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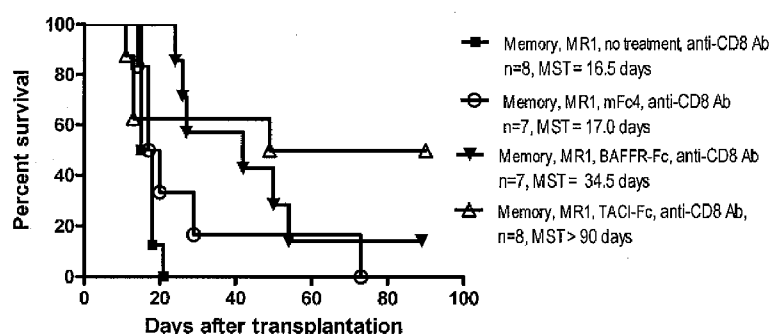
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(54) Title: METHODS AND COMPOSITIONS TO TREAT IMMUNE-MEDIATED DISORDERS

Figure 3



(57) Abstract: Methods and compositions for treating or preventing immune-mediated conditions are provided. The methods of the invention comprise administering to a subject a therapeutically effective amount of a BLYS/APRIL antagonist (e.g. TACI-Ig) in combination with an antagonist of the CD40 pathway (e.g. an anti-CD40 or an anti-CD154 antibody). The combination of the BLYS/APRIL antagonist and the antagonist of the CD40 pathway promotes a desired therapeutic response. The BLYS/APRIL antagonist and the CD40 pathway antagonist may be administered simultaneously or sequentially as a single pharmaceutical composition or as separate pharmaceutical compositions, each comprising a BLYS/APRIL antagonist and an antagonist of the CD40 pathway. Methods of the invention find use in treating or preventing a variety of immune-mediated conditions including, for example, treating or preventing transplant rejections or graft-versus-host disease or reducing the level of an immune response or reducing the level of immunoglobulins in a subject in need thereof.

METHODS AND COMPOSITIONS TO TREAT IMMUNE-MEDIATED DISORDERS

FIELD OF THE INVENTION

5 The present invention relates to methods and compositions for modulating an immune response.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS
A TEXT FILE VIA EFS-WEB

10 The official copy of the sequence listing is submitted concurrently with the specification as a text file via EFS-Web, in compliance with the American Standard Code for Information Interchange (ASCII), with a file name of 402673seqlist.txt, a creation date of February 25, 2011, and a size of 20 Kb. The sequence listing filed via EFS-Web is part of the specification and is hereby incorporated in its entirety by reference herein.

BACKGROUND OF THE INVENTION

15 The success of surgical transplantation of organs and tissue is largely dependent on the ability of the clinician to modulate the immune response of the transplant recipient. Specifically, the immunological response directed against the transplanted foreign tissue must be controlled if the tissue is to survive and function. It is known that the normally functioning immune system of the transplant recipient recognizes the transplanted organ as "non-self" tissue and thereafter mounts an immune response to the presence of the transplanted organ. Left unchecked, the immune response will generate a multitude of cells and proteins that will ultimately result in loss of biological functioning or death of the transplanted organ. Transplant rejection remains the leading impediment to long term graft survival in humans.

20 In this regard, the development of immunosuppressive medications has brought about exceptional advances in the transplantation of organs and tissues and the treatment of autoimmune diseases and has made a great contribution to the study of the *in vivo* mechanism of immune responses to the transplanted organ or tissue.

25 Immunosuppressive drugs were developed to inhibit or attenuate transplant rejection.

An example is cyclosporine A (US Patent 4,117,118) produced from *Tolypocladium inflatum*, a soil fungus. FK-506 has also been discovered to be an immunosuppressant and has been commercialized, as has the calcineurin inhibitors-cyclosporine, which has become the mainstay of immunosuppressive therapy in solid organ transplantation.

5 However, each of these drugs produces severe adverse drug effects (ADEs) such as nephrotoxicity, posttransplantation diabetes mellitus, and hypertension.

Further methods and compositions are needed in the art to improve the treatment of immune-mediated conditions, such as, transplant rejections.

10 BRIEF SUMMARY OF THE INVENTION

Methods and compositions for treating or preventing immune-mediated conditions are provided. The methods of the invention comprise administering to a subject a therapeutically effective amount of a BLyS/APRIL antagonist in combination with an antagonist of the CD40 pathway. The combination of the BLyS/APRIL
15 antagonist and the CD40 pathway antagonist promotes a desired therapeutic response. The BLyS/APRIL antagonist and the CD40 pathway antagonist may be administered simultaneously or sequentially as a single pharmaceutical composition or as separate pharmaceutical compositions, each pharmaceutical composition comprising a
20 BLyS/APRIL antagonist and an antagonist of the CD40 pathway. Methods of the invention find use in treating or preventing or delaying the development of a variety of immune-mediated conditions including, for example, treating or preventing or delaying the development of transplant rejections, graft-versus-host disease, reducing or delaying anti-graft responses in a subject, reducing the level of an immune response or reducing the level of immunoglobulins, including alloantibodies or xenoantibodies, in a subject in
25 need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows donor reactive IgG alloantibody serum titers on day 10 post transplantation.

Figure 2 shows donor reactive IgG alloantibody serum titers on day 14 post transplantation.

Figure 3 shows allograft survival post transplantation following BAFF and APRIL neutralization.

DETAILED DESCRIPTION OF THE INVENTION

The present inventions now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the inventions are shown. Indeed, these inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

Donor-reactive antibodies initiate episodes of acute rejection and may lead to chronic pathology of transplant organs. Production of pathogenic alloantibodies is dependent on helper signals provided to B cells by activated donor-reactive CD4 T cells through CD40/CD154 interactions. The instant invention demonstrates that memory CD4 T cells can utilize a pathway other than CD40/CD154 to induce donor-reactive IgG alloantibodies. As demonstrated herein, B lymphocyte stimulator (BLyS), also known as B-cell activating factor of the TNF (Tumor Necrosis Factor) family (BAFF), and A Proliferation-Inducing Ligand (APRIL) signaling is required for memory CD4⁺ T cell induced alloantibody production when the CD40-CD154 pathway is compromised. These findings provide improved methods to decrease an immune response or decrease

antibodies in a subject by the combined administration of a BLyS/APRIL antagonist and a CD40 pathway antagonist. In specific embodiments, the combination of the BLyS/APRIL antagonist and the antagonist of the CD40 pathway promotes a desired therapeutic response comprising a reduction in serum immunoglobulin levels. In still other embodiments, the therapeutic response comprises a reduction in donor-reactive immunoglobulins. Such reductions in an immune response find use in reducing an anti-graft response, promoting immune tolerance, and reducing an immune response in a subject in need thereof. In specific embodiments, the methods for treating or preventing various immune-mediated disorders are provided, including but not limited to, transplant rejections and graft-vs.-host disease.

As used herein, a “reduction in serum immunoglobulin levels” comprises any statistically significant decrease in the level of serum antibody titers of any one or all of the immunoglobulin classes (i.e. IgE, IgG, IgM, IgD and/or IgA) in a subject. Such a reduction can comprise a decrease in serum IgE, IgM, IgD, or IgA antibody titers of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater in the serum of a subject following the administration of the combination therapy. In other embodiments, such a reduction comprises a decrease in serum IgG antibody titers of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater in the serum of a subject following the administration of the therapy. In still further embodiments, the reduction in serum immunoglobulin levels can comprises a statistically significant reduction in the level of any one of the subtypes of IgG, including a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater in the serum of any one or any combination of IgG1, IgG2, IgG3 or IgG4.

In still further embodiments, the reduction in serum immunoglobulin levels can comprise any statistically significant decrease in the level of donor-reactive immunoglobulins. As used herein, a “donor-reactive immunoglobulin(s)” include and “xenoantibody (ies)” and “alloantibody(ies)”. Donor-reactive immunoglobulins refer to antibodies that are generated in a subject against non-self (allo or xeno) antigens. It is further noted that the alloantibodies or the xenoantibodies can further include subject-host reactive antibodies that arise from a graft transplant (e.g., heart allograft) or GVHD. Thus, an alloantibody or a xenoantibody can arise from a response in either direction (graft-vs-host or host-vs-graft). A reduction in the level of donor-reactive immunoglobulins comprises any statistically significant decrease in antibody titers of any one or all donor-reactive immunoglobulin classes (i.e. IgE, IgG, IgM, IgD and/or

IgA) in a subject. Such a reduction can comprise a decrease in donor-reactive IgE, IgM, IgD, or IgA antibody titers in the serum of a subject of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater in a subject following the administration of the combination therapy. In other embodiments, such a reduction in donor-reactive immunoglobulins comprise a decrease in serum donor-reactive IgG antibody titers of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater in a subject following the administration of the therapy. In still further embodiments, the reduction in donor-reactive immunoglobulin levels in the serum can comprise a statistically significant reduction in the level of any one of the subtypes of donor-reactive IgG, including a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater in the serum of any one or any combination of donor-reactive IgG1, IgG2, IgG3 or IgG4.

It is noted that the methods employed herein do not require that the biological sample from the subject be analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, or BCMA prior to administration of the combination therapy. In further embodiments, a biological sample from the subject is not analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, or BCMA prior to administration of the combination therapy. In still further embodiments, the methods employed herein do not require that the biological sample from the subject be analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, BCMA, TACI, BAFF-R, and/or BLYS prior to administration of the combination therapy. In further embodiments, a biological sample from the subject is not analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, BCMA, TACI, BAFF-R, and/or BLYS prior to administration of the combination therapy. It is recognize that in any of these embodiments, the elevated level of APRIL or BLYS can be detected by either detecting the homotrimer of each protein form, or alternatively, by detecting the level of the individual subunit of each homotrimer.

I. BLYS/APRIL Antagonists

The terms "BLYS" or "BLYS polypeptide," "TALL-1" or "TALL-1 polypeptide," or "BAFF" or "BAFF polypeptide" when used herein encompass native BLYS polypeptides and BLYS variants. "BLYS" is a designation given to those polypeptides which are encoded by the human BLYS sequence (SEQ ID NO: 1) or the mouse BLYS sequence (SEQ ID NO: 2). Variants or fragments of the BLYS polypeptides which show

BLyS biological activity are encompassed within this designation as well. For example, a biologically active BLyS polypeptide can bind three members of the TNF receptor family, namely the transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI), B-cell maturation antigen (BCMA), and B-cell activation factor of the TNF family receptor (BAFF-R) and thereby activate downstream pathway signaling in these pathways. Alternately, a biologically active BLyS potentiates any one or combination of the following events *in vitro* or *in vivo*: an increased survival of B cells, an increased level of IgG and/or IgM, an increased numbers of plasma cells, and processing of NF- κ B2/100 to p52NF- κ B in splenic B cells (e.g., Batten *et al.* (2000) *J. Exp. Med.* 192: 1453-1465; Moore *et al.* (1999) *Science* 285: 260-263; Kayagaki *et al.* (2002) 10: 515-524).

The terms "APRIL", "APRIL polypeptide", "A PRoliferation-Inducing Ligand", "TNSF13A", "Tall-2", and "TRDL-1" all refer to the same polypeptide and when used herein encompass native APRIL polypeptides and APRIL variants. "APRIL" is a designation given to polypeptides having the sequence shown in SEQ ID NO:3. Variants and fragments of the APRIL polypeptides which show APRIL biological activity are encompassed within this designation as well. For example, a biologically active APRIL polypeptide can bind two members of the TNF receptor family, namely the transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA), as well as heparin sulfate proteoglycans and thereby activate downstream pathway signaling in these pathways. Alternately, a biologically active APRIL potentiates any one or combination of the following events *in vitro* or *in vivo*: promote B-cell proliferation by binding to BCMA and TACI receptors (Gross *et al.* (2000) *Nature*. 27;404(6781):995-9; Marsters *et al.* (2000) *Curr Biol* 29;10(13):785-8 and Wu *et al.* (2000) *J Biol Chem.* 10;275(45):35478-85), which are expressed on resting and activated B cells; in addition, TACI has been found on a small subset of activated T cells. The biological role of APRIL is not restricted to proliferation induction (Hahne *et al.* (1998) *J Exp Med.* 21;188(6):1185-90), but APRIL has also been shown to have an anti-apoptotic effect (Kimberley *et al.* (2009) *Results Probl Cell Differ.* 49:161-82 and Kimberley *et al.* (2009) *J Cell Physiol.* 218:1-8). Non-limiting examples of active variants and fragments of APRIL are further described in Hahne *et al.* (1998) *J. Exp. Med.* 188:1185-1190; GenBank Accession No. AF046888; WO 99/00518; WO 99/12965; WO 99/33980; WO 97/33902; WO 99/11791; EP 911,633; Wang *et al.* (2009)

Veterinary Immunology and Immunopathology 128:407-412 and WO99/50416, each of which is herein incorporated by reference in their entirety.

Methods and compositions described herein employ agents that act to antagonize the activity of both the BLyS polypeptide and the APRIL polypeptide. As used herein, the term “BLyS/APRIL” antagonist refers to a compound or a combination of compounds, including for example, an antibody, a synthetic or native sequence peptide and/or a small molecule antagonist, that can function in a direct or indirect manner to partially or fully block, inhibit or neutralize BLyS and APRIL signaling *in vitro* or *in vivo*. A BLyS/APRIL antagonist can comprise a single antagonistic compound or can comprise multiple, distinct compounds, one acting as a BLyS antagonist and one acting as an APRIL antagonist.

Antagonism using a BLyS/APRIL antagonist does not necessarily indicate a total elimination of BLyS/APRIL activity. Instead, the activity of both BLyS and APRIL signaling could decrease by a physiologically relevant amount including, for example, a decrease in both BLyS signaling and APRIL signaling of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% when compared to an appropriate control which has not been treated with the antagonist.

In specific embodiments, the BLyS/APRIL antagonist directly binds BLyS and/or APRIL and thereby blocks/inhibits their activity or, alternatively, the BLyS/APRIL antagonist binds to the BLyS and/or APRIL receptors (i.e., BAFF-R, BCMA, and TACI) and thereby block the activity of BLyS and APRIL. In one embodiment, the BLyS/APRIL antagonist comprise an soluble form of a BAFF-R, BCMA and/or TACI receptor. In another embodiment, an antagonist that directly binds BLyS and APRIL comprises, for example, a polypeptide comprising the extracellular domain (ECD) of TACI. Such extracellular domains of TACI or active variants and fragments thereof are disclosed in further detail elsewhere herein. In still other embodiments, the BLyS/APRIL antagonist can comprise multiple, distinct compounds, one acting as a BLyS antagonist and one acting as an APRIL antagonist. In such embodiments, the BLyS/APRIL antagonist can comprise an effective combination of any of the extracellular domains of the BLyS and APRIL receptors (i.e. TACI, BCMA, or BAFF-R) or an active fragment thereof, such that the activity of both BLyS and APRIL is disrupted in the presence of the combination. In still further embodiments, the APRIL/BLyS antagonist comprises an antagonistic antibody or other peptide or small

molecule, wherein the antibody or small molecule blocks activity of both APRIL and BLyS. Alternatively, a combination of antagonistic antibodies and/or small molecules and/or peptides wherein one compound antagonizes APRIL and the other compound antagonizes BLyS can be used. Several assays useful for testing BLyS and/or APRIL antagonists such as the B cell proliferation assay described in WO 00/40716 among others are well known to one of ordinary skill in the art.

i. Extracellular Domains of BLyS and APRIL Receptors

In one embodiment, the BLyS/APRIL antagonist comprises at least the extracellular domain of a BLyS or an APRIL receptor (i.e., TACI, BAFF-R, and/or BCMA). In one embodiment, the BLyS/APRIL antagonist comprises at least the extracellular domain of the TACI polypeptide or an active fragment or variant thereof. The general term "TACI" includes the TACI polypeptides described in WO 98/39361, WO 00/40716, WO 01/85782, WO 01/87979, WO 01/81417, and WO 02/094852, each of which is herein incorporated by reference. One form of the TACI polypeptide is set forth herein and comprises or consists of amino acids 1-246 of SEQ ID NO: 4. TACI polypeptides or fragments and variants thereof that can be employed as BLyS/APRIL antagonists can be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

In other embodiments, the BLyS/APRIL antagonist comprises an extracellular domain of the BCMA polypeptide or an active fragment or active variant thereof. The general term "BCMA" includes the BCMA polypeptides described in Laabi *et al.* (1992) *EMBO J.* 11: 3897-3904; Laabi *et al.* (1994) *Nucleic Acids Res.* 22: 1147-1154; Gras *et al.* (1995) *Int. Immunology* 7: 1093-1106; and Madry *et al.* (1998) *Int. Immunology* 10: 1693-1702, each of which is herein incorporated by reference. One form of the BCMA polypeptide is set forth herein and comprises or consists of amino acid residues 1-184 of SEQ ID NO:5. The BCMA polypeptides of the invention can be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

In still other embodiments, a BLyS antagonist can comprise an extracellular domain of the BAFF-R polypeptide or an active fragments or variants thereof. The general term "BAFF-R" includes the BAFF-R polypeptides described in WO 02/24909 and WO 03/14294, each of which are herein incorporated by reference. One form of the BAFF-R polypeptide is set forth herein and comprises or consists of amino acid residues

1 to 184 of SEQ ID NO:6. The BAFF-R polypeptides of the invention can be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

For the purposes of functioning as a BLyS/APRIL antagonist, the extracellular domain of these various receptors comprises a polypeptide essentially free of the transmembrane or cytoplasmic domains and retains the ability to bind and inhibit BLyS and/or APRIL. Specifically, the extracellular domain of TACI can comprise amino acids 1 to 154 of the TACI polypeptide sequence (SEQ ID NO:4) or active variants and fragments thereof. Exemplary, but non-limiting extracellular domains of TACI that can be employed as a BLyS/APRIL antagonist are described in von Bulow *et al.*, supra, WO 98/39361, WO 00/40716, WO 01/85782, WO 01/87979, WO 01/81417, and US Patent No. 7,501,497 each of which is herein incorporated by reference. Extracellular domains of TACI and variants and fragment thereof will continue to bind at least one of ZTNF2 (APRIL) or ZTNF4 (BLyS). In particular embodiments, the extracellular domains can comprise amino acids 1-106 of SEQ ID NO:4, amino acids 1-142 of SEQ ID NO:4, amino acids 30-154 of SEQ ID NO:4, amino acids 30-106 of SEQ ID NO:4, amino acids 30-110 of SEQ ID NO:4, amino acids 30-119 of SEQ ID NO:4, amino acids 1-166 of SEQ ID NO:4, amino acids 1-165 of SEQ ID NO:4, amino acids 1-114 of SEQ ID NO:4, amino acids 1-119 of SEQ ID NO:4, amino acids 1-120 of SEQ ID NO:4, and amino acids 1-126 of SEQ ID NO:4. In further embodiments, the BLyS/APRIL antagonist comprise amino acid residues 34-66 and 71-104 of SEQ ID NO:4 or amino acids 30-110 of SEQ ID NO:4. In other embodiments, the TACI extracellular domain can comprise those molecules having only one cysteine rich domain, i.e., comprising either amino acid residues 34-66 or amino acid residues 71-104 of SEQ ID NO: 4.

Extracellular domains of BAFF-R include polypeptides comprising amino acids 1-71 of the BAFF-R polypeptide sequence (SEQ ID NO:6) or active fragments and variants thereof which have the ability to act as BLyS/APRIL antagonists. Non-limiting examples, of variants and fragments of the extracellular domain of BAFF-R are described in WO 02/24909, WO 03/14294, and WO 02/38766, each of which is herein incorporated by reference. In particular, these extracellular domains can comprise amino acids 1-77 of SEQ ID NO: 6, amino acids 7-77 of SEQ ID NO:6, amino acids 1-69 of SEQ ID NO:6, amino acids 7-69 of SEQ ID NO:6, amino acids 2-62 of SEQ ID NO:6, amino acids 2-71 of SEQ ID NO:6, amino acids 1-61 of SEQ ID NO:6, amino acids 2-63 of SEQ ID NO:6, amino acids 1-45 of SEQ ID NO:6, amino acids 1-39 of

SEQ ID NO:6, amino acids 7-39 of SEQ ID NO:6, amino acids 1-17 of SEQ ID NO:6, amino acids 39-64 of SEQ ID NO:6, amino acids 19-35 of SEQ ID NO:6, and amino acids 17-42 of SEQ ID NO:6. In addition, the BAFF-R extracellular domain polypeptide can comprise those molecules having a cysteine rich domain, for example, amino acids 1-35 of SEQ ID NO:6. See, Thompson *et al.* (2001) *Science* 293:2108-2111, herein incorporated by reference.

Extracellular domains of BCMA include polypeptides comprising amino acids 1-48 of the BCMA polypeptide sequence (SEQ ID NO:5). Additionally, the extracellular domain can be fragments or variants of this sequence, such as extracellular domains as described in WO 00/40716 and WO 05/075511, each of which is herein incorporated by reference. In particular, these extracellular domains of BCMA can comprise amino acids 1-150 of SEQ ID NO:5, amino acids 1-48 of SEQ ID NO:5, amino acids 1-41 of SEQ ID NO:5, amino acids 8-41 of SEQ ID NO:5, amino acids 8-37 of SEQ ID NO:5, amino acids 8-88 of SEQ ID NO:5, amino acids 41-88 of SEQ ID NO:5, amino acids 1-54 of SEQ ID NO:5, amino acids 4-55 of SEQ ID NO:5, amino acids 4-51 of SEQ ID NO:5, and amino acids 21-53 of SEQ ID NO:5. In addition, the BCMA extracellular domain can comprise those molecules having only a partial cysteine rich domain, including for example, amino acids 6-45 of SEQ ID NO:5. See, also U.S. Patent No. 7,507,580, herein incorporated by reference.

ii. Immunoglobulin Fusion Proteins

The various extracellular domains or active fragments and variants thereof of the various APRIL and BLyS receptors discussed above can further be employed as fusion proteins comprising the extracellular domain or active fragment or variant thereof operably linked to a region of a constant region domain of an immunoglobulin. Within the context of a fusion protein, the term "operably linked" is intended to indicate that the two heterologous proteins are fused in-frame to allow for the expression of a single polypeptide. For example, a region of the APRIL or BLyS receptor can be fused in-frame to at least one constant region domain of an immunoglobulin. Any such fusion protein is referred to herein as Ig-fusion proteins.

As used herein, the term "immunoglobulin-fusion protein" or "Ig-fusion protein" refers to a fusion protein comprising a first polypeptide having a desired antagonist activity (i.e., a BLyS/APRIL antagonist or CD40 pathway antagonist) operably linked to at least one constant region domain of an immunoglobulin. Generally, an

immunoglobulin heavy chain comprises a variable region and a constant region. The constant region is comprised of distinct domains including, for example, the C_{H1} domain, the C_{H2} domain, and the C_{H3} domain. As used herein, in one embodiment, an Fc fragment of an immunoglobulin heavy chain comprises the disulfide heavy chain hinge region, a C_{H2} domain and a C_{H3} domain. Other exemplary Fc fragments of an immunoglobulin heavy chain include, with or without a hinge region, an Fc fragment comprising a C_{H3} domain, or an Fc fragment comprising a C_{H3} domain and a C_{H2} domain. The various domains of the heavy chain constant region can be obtained from any immunoglobulin, such as IgG₁, IgG₂, IgG₃, or IgG₄ subtypes, IgA (including IgA₁ and IgA₂), IgE, IgD or IgM. For example, useful Fc-fusion proteins according to this invention are polypeptides that comprise the BLyS/APRIL binding portions of a BLyS/APRIL receptor (i.e., TACI, BAFF-R, or BCMA) without the transmembrane or cytoplasmic sequences of the BLyS/APRIL receptor. In one embodiment, the extracellular domain of BAFF-R, TACI or BCMA or active variant or fragment thereof is fused to the Fc fragment of an immunoglobulin.

Modified version of human IgG₁ Fc have been generated for Fc fusion proteins. Briefly, Fcγ1, Fc4, Fc5, and Fc6 contain mutations in human IgG₁ Fc to reduce effector functions mediated by the Fc by reducing FcγRI binding and complement C1q binding. The sequences of these modified Fc regions are set forth in US Patent No. 7,501,497 which is herein incorporated by reference.

In one embodiment, the BLyS/APRIL antagonist comprises a TACI-Ig fusion protein. A non-limiting example of a TACI-Ig fusion protein comprises the following components: 1) a polypeptide that comprises the extracellular domain of TACI or a active variant and/or fragment thereof; and 2) at least one immunoglobulin constant region domain. In one embodiment, the methods of the invention utilize a fusion protein comprising at least one human immunoglobulin constant region domain and any polypeptide with at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to the TACI extracellular domain or an active fragment or variant thereof. U.S. Patent Nos. 5,969,102, 6,316,222 and 6,500,428 and U.S. patent applications 091569,245 and 091627,206 (teachings of which are incorporated herein in their entirety by reference) disclose sequences for the extracellular domain of TACI as well as specific fragments of the TACI extracellular domain that interact with BLyS and APRIL. Thus, the TACI-Ig fusion protein can

comprise, for example, any of the following regions of TACI operably linked to at least one immunoglobulin constant region domain: amino acids 1-106 of SEQ ID NO:4, amino acids 1-142 of SEQ ID NO:4, amino acids 30-154 of SEQ ID NO:4, amino acids 30-106 of SEQ ID NO:4, amino acids 30-110 of SEQ ID NO:4, amino acids 30-119 of SEQ ID NO:4, amino acids 1-166 of SEQ ID NO:4, amino acids 1-165 of SEQ ID NO:4, amino acids 1-114 of SEQ ID NO: 4, amino acids 1-119 of SEQ ID NO:4, amino acids 1-120 of SEQ ID NO:4, amino acids 1-126 of SEQ ID NO:4, or amino acid residues 34-66 and 71-104 of SEQ ID NO:4.

In one embodiment, the BLyS/APRIL antagonist comprising the TACI-Ig fusion protein comprises TACI-Fc γ 1, TACI-Fc4, or TACI-Fc6. In a specific embodiment, TACI-Fc5 is employed and comprises a recombinant fusion polypeptide comprising the extracellular domain of TACI or active variant or fragment thereof fused in-frame to Fc5. The amino acid sequence of Fc5 is set forth in SEQ ID NO: 7. Any of the various extracellular domains of TACI described herein can be operably linked to Fc5. In one non-limiting embodiment, the TACI-Fc5 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 8 or an active fragment or variant thereof. For example, a variant of TACI-Fc5 can comprise at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

In another embodiment, the BLyS/APRIL antagonist comprises a BAFF-R-Ig fusion protein. A non-limiting example of a BAFF-R-Ig fusion protein comprises the following components: 1) a polypeptide that comprises the extracellular domain of BAFF-R or a active variant and/or fragment thereof; and 2) at least one immunoglobulin constant region domain. In one embodiment, the methods of the invention utilize a fusion protein comprising at least one human immunoglobulin constant region domain and any polypeptide with at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to the BAFF-R extracellular domain or an active fragment or variant thereof. Non-limiting examples, of variants and fragments of the extracellular domain of BAFF-R are described in WO 02/24909, WO 03/14294, and WO 02/38766, each of which is herein incorporated by reference. Thus, the BAFF-R-Ig fusion protein can comprise, for example, any of the following regions of BAFF-R operably linked to at least one immunoglobulin constant region domain: amino acids 1-77 of SEQ ID NO: 6, amino acids 7-77 of SEQ ID NO:6, amino acids 1-69 of SEQ ID NO:6, amino acids 7-69 of SEQ ID NO:6, amino acids 2-62

of SEQ ID NO:6, amino acids 2-71 of SEQ ID NO:6, amino acids 1-61 of SEQ ID NO:6, amino acids 2-63 of SEQ ID NO:6, amino acids 1-45 of SEQ ID NO:6, amino acids 1-39 of SEQ ID NO:6, amino acids 7-39 of SEQ ID NO:6, amino acids 1-17 of SEQ ID NO:6, amino acids 39-64 of SEQ ID NO:6, amino acids 19-35 of SEQ ID NO:6, and amino acids 17-42 of SEQ ID NO:6. In addition, the BAFF-R extracellular domain polypeptide can comprise those molecules having a cysteine rich domain, for example, amino acids 1-35 of SEQ ID NO:6. See, Thompson *et al.* (2001) *Science* 293:2108-2111, herein incorporated by reference. In one embodiment, the BLyS/APRIL antagonist comprising the TACI-Ig fusion protein comprises BAFF-R-Fc γ 1, BAFF-R-Fc4, BAFF-R-Fc5, or BAFF-R- Fc6.

In still another embodiment, the BLyS/APRIL antagonist comprises a BCMA-Ig fusion protein. A non-limiting example of a BCMA-Ig fusion protein comprises the following components: 1) a polypeptide that comprises the extracellular domain of BCMA or a active variant and/or fragment thereof; and 2) at least one immunoglobulin constant region domain. In one embodiment, the methods of the invention utilize a fusion protein comprising at least one human immunoglobulin constant region domain and any polypeptide with at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to the BCMA extracellular domain or an active fragment or variant thereof. Extracellular domains of BCMA include polypeptides comprising amino acids 1-48 of the BCMA polypeptide sequence (SEQ ID NO:5). Additionally, the extracellular domain can be fragments or variants of this sequence, such as extracellular domains as described in WO 00/40716 and WO 05/075511, each of which is herein incorporated by reference. Thus, the BCMA-Ig fusion protein can comprise, for example, any of the following regions of BCMA operably linked to at least one immunoglobulin constant region domain: In particular, these extracellular domains of BCMA can comprise amino acids 1-150 of SEQ ID NO:5, amino acids 1-48 of SEQ ID NO:5, amino acids 1-41 of SEQ ID NO:5, amino acids 8-41 of SEQ ID NO:5, amino acids 8-37 of SEQ ID NO:5, amino acids 8-88 of SEQ ID NO:5, amino acids 41-88 of SEQ ID NO:5, amino acids 1-54 of SEQ ID NO:5, amino acids 4-55 of SEQ ID NO:5, amino acids 4-51 of SEQ ID NO:5, and amino acids 21-53 of SEQ ID NO:5. In addition, the BCMA extracellular domain can comprise those molecules having only a partial cysteine rich domain as disclosed elsewhere herein.

In one embodiment, the BLyS/APRIL antagonist comprising the BCMA-Ig fusion protein comprises BCMA-Fc γ 1, BCMA-Fc4, BCMA-Fc5 or BCMA-Fc6.

iii. Antagonist Antibodies

5 In still further embodiments, the APRIL/BLyS antagonist comprises an antagonistic antibody. The antagonistic antibody can block the activity of both APRIL and BLyS or alternatively, the antagonist antibody can comprise a combination of antagonistic antibodies wherein one antibody antagonizes APRIL and the other antibody antagonizes BLyS.

10 In one embodiment, the antagonist antibody is specific for BLyS and binds within a region of human BLyS (SEQ ID NO: 1) comprising residues 162-275 of SEQ ID NO:1 and/or a neighboring amino acid or amino acids selected from the group consisting of 162, 163, 206, 211, 231, 233, 264 and 265 of human BLyS. The binding of the antibody is such that the antibody sterically hinders BLyS binding to one or more of its receptors.
15 Such antibodies are described in WO 02/02641 and WO 03/055979. A particularly preferred antibody is the one described as Lymphostat-B™ (belimumab) (Baker *et al.* (2003) *Arthritis Rheum* 48, 3253-3265).

In another embodiment, the antagonist antibody is specific for APRIL and bind within a region of human APRIL (SEQ ID NO: 3). The binding of the antibody is such
20 that the antibody sterically hinders APRIL binding to one or more of its receptors.

Other BLyS/APRIL antagonistic antibodies include, for example, indirect antagonists which interact with TACI, BCMA, and BAFF-R and thereby prevent BLyS and APRIL from activating their receptors. Such antagonistic antibodies include a TACI antibody that binds in a region of TACI (SEQ ID NO:4) such that the binding of BLyS is
25 sterically hindered. For example, binding at amino acids 72-109 of SEQ ID NO:4 or a neighboring region of TACI is believed to block BLyS binding. In addition, APRIL binding to TACI is believed to occur in the region of amino acids 82-222 of SEQ ID NO:4. Thus, binding of APRIL to this region of TACI can also be blocked.

Another BLyS/APRIL antagonist is a BAFF-R antibody that binds in a region of
30 BAFF-R such that binding of human BAFF-R (SEQ ID NO:6) to BLyS is sterically hindered. For example, binding at amino acids 23-38 or amino acids 17-42 of SEQ ID NO:6 or a neighboring region of BAFF-R is believed to block BLyS binding.

Finally, a further indirect antagonist would be a BCMA antibody that binds in a region of BCMA (SEQ ID NO:5) such that the binding of BLyS is sterically hindered. For example, binding at amino acids 5-43 of SEQ ID NO:5 or a neighboring region of BCMA is believed to block BLyS (or APRIL) binding.

When employing an antagonistic antibody that directly interacts with the various APRIL and BLyS receptors, a combination of antibodies which block activation of all receptors (BAFF-R, TACI and BCMA) is preferred.

iv. Other BLyS/APRIL Antagonists

In still further embodiments, a BLyS/APRIL antagonist can include BLyS or APRIL binding polypeptides that are not native sequences or variants of BLyS or APRIL. Some examples of BLyS antagonistic polypeptides are those having the sequence of Formula I, Formula II, Formula III as described in WO 05/000351. In particular, some binding polypeptides include ECFDLLVRAWVPCSVLK (SEQ ID NO:9), ECFDLLVRHWVPCGLLR (SEQ ID NO:10), ECFDLLVRRWVPCMLG (SEQ ID NO:11), ECFDLLVRSWVPCHMLR (SEQ ID NO:12), ECFDLLVRHWVACGLLR (SEQ ID NO:13), or sequences listed in FIG. 32 of WO 05/000351, herein incorporated by reference..

Other BLyS/APRIL antagonistic peptides or small molecules include, for example, indirect antagonists which interact with TACI, BCMA, and BAFF-R and thereby prevent BLyS and APRIL from activating their receptors. Such antagonistic peptides or small molecules can bind TACI in a region such that the binding of BLyS and/or APRIL is sterically hindered. For example, binding at amino acids 72-109 of SEQ ID NO:4 or a neighboring region of TACI is believed to block BLyS binding.

Another BLyS/APRIL antagonist is an antagonist peptide or small molecule that binds in a region of BAFF-R such that binding of human BAFF-R to BLyS is sterically hindered. For example, binding at amino acids 23-38 or amino acids 17-42 of SEQ ID NO:6 or a neighboring region of BAFF-R is believed to block BLyS binding. Finally, a further indirect antagonist would be an antagonistic peptide that binds in a region of BCMA such that the binding of BLyS and APRIL is sterically hindered. For example, binding at amino acids 5-43 of SEQ ID NO:5 or a neighboring region of BCMA is believed to block BLyS (or APRIL) binding. It is recognized when employing an antagonistic peptide or small molecule that directly interacts with the various APRIL and BLyS

receptors, a combination of molecules which block activation of all receptors (BAFF-R, TACI and BCMA) is preferred.

II. CD40 Pathway Antagonists

5 The cell surface molecule CD40 is a member of the tumor necrosis factor receptor superfamily and is broadly expressed by immune, hematopoietic, vascular, epithelial, and other cell types. As used herein, the term “CD40 pathway” antagonist refers to a compound, including for example, an antibody, a synthetic or native sequence peptide and/or a small molecule antagonist, that functions in a direct or indirect manner
10 to partially or fully block, inhibit or neutralize CD40 signaling *in vitro* or *in vivo*. Antagonism using the CD40 pathway antagonist does not necessarily result in the total elimination of CD40 signaling activity. Instead, the activity of the CD40 pathway could decrease by a physiological relevant amount including, for example, a decrease of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,
15 75%, 80%, 85%, 95% or 100% of the activity of CD40 signaling compared to an appropriate control.

 In one embodiment, the CD40 pathway antagonist comprises a “CD40 antagonist”. As used herein CD40 comprises a member of the TNF-receptor superfamily and is a co-stimulatory protein found on antigen presenting cells and is required for their
20 activation. The binding of CD154 (CD40L) on T_H cells to CD40 activates antigen presenting cells and induces a variety of downstream effects. The CD40 receptor has been found to be essential in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation. A CD40 antagonist inhibits/reduces,
25 directly or indirectly, CD40 activity, interactions of CD40 with the CD40 ligands, or the functional responses mediated through CD40 signaling. Preferably, a CD40 antagonist will inhibit or reduce such activities by a physiologically relevant amount, including, at least by about 10% or 20%, more preferably by at least about 30% or 40%, still more preferably by at least about 50% or 60%, yet more preferably by at least about 70% or
30 80% and most preferably by at least about 90%. Such antagonists can be identified by a number of art recognized APC- and/or T-cell functional assays, such as T cell proliferation and/or effector function, antibody production, cytokine production, and phagocytosis. In other embodiments, the biological activity of the CD40 antagonist is determined, for example, by competition for binding to the ligand binding domain of

CD40 (*i.e.* competitive binding assays). Both murine CD154 and human CD154 bind to human CD40. In specific embodiments, CD40 antagonist will disrupt the CD40/CD154 interaction.

In one embodiment, the CD40 pathway antagonist comprises an antagonist antibody that binds directly to CD40. Non-limiting examples of CD40 antagonistic antibodies include, but are not limited to, F33 (Ellmark *et al.* (2002) *Immunology* 106: 456-463), 4D11 (Aoyagi *et al.* (2009) *Am J Transplant.* 9(8):1732-41), HCD122 (Luqman *et al.* (2008) *Blood* 112(3):711-20), 15B8 (US Patent 7445780), and 5D12 (US Patent 7361345). Additional antagonistic antibodies that bind to CD40 and block/inhibit its function, include, but are not limited to, Mab5D12 and variant thereof as disclosed in US Publication 2008/0085531 and the antagonist anti-CD40 antibodies disclosed in US Publication 2007/0292439. The contents of each of these references is herein incorporated by reference.

In other embodiment, the CD40 pathway antagonist comprises a “CD154” or “CD40L” antagonist. As used herein, CD40L or sCD40L is interchangeable with CD154 or sCD154. CD154 represents a ligand for CD40 and the mammalian form of CD154 and various variants thereof are characterized in US Publication 2003/0091564, which is herein incorporated by reference. Such an antagonist inhibits/reduces, directly or indirectly, the ability of CD154 from binding to the CD40 receptor or inhibits/reduces, directly or indirectly, the ability of CD154 to activate CD40. Preferably, a CD154 antagonist will inhibit or reduce the production, secretion, release, binding, uptake or biological activity of CD154 by a physiological relevant amount, for example, by at least about 10% or 20%, by at least about 30% or 40%, by at least about 50% or 60%, by at least about 70% or 80%, or by at least about 90%.

In one embodiment, the CD40 pathway antagonist comprises an antagonist antibody that binds directly to CD154. Non-limiting examples of CD154 antagonistic antibodies include BG9588 (Boumpas *et al.* (2003) *Arthritis Rheum* 48(3):719-27), hu5C8 (Huang *et al.* (2002) *Arthritis Rheum* 46(6):1554-62), IDEC-131 (Brams *et al.* (2001) *Int Immunopharmacol* 1(2):277-94), MR-1 (Stein (1998) *Gene Ther* 5(4):431-9), each of these references is herein incorporated by reference. Small molecule antagonists of CD40/CD154 are also known. See, for example, Margolles-Clark *et al.* (2009) *Journal of Molecular Medicine* 87:1133-1143.

In still further embodiments, the CD40 pathway antagonist comprises an immunoglobulin fusion protein which comprises a first polypeptide having an

antagonistic activity for the CD40 pathway operably linked to at least one constant region domain of an immunoglobulin. As described in more extensive detail elsewhere herein, at least the extracellular domains or active fragments or variants thereof of receptor molecules can be employed as the antagonist polypeptides in these fusion proteins. Thus, in one embodiment, the CD40 antagonist comprises a CD40 pathway antagonist polypeptide-Ig fusion protein. In still further embodiments, the CD40 pathway antagonist polypeptide comprises at least the extracellular domain or active variant or fragment thereof of receptors found in the CD40 pathway, including, the extracellular domain from CD40 or from CD154.

Various constant region domains of immunoglobulins that can be employed in these CD40 pathway antagonist-Ig fusion proteins and can comprise, for example, a C_{H1} domain, a C_{H2} domain, or a C_{H3} domain, or an Fc fragment of an immunoglobulin heavy chain. [Exemplary Fc fragments of an immunoglobulin heavy chain include, with or without a hinge region, an Fc fragment comprising a C_{H3} domain, or a C_{H3} domain and a C_{H2} domain.] The various domains of the heavy chain constant region can be obtained from any immunoglobulin, such as IgG₁, IgG₂, IgG₃, or IgG₄ subtypes, IgA (including IgA₁ and IgA₂), IgE, IgD or IgM. Modified version of human IgG₁ Fc can also be used, such as, Fcγ1, Fc4, Fc5, and Fc6, which are described elsewhere herein.

A CD40 pathway antagonist-Ig fusion protein can be produced by recombinant methods according to standard techniques (e.g., see Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). In one embodiment, the CD40 antagonistic polypeptide comprises of a portion of the CD40 polypeptide (e.g., a portion after cleavage of the signal sequence) that is sufficient to continue to bind the ligand, CD154. The second peptide can include an immunoglobulin constant region domain (see, for example, U.S. Pat. Nos. 5,116,964; 5,580,756; 5,844,095); a GST peptide, or an influenza hemagglutinin epitope tag (HA) (e.g. Herrsher *et al.* (1995) *Genes Dev.* 9:3067-3082). Such fusion proteins can be monovalent or bivalent as is recognized in the art. The immunoglobulin constant region may contain genetic modifications which reduce or eliminate effector activity inherent in the immunoglobulin structure. See, for example, WO 97/28267.

III. Antibodies and Antibody Fragments

As outlined above, in some embodiments, the BLyS/APRIL antagonist and/or the CD40 pathway antagonist comprises an antagonist antibody. As discussed herein, these antagonist antibodies are collectively referred to as “antagonistic BLyS/APRIL antibodies” and “antagonistic CD40 pathway antibodies”. All of these antibodies are encompassed by the discussion herein. The respective antibodies can be used alone or in combination in the methods of the invention. The term “antibody” is used in the broadest sense and specifically covers, for example, monoclonal antibodies, polyclonal antibodies, antibodies with polyepitopic specificity, single chain antibodies, and fragments of antibodies. “Antibodies” also include fragments of the above (e.g., CDR’s, CDR3, single-chain antibodies, FAV, and the like which confer the binding specificity of the antibody to the target. The antibody employed herein can be chimeric, humanized, or human antibody.

By “antibodies that specifically bind” is intended that the antibodies will not substantially cross-react with another polypeptide. By “not substantially cross react” is intended that the antibody or fragment has a binding affinity for a non-homologous protein which is less than 10%, more preferably less than 5%, and even more preferably less than 1%, of the binding affinity for its given target.

The antagonistic antibodies disclosed herein and for use in the methods of the present invention can be produced using any antibody production method known to those of skill in the art. Thus, polyclonal sera may be prepared by conventional methods. In general, a solution containing the desired antigen is first used to immunize a suitable animal, preferably a mouse, rat, rabbit, or goat. Rabbits or goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies.

Polyclonal sera can be prepared in a transgenic animal, preferably a mouse bearing human immunoglobulin loci. Immunization can also be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund’s complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously, intraperitoneally, or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund’s incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using

methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera are obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*e.g.*, 1,000 x g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that can be present in minor amounts.

Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256: 495, or may be made by recombinant DNA methods (see, *e.g.*, U. S. Patent No. 4,816, 567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) *Nature* 352: 624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597, for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain (s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816, 567; Morrison *et al.* (1984) *Proc. Natl. Acad. Sci. USA*, 81: 6851-6855). Methods of making chimeric antibodies are known in the art.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin.

5 For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are
10 replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or
15 substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no
20 more than 3. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.* (1986) *Nature* 321: 522-525; Reichmann *et al.* (1988) *Nature* 332: 323-329; and Presta (1992) *Curr. Op. Struct. Biol.* 2: 593-596. The humanized antibody includes a PRIMATIZED antibody wherein the antigen-binding
25 region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest. Methods of making humanized antibodies are known in the art.

Human antibodies can also be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter (1991) *J. Mol. Biol.* 227: 381; Marks *et al.* (1991) *J. Mol. Biol.* 222: 581. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77; and, Boerner *et al.* (1991) *J. Immunol.* 147(1):86-95.

IV. Variants and Fragments

Depending on the context, “fragment” refers to a portion of the amino acid sequence of a polypeptide or protein, or polynucleotide encoding a portion of the amino acid sequence of a polypeptide or protein. Fragments may retain the activity of the original protein and hence, such “active” fragments include, for example, fragments of an extracellular domain of TACI, BCMA, BAFF-R, CD40 or CD154 which continue to bind their ligand, and thereby act as BLyS/APRIL antagonists. Thus, active fragments of an extracellular domain of TACI, BCMA, BAFF-R, CD40 or CD154 can encode at least 15, 25, 30, 50, 100, 150, 200, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length extracellular domain. Similarly, active fragments of a nucleotide sequence can range from at least 20 nucleotides, 50 nucleotides, 100 nucleotides, and up to the full-length polynucleotide encoding an active extracellular domain of TACI, BCMA, BAFF-R, CD40 or CD154.

“Variant” protein is intended to mean a protein derived from a native and/or original protein by deletion (so-called truncation) of one or more amino acids at the N-terminal and/or C-terminal end of the protein; deletion and/or addition of one or more amino acids at one or more internal sites in the protein; or substitution of one or more amino acids at one or more sites in the protein. Variant proteins encompassed by the present invention are active. Active variants continue to possess the desired activity, i.e., having antagonistic activity against BLyS and/or APRIL. Thus, active variants of an antagonistic polypeptide of the invention will have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs and parameters described elsewhere herein. An active variant of a polypeptide may differ from that polypeptide by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

The extracellular domain of TACI, BCMA, BAFF-R, CD40 or CD154 may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, active amino acid sequence variants and fragments of the extracellular domains of the TACI, BCMA and BAFF-R, CD40 or CD154 can be prepared by mutations in the encoding polynucleotide. Methods for mutagenesis and polynucleotide alterations are

well known in the art. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82: 488-492; Kunkel *et al.* (1987) *Methods in Enzymol.* 154: 367-382; U.S. Patent No. 4,873,192; Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York) and the references cited therein. Guidance
5 as to amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be made. One skilled in the art will appreciate that the
10 activity of an antagonist polypeptide disclosed herein can be evaluated by routine screening assays.

As used herein, a "native sequence" polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding polypeptide derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by
15 recombinant and/or synthetic means. The term "native sequence" specifically encompasses naturally-occurring truncated, soluble or secreted forms (e. g., an extracellular domain sequence), naturally-occurring variant forms (e. g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide.

As used herein, "sequence identity" or "identity" in the context of two
20 polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for
25 other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or
30 "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1.

The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

V. Pharmaceutical Compositions

The BLyS/APRIL antagonist and the CD40 pathway antagonist (also referred to herein as "active compounds") disclosed herein can be incorporated into pharmaceutical compositions suitable for administration. The BLyS/APRIL antagonist and the CD40 pathway antagonist can be formulated in separate pharmaceutical compositions or can be formulated within a single pharmaceutical composition for simultaneous administration. Such compositions typically comprise each antagonist separately or in combination and a pharmaceutically acceptable carrier. As used herein, the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and

agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, or subcutaneous administration. In addition, it may be desirable to administer a therapeutically effective amount of the pharmaceutical composition locally to an area in need of treatment (e.g., where it is desirable to have a reduced immune response, such as to an area of the body where the graft transplantation occurred). This can be achieved by, for example, local or regional infusion or perfusion during surgery, injection, catheter, or implant (for example, implants formed from porous, non-porous, or gelatinous materials, including membranes, such as sialastic membranes or fibers), and the like. In one embodiment, administration can be by direct injection at the site (or former site) of a graft transplant. In another embodiment, the therapeutically effective amount of the pharmaceutical composition is delivered in a vesicle, such as liposomes (see, e.g., Langer, *Science* 249:1527-33, 1990 and Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez Berestein and Fidler (eds.), Liss, N.Y., pp. 353-65, 1989).

In yet another embodiment, the therapeutically effective amount of the pharmaceutical composition can be delivered in a controlled release system. In one example, a pump can be used (see, e.g., Langer, *Science* 249:1527-33, 1990; Sefton, *Crit. Rev. Biomed. Eng.* 14:201-40, 1987; Buchwald *et al.*, *Surgery* 88:507-16, 1980; Saudek *et al.*, *N. Engl. J. Med.* 321:574-79, 1989). In another example, polymeric materials can be used (see, e.g., Levy *et al.*, *Science* 228:190-92, 1985; During *et al.*, *Ann. Neurol.* 25:351-56, 1989; Howard *et al.*, *J. Neurosurg.* 71:105-12, 1989). Other controlled release systems, such as those discussed by Langer (*Science* 249:1527-33, 1990), can also be used.

Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and

agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

5 Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL9 (BASF; Parsippany, NJ), or phosphate buffered saline (PBS). In
10 all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid
15 polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,
20 ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

25 Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated
30 above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated with each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

The various formulations of the APRIL/BLyS antagonist and the CD40 pathway antagonist described above can be used in combination with other medicaments. Such medicaments include but are not limited to NSAIDS (nonsteroidal anti-inflammatory drugs) both over the counter and those requiring a prescription such as diclofenac sodium, indomethacin, diflunisal and nabumetone; anti-malarials such as hydroxychloroquine sulfate and chloroquine; corticosteroids such as prednisone, hydrocortisone, and methylprednisolone; and immunosuppressives such as azathioprine, cyclophosphamide, methotrexate, cyclosporine, and mycophenolate mofetil, and IVIg, DHEA, and thalidomide.

In one embodiment, a pharmaceutical composition comprises a therapeutically effective amount of a combination therapy comprising the polypeptide set forth in SEQ ID NO:8 or an active variant or fragment thereof and the MR1 antibody.

VI. Methods of Treatment

Methods are provided that promote immune tolerance and act to reduce an immune response in a subject in need thereof. The methods comprise administering to the subject a therapeutically effective amount of a combination therapy comprising a BLYS/APRIL antagonist (e.g., TACI-Ig fusion protein, including for example, the TACI-Ig fusion protein set forth in SEQ ID NO :8 or an active variant or fragment thereof) and a CD40 pathway antagonist. Such methods find use in reducing the level of immunoglobulins in the serum of the subject and can be employed to reduce an immune response to a foreign antigen (i.e., a decrease in alloantibody levels or xenoantibody levels) and/or in treating a subject having an immune-mediated disorder, such as an autoimmune disorder or an inflammatory disorder. Thus, the combination therapy comprising a BLYS/APRIL antagonist and a CD40 pathway antagonist prevents or treats graft transplant rejection, reduces anti-graft responses in a subject in need thereof, prolongs graft transplant survival and/or treats or prevents graft-vs.-host disease.

Additional immune and inflammatory disorders that can be treated or prevented include, but are not limited to, any "autoimmune disease", which is defined herein a non-malignant disease or disorder arising from and directed against an individual's own tissues. Non-limiting autoimmune diseases include arthritis (rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), psoriasis, dermatitis including atopic dermatitis; chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, systemic scleroderma and sclerosis, responses associated with inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis), respiratory distress syndrome, adult respiratory distress syndrome (ARDS), meningitis, allergic rhinitis, encephalitis, uveitis, colitis, glomerulonephritis, allergic conditions, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE), lupus (including nephritis, non-renal, discoid, alopecia), juvenile onset diabetes, multiple sclerosis, allergic encephalomyelitis, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis, agranulocytosis, vasculitis (including ANCA), aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia,

pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet disease, Castleman's syndrome, Goodpasture's Syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens- Johnson syndrome, solid organ transplant rejection (including pretreatment for high panel reactive antibody titers, IgA deposit in tissues, etc), graft versus host disease (GVHD), pemphigoid bullous, pemphigus (all including vulgaris, foliaceus), autoimmune polyendocrinopathies, Reiter's disease, stiff-man syndrome, giant cell arteritis, immune complex nephritis, IgA nephropathy, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism; autoimmune endocrine diseases including autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM) and Sheehan's syndrome; autoimmune hepatitis, Lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre'Syndrome, Large Vessel Vasculitis (including Polymyalgia Rheumatica and Giant Cell (Takayasu's) Arteritis), Medium Vessel Vasculitis (including Kawasaki's Disease and Polyarteritis Nodosa), ankylosing spondylitis, Berger's Disease (IgA nephropathy), Rapidly Progressive Glomerulonephritis, Primary biliary cirrhosis, Celiac sprue (gluten enteropathy), Cryoglobulinemia, ALS, coronary artery disease.

In non-limiting embodiments, methods to reduce antibody levels (including IgG antibody levels) in a mammal in need thereof; method to reduce alloantibody levels (including IgG alloantibody levels), method to reduce xenoantibody levels (including IgG xenoantibody levels), method to reduce donor-reactive antibody levels (including IgG donor-reactive antibody levels), methods to reduce anti-graft responses, methods to prevent or treat graft transplant rejection; or methods to treat or prevent graft-vs.-host disease, method to reduce subject host-reactive antibody levels (including IgG subject host-reactive antibody levels), are provided. The methods comprise administering to the

mammal a therapeutically effective amount of a combination therapy comprising (a) a TACI-immunoglobulin fusion protein comprising the extracellular domain of TACI or an active fragment thereof; and, (b) an antagonist of the CD40 pathway. In further embodiments, the TACI-Ig fusion protein comprises the sequence set forth in SEQ ID NO:8 or an active variant or fragment thereof. In still further embodiments, the combination therapy comprises the TACI-Ig fusion protein and the MR1 anti-CD154 antibody. In such methods, the combination therapy can decrease antibody titers (including IgG antibody titers, or other combination of antibody classes as discussed herein) in the serum of the mammal by a statistically significant amount including a reduction of about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%. In still other embodiments, the combination therapy can decrease alloantibody titers (including IgG alloantibody titers, or other combination of alloantibody classes as discussed herein) in the serum of the mammal by a statistically significant amount including a reduction of about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%. In still other embodiments, the combination therapy can decrease xenoantibody titers (including IgG xenoantibody titers, or other combination of xenoantibody classes as discussed herein) in the serum of the mammal by a statistically significant amount including a reduction of about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%. In further embodiments, a biological sample from the subject is not analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, or BCMA prior to administration of the combination therapy. In still other embodiments, a biological sample from the subject need not be analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, or BCMA prior to administration of said combination therapy. In still further embodiments, the methods employed herein do not require that the biological sample from the subject be analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, BCMA, TACI, BAFF-R, and/or BLYS prior to administration of the combination therapy. In further embodiments, a biological sample from the subject is not analyzed for an elevated level of BLYS/APRIL heterotrimer (HT), APRIL, BCMA, TACI, BAFF-R, and/or BLYS prior to administration of the combination therapy. It is recognize that in any of these embodiments, the elevated level of APRIL or BLYS can be detected by either detecting the homotrimer of each protein form, or alternatively, by detecting the level of the individual subunit of each homotrimer.

“Treatment” is herein defined as curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving, or affecting the condition or the symptoms of a subject with an immune-mediated disorder, including for example, transplant rejections or graft-vs-host disease. The subject to be treated can be suffering from or at risk of developing an immune-mediated disorder, including, for example, be in need of a reduction in immunoglobulin levels, being prepared for a graft transplantation procedure, has undergone a graft transplantation procedure, or is suffering from or is at risk of suffering from host-vs-graft disease. By “treatment” is also intended that the combination of the BLyS/APRIL antagonist and the CD40 pathway antagonist is administered to the subject as part of a single pharmaceutical composition, or alternatively as part of individual pharmaceutical compositions administered simultaneously or consecutively, each comprising either the BLyS/APRIL antagonist or the CD40 pathway antagonist.

Administration of the BLyS/APRIL antagonist in combination with the CD40 pathway antagonist can be for either a prophylactic or therapeutic purpose. By “preventing” is intended that the combination of agents is provided prophylactically, i.e., the combination is provided in advance of any symptom. The prophylactic administration of the combination of agents serves to prevent or attenuate any subsequent symptom. When provided therapeutically, the substance is provided at (or shortly after) the onset of a symptom. The therapeutic administration of the substance serves to attenuate any actual symptom.

In one embodiment, methods are provided to treat or prevent or prolong the development of graft transplantation rejection or host-vs-graft disease in a subject. A “graft” as used herein refers to biological material derived from a donor for transplantation into a recipient. Grafts include such diverse material as, for example, isolated cells such as islet cells; tissue such as the amniotic membrane of a newborn, bone marrow, hematopoietic precursor cells, stem cells, neuronal or embryonic cells, and ocular tissue, such as corneal, macular or retinal, tissue; and organs such as heart, skin, liver, spleen, pancreas, thyroid lobe, lung, kidney, tubular organs (e.g., intestine, blood vessels, or esophagus), etc. The tubular organs can be used to replace damaged portions of esophagus, blood vessels, or bile duct. The skin grafts can be used not only for burns, but also as a dressing to damaged intestine or to close certain defects such as diaphragmatic hernia. The graft is derived from any mammalian source, including human, whether from cadavers or living donors. In specific embodiments, the graft is

bone marrow or an organ such as heart and the donor of the graft and the host are matched for HLA class II antigens. A "donor" as used herein refers to the mammalian species, dead or alive, from which the graft is derived.

By "subject" is intended mammals, e.g., primates, humans, agricultural and domesticated animals such as, but not limited to, dogs, cats, cattle, horses, pigs, sheep, and the like. In specific embodiments, the subject undergoing treatment with the pharmaceutical formulations of the invention is a human. The subjects of the invention may be suffering from the symptoms of transplant rejection or graft-vs-host disease or may be at risk for transplant rejection or graft-vs-host disease (e.g. a subject that will undergo transplantation procedures). A "mammalian subject host" refers to any compatible transplant recipient. By "compatible" is meant a mammalian subject host that will accept the donated graft (e.g., heart allograft). If both the donor of the graft and the host are human, they are preferably matched for HLA class II antigens so as to improve histocompatibility.

The term "transplant" or "transplantation" and variations thereof refers to the insertion of a graft into a host, whether the transplantation is syngeneic (where the donor and recipient are genetically identical), allogeneic (where the donor and recipient are of different genetic origins but of the same species), or xenogeneic (where the donor and recipient are from different species). Thus, in a typical scenario, the host is human and the graft is an allograft, derived from a human of the same or different genetic origins. In some cases, the host is human and the graft is a heart allograft, derived from a human of the same or different genetic origins. In another scenario, the graft is derived from a species different from that into which it is transplanted, such as a baboon heart transplanted into a human recipient host, and including animals from phylogenically widely separated species, for example, a pig heart valve, or animal beta islet cells or neuronal cells transplanted into a human host.

The methods of the invention can be used to treat or prevent various forms of graft rejections. For example, the methods can be used to treat or prevent acute rejection, hyperacute rejection and/or chronic rejection. The term "acute rejection" refers to onset of a primary immune response to a graft, generally within days or weeks, and up to about 6 to 12 months, after transplantation. The immune response is caused by T cell recognition of the transplanted tissue associated with e.g., prominent local cytokine production, widespread pro-inflammatory activation of vascular endothelia, intense leukocyte infiltration, and development of graft-reactive, cytolytic T cells (CTL) that has

traditionally been associated with the acute loss of graft function. In other embodiments, the methods of the invention treat or prevent hyperacute rejection. A "hyperacute rejection" is a type of rejection that occurs very rapidly, resulting in necrosis of the transplanted tissue within minutes or a few hours of contact, and is caused by reactivity of the donor cells with pre-existing antibody. As used herein, the terms "chronic rejection" refers to indolent, progressive immune responses that often occur one or more years after transplantation. Chronic rejection usually manifests in vascularized solid organ allografts as obliterative arteriopathy or graft vascular disease (GVD), infiltration of immunocytes, interstitial and tubular atrophy, graft arteriosclerosis, and a marked fibrosis.

The method disclosed herein further can be used to treat or prevent graft-versus-host disease. As used herein, "graft-versus-host reaction or disease (GVH)" refers to the pathologic consequences of a response initiated by transplanted immunocompetent T lymphocytes into an allogeneic or xenogeneic, immunologically incompetent host. The host is unable to reject the grafted T cells and the transplanted T lymphocytes attack the tissues of the recipient due to recognition of recipient's antigens on recipient's MHC molecules (not necessarily by recipient's tissues).

The administration of the BLyS/APRIL antagonist in combination with the CD40 pathway antagonist results in a statistically significant decrease in an anti-graft response of the subject having undergone treatment (e.g., a heart allograft transplantation) compared to a subject not having undergone the treatment. A decreased anti-graft response can be measured by a number of clinical symptoms known to those of skill in the art and can be reflected in a reduction in the level of immunoglobulins in the serum of the patient, including, for example, a reduction in the level of alloantibody or xenoantibody production against the graft tissue (e.g., heart allograft) or a reduction in the level of donor-reactive antibody production against the graft tissue, including a reduction in any of the antibody or alloantibody or xenoantibody classes or combinations thereof as described elsewhere herein. The decrease in the anti-graft response can occur for at least at least about 1 week, 1 month, 6 months, 1 year or more. In still other embodiments, the therapy disclosed herein can be used to achieve at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 years or greater of a decrease in an anti-graft response. The decrease in the anti-graft response can be achieved through repeated administration of the therapy over this time period or it can be achieved after the termination of the treatment. As used herein, the phrase "long-term decrease or prevention of an anti-graft response" refers to a

significant decrease in the anti-graft response in a subject for an extensive period of time, such as one or more years, preferably several years, and more preferably life. In other embodiments, the methods disclosed here provide for delaying the onset of an anti-graft response by a statistically significant amount when compared to an appropriate control.

5 The delayed onset can be about a 5%, 10%, 15%, 20%, 25%, 30%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or a greater increase in time compared to an appropriate control (i.e., a patient not receiving the combination therapy disclosed herein). In other embodiments, the graft survival time can be increased employing the methods disclosed herein by at least 5%, 10%, 15%, 20%, 25%, 30%, 50%, 60%, 70%, 80%, 90%, 100%,
10 150%, 200% or greater increase in time compared to an appropriate control (i.e., a patient not receiving the combination therapy disclosed herein).

Moreover, when the combination therapy is administered to treat or prevent transplantation rejection or graft-vs.-host disease, the combination therapy can result in a decrease in serum antibody titers or a decrease in serum alloantibody titers or a decrease
15 in serum xenoantibody titers, including serum IgG antibody and/or IgG alloantibody and/or IgG xenoantibody titers, within at least 30 days, 25 days, 20 days, 15 days, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 day post-graft transplantation.

In other embodiments, the methods disclosed herein provide for delaying the onset of transplantation rejection or graft-vs.-host disease by a statistically significant
20 amount when compared to an appropriate control. The delayed onset can be by about 5%, 10%, 15%, 20%, 25%, 30%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or a greater increase in time compared to an appropriate control (i.e., a patient not receiving the combination therapy disclosed herein). In still further embodiments, the methods provided herein find use in delaying memory CD4 T cell induced alloantibody responses
25 in heart allograft recipients. This delay may attenuate antibody-mediated allograft injury, but, in some embodiments, may also hinder the kinetics of T cell priming and/or impair T cell trafficking into the graft, thus leading to prolonged allograft survival.

The methods of the invention comprise using a combination therapy. The term “combination” is used in its broadest sense and means that a subject is treated with at
30 least two therapeutic agents, more particularly a BLYS/APRIL antagonist and a CD40 pathway. The timing of administration of the BLYS/APRIL antagonist and the CD40 pathway antagonist can be varied so long as the beneficial effects of the combination of these agents are achieved. The phrase “in combination with” refers to the administration of a BLYS/APRIL antagonist with a CD40 pathway antagonist either simultaneously,

sequentially, or a combination thereof. Therefore, a subject undergoing a combination therapy of the invention can receive the BLyS/APRIL antagonist and the CD40 pathway antagonist at the same time (i.e., simultaneously) or at different times (i.e., sequentially, in either order, on the same day or on different days), so long as the therapeutic effect of the combination of both agents is achieved in the subject undergoing therapy. Where the BLyS/APRIL antagonist and the CD40 pathway antagonist are administered simultaneously, they can be administered as separate pharmaceutical compositions, each comprising either a BLyS/APRIL antagonist or a CD40 pathway antagonist, or can be administered as a single pharmaceutical composition comprising both agents.

The methods of the invention comprise administering to a subject a therapeutically effective amount of a BLyS/APRIL antagonist in combination with a CD40 pathway antagonist. Any method for administering the BLyS/APRIL antagonist and/or the CD40 pathway antagonist to a subject may be used in the practice of the invention. Examples of possible routes of administration include parenteral, (e.g., intravenous (IV), intramuscular (IM), intradermal, subcutaneous (SC), or infusion) administration. By “therapeutically effective dose,” “therapeutically effective amount,” or “effective amount” is intended an amount of the BLyS/APRIL antagonist that, when administered in combination with an amount of a CD40 pathway antagonist, brings about a positive therapeutic response with respect to treatment or prevention. “Positive therapeutic response” refers to, for example, improving the condition of at least one of the symptoms of an immune mediated disorder.

Moreover, the administration may be by continuous infusion or by single or multiple boluses. In specific embodiments, one or both of the agents is infused over a period of less than about 4 hours, 3 hours, 2 hours or 1 hour. In still other embodiments, the infusion occurs slowly at first and then is increased over time.

Generally, the dosage of the combination of agents will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. In specific embodiments, it may be desirable to administer the BLyS/APRIL antagonist in the range of from about 1 to 20 mg/kg, 5 to 10 mg/kg, 2 to 10 mg/kg, 10 to 20 mg/kg, 5 to 15 mg/kg, 1 to 10 mg/kg, 0.1 to 5 mg/kg, 1 to 5 mg/kg, 1 to 3 mg/kg, 2 to 5 mg/kg or any range in between 1 and 20 mg/kg. When the BLyS/APRIL antagonist comprises the TACI-Ig polypeptide or the TACI-Ig polypeptide set forth in SEQ ID NO:8 the range of administration can be from about 1 to 20 mg/kg, 5 to 10

mg/kg, 2 to 10 mg/kg, 10 to 20 mg/kg, 5 to 15 mg/kg, 1 to 10 mg/kg, 0.1 to 5 mg/kg, 1 to 5 mg/kg, 1 to 3 mg/kg, 2 to 5 mg/kg or any range in between 1 and 20 mg/kg.

In specific embodiments, it may be desirable to administer the CD40 pathway antagonist in the range of from about 1 to 100 mg/kg, 20 to 30mg/kg, 30 to 40 mg/kg, 40 to 50 mg/kg, 50 to 60 mg/kg, 60 to 70 mg/kg, 70 to 80 mg/kg, 80 to 100mg/kg, 1 to 20 mg/kg, 5 to 10 mg/kg, 2 to 10 mg/kg, 10 to 20 mg/kg, 5 to 15 mg/kg, 1 to 10 mg/kg, 1 to 5 mg/kg, 2 to 5 mg/kg or any range in between 1 and 20 mg/kg. When the CD40 pathway antagonist comprises an anti-CD154 antagonistic antibody or the anti-CD154 antibody MR1, the range of administration can be from about 1 to 100 mg/kg, 20 to 30mg/kg, 30 to 40 mg/kg, 40 to 50 mg/kg, 50 to 60 mg/kg, 60 to 70 mg/kg, 70 to 80 mg/kg, 80 to 100mg/kg, 5 to 10 mg/kg, 2 to 10 mg/kg, 10 to 20 mg/kg, 5 to 15 mg/kg, 1 to 10 mg/kg, 1 to 5 mg/kg, 2 to 5 mg/kg or any range in between 1 and 100 mg/kg.

The timing of administration of the BLyS/APRIL antagonist and the CD40 pathway antagonist can vary. Each agent can be administered simultaneously or separately pre or post-graft transplantation. For example, both agents can be administered simultaneously prior to graft transplantation, during the graft transplantation procedure, following graft transplantation and prior to the development of graft rejection symptoms, and/or following the development of the graft rejection symptoms. Alternately, the agents can be administered consecutively but prior to graft transplantation, during the graft transplantation procedure, following graft transplantation and prior to the development of graft rejection symptoms, and/or following the development of the graft rejection symptoms. In specific embodiments, the antagonist of the CD40 pathway is administered prior to transplantation and the TACI immunoglobulin fusion protein is administered following transplantation.

Administration of the BLyS/APRIL antagonist and/or CD40 pathway antagonist prior to the graft transplantation procedure can act to "desensitize" a mammal awaiting transplantation and thereby reduce or abolish allergic sensitivity or reactivity to a graft transplant, prior to administration of the transplant to the mammal. Thus, in specific embodiments, one or both of the BLyS/APRIL antagonist and CD40 pathway antagonist are administered prior to graft transplantation.

In some embodiments of the invention, the method comprises administration of multiple doses of a BLyS/APRIL antagonist in combination with multiple doses of a CD40 pathway antagonist. The method may comprise administration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, or more therapeutically effective doses of a

pharmaceutical composition comprising either a BLyS/APRIL antagonist or a CD40 pathway antagonist, or both. The frequency and duration of administration of multiple doses of the pharmaceutical compositions is such as to reduce an immune response and thereby treat or prevent immune-mediated disorders (e.g., transplant rejection or graft-vs-host disease) or decrease the level of immunoglobulins in a subject in need thereof. Moreover, treatment of a subject with a therapeutically effective amount of a combination of a BLyS/APRIL antagonist and a CD40 pathway antagonist can include a single treatment or can include a series of treatments. It will also be appreciated that the effective dosage of a BLyS/APRIL antagonist or CD40 pathway antagonist used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays known in the art.

The BLyS/APRIL antagonist and/or the CD40 antagonist can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

The exact formulation, route of administration, and dosage of the BLyS/APRIL antagonist and the CD40 pathway antagonist can be chosen by the individual physician in view of the patient's condition. Dosage amount and dosing intervals can be adjusted individually to provide plasma levels of the BLyS/APRIL antagonist and the CD40 pathway antagonist that are sufficient to maintain positive therapeutic effects.

One of skill in the art will appreciate that the methods disclose herein can be combined with any other therapy. Such therapies include but are not limited to therapies employed to treat or prevent various immune-mediated disorders, including, transplant rejection or graft-vs-host disease including for example, inhaled cyclosporine, corticosteroids, calcineurin inhibitors, anti-proliferative agents, and/or mTOR inhibitors.

A physician of ordinary skill in the art can determine when treatment of an immune-mediated disorder should be initiated and for how long the treatment should continue. Such treatment decisions may be supported by standard clinical laboratory results which monitor the clinical manifestations of an immune mediated disorder. The methods of the invention may be practiced by continuously or intermittently administering a therapeutically effective dose of the BLyS/APRIL antagonist in

combination with a CD40 pathway antagonist for as long as deemed efficacious. The decision to end therapy by the method of the invention may also be supported by standard clinical laboratory results indicating the disappearance of at least one of the clinical symptoms associated with transplant rejection or host-vs-graft disease.

Alternatively, a physician may choose to initiate the therapeutic methods described herein for a patient at risk of developing prior to the appearance of clinical symptoms associated with transplant rejection.

Factors influencing the mode of administration and the respective amount of the combination of agents disclosed herein include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, medical history (e.g., existence of other diseases such as diabetes, kidney or liver disease, and other drugs or treatments the patient is currently taking or has taken in the past), and physical condition of the individual undergoing therapy. Similarly, the amount of the combination of therapeutic agents disclosed herein to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of these recited APRIL/BLyS antagonist and CD40 pathway antagonist. Generally, a higher dosage of is preferred with increasing weight of the patient undergoing therapy.

Treatment regimens will be based on doses and dosing schedules that maximize therapeutic effects. The therapeutically effective amount of a combination of a BLyS/APRIL antagonist and a CD40 pathway antagonist can be determined. In particular embodiments, the therapeutically effective dose of a combination of a BLyS/APRIL antagonist and the CD40 pathway antagonist may comprise doses of the individual agents that, when administered alone, would not be therapeutically effective or would be less therapeutically effective than when administered in combination with each other. Thus, when a BLyS/APRIL antagonist and the CD40 pathway antagonist are administered in combination, a synergistic therapeutic effect may be observed.

“Synergistic therapeutic effect” refers to a therapeutic effect observed with a combination of two or more therapies (in this case, the BLyS/APRIL antagonist and the CD40 pathway antagonist) wherein the therapeutic effect (as measured by any of a number of parameters including a synergistic decrease in the level of the serum immunoglobulins, including any one or combination of IgE, IgG, IgM, IgD, and/or IgA or as measured by a synergistic decrease in the level of the donor-reactive immunoglobulins, including any one or combination of donor-reactive IgE, IgG, IgM,

IgD, and/or IgA) is greater than the sum of the respective individual therapeutic effects observed with the respective individual therapies. The combination of a BLyS/APRIL and the CD40 pathway antagonist may produce a synergistic effect that permits a reduction in the dosages of these agents and an improvement of the clinical outcome of the subject being treated. A reduced dose of the BLyS/APRIL antagonist and the CD40 pathway antagonist may in turn reduce unwanted side effects associated with each agent.

The present invention also provides for the use of a BLyS/APRIL antagonist in the manufacture of a medicament for treating, decreasing an anti-graft response, preventing an immune mediated disorder such as graft-vs-host-disease and/or transplant rejection or reducing the level of serum immunoglobulins or donor-reactive immunoglobulins in a subject in need thereof, wherein the medicament is coordinated with treatment using a CD40 pathway antagonist. By “coordinated” is intended that the medicament comprising the BLyS/APRIL antagonist is to be used either prior to, during, or after treatment of the subject using a CD40 pathway antagonist. In some embodiments, the combination of agents is a synergistic combination that produces a synergistic therapeutic effect.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1

Despite advances in immunosuppression and routine PRA screening prior to transplantation, alloreactive antibodies mediate an unacceptably high incidence of acute allograft rejection episodes and contribute to the development of chronic rejection. Production of pathogenic alloantibody isotypes requires interactions between B cells and helper CD4 T cells specific for the same set of donor antigens. During primary immune responses, this help is delivered by activated helper CD4⁺ T cells in germinal centers within secondary lymphoid organs and is largely dependent on the CD40/CD154 co-stimulatory pathway. While primary T cells responses are efficiently controlled by current graft-prolonging therapies, the T cell repertoire of many human transplant recipients contains alloreactive memory CD4⁺ T cells. Due to their enhanced survival, activation and trafficking properties, memory T cells precipitate allograft rejection despite immunosuppression or conventional costimulatory blockade.

We previously reported that in contrast to primary effector CD4⁺ T cells, memory CD4⁺ T cells can induce donor-reactive IgG alloantibody in CD40/CD154 independent fashion suggesting alternative molecular mechanisms of help (1-3). The goal of these experiments was to test whether combination of anti-CD154 antibody MR1 and reagents neutralizing B-lymphocyte stimulator (BLyS), aka, cell activating factor (BAFF, aka BLyS) and a proliferation-inducing ligand (APRIL) inhibits alloantibody production induced by donor-reactive memory CD4 T cells.

Methods:

Generation of donor-reactive memory CD4⁺ T cells. We used TCR transgenic MAR mice on C57BL/6 RAG2^{-/-} background as a source of donor-reactive CD4⁺ T cells. MAR cells are specific for male HY_{Dby} peptide presented by I-A^b. To generate memory CD4⁺ T cells, spleen cells from MAR mice were cultured with 3 uM HY_{Dby} peptide for 5 days followed by an adoptive transfer into naïve female C57BL/6 (H-2^b) mice. We have demonstrated that 3-4 weeks *in vivo* without re-stimulation are sufficient to generate CD4⁺ T cells with a memory CD44^{hi}CD25^{lo}CD69^{lo}CD62^{hi/lo} phenotype (1, 4).

Heart transplantation and treatment. Three weeks after MAR cell injection, mice were transplanted with cardiac allografts from male C3H (H-2^k) donors. In these settings, transferred MAR T cells recognize donor male antigen through the indirect pathway and behave similarly to polyclonal memory CD4 T cells providing help for donor-specific alloAb production (1, 2). All recipients were treated with anti-CD154 antibody (clone MR1, BioXCell, West Lebanon NH) at 0.5 mg/mouse intravenously 1 day prior to transplantation. To neutralize BLyS and APRIL, we used murine (m) fusion proteins containing effector function negative Fc derived from C57BL/6 mice: mBAFFR-Fc (blocks only BLyS), mTACI-Fc (blocks both BAFF and APRIL) and control mFc4 (all produced by ZymoGenetics, Seattle WA). Fusion proteins were administered intraperitoneally three times for a week for 2 weeks post transplant at 100 ug/mouse/injection. Heart allograft recipients containing memory CD4⁺ T cells and treated with anti-CD154 antibody were divided into 4 groups of 3-4 mice: 1) no further treatment; 2) control mFc4; 3) mBAFFR-Fc and 4) mTACI-Fc.

Alloantibody analysis. Samples of peripheral blood were collected prior to transplantation and on d. 10, 14 and 21 post transplantation. The numbers of B220⁺ circulating cells were determined by flow cytometry and calculated as percentage of CD45⁺ cells. Donor C3H or third party BALB/c (H-2^d) thymocytes were isolated and

1x10⁶ aliquots were incubated with serially diluted recipient serum followed by fluorescently labeled goat anti-mouse IgG antibody isotypes. The intensity of staining was analyzed by flow cytometry, and the titers of C3H-reactive IgG alloantibody was calculated as previously published (3, 5). For every sample, the mean channel
5 fluorescence (MCF) of each dilution was determined. The dilution that returned the MCF to the level observed when C3H thymocytes were stained with 1:10 dilution of naïve B6 serum was divided by two and reported as a titer.

Statistical analysis. The comparison of B cell percentages in peripheral blood was performed by one-way ANOVA followed by Bonferroni's multiple comparison test. For
10 the alloantibody titers, we used non-parametric equivalent of one-way ANOVA, the Kruskal-Wallis test. When overall p value was <0.05, pairwise comparisons were carried out using Dunn's post test. A value of p<0.05 was considered statistically significant. The data are presented as mean ± standard deviation.

15 *Results:*

The numbers of B220⁺ cells in peripheral blood were comparable in all groups on d. 10 post-transplant (Table 1). Treatment with either mBAFFR-Fc or mTACI-Fc significantly reduced the percentage of circulating B cells by d. 14 after transplantation. Recipients treated with mTACI-Fc, but not with mBAFFR-Fc, revealed further decline in
20 numbers of peripheral B cells on d. 21 post transplant. Neither treatment entirely depleted B220⁺ cells from circulation.

Consistent with our previous reports, recipients that received no treatment of mFc4 had high titers of all anti-donor IgG isotypes despite treatment with anti-CD154 antibody (Tables 2 and 3). The combination of anti-CD154 antibody and mTACI-Fc treatment
25 resulted in complete abrogation of anti-donor IgG antibody production. In contrast, mice treated with MR1 and mBAFF-R-Fc had high titers of IgG1, intermediate titers of IgG_{2c} and low titers of IgG_{2b} and IgG₃ on d. 10 post-transplant. The titers of IgG_{2c}, IgG_{2b} and IgG₃ tended to decline in group by d. 14 post-transplant, but the decrease did not reach statistical significance. The temporal analyses of donor-reactive IgG production and B
30 cell numbers suggest that the decline in anti-donor Ab caused by anti-CD154/mTACI-Fc treatment can be attributed to a disruption in BAFF- and APRIL-mediated B cell activation and/or generation and function of antibody-secreting cells rather than to B cell depletion.

Table 1. Percentage of CD45⁺B220⁺ cells in peripheral blood.

Treatment	d. -2	d. 10	d. 14	d. 21
none	45.0 ± 5.5	27.2 ± 7.9	49.5 ± 10.7	62.3 ± 5.4
mFc4	52.5 ± 7.8	43.7 ± 6.8	49.7 ± 9.9	59.7 ± 6.8
mBAFFR-Fc	39.2 ± 6.0	34.3 ± 10.0	24.3 ± 4.1 ^{a, b}	31.0 ± 2.6 ^{a, b}
mTACI-Fc	34.8 ± 13.4	27.5 ± 7.2	23.5 ± 8.0 ^{a, b}	17.5 ± 2.9 ^{a, b, c}

^a – p < 0.05 compared to no treatment group; ^b – p < 0.05 compared to mFc4-treated group; ^c – p < 0.05 compared to mBAFFR-Fc-treated group

Table 2. Donor reactive IgG alloantibody serum titers on d. 10 post transplant.

Treatment	IgG2c	IgG2b	IgG1	IgG3
none	2745 ± 1800	2430 ± 1403	1350 ± 1597	2835 ± 1620
mFc4	2025 ± 1403	555 ± 599	1365 ± 1983	855 ± 624
mBAFFR-Fc	1365 ± 1983	1485 ± 1871	75 ± 52	1275 ± 2053
mTACI-Fc	45 ± 0	75 ± 52 ^a	45 ± 0	45 ± 0

^a – p < 0.05 compared to no treatment group

Table 3. Donor reactive IgG alloantibody serum titers on d. 14 post transplant.

Treatment	IgG2c	IgG2b	IgG1	IgG3
none	1935 ± 1980	4860 ± 4209	3060 ± 5279	3038 ± 1215
mFc4	2025 ± 1403	2835 ± 1403	2475 ± 2026	1365 ± 1983
mBAFFR-Fc	405 ± 0	75 ± 52	2835 ± 1403	101 ± 58
mTACI-Fc	45 ± 0 ^b	45 ± 0	67 ± 45 ^a	45 ± 0 ^a

^a – p < 0.05 compared to no treatment group; ^b – p < 0.05 compared to mFc4-treated group

- Chen, Y., P. S. Heeger, and A. Valujskikh. 2004. *In vivo* helper functions of alloreactive memory CD4⁺ T cells remain intact despite donor-specific transfusion and anti-CD40 ligand therapy. *J Immunol* 172:5456-5466.
- Zhang, Q., Y. Chen, R. L. Fairchild, P. S. Heeger, and A. Valujskikh. 2006. Lymphoid sequestration of alloreactive memory CD4 T cells promotes cardiac allograft survival. *J Immunol* 176:770-777.
- Zhang, Q. W., M. Rabant, A. Schenk, and A. Valujskikh. 2008. ICOS-Dependent and -independent functions of memory CD4 T cells in allograft rejection. *Am J Transplant* 8:497-506.

4. Dutton, R. W., L. M. Bradley, and S. L. Swain. 1998. T cell memory. *Annu Rev Immunol* 16:201-223.
5. Amano, H., A. Bickerstaff, C. G. Orosz, A. C. Novick, H. Toma, and R. L. Fairchild. 2005. Absence of recipient CCR5 promotes early and increased allospecific antibody responses to cardiac allografts. *J Immunol* 174:6499-6508.

Example 2

Donor-reactive antibodies initiate acute rejection and may lead to chronic pathology of transplanted organs. Alloreactive memory CD4 T cells can use pathways other than CD40/CD154 to induce anti-donor IgG alloantibodies (alloAb) and accelerate rejection. The combination of anti-CD154 antibody MR1 and reagents neutralizing B-cell activating factor (BAFF, aka BLyS) and a proliferation inducing ligand (APRIL) inhibits alloantibody production induced by donor-reactive memory CD4 T cells in murine cardiac allograft recipients. The purpose of these experiments was to determine whether inhibition of anti-donor antibody responses via BAFF/APRIL neutralization in combination with anti-CD154 therapy prolongs heart graft survival in the presence of donor-specific memory CD4 T cells.

Generation of donor-reactive memory CD4 T cells. We used TCR transgenic MAR mice on C57BL/6 RAG2^{-/-} background as a source of donor-reactive CD4 T cells. MAR cells are specific for male HY_{Dby} peptide presented by I-A^b. To generate memory CD4 T cells, spleen cells from MAR mice were cultured with 3 μM HY_{Dby} peptide for 5 days followed by an adoptive transfer into naïve female C57BL/6 (H-2^b) mice.

Heart transplantation and treatment. Three weeks after MAR cell injection, mice were transplanted with cardiac allografts from male BALB/c (H-2^d) donors. In these settings, transferred MAR T cells recognize donor male antigen through the indirect pathway and behave similarly to polyclonal memory CD4 T cells providing help for donor-specific alloAb production¹⁻². All recipients were treated with anti-CD154 antibody (clone MR1, BioXCell, West Lebanon NH) at 1 mg/mouse intravenously 1 day prior to transplantation. It was previously demonstrated that the recipients containing donor-reactive memory CD4 T cells and treated with anti-CD154 antibody generate potent anti-donor CD8 T cell responses. In these settings, CD8 T cells are the main mechanism of heart allograft rejection¹. In order to evaluate the effects of BAFF and APRIL neutralization in the rejection that is at least partially mediated by alloantibody, all recipients were depleted of CD8 T cells. This was achieved by injecting a cocktail of

anti-mouse CD8 antibodies (clones YTS169 and TIB105, 0.2 mg of each on d. -3, -2, and -1 before transplantation, and every 5 days thereafter for the duration of the experiment). The efficiency of CD8 T cell depletion was confirmed by flow cytometry analyses of peripheral blood and spleen cells at the time of graft rejection.

5 To neutralize BAFF and APRIL, we used fusion proteins containing effector function negative Fc derived from C57BL/6 mice: mBAFFR-Fc (blocks only BAFF), mTACI-Fc (blocks both BAFF and APRIL) and control mFc4 (all provided by ZymoGenetics, Seattle WA). Fusion proteins were administered intraperitoneally three times a week for 2 weeks post transplant at 100 µg/mouse/injection. Heart allograft recipients containing memory CD4 T cells and treated with anti-CD154 and anti-CD8 antibody were divided into 4 groups of mice: 1) no further treatment; 2) control mFc4; 3) mBAFFR-Fc and 4) mTACI-Fc. The transplanted hearts were monitored daily by abdominal palpation, the rejection was defined as the absence of heartbeat and confirmed by laparotomy. The recipients were sacrificed at the time of rejection or on d. 90 after transplantation if rejection did not occur earlier. For morphology examination, portions of the grafts were fixed with 10% formalin, paraffin embedded sections were prepared and stained with hematoxyline and eosin (H&E). To assess antibody-mediated injury to the graft tissue, immunohistochemistry staining for C4d complement component was performed on paraffin embedded sections.

20 *Analyses of T cell alloresponses.* Spleen cells were isolated from the recipients at the time of rejection or on d. 90 after transplantation if rejection did not occur earlier and tested in a recall IFN γ ELISPOT assay in response to donor (BALB/c) or third party (C3H, H-2^k) stimulator cells as previously published by our laboratory¹⁻³. The resulting spots were analyzed using an ImmunoSpot Series 2 Analyzer (Cellular Technology Ltd., Cleveland, OH).

25 *Statistical analysis.* The survival fractions were calculated using Kaplan-Meier method and compared by the log-rank test. The results of ELISPOT assays were analyzed using one-way ANOVA, followed by Bonferroni's multiple comparison post-test. A value of $p < 0.05$ was considered statistically significant. The data are presented as mean $\pm$ SEM.

30 *Results:* Despite the presence of donor-reactive memory CD4 T cells, the combination treatment with mTACI-Fc and anti-CD154 antibody resulted in the

significant prolongation of graft survival in 5 out of 8 CD8 depleted recipients, with 4 heart allografts surviving longer than 90 days post transplant. Incorporation of anti-CD8 depleting Abs into the treatment regiment resulted in the significant prolongation in graft survival in the mTACI-Fc treated group (MST>90 d. vs. 18 d. in mFc4-treated recipients, n=6-7 per group). mBAFFR-Fc treatment was less efficient in prolonging graft survival in CD8 depleted recipients (MST=34 d, n=6) (Table 4). The priming of anti-donor IFN γ producing spleen CD4 T cells was comparable between groups at the time of rejection and at d. 90 post transplant in non-rejecting mice. Cardiac allografts rejected by mFc4-treated recipients were heavily infiltrated with mononuclear cells and had high intensity diffuse C4d deposition. The rejection by mTACI-Fc or mBAFFR-Fc treated recipients was characterized by moderate cellular infiltrate and focal C4d staining. The levels of C4d deposition and leukocyte infiltrate directly correlated with the time after transplantation. These results indicate that BAFF and APRIL neutralization in combination with anti-CD154 treatment delays memory CD4 T cell induced alloantibody responses in heart allograft recipients. It is proposed that this delay not only attenuates antibody-mediated allograft injury, but also hinders the kinetics of T cell priming and/or impairs T cell trafficking into the graft, thus leading to prolonged allograft survival.

Table 4. Graft survival following BAFF and APRIL neutralization

Group	Treatment ^a	Time to rejection, days	Median survival time, days	Number of IFN γ -secreting donor-reactive cells per 5 x 10 ⁵ splenocytes
1	none	15, 15, 15, 15, 18, 18, 18, 21	16.5	83 $\pm$ 18
2	mFc4	14, 17, 17, 20, 29, 73	17.0	260 $\pm$ 157
3	mBAFFR-Fc	24, 26, 27, 42, 50, 54, >89	34.5 ^b	335 $\pm$ 143
4	mTACI-Fc	11, 13, 13, 49, >90, >90, >90, >90	>90 ^b	355 $\pm$ 149

^a - all recipients contained donor-reactive memory CD4 T cells, were treated with anti-CD154 antibody and depleted of CD8 T cells.

^b - p < 0.05 compared to mFc4-treated group

1. Chen Y, Heeger PS, Valujskikh A: In vivo helper functions of alloreactive memory CD4⁺ T cells remain intact despite donor-specific transfusion and anti-CD40 ligand therapy, *J Immunol* 2004, 172:5456-5466
- 5 2. Zhang Q, Chen Y, Fairchild RL, Heeger PS, Valujskikh A: Lymphoid sequestration of alloreactive memory CD4 T cells promotes cardiac allograft survival, *J Immunol* 2006, 176:770-777
- 10 3. Zhang QW, Rabant M, Schenk A, Valujskikh A: ICOS-Dependent and -independent functions of memory CD4 T cells in allograft rejection, *Am J Transplant* 2008, 8:497-506

Table 5. Summary of SEQ ID NOS

SEQ ID NO	Sequence	Description
1	Human BLyS	polypeptide
2	Murine BLyS	polypeptide
3	Human APRIL	polypeptide
4	Human TACI	polypeptide
5	Human BCMA	polypeptide
6	Human BAFF-R	polypeptide
7	Fc5	polypeptide
8	amino acids 30-110 of TACI (SEQ ID NO:4) fused in frame to Fc5 (SEQ ID NO:8)	polypeptide
9	BLyS antagonistic polypeptide	polypeptide
10	BLyS antagonistic polypeptide	polypeptide
11	BLyS antagonistic polypeptide	polypeptide
12	BLyS antagonistic polypeptide	polypeptide
13	BLyS antagonistic polypeptide	polypeptide

The article "a" and "an" are used herein to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one or more element.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same

extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

5 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

THAT WHICH IS CLAIMED

1. A method to reduce IgG alloantibody or IgG xenoantibody production in a mammal in need thereof comprising administering to said mammal a therapeutically effective amount of a combination therapy comprising

a) a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI) immunoglobulin fusion protein comprising the extracellular domain of TACI or an active fragment thereof; and,

b) an antagonist of the CD40 pathway,

wherein said combination therapy decreases IgG alloantibody or IgG xenoantibody titers in the serum of said mammal by at least 50% percent; and,

wherein a biological sample from said mammal is not required to be analyzed for an elevated level of BLyS/APRIL heterotrimer (HT), APRIL, or BCMA prior to administration of said combination therapy.

2. The method of claim 1, wherein said combination therapy decreases IgG alloantibody titers in the serum of said mammal by at least 90% percent.

3. The method of claim 1, wherein said combination therapy decreases IgG xenoantibody titers in the serum of said mammal by at least 90% percent.

4. The method of claim 1, 2 or 3, wherein said mammal has undergone a graft transplantation.

5. The method of claim 4, wherein said transplantation comprises an allogeneic transplantation.

6. The method of claim 4, wherein said transplantation comprises a xenogeneic transplantation.

7. A method of treating or preventing graft-versus-host disease in a mammalian subject having undergone a graft transplantation comprising administering to the mammalian subject a therapeutically effective amount of a combination therapy comprising

a) a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI) immunoglobulin fusion protein comprising the extracellular domain of TACI or an active fragment thereof; and,

b) an antagonist of the CD40 pathway,

5 wherein said combination therapy decreases IgG alloantibody or IgG xenoantibody titers in the serum of said mammalian subject by at least 50% percent and thereby treats or prevents said graft-versus-host disease;

wherein a biological sample from said mammalian subject is not analyzed for an elevated level of a BLyS/APRIL heterotrimer (HT), APRIL, or BCMA prior to
10 administration of said combination therapy.

8. The method of claim 7, wherein said combination therapy decreases IgG alloantibody titers or IgG xenoantibody titers in the serum of said mammal by at least 90% percent.

15 9. A method for preventing or treating graft rejection in a mammalian subject having undergone a graft transplantation comprising administering to the mammalian subject a therapeutically effective amount of a combination therapy comprising

20 a) a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI) immunoglobulin fusion protein comprising the extracellular domain of TACI or an active fragment thereof; and,

b) an antagonist of the CD40 pathway,

25 wherein said combination therapy decreases IgG alloantibody or IgG xenoantibody titers in the serum of said mammalian subject by at least 50% percent and thereby treats or prevents said transplant rejection in said mammalian subject;

wherein a biological sample from said mammalian subject is not analyzed for an elevated level of a BLyS/APRIL heterotrimer (HT), APRIL, or BCMA prior to
administration of said combination therapy.

30 10. The method of claim 9, wherein said combination therapy decreases IgG alloantibody titers in the serum of said mammal by at least 90% percent.

11. The method of claim 9, wherein said combination therapy decreases IgG xenoantibody titers in the serum of said mammal by at least 90% percent.

12. The method of any one of claims 7-10, wherein said mammalian subject has undergone a transplantation comprising an allogenic transplantation.

13. The method of claim any one of claims 7-9 and 11, wherein said mammalian subject has undergone a transplantation comprising a xenogeneic transplantation.

14. The method of any one of claims 3-13, wherein said decrease in IgG alloantibody or IgG xenoantibody titers in the serum of said mammal occurs within 14 days of said transplantation.

15. The method of any one of claims 1-14, wherein said combination therapy comprises the simultaneous administration of the TACI immunoglobulin fusion protein and the antagonist of the CD40 pathway.

16. The method of any one of claims 1-14, wherein said combination therapy comprises the sequential administration of the TACI immunoglobulin fusion protein and the antagonist of the CD40 pathway.

17. The method of claim 16, wherein said antagonist of the CD40 pathway is administered prior to transplantation and the TACI immunoglobulin fusion protein is administered following transplantation.

18. The method of any one of claims 1-14, wherein at least of the TACI immunoglobulin fusion protein or the antagonist of the CD40 pathway is administered prior to graft transplantation.

19. The method of any one of claims 1-18, wherein the extracellular domain of TACI or an active fragment thereof of said TACI immunoglobulin fusion protein comprises amino acid residues 34-66 and 71-104 of SEQ ID NO:4.

20. The method of claim 19, wherein said TACI-immunoglobulin fusion protein comprises:

(a) the extracellular domain of TACI or an active fragment thereof having:

i) a polypeptide comprising amino acid residues 30-110 of SEQ ID

NO:4; or,

ii) a polypeptide comprising amino acid residues 34-66 and 71-104 of SEQ ID NO:4;

wherein the extracellular domain of TACI or the active fragment binds at least one of ZTNF2 or ZTNF4, and

(b) an immunoglobulin moiety comprising a heavy chain constant region domain.

21. The method of claim 20, wherein the immunoglobulin moiety is an IgG₁ immunoglobulin moiety.

22. The method of claim 21, wherein the immunoglobulin moiety is an IgG₁ Fc fragment that comprises a disulfide linked heavy chain hinge region, a C_H2 domain and a C_H3 domain.

23. The method of claim 19, wherein the TACI-immunoglobulin fusion protein comprises an amino acid sequence as set forth in SEQ ID NO:8.

24. The method of any one of claims 19-23, wherein the TACI-immunoglobulin fusion protein is a dimer.

25. The method of any one of claims 1-24, wherein said antagonist of the CD40 pathway comprises an antibody or a small molecule.

26. The method of claim 25, wherein said antibody binds to CD40 or CD154.

27. The method of claim 26, wherein said antibody that binds to CD154 comprises the MR1 antibody.

28. A pharmaceutical composition comprising a therapeutically effective amount of a combination therapy comprising SEQ ID NO:8 and the MR1 antibody.

Figure 1

1/3

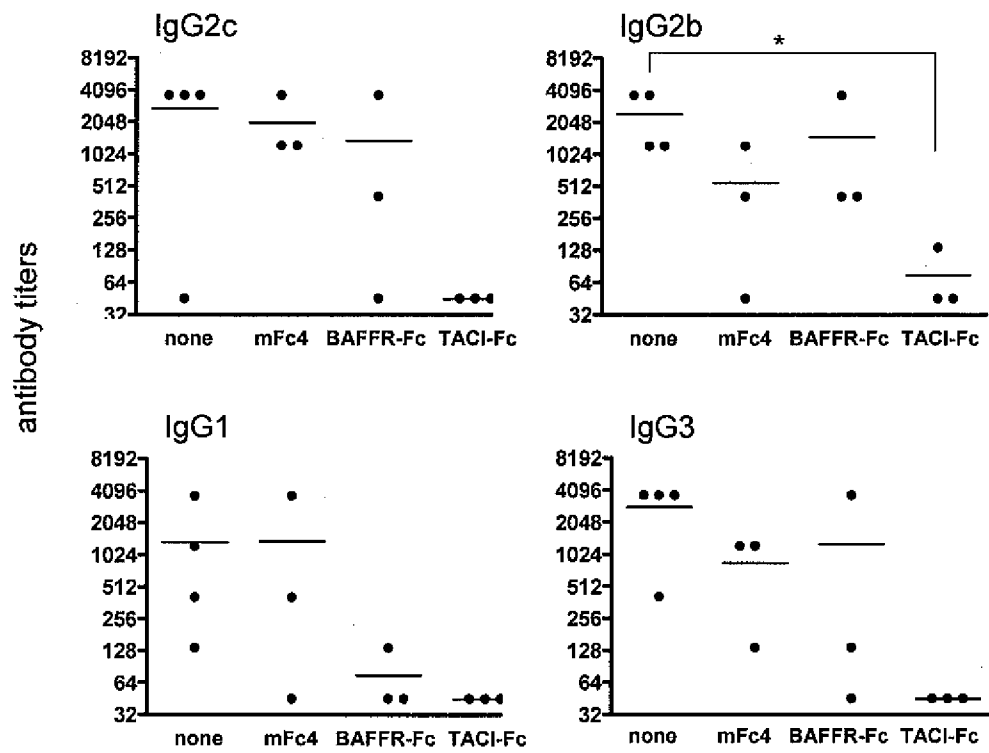


Figure 2

2/3

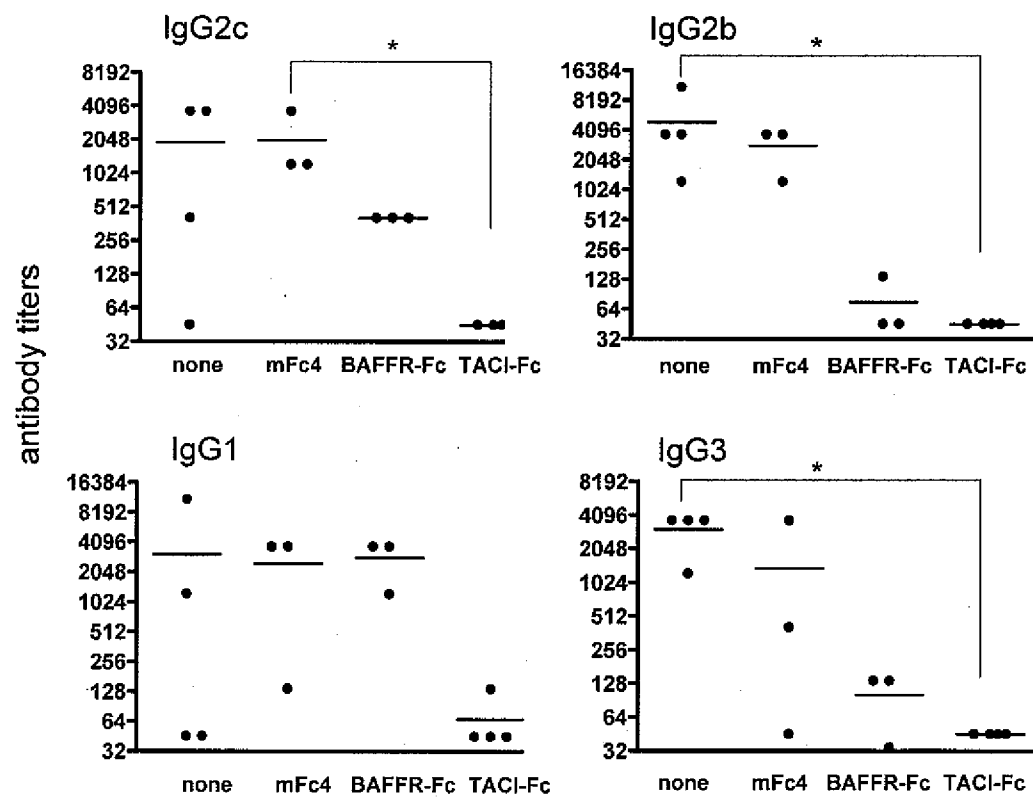
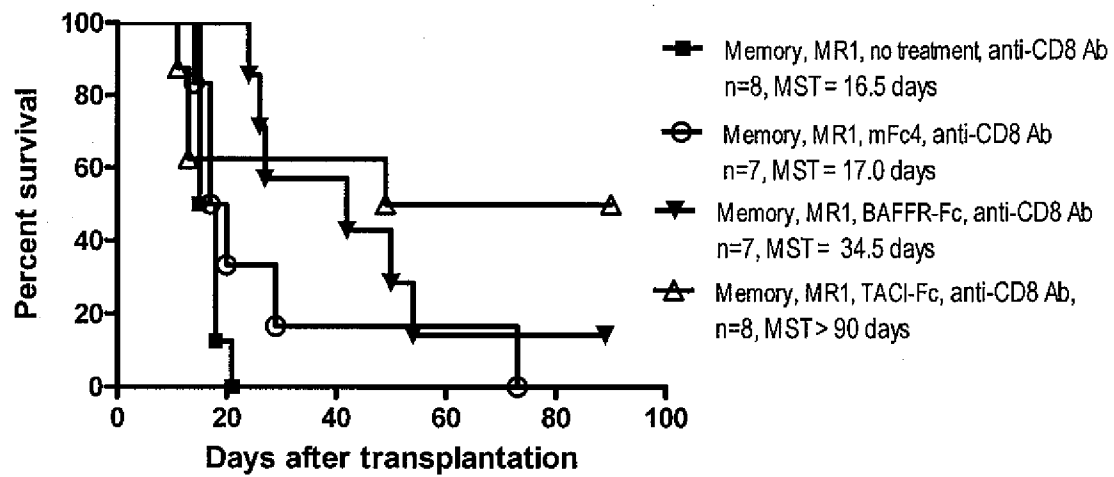


Figure 3

3/3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/026445

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 A61K38/17 A61P37/06
ADD. C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOSKOVIC S ET AL.: "Inhibitory effect of atacicept (TACI-Ig) on circulating antibodies, B cells and plasma cells in allosensitized cynomolgus monkeys", AMERICAN JOURNAL OF TRANSPLANTATION, vol. 8, no. Suppl2, May 2008 (2008-05), page 268, XP002637793, paragraph [0339]	1-6, 14-27
Y	----- -/--	9-11,28



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

19 May 2011

Date of mailing of the international search report

06/06/2011

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Authorized officer

Bumb, Peter

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2011/026445

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEUNG E ET AL: "ALLOGENEIC HEMATOPOIETIC CHIMERISM IN MICE TREATED WITH SUBLETHAL MYELOABLATION AND ANTI-CD154 ANTIBODY: ABSENCE OF GRAFT-VERSUS-HOSTDISEASE, INDUCTION OF SKIN ALLOGRAFT TOLERANCE, AND PREVENTION OF RECURRENT AUTOIMMUNITY IN ISLET-ALLOGRAFTED NOD/LT MICE", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 95, no. 6, 15 March 2000 (2000-03-15), pages 2175-2182, XP000943493, ISSN: 0006-4971 the whole document	7,8, 12-27
Y	BURNS AUDREA M ET AL: "Memory alloreactive B cells and alloantibodies prevent anti-CD154-mediated allograft acceptance.", JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 FEB 2009 LNKD- PUBMED:19155477, vol. 182, no. 3, 1 February 2009 (2009-02-01), pages 1314-1324, XP002637794, ISSN: 1550-6606 the whole document	9-11,28
A	COLVIN ROBERT B: "Pathology of chronic humoral rejection", CONTRIBUTIONS TO NEPHROLOGY, KARGER, BASEL, CH, vol. 162, 1 January 2009 (2009-01-01), pages 75-86, XP008136691, ISSN: 0302-5144 page 82	1-28
A	KHUSH KIRAN K ET AL: "New developments in immunosuppressive therapy for heart transplantation", EXPERT OPINION ON EMERGING DRUGS, ASHLEY PUBLICATIONS, GB, vol. 14, no. 1, 1 March 2009 (2009-03-01), pages 1-21, XP008136641, ISSN: 1472-8214, DOI: DOI:10.1517/1472282109022791605 page 13	1-28
A	WO 2007/134326 A2 (ARES TRADING SA [CH]; ZYMOGENETICS INC [US]; BROLY HERVE [FR]; BUSBY S) 22 November 2007 (2007-11-22) paragraph [0014]	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2011/026445

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007134326 A2	22-11-2007	AR 060935 A1	23-07-2008
		AU 2007249223 A1	22-11-2007
		CA 2651791 A1	22-11-2007
		CN 101489573 A	22-07-2009
		EA 200870535 A1	28-04-2009
		EC SP088982 A	30-01-2009
		EP 2035028 A2	18-03-2009
		JP 2009537563 T	29-10-2009
		KR 20090016707 A	17-02-2009
		US 2007274984 A1	29-11-2007
		ZA 200809202 A	27-01-2010
