEQ ID NO:)**	**CDR-L2 (SEQ ID NO:)**	**CDR-L3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
L18	M40 parent mouse	KASQSVDYAGDSYMN (24)	AASNLES (37)	QQSNEDPYT (41)
L35	HFR (M69)	KASQSVDYAGDSYMN (24)	AASNLES (37)	QQSNEDPYT (41)
L255	M600; M678; M680	KASQSVDYAGDS**F**MN (25)	AASNLES (37)	QQSNEDPYT (41)
L256		KASQSVDYAGDS**W**MN (26)	**V**ASNLES (38)	QQSNEDPYT (41)
L257	M596	KASQSVDYAGDS**W**MN (26)	**T**ASNLES (39)	QQSNEDPYT (41)
L258		KASQSVDYAG**S**S**F**MN (27)	**T**ASNLES (39)	QQSNEDPYT (41)
L260		KASQSVD**W**AG**H**S**W**MN (28)	**T**ASNLES (39)	QQSNEDPYT (41)
L261		KASQSVDYAG**S**S**F**MN (27)	**E**ASNLES (40)	QQSNEDPYT (41)
L267	M703=M709; M706=M710	KASQSVDYAG**E**S**F**MN (29)	AASNLES (37)	QQSNEDPYT (41)
		KASQSVDX_1AGX_2SX_3MN (153)	X_1ASNLES (154)	

For SEQ ID NO:153, X_1 is W or Y; X_2 is D, E, S or H; X_3 is F, W, or Y.

For SEQ ID NO:154, X_1 is A, E, T, or V;

Table 33. 2177 path 2

VL		**CDR-L1 (SEQ ID NO:)**	**CDR-L2 (SEQ ID NO:)**	**CDR-L3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
L18	M40 parent mouse	KASQSVDYAGDSYMN (24)	AASNLES (37)	QQSNEDPYT (41)
L36	HFR (M55; M50)	KASQSVDYAGDSYMN (24)	AASNLES (37)	QQSNEDPYT (41)
L216	M156; M167	KASQSVDY**FSE**SYMN (30)	AASNLES (37)	QQSNEDPYT (41)
L217	M155; M166; M170; M672; M673	KASQSVDYA**HE**S**F**MN (31)	AASNLES (37)	QQSNEDPYT (41)
L218	M150; M161	KASQSVDY**F**GDS**L**MN (32)	AASNLES (37)	QQSNEDPYT (41)
L219	M149; M160; M169	KASQSVDYAGDS**F**MN (25)	AASNLES (37)	QQSNEDPYT (41)
L266	M668=M713; M669; M670; M671=M711;	KASQSVDYAG**E**SFMN (31)	AASNLES (37)	QQSNEDPYT (41)
L220	M157; M171; M158; M159	KASQSVDYAGDSYMN (24)	AASNLES (37)	QQSNEDPYT (41)
L221	M154	KASQSVDY**FRT**S**F**MN (33)	AASNLES (37)	QQSNEDPYT (41)
L222	M153	KASQSVDY**V**G**T**S**F**MN (34)	AASNLES (37)	QQSNEDPYT (41)
L223	M152; M163	KASQSVDY**WS**DS**F**MN (35)	AASNLES (37)	QQSNEDPYT (41)
L224	M151; M162	KASQSVDY**YNS**S**F**MN (36)	AASNLES (37)	QQSNEDPYT (41)

		KASQSVDYX$_1$X$_2$X$_3$MSX$_4$MN (155)		

For SEQ ID NO:155, X_1 is A, F, V, W or Y; X_2 is G, H, N, R, or S; X_3 is D, E, S, or T; X_4 is F, L, or Y.

Table 34. 2191 path 1

VH		**CDR-H1 (SEQ ID NO:)**	**CDR-H2 (SEQ ID NO:)**	**CDR-H3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
H10	M41 parent	SYTMS (42)	YISSGGGNTYYPDSVKG (46)	HRGNPFDY (58)
		Extended CDR-H1	**Kabat**	**Kabat**
H31	HFR (M91)	GFTFSSYTMS (43)	YISSGGGNTYYPDSVKG (46)	HRGNPFDY (58)
H227	M427, M429=M696; M488=M694; M489	GFTFSSY**G**MS (44)	YI**DE**GGG**Q**T**I**YPDSVKG (47)	HRGNPFDY (58)
H228	M492=M695; M493	GFTFSSY**S**MS (45)	YI**DA**GGG**F**T**I**YPDSVKG (48)	HRGNPFDY (58)
H231	M501	GFTFSSY**S**MS (45)	**H**I**DA**GGG**R**T**W**YPDSVKG (49)	HRGNPFDY (58)
H222	M526	GFTFSSY**G**MS (44)	YI**DR**GGG**V**T**I**YPDSVKG (50)	HRGNPFDY (58)
H227	Kabat defined CDR-H1	SYGMS (161)		
			X$_1$IX$_2$X$_3$GGGX$_4$TX$_5$YPDSVKG (156)	

For SEQ ID NO:156, X_1 is H or Y; X_2 is D or S; X_3 is A, E, R, or S; and X_4 is I, W, or Y.

Table 35. 2191 path 2

VH		**CDR-H1 (SEQ ID NO:)**	**CDR-H2 (SEQ ID NO:)**	**CDR-H3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
H10	M41 parent	SYTMS (42)	YISSGGGNTYYPDSVKG (46)	HRGNPFDY (58)
		Extended CDR-H1	**Kabat**	**Kabat**
H39	HFR ; M131; M136-138	GFTFSSYTMS (43)	YISSGGGNTYYPDSVKG (46)	HRGNPFDY (58)
H40	M132; M139; M140	GFTFSSYTMS (43)	**A**I**DH**GGG**R**TYYPDSVKG (51)	HRGNPFDY (58)
H41		GFTFSSYTMS (43)	**A**I**DH**GGG**RTW**YPDSVKG (52)	HRGNPFDY (58)

H79	M133; M141=M707; M142	GFTFSSYTMS (43)	**A**I**DH**GGG**Q**T**L**YPDSVKG (53)	HRGNPFDY (58)
H145		GFTFSSYTMS (43)	YIS**G**GGG**Q**T**L**YPDSVKG (54)	HRGNPFDY (58)
H190		GFTFSSYTMS (43)	**T**I**DR**GGG**S**T**W**YPDSVKG (55)	HRGNPFDY (58)
H191	M134; M143; M144;	GFTFSSYTMS (43)	**A**I**DG**GGG**A**TYYPDSVKG (56)	HRGNPFDY (58)
H192	M135; M145; M146; M408=M708	GFTFSSYTMS (43)	**V**I**DH**GGG**S**T**H**YPDSVKG (57)	HRGNPFDY (58)
			X_1IX$_2$X$_3$GGGX$_4$TX$_5$YPDSVKG (157)	

For SEQ ID NO:157, X_1 is A, T, V, or Y; X_2 is D or S; X_3 is G, H, R, or S; X_4 is A, N, Q, R, or S; and X_5 is H. L, W, or Y.

Table 36. 2191 path 1

VL		**CDR-L1 (SEQ ID NO:)**	**CDR-L2 (SEQ ID NO:)**	**CDR-L3 (SEQ ID NO:)**
		Kabat	**Kabat**	**Kabat**
L20	M41 parent mouse	RASKSVSTSGYSFMH (59)	LASNLES (67)	QHSRELPWT (75)
L42	HFR (M91)	RASKSVSTSGYSFMH (59)	LASNLES (67)	QHSRELPWT (75)
L244	M427	RASKSVS**AW**GYSFMH (60)	**V**AS**R**LES (68)	QHSRELPWT (75)
L245	M429=M696	RASKSVS**HVRW**SFMH (61)	LAS**K**LES (69)	QHSRELPWT (75)
L249	M488=M694; M492=M695; M526	RASKSVS**EGRW**SFMH (62)	**V**AS**R**LES (68)	QHSRELPWT (75)
L250	M489; M493; M501	RASKSVS**LDRW**SFMH (63)	LASNLES (67)	QHSRELPWT (75)
		RASKSVSX$_1$X$_2$X$_3$X$_4$SFMH (158)		

For SEQ ID NO:158, X_1 is A, E, H, L, T, or Y; X_2 is D, G, I, S, V, or W; X_3 is G or R; and X_4 is W or Y.

Table 37. 2191 path 2

VL		CDR-L1 (SEQ ID NO:)	CDR-L2 (SEQ ID NO:)	CDR-L3 (SEQ ID NO:)
		Kabat	Kabat	Kabat
L20	M41 parent mouse	RASKSVSTSGYSFMH (59)	LASNLES (67)	QHSRELPWT (75)
L59		RASKSVSTSGYSFMH (59)	VGNRLED (70)	QHSRELPWT (75)
L62		RASKSVSTSGYSFMH (59)	VGDRRQE (71)	QHSRELPWT (75)
L124		RASKSVSTSGYSFMH (59)	VGSRMAF (72)	QHSRELPWT (75)
L131		RASKSVSTSGYSFMH (59)	VGDRANW (73)	QHSRELPWT (75)
L137	M138; M408=M708	RASKSVSTSGYSFMH (59)	VGSRLDY (74)	QHSRELPWT (75)
L160	M137; M160; M142; M143; M146	RASKSVS**YVRW**SFMH (64)	LASNLES (67)	QHSRELPWT (75)
L172		RASKSVS**HIRW**SFMH (65)	LASNLES	QHSRELPWT (75)
L173	M136; M139; M141=M707; M144; M145	RASKSVS**HVRW**SFMH (66)	LASNLES	QHSRELPWT (75)

Protein and Antibody Variable Region Sequences

Table 38.

SEQ ID NO	Clone	Sequence (CDR sequences underlined)	Features or Origin	Comments
174	Human CD27 ECD	TPAPKSCPERHYWAQGKLCCQMCEPGTFLVKDCDQHR KAAQCDPCIPGVSFSPDHHTRPHCESCRHCNSGLLVR NCTITANAECACRNGWQCRDKECTECDPLPNPSLTAR SSQALSPHPQPTHLPYVSEMLEARTAGHMQTLADFRQ LPARTLSTHWPPQRSLCSSDFIRILHHHHHH	ECD: 1-173, His6	

175	Human CD27 ECD truncated	TPAPKSCPERHYWAQGKLCCQMCEPGTFLVKDCDQHR KAAQCDPCIPGVSFSPDHHTRPHCESCRHCNSGLLVR NCTITANAECACRNGWQCRDKECTECDPLPNPSLTAR SSQALSPHPQLEVLFQGPHHHHHH	ECD: 1-121, cleavage site, His6	
176	Human CD27 ECD truncated	TPAPKSCPERHYWAQGKLCCQMCEPGTFLVKDCDQHR KAAQCDPCIPGVSFSPDHHTRPHCESCRHCNSGLLVR NCTITANAECACRNGWQCRDKECTECDGGHHHH	ECD:1-101	Proteos
150	Human CD70 ECD	MPEEGSGCSVRRRPYGCVLRALVPLVAGLVICLVVCI QRFAQAQQQLPLESLGWDVAELQLNHTGPQQDPRLYW QGGGPALGRSFLHGPELDKGQLRIHRDGIYMVHIQVTL AICSSTTASRHHPTTLAVGICSPASRSISLLRLSFHQ GCTIASQRLTPLARGDTLCTNLTGTLLPSRNTDETFF GVQWVRP	ECD	
76	**C2186** Heavy Chain (HC) Variable Region	QVQLQQPGAELVKPGASVKLSCKASGYTFTNYWMNWV KQRPGRGLEWIGRIHPSDSETHYNQNFKSKATLTVDK SSSTAYIQLSSLTSEDSAVYYCARPVLYGDYGFPCWG QGTLVTVSA		Murine
77	**C2186** Light Chain (LC) Variable Region	DIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYM NWYQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTD FTLNIHPVEEEDAATYYCQQSNEDPYTFGGGTKLEIK		Murine
78	**C2192** Heavy Chain (HC) Variable Region	QVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWV KQRPGKGLEWIGRIYPGDGDTNYNGKFKGKATLTADK SSSTAYMQLSSLTSEDSAVYFCARRWDGGNYFFDYWG QGTTLTVSS		Murine
79	**C2192** Light Chain (LC) Variable Region	DIVMTQSHKFMSTSVGDRVSITCMASQDVGTAVAWYQ RRPGQSPKLLIYWTSTRHTGVPDRFTGSGSGTDFTLT ISNVQSEDLADYFCQQYSSYPLTFGSGTKLEIK		Murine
80	**C2177** Heavy Chain (HC) Variable Region	QVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWV KQRPGKGLEWIGRIYPGDGDTNYNGKFKGKATLTADK SSSTAYMQLSSLTSEDSAVYFCARSDYYGDYGFAYWG QGTLVTVSA	H7	Murine
81	**C2177** Light Chain (LC) Variable Region	DIVLTQSPASLAVSLGQRATISCKASQSVDYAGDSYM NWYQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTD FTLNIHPVEEEDAATYYCQQSNEDPYTFGGGTKLEIK	L18	Murine

82	L35	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGDSYM NWYQQKPGQPPKLLIYAASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK	4-1/2	HFR selected for C2177 AM path 1
83	L255	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGDSFM NWYQQKPGQPPKLLIYAASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK		VL in M584, M600 AM clones; in M678, M680 PTM variants
84	L256	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGDSWM NWYQQKPGQPPKLLIYVASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK		C2177 affinity maturation path 1
85	L257	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGDSWM NWYQQKPGQPPKLLIYTASNLESGVPDRFSGSGSGTD FTLTISSLLAEDVAVYYCQQSNEDPYTFGQGTKLEIK		VL in M558, M596 AM clones
86	L258	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGSSFM NWYQQKPGQPPKLLIYTASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK		C2177 affinity maturation path 1
87	L260	DIVMTQSPDSLAVSLGERATINCKASQSVDWAGHSWM NWYQQKPGQPPKLLIYTASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK		C2177 affinity maturation path 1
88	L261	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGSSFM NWYQQKPGQPPKLLIYEASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK		C2177 affinity maturation path 1
89	L267	DIVMTQSPDSLAVSLGERATINCKASQSVDYAGESFM NWYQQKPGQPPKLLIYAASNLESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEIK		VL in M703, M706 PTM variants
90	L36	DIQMTQSPSSLSASVGDRVTITCKASQSVDYAGDSYM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKLEIK	O12/2	HFR selected for C2177 AM path 2
91	L216	DIQMTQSPSSLSASVGDRVTITCKASQSVDYFSESYM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		C2177 AM path 2
92	L217	DIQMTQSPSSLSASVGDRVTITCKASQSVDYAHESFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		VL in M166 AM clone
93	L218	DIQMTQSPSSLSASVGDRVTITCKASQSVDYFGDSLM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		C2177 AM path 2

94	L219	DIQMTQSPSSLSASVGDRVTITCKASQSVDYAGDSFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		VL in M160, M169 AM clones
95	L219 PTM	DIQMTQSPSSLSASVGDRVTITCKASQSVDYAGESFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		VL in M668, M669, M670, M671 PTM variants
96	L266	DIQMTQSPSSLSASVGDRVTITCKASQSVDYAGeSFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		VL in M668, M669, M670, M671 PTM variants
97	L220	DIQMTQSPSSLSASVGDRVTITCKASQSVDYAGDSYM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		VL in M158 AM clone
98	L221	DIQMTQSPSSLSASVGDRVTITCKASQSVDYFRTSFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		C2177 AM path 2
99	L222	DIQMTQSPSSLSASVGDRVTITCKASQSVDYVGTSFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		C2177 AM path 2
100	L223	DIQMTQSPSSLSASVGDRVTITCKASQSVDYWSDSFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		C2177 AM path 2
101	L224	DIQMTQSPSSLSASVGDRVAITCKASQSVDYYNSSFM NWYQQKPGKAPKLLIYAASNLESGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQSNEDPYTFGQGTKVEIK		C2177 AM path 2
102	H24	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYPGDGDTNYNGKFKGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS	IGHV5-51/4	HFR selected C2177 AM path 2
103	H221	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYSGDGDTNYNGKFKGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M169 AM clones
104	H257	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYSGDaDTNYaqKFKGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M670 PTM variant
105	H258	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYSGDaDTNYNqKFKGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M671 PTM variant

106	H25	QVQLVQSGAEVKKPGASVKVSCKASGYAFSSSWMNWV RQAPGQGLEWMGRIYPGDGDTNYNGKFKGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARSDYYGDYGFAYWG QGTLVTVSS	IGHV1-46/4	HFR selected for C2177 AM, path 2
107	H196	QVQLVQSGAEVKKPGASVKVSCKASGYAFSSSWMNWV RQAPGQGLEWMGRIYSGDGDTNYNGKFKGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M158, M160 AM clones
108	H255	QVQLVQSGAEVKKPGASVKVSCKASGYAFSSSWMNWV RQAPGQGLEWMGRIYSGDADTNYAQKFKGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M668, M672 PTM variants
109	H256	QVQLVQSGAEVKKPGASVKVSCKASGYAFSSSWMNWV RQAPGQGLEWMGRIYSGDADTNYNQKFKGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M669, M673 PTM variants
110	H197	QVQLVQSGAEVKKPGASVKVSCKASGYAFSSSWMNWV RQAPGQGLEWMGRIYQGDGDTNYNGKFKGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone
111	H28	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYPGDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS	IGHV5-51c/4	HFR selected for C2177 affinity maturation (AM) path 1
112	H236	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYPGDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARADYYGDYGFGYWG QGTLVTVSS		VH in M584 AM clone path 1
113	H237	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIFVRDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone path 1
114	H238	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYVGDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone path 1
115	H239	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYAGDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M596 AM clone path 1

116	H240	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYARDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M558, M600 AM clones, path 1
117	H241	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYGRDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone path 1
118	H242	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYANDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone path 1
119	H243	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYGGDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone path 1
120	H244	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYSGDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		V2177 AM clone path 1
121	H245	EVQLVQSVAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYSRDGDTNYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		C2177 AM clone path 1
122	H259	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYAGDGDTAYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M678 PTM mutant
123	H260	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYAGDGDTNYAPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M680 PTM mutant
124	H270	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYAGDADTAYSPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M703 PTM mutant
125	H272	EVQLVQSGAEVKKPGESLKISCKGSGYAFSSSWMNWV RQMPGKGLEWMGRIYAGDADTNYAPSFQGQVTISADK SISTAYLQWSSLKASDTAMYYCARSDYYGDYGFAYWG QGTLVTVSS		VH in M706 PTM mutant

Table 39.

SEQ ID	Clone	Sequence	Features or Origin	Comments
126	C2191 Heavy Chain (HC) Variable Region	EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQ TPEKRLEWVAYISSGGGNTYYPDSVKGRFTISRDNARNT LYLQMSSLRSEDTAMYYCSRHRGNPFDYWGQGTTLTVSS	H10	Murine
127	C2191 Light Chain (LC) Variable Region	DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSFMHW YQQKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLN IHPVEEEDAATYYCQHSRELPWTFGGGTKLEIK	L20	Murine
128	H30	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRQ APGKGLEWVSYISSGGGNTYYPDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAKHRGNPFDYWGQGTLVTVSS	IGHV3-23/4	HFR selected for C2191 AM path 2
129	H79	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRQ APGKGLEWVSAIDHGGGQTLYPDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M141 AM variant, path 2. Table 29
130	H192	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRQ APGKGLEWVSVIDHGGGSTHYPDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M408 AM variant, path 2. Table 29
131	H31	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYTMSWIRQ APGKGLEWVSYISSGGGNTYYPDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS	IGHV3-11/4	HFR selected for C2191 AM path 1
132	H222	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYGMSWIRQ APGKGLEWVSYIDRGGGVTIYPDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M526 AM variant, path 1

133	H227	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYGMSWIRQ APGKGLEWVSYIDEGGGQTIYPDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M427, M429, M488, M489 AM variants, path 1
134	H228	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMSWIRQ APGKGLEWVSYIDAGGGFTIYPDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M492, M493 AM variants, path 1
135	H231	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMSWIRQ APGKGLEWVSHIDAGGGRTWYPDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M501 AM variant, path 1
136	H232	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYPMSWIRQ APGKGLEWVSHIATGGGNTYYPDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		C2191 AM clone, path 1
137	L40	DIQMTQSPSSLSASVGDRVTITCRASKSVSTSGYSFMHW YQQKPGKAPKLLIYLASNLESGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQHSRELPWTFGQGTKVEIK	IGKVO12/1	HFR selected for C2191 AM, path 2
138	L137	DIQMTQSPSSLSASVGDRVTITCRASKSVSTSGYSFMHW YQQKPGKAPKLLIYVGSRLDYGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQHSRELPWTFGQGTKVEIK		VL in M408 AM variant, path 2. Table 29
139	L173	DIQMTQSPSSLSASVGDRVTITCRASKSVSHVRWSFMHW YQQKPGKAPKLLIYLASNLESGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQHSRELPWTFGQGTKVEIK		VL in M141 AM variant, path 2. Table 29
140	L42	DIQMTQSPSSLSASVGDRVTITCRASKSVSTSGYSFMHW YQQKPGKAPKLLIYLASNLESGVPSRFSGSGSGTDFTFT ISSLQPEDIATYYCQHSRELPWTFGQGTKVEIK	IGKVO8/1	HFR selected for C2191 AM path 1

141	L244	DIQMTQSPSSLSASVGDRVTITCRASKSVSAWGYSFMHW YQQKPGKAPKLLIYVASRLESGVPSRFSGSGSGTDFTFT ISSLQPEDIATYYCQHSRELPWTFGQGTKVEIK		VL in M427 AM variant , path 1
142	L245	DIQMTQSPSSLSASVGDRVTITCRASKSVSHVRWSFMHW YQQKPGKAPKLLIYLASKLESGVPSRFSGSGSGTDFTFT ISSLQPEDIATYYCQHSRELPWTFGQGTKVEIK		VL in M429 AM variant , path 1
143	L249	DIQMTQSPSSLSASVGDRVTITCRASKSVSEGRWSFMHW YQQKPGKAPKLLIYVASRLESGVPSRFSGSGSGTDFTFT ISSLQPEDIATYYCQHSRELPWTFGQGTKVEIK		VL in M488, M492, M526 AM variant s, path 1
144	L250	DIQMTQSPSSLSASVGDRVTITCRASKSVSLDRWSFMHW YQQKPGKAPKLLIYLASNLESGVPSRFSGSGSGTDFTFT ISSLQPEDIATYYCQHSRELPWTFGQGTKVEIK		VL in M489, M493, M501 AM variant s, path 1
145	H39	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRQ APGKGLEWVSYISSGGGNTYYPDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		HFR; VH in M131; M136-138 AM clones, path 2
146	H40	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRQ APGKGLEWVSAIDHGGGRTYYPDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M132; M139; M140 AM clones, path 2
147	H191	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMSWVRQ APGKGLEWVSAIDGGGGATYYPDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARHRGNPFDYWGQGTLVTVSS		VH in M134; M143; M144; AM clones, path 2
148	L160	DIQMTQSPSSLSASVGDRVTITCRASKSVSYVRWSFMHW YQQKPGKAPKLLIYLASNLESGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQHSRELPWTFGQGTKVEIK		VL in M137; M160; M142; M143; M146 clones, path 2

Table 40. CD27 and CD70 Proteins

SEQ ID NO:	DESCRIPTION	Features, Abbreviations
149	Human CD27 propolypeptide	1-20 Signal, 21-191 Extracellular domain, 192-212 Transmembrane, 213-260 Intracellular TPAPKSCPER HYWAQGKLCC QMCEPGTFLV KDCDQHRKAA QCDPCIPGVS FSPDHHTRPH CESCRHCNSG LLVRNCTITA NAECACRNGW QCRDKECTEC DPLPNPSLTA RSSQALSPHP QPTHLPYVSE MLEARTAGHM QTLADFRQLP ARTLSTHWPP QRSLCSSDFI RILVIFSGMF LVFTLAGALF LHQRRKYRSN KGESPVEPAE PCRYSCPREE EGSTIPIQED YRKPEPACSP
150	Human CD70 propolypeptide	1-17 intracellular, 18-38 transmembrane, and 39-193 extracellular MPEEGSGCSV RRRPYGCVLR AALVPLVAGL VICLVVCIQR FAQAQQQLPL ESLGWDVAEL QLNHTGPQQD PRLYWQGGPA LGRSFLHGPE LDKGQLRIHR DGIYMVHIQV TLAICSSTTA SRHHPTTLAV GICSPASRSI SLLRLSFHQG CTIASQRLTP LARGDTLCTN LTGTLLPSRN TDETFFGVQW VRP

Table 41. Antibody constant region sequences

Description	**Sequence**	**SEQ ID NO:**

Heavy chain human IgG4 Ala/Ala Ser to Pro constant region	**astkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslslgk**	**159**
Light chain human kappa constant region	**rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec**	**160**

Table 42. Antibody nucleotide sequences

Description	Sequence	SEQ ID NO:
Light Chain encoding sequence (encodes the light chain sequence of SEQ ID NO: 160)	GACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGCGACCGGGTGACCATCACCTGCCGGGCCAGCAAGAGCGTGAGCGAGGGGCGATGGAGCTTCATGCACTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACGTGGCCAGCAGACTGGAGAGCGGCGTGCCCAGCCGGTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCTTCACCATCAGCAGCCTGCAGCCCGAGGACATCGCCACCTACTACTGCCAGCACAGCCGGGAGCTGCCCTGGACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT	163

Heavy Chain encoding sequence (encodes the heavy chain sequence of SEQ ID NO: 159)	CAGGTGCAGCTGGTGGAGAGCGGCGGCGGCCTGGTGAAGCCCGGCGGCAGCCTGC GGCTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGGGATGAGCTGGAT CCGGCAGGCCCCCGGCAAGGGCCTGGAGTGGGTGAGCTACATCGATGAGGGCGGC GGCCAGACCATCTACCCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACA ACGCCAAGAACAGCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGC CGTGTACTACTGCGCCCGGCACCGGGGCAACCCCTTCGACTACTGGGGCCAGGGC ACCCTGGTGACCGTGAGCAGCGCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGG CGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCA GCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAAACCTACACCTGCAACGT AGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCCATGCCCACCATGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCC TGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCAC GTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTAC GTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCA ACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAA CGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGC CCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA AGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT ACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATG CTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTG TCTCTGGGTAAA	164

CLAIMS

1. An isolated human CD27 antibody or antigen-binding fragment thereof, comprising a light chain variable amino acid sequence and a heavy chain variable amino acid sequence, wherein the light chain variable amino acid sequence comprises SEQ ID NO: 143 and the heavy chain variable amino acid sequence comprises SEQ ID NO: 133 for use in inhibiting T-cell mediated B-cell antibody production in a subject, wherein the antibody or antigen binding portion thereof inhibits the activation of CD27 on human T-cells in the presence of human CD70 protein or a portion of human CD70 protein.

2. Use of an isolated human CD27 antibody or antigen-binding fragment thereof, comprising a light chain variable amino acid sequence and a heavy chain variable amino acid sequence, wherein the light chain variable amino acid sequence comprises SEQ ID NO: 143 and the heavy chain variable amino acid sequence comprises SEQ ID NO: 133 in the manufacture of a medicament for the inhibition of T-cell mediated B-cell antibody production in a subject, wherein the antibody or antigen binding portion thereof inhibits the activation of CD27 on human T-cells in the presence of human CD70 protein or a portion of human CD70 protein.

3. The use of claim 2, wherein the subject is suffering from an inflammatory disorder.

4. The use of claim 3, wherein the inflammatory disorder is systemic lupus erythematosus.

5. The use of claim 2, wherein the subject exhibits an antigen-specific humoral immune response associated with a disorder selected from the group consisting of a disease of the pulmonary or pleural system, a disease of the central or peripheral nervous system, a disease of the eye or visual system, a disease of the gastrointestinal and digestive system, a disease of the cardiovascular system, an infectious disease, and a parasitic infection.

6. The use of claim 2, wherein the antibody or antigen binding portion thereof is to be administered systemically to the subject.

7. An isolated human antibody or antigen-binding fragment thereof, comprising an antibody variable region binding to an epitope on human CD27 protein bound by an antibody selected from the group consisting of antibodies C2177, CD2186, CD2191, and CD2192.

8. An isolated human antibody or antigen-binding fragment thereof, comprising an antibody variable region competing for binding to human CD27 protein with an antibody selected from the group consisting of antibodies C2177, CD2186, CD2191, and CD2192.

9. An isolated human CD27 antibody or antigen-binding fragment thereof, comprising a light chain variable region, said light chain variable region comprising:

    a complementarity determining region light chain 1 (CDRL1) amino acid sequence selected from the group consisting of SEQ ID NOs: 24-36, 153, and 155;

    a CDRL2 amino acid sequence selected from the group consisting of SEQ ID NOs: 37-40 and 154; and

a CDRL3 amino acid sequence of SEQ ID NO: 41.

10. An isolated human CD27 antibody or antigen-binding fragment thereof, comprising a heavy chain variable region, said heavy chain variable region comprising:

    a complementarity determining region heavy chain 1 (CDRH1) amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 2;

    a CDRH2 amino acid sequence selected from the group consisting of SEQ ID NOs: 3-22, 151, and 152; and

    a CDRH3 amino acid sequence selected from the group consisting of SEQ ID NOs: 23 and 162.

11. An isolated human CD27 antibody or antigen-binding fragment thereof, comprising a light chain variable region and a heavy chain variable region, said light chain variable region comprising:

    a complementarity determining region light chain 1 (CDRL1) amino acid sequence selected from the group consisting of SEQ ID NOs: 24-36, 153, and 155;

    a CDRL2 amino acid sequence selected from the group consisting of SEQ ID NOs: 37-40 and 154; and

    a CDRL3 amino acid sequence of SEQ ID NO: 41, and said heavy chain variable region comprising:
    a CDRH1 amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 2;

    a CDRH2 amino acid sequence selected from the group consisting of SEQ ID NOs: 3-22, 151, and 152; and

    a CDRH3 amino acid sequence selected from the group consisting of SEQ ID NOs: 23 and 162.

12. An isolated human CD27 antibody or antigen-binding fragment thereof, comprising a light chain variable amino acid sequence selected from the group consisting of SEQ ID NOs: 82-101.

13. The isolated antibody or antigen-binding fragment thereof of claim 12, further comprising a heavy chain variable amino acid sequence selected from the group consisting of SEQ ID NOs: 102-125.

14. An isolated human CD27 antibody or antigen-binding fragment thereof, comprising a heavy chain variable amino acid sequence selected from the group consisting of SEQ ID NOs: 102-125.

15. An isolated CD27 antibody or antigen-binding fragment thereof, comprising a light chain variable amino acid sequence selected from the group consisting of SEQ ID NOs: 77, 79, 81, and 127.

16. The isolated antibody or antigen-binding fragment thereof of claim 15, further comprising a heavy chain variable amino acid sequence selected from the group consisting of SEQ ID NOs: 76, 78, 80, and 126.

17. An isolated CD27 antibody or antigen-binding fragment thereof, comprising a heavy chain variable amino acid sequence selected from the group consisting of SEQ ID NOs: 76, 78, 80, and 126.