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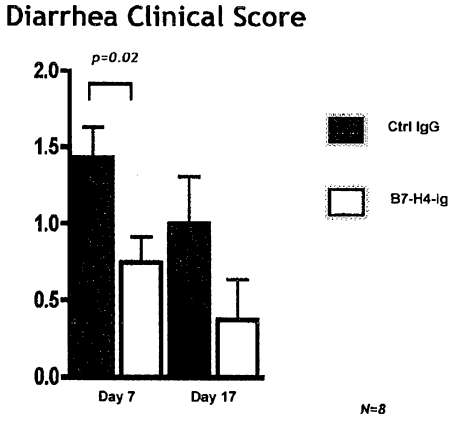

FIGURE 4A

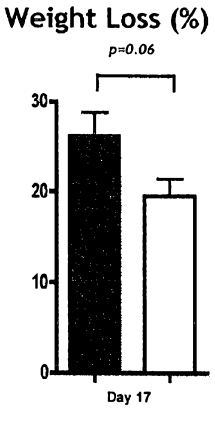

FIGURE 4B

(57) Abstract: Methods for modulating immune responses in a subject are provided. A preferred embodiment provides methods and compositions for reducing or inhibiting transplant rejection in a subject, preferably a human subject. Transplant rejection can be inhibited or reduced in a subject by administering an effective amount of B7-H4 polypeptide, fragments or fusions thereof to inhibit or reduce the biological activity of an immune cell or to reduce the amounts of proinflammatory molecules at a site of transplant. Th1, Th17 and Th22 cells are exemplary T cells that can be targeted for inhibition by B7-H4 polypeptides, fusion proteins or fragments thereof to inhibit or reduce inflammation.

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METHODS AND COMPOSITIONS FOR THE INHIBITION OF TRANSPLANT REJECTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisonal Patent Application No. 61/238,605 filed on August 31, 2009, U.S. Provisional Patent Application No. 61/266,854, filed on December 4, 2009, U.S. Provisional Patent Application No. 61/254,930 filed on October 26, 2009, U.S. Provisional Patent Application No. 61/286,537 filed on December 15, 2009, and U.S. Provisional Patent Application No. 61/378,361 filed August 30, 2010.

FIELD OF THE INVENTION

The field of the invention is generally in area of immunology, in particular to methods and compositions for treating transplant rejection.

BACKGROUND OF THE INVENTION

Continued advances in surgical techniques and immunosuppressive therapy have allowed organ transplantation to become an extremely successful treatment option for patients in need of transplants. Beginning with the revolutionary discovery of cyclosporine in the 1970s, immunosuppressive regimens have evolved greatly and lead to a significant increase in the survival rates for many types of organ and tissue transplants, such as heart, kidney and bone marrow. For example, current statistics confirm one-year liver graft survival rates in excess of 80% (Pillai *et al.*, *World J Gastroenterol*, 15(34): 4225-4233 (2009)).

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. The immunological response directed against the transplanted foreign tissue must be controlled if the tissue is to survive and function. During transplant rejection, 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 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 and can also lead to other severe toxic side effects in the transplant recipient.

Transplant rejection remains the leading impediment to long term graft survival in humans.

T cells play a central role in transplant rejection (Gandhi, A., *et al.*, *Curr Opin Organ Transplant*, 13:622-626 (2008)). Most therapies for preventing transplant rejection focus on inhibiting T cell activation. Naïve T cells require 2 signals for their full activation (Vincennti, F., *et al.*, *J Allergy Clin Immunol*, 121(2):299-306). The first signal is antigen-specific and is provided by the T cell receptor interacting with the MHC and antigenic peptide complex on the antigen presenting cell (APC). The second signal, or costimulatory signal, is provided by the interactions between molecules such as CD80 and CD86 on the APC with cognate receptors on naïve T cells. The presence of cytokines including IL-2 stimulates the process of activation resulting in T cell proliferation. If the T cell does not receive a costimulation signal because of blockade of this pathway, it becomes anergic and undergoes apoptosis.

CD80 (B7-1) and CD86 (B7-2) are members of the B7 superfamily of co-stimulatory molecules that each can engage the stimulatory CD28 receptor and the inhibitory CTLA-4 (CD152) receptor. In conjunction with signaling through the T cell receptor, CD28 ligation increases antigen-specific proliferation of T cells, enhances production of cytokines, stimulates differentiation and effector function, and promotes survival of T cells (Lenshow, et al., *Annu. Rev. Immunol.*, 14:233-258 (1996); Chambers and Allison, *Curr. Opin. Immunol.*, 9:396-404 (1997); and Rathmell and Thompson, *Annu. Rev. Immunol.*, 17:781-828 (1999)). In contrast, signaling through CTLA-4 is thought to deliver a negative signal that inhibits T cell proliferation, IL-2 production, and cell cycle progression (Krummel and Allison, *J. Exp. Med.*, 183:2533-2540 (1996); and Walunas, et al., *J. Exp. Med.*, 183:2541-2550 (1996)).

Current immunosuppressive agents include calcineurin inhibitors such as cyclosporine and tacrolimus. These agents inhibit the intracellular protease calcineurin which is not exclusive to T cells. Because calcineurin in present in other cell types, calcieurin inhibitors cause side effects in other

tissues. mTOR inhibitors such as sirolimus similarly have side effects in other tissues because they inhibit a signal transduction pathway which is not specific to T cells. (Emamaullee, J. *et al.*, *Expert Opin. Biol. Ther.* 9(6):789-796 (2009)). Antibody-based agents that bind to receptors on T cells provide more selectivity than calcineurin inhibitors but often provide a more potent inhibitory effect on the immune system that can last for several months.

CTLA-4 is expressed after T cell activation and acts to downregulate the T cell response. CTLA-4 binds with higher avidity to the CD80 and CD86 ligands than CD28 and competes with CD28 to downregulate T cell activity. Fusion proteins with the extracellular domain of CTLA-4 have been shown to selectively prevent activation of T cells through CD28 (Alegre, M., *et al.*, *Current Pharmacoloical Design*, 12:149-160).

Therefore, it is an object of the invention to provide methods and compositions for inhibiting or reducing transplant rejection.

It is another object of the invention to provide methods and compositions for prolonging allograft survival.

It is another object of the invention to provide methods and compositions for inhibiting or reducing graft versus host disease.

It is another object of the invention to provide immunosuppressive agents specific for T cells.

It is another object of the invention to provide methods and compositions for inhibiting transplant rejection by inhibiting the development of naïve T cells into any of Th1, Th17 or Th22 cells.

It is another object of the invention to provide methods and compositions for inhibiting transplant rejection by inhibiting or reducing the production of proinflammatory molecules and thereby inhibit or reduce transplant rejection.

It is an object of the invention to provide methods and compositions for inhibiting transplant rejection that inhibit the proliferation of T cells, decrease or inhibit production of pro-inflammatory molecules by T cells, decrease or inhibit differentiation and effector function of Th1, Th17 or Th22 cells, and decrease or inhibit survival of Th1, Th17 or Th22 cells.

It is another object of the invention to provide methods and compositions for inhibiting transplant rejection by enhancing the suppressive effect of Tregs.

It is another object of the invention to provide methods and compositions for inhibiting transplant rejection by enhancing the suppressive effect of Tregs on the Th1, Th17 and Th22 pathways, to reduce the level of IFN-gamma, IL-17 and other inflammatory molecules produced and thereby inhibit or reduce inflammation, alloreactivity and transplant rejection.

It is another object of the invention to provide methods and compositions for inhibiting transplant rejection by administering an agent that acts directly on Tregs to promote or enhance production of IL-10 to further suppress the production of inflammatory molecules and thereby inhibit or reduce transplant rejection.

It is another object of the invention to provide methods and compositions for inhibiting transplant rejection that increase or promote the activity of Tregs, increase the differentiation of naïve T cells intoTregs, increase the number of Tregs, or increase the survival of Tregs.

It is another object of the invention to provide compositions and methods for modulating the proinflammatory activity of Th1, Th17 or Th22 T cells while simultaneously increasing or promoting the activity of Tregs.

It is another object of the invention to provide combination therapies for inhibiting transplant rejection.

SUMMARY OF THE INVENTION

Methods for modulating immune responses in a subject are provided. A preferred embodiment provides methods and compositions for reducing or inhibiting transplant rejection in a subject, preferably a human subject, by administering an effective amount of a B7-H4 polypeptide or fragment, or fusion protein thereof to inhibit or reduce the biological activity of an immune cell or to reduce the amounts of proinflammatory molecules, for example, proinflammatory cytokines. The most preferred embodiment is a B7-H4-Ig fusion protein. Exemplary proinflammatory molecules include, but are not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. Th1, Th17 and Th22 are exemplary T cells that can

be targeted for inhibition by B7-H4 polypeptides, fusion proteins or fragments thereof to inhibit or reduce inflammation. B7-H4 polypeptides or fragments, or fusion proteins thereof can also be used to target other cells that secrete, or cause other cells to secrete, inflammatory molecules. Additionally, B7-H4 polypeptides, fusion proteins or fragments thereof can target Tregs to cause an enhanced suppressive effect on production of inflammatory molecules such as IL-17. B7-H4 polypeptides or fragments or fusions thereof can also act directly on Tregs to promote or enhance production of IL-10 to further suppress the production of inflammatory molecules, or to enhance the suppressive effects of Tregs and thereby inhibit or reduce transplant rejection.

B7-H4 polypeptides or fragments, or fusions thereof act at multiple points in multiple pathways. For example, they can inhibit the development of naïve T cells into Th1, Th17, Th22 or other cells that secrete, or cause other cells to secrete, inflammatory molecules. Alternatively, they can interact with Th1, Th17, Th22 and/or other cells, to inhibit or reduce the production of proinflammatory molecules or inhibit proliferation of Th1, Th17 and Th22 cells. Additionally, they can work with Tregs to cause an enhanced suppressive effect on Th1, Th17 and/or Th22 cells to reduce the level of IFN-γ and/or IL-17 produced. They can also act directly on Tregs to promote or enhance production of IL-10 to suppress the Th1 and/or Th17 pathway. Additionally they can work by enhancing the differentiation, recruitment and/or expansion of Treg cells in the region of engrafted tissue.

The transplanted material to be treated can be cells, tissues, organs, limbs, digits or a portion of the body, preferably the human body. The transplants are typically allogenic or xenogenic. B7-H4 polypeptides or fragments, or fusions thereof can be administered systemically or locally. In some embodiments, B7-H4 polypeptides, fragments, or fusions thereof are administered to a site of transplantation prior to, at the time of, or following transplantation. In one embodiment, the B7-H4 polypeptides, fragments or fusions thereof are administered to a site of transplantation parenterally, such as by subcutaneous injection. In other embodiments, B7-H4 polypeptides or fragments, or fusions thereof are administered *ex vivo* directly to cells, tissue

or organs to be transplanted. In one embodiment, the transplant material is contacted with B7-H4 polypeptides or fragments, or fusions thereof prior to transplantation, after transplantion, or both. In other embodiments, B7-H4 polypeptides or fragments, or fusions thereof are administered to immune tissues or organs, such as lymph nodes or the spleen. Vectors encoding B7-H4 polypeptides are also provided. These vectors can be used to deliver B7-H4 locally *in vivo* or *ex vivo*, for example to islet cells. An exemplary vector is an adenoviral vector.

B7-H4 polypeptides or fragments, or fusions thereof can be administered in combination with one or more additional therapeutic agents, including, but not limited to, antibodies against other lymphocyte surface markers (e.g., CD40) or against cytokines, other fusion proteins, e.g., CTLA-4-Ig (Orencia®), TNFR-Ig (Enbrel®), or other immunosuppressive drugs, anti-proliferatives, cytotoxic agents, or other compounds that may assist in immunosuppression. In one embodiment, the additional therapeutic agent is a CTLA-4 fusion protein, such as CTLA-4 Ig (abatacept). In a preferred embodiment, the additional therapeutic agent is a CTLA-4-Ig fusion protein known as belatacept that contains two amino acid substuitutions (L104E and A29Y) that markedly increase its avidity to CD86 *in vivo*.

Still another embodiment provides methods and compositions for treating one or more symptoms of graft versus host disease (GVHD) in a subject in need thereof by administering an effective amount of B7-H4 polypeptides or fragments, or fusions thereof to alleviate one or more symptoms associated with GVHD.

Another embodiment provides a method for treating diabetes by transplanting insulin producing cells into a subject in need thereof and administering an effective amount of B7-H4 polypeptides, fusions thereof, or vectors encoding the B7-H4 polypeptides or B7-H4 fusion proteins to inhibit or reduce transplant rejection of the insulin-producing cells.

A method for inhibiting or reducing chronic transplant rejection is also provided. The method includes administering to a subject in need thereof an effective amount of a B7-H4 polypeptides or fragments, or fusions

thereof thereof to inhibit or reduce chronic rejection of a transplant relative to a control.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of diabetes induction and treatment with allogeneic islet transplantation and administration of B7-H4-Ig (0.25 mg) on Days 0, 2, 4, 6, and 8.

Figure 2 is a line graph of percent islet survival versus days post transplant in mice treated with B7-H4-Ig (solid line) or a contol (dashed line).

Figure 3 is a schematic diagram of an experimental design for a graft versus host disease assay. Four groups of mice were subjected to bone marrow transplants. Group I had syngeneic bone marrow transplants. Group II had allogenic bone marrow transplants and were treated with control IgG (0.5 mg) on Days 0, 1, 7 and 14. Group III had allogenic bone marrow transplants and were treated with B7-H4-Ig (0.5 mg) on Days 0, 1, 7, and 14. Group IV had allogenic bone marrow transplants and were treated with phosphate buffered saline (PBS) on Days 0, 1, 7, and 14.

Figure 4A is a bar graph showing diarrhea clinical score of the Group II and III mice treated in Figure 3. Control IgG (solid shading). B7-H4-Ig (open box). Figure 4B is a bar graph showing percent weight loss of the mice treated in Figure 3 Control IgG (solid shading).

Figure 5 is a line graph showing percent survival versus days post bone marrow transplant of mice treated in Figure 3. Syngeneic transplant (solid square), allogenic transplant treated with control IgG (open triangle), allogenic transplant treated with B7-H4-Ig (solid triangle), allogenic transplant treated with phosphate buffered saline (open circle). The indicated protein was injected at 0.5 mg on Days 0, 1, 7, and 14 as indicated by the arrows.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The term "syngeneic" refers to genetically identical or closely related organisms, cells, tissues, organs, and the like.

The term "allogeneic" refers to organisms, cells, tissues, organs, and the like from, or derived from, individuals of the same species, but wherein the organisms, cells, tissues, organs, and the like are genetically different one from another.

The term "xenograft" refers to a transplant in which the donor and recipient are of different species.

The term "transplant rejection" refers to a partial or complete destruction of a transplanted cell, tissue, organ, or the like on or in the transplant recipient.

The term "host" or "subject" refers to an organism (preferably the organism is a mammal), a tissue, organ, or the like that is the recipient of a transplanted cell, tissue, organ, or the like. The terms "host", "recipient", or "subject" when referring to transplant hosts, subjects or recipients are used interchangeably herein.

An "amount therapeutically effective to inhibit transplant rejection" refers to an amount of B7-H4-Ig that when administered to a transplant recipient, inhibits, either partially or completely, rejection of the transplant relative to an untreated control.

As used herein, "inflammatory molecules" refers to cytokines, metelloproteases and other molecules including, but not limited to IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

II. B7-H4 and Fusion Proteins Thereof

A. B7-H4

B7-H4 is a member of the B7 family of proteins and is involved in T cell signaling pathways. Naïve T cells require 2 signals for their full activation (Vincennti, F., et al., J Allergy Clin Immunol, 121(2):299-306). The first signal is antigen-specific and is provided by the T cell receptor interacting with the MHC and antigenic peptide complex on the antigen presenting cell (APC). The second signal, or costimulatory signal, is provided by the interactions between molecules such as CD80 and CD86 on the APC with cognate receptors such as CD28 on naïve T cells. The most extensively characterized T cell costimulatory pathway is B7-CD28, in which B7-1 (CD80) and B7-2 (CD86) each can engage the stimulatory CD28 receptor and the inhibitory CTLA-4 (CD152) receptor. In

conjunction with signaling through the T cell receptor, CD28 ligation increases antigen-specific proliferation of T cells, enhances production of cytokines, stimulates differentiation and effector function, and promotes survival of T cells (Lenshow, et al., *Annu. Rev. Immunol.*, 14:233-258 (1996); Chambers and Allison, *Curr. Opin. Immunol.*, 9:396-404 (1997); and Rathmell and Thompson, *Annu. Rev. Immunol.*, 17:781-828 (1999)). In contrast, signaling through CTLA-4 is thought to deliver a negative signal that inhibits T cell proliferation, IL-2 production, and cell cycle progression (Krummel and Allison, *J. Exp. Med.*, 183:2533-2540 (1996); and Walunas, et al., *J. Exp. Med.*, 183:2541-2550 (1996)). Other members of the B7 family include B7-H1 (Dong, et al., *Nature Med.*, 5:1365-1369 (1999); and Freeman, et al., *J. Exp. Med.*, 192:1-9 (2000)), B7-DC (Tseng, et al., *J. Exp. Med.*, 193:839-846 (2001); and Latchman, et al., *Nature Immunol.*, 2:261-268 (2001)), B7-H2 (Wang, et al., *Blood*, 96:2808-2813 (2000); Swallow, et al., *Immunity*, 11:423-432 (1999); and Yoshinaga, et al., *Nature*, 402:827-832 (1999)), and B7-H3 (Chapoval, et al., *Nature Immunol.*, 2:269-274 (2001)) and B7-H4 (Choi, et al., *J. Immunol.*, 171:4650-4654 (2003); Sica, et al., *Immunity*, 18:849-861 (2003); Prasad, et al., *Immunity*, 18:863-873 (2003); and Zang, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 100:10388-10392 (2003)). B7-H1 and B7-DC are ligands for PD-1, B7-H2 is a ligand for ICOS, and B7-H3 and B7-H4 remain orphan ligands at this time (Dong, et al., *Immunol. Res.*, 28:39-48 (2003)).

B7-H4 is a negative regulator of T cell responses. Human and mouse B7-H4 share 87% amino acid identity suggesting an important evolutionarily conserved function. Human and mouse B7-H4 mRNAs are expressed broadly in both lymphoid (spleen and thymus) and nonlymphoid organs (including lung, liver, testis, ovary, placenta, skeletal muscle, pancreas, and small intestine), however B7-H4 protein is not detected in normal human tissues by immunohistochemistry. Limited studies of B7-H4 protein expression indicate that B7-H4 is not expressed on freshly isolated human T cells, B cells, DC, and monocytes, but it can be induced on these cell types after *in vitro* stimulation. Immunohistochemical staining shows that B7-H4 is highly expressed in tumors of the lung, ovaries, and head and neck, and reverse-transcriptase polymerase chain reaction (RT-PCR) analyses indicate

that mouse B7-H4 also is highly expressed in a number of tumor cell lines, including prostate, lung, and colon carcinomas.

Functional studies using B7-H4 transfectants and immobilized B7-H4-Ig fusion proteins demonstrate that B7-H4 delivers a signal that inhibits TCR-mediated $CD4^+$ and $CD8^+$ T proliferation, cell-cycle progression and IL-2 production. B7-1 costimulation cannot overcome B7-H4-Ig-induced inhibition. In agreement with the *in vitro* activity, B7-H4 knock-out mice develop autoimmunity.

B. Exemplary B7-H4 Polypeptides and Fusion Proteins

Fusion proteins containing B7-H4 polypeptides coupled to other polypeptides to form fusion proteins are provided. B7-H4 fusion polypeptides have a first fusion partner comprising all or a part of a B7-H4 protein fused (i) directly to a second polypeptide or, (ii) optionally, fused to a linker peptide sequence that is fused to the second polypeptide. The fusion proteins optionally contain a domain that functions to dimerize or multimerize two or more fusion proteins. The peptide/polypeptide linker domain can either be a separate domain, or alternatively can be contained within one of one of the other domains (B7-H4 polypeptide or second polypeptide) of the fusion protein. Similarly, the domain that functions to dimerize or multimerize the fusion proteins can either be a separate domain, or alternatively can be contained within one of one of the other domains (B7-H4 polypeptide, second polypeptide or peptide/polypeptide linker domain) of the fusion protein. In one embodiment, the dimerization/multimerization domain and the peptide/polypeptide linker domain are the same.

Fusion proteins disclosed herein are of formula I:

$$N\text{-}R_1\text{-}R_2\text{-}R_3\text{-}C$$

wherein "N" represents the N-terminus of the fusion protein, "C" represents the C-terminus of the fusion protein, "R_1" is a B7-H4 polypeptide, "R_2" is an optional peptide/polypeptide linker domain, and "R_3" is a second

polypeptide. Alternatively, R_3 may be the B7-H4 polypeptide and R_1 may be the second polypeptide.

The fusion proteins can be dimerized or multimerized. Dimerization or multimerization can occur between or among two or more fusion proteins through dimerization or multimerization domains. Alternatively, dimerization or multimerization of fusion proteins can occur by chemical crosslinking. The dimers or multimers that are formed can be homodimeric/homomultimeric or heterodimeric/heteromultimeric.

A. B7-H4 polypeptides

In a preferred embodiment the B7- H4 polypeptide is from a mammalian species. In the most preferred embodiment, the B7- H4 polypeptide is of murine, non-human primate (*Pan troglodytes*, *Macaca mulatta* or *Macaca fascicularis*), or human origin. Useful murine B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide encoded by the nucleic acid having GenBank Accession Number NM_178594 or AY280973. Useful murine B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide according to GenBank Accession Number AAH32925.1 or NP_848709.2. Useful human B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide encoded by the nucleic acid having GenBank Accession Number AK026071. Useful human B7-H4 polypeptides have at least about 80, 85, 90, 95 or 100% sequence identity to the B7-H4 polypeptide according to GenBank Accession Number NP_078902.2 or BAB15349.1.

Murine B7-H4 polypeptides can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct    60
ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg   120
accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct   180
gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggtc   240
cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc   300
acagcagtgt ttgctgatca ggtggtagtt ggcaatgctt ccctgagact gaaaaacgtg   360
cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat   420
gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat   480
gccagttcag agagtttacg ctgcgaggct cctcggtggt tcccccagcc cacagtggcc   540
tgggcatctc aagtcgacca aggagccaat ttctcagaag tctccaacac cagctttgag   600
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ttgaactctg agaatgtgac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac    660
aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagtg    720
acagattcag aggtcaaaag gcgaagtcag ctgcagttgc tgaactctgg gccttccccg    780
tgtgtttttt cttctgcctt tgtggctggc tgggcactcc tatctctctc ctgttgcctg    840
atgctaagat ga                                                       852
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(SEQ ID NO:1).

Murine B7-H4 polypeptides can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRSSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGPSP CVSSSAFVAG WALLSLSCCL MLR                      283
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(SEQ ID NO:2),

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MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRSSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGP SPCVSSSAFV AGWALLSLSC CLMLR                               275
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(SEQ ID NO:3),

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG PSPCVSSSAF    240
VAGWALLSLS CCLMLR                                                    256
```

(SEQ ID NO:4),

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRTSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGPSP CVFSSAFVAG WALLSLSCCL MLR                      283
```

(SEQ ID NO:5),

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRTSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGP SPCVFSSAFV AGWALLSLSC CLMLR                               275
```

(SEQ ID NO:6), or

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT    120
```

```
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG PSPCVFSSAF   240
VAGWALLSLS CCLMLR                                                   256
```

(SEQ ID NO:7)

Human B7-H4 polypeptides can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct    60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact   120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct   180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc   240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg   300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg   360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat   420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat   480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc   540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag   600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac   660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg   720
acagaatcgg agatcaaaag gcggagtcac ctacagctgc taaactcaaa ggcttctctg   780
tgtgtctctt ctttctttgc catcagctgg gcacttctgc ctctcagccc ttacctgatg   840
ctaaaataa                                                           849
```

(SEQ ID NO:8).

Human B7-H4 polypeptides can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP    60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV   120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV   180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV   240
TESEIKRRSH LQLLNSKASL CVSSFFAISW ALLPLSPYLM LK                       282
```

(SEQ ID NO:9),

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV    60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY   120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG   180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR   240
SHLQLLNSKA SLCVSSFFAI SWALLPLSPY LMLK                                274
```

(SEQ ID NO:10),

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFFA    240
ISWALLPLSP YLMLK                                                     255
```

(SEQ ID NO:11),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKASL CVSSFFAISW ALLPLSPYLM LK 282

(SEQ ID NO:12),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKA SLCVSSFFAI SWALLPLSPY LMLK 274

(SEQ ID NO:13), or

GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFFA 240
ISWALLPLSP YLMLK 255

(SEQ ID NO:14).

Non-human primate B7-H4 polypeptides can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL 60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD 120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ 180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE 240
IKRRSHLQLL NSKASLCVSS FFAISWALLP LSPYLMLK 278

(SEQ ID NO:15), or

GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFFA 240
ISWALLPLSP YLMLK 255

(SEQ ID NO:16), or

MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKASL CVSSFLAISW ALLPLAPYLM LK 282

(SEQ ID NO:17), or

GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120

GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFLA 240
ISWALLPLAP YLMLK 255

(SEQ ID NO:18), or

MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKASL CVSSFLAISW ALPPLAPYLM LK 282

(SEQ ID NO:19), or

GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT 180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK ASLCVSSFLA 240
ISWALPPLAP YLMLK 255

(SEQ ID NO:20),

where SEQ ID NOs:15 and 16 are chimapanzee (*Pan troglodytes*) polypeptide sequences, SEQ ID NOs:17 and 18 are rhesus monkey (*Macaca mulatta*) polypeptide sequences, and SEQ ID NOs:19 and 20 are cynomolgus monkey (*Macaca fascicularis*) polypeptide sequences.

Nucleic acids encoding B7-H4 polypeptides may be optimized for expression in the expression host of choice. Codons may be substituted with alternative codons encoding the same amino acid to account for differences in codon usage between the mammal from which the B7-H4 nucleic acid sequence is derived and the expression host. In this manner, the nucleic acids may be synthesized using expression host-preferred codons.

1. Fragments of B7-H4 polypeptides

The B7-H4 proteins contain two immunoglobulin domains within the extracellular, the IgV domain (or V domain) and the IgC domain (or C domain), which are related to the variable and constant domains of antibodies. The domains can be identified by anyone skilled in the art by searching against family and domain databases. The IgV domain is believed to be responsible for receptor binding, based on functional data from the isolated IgV domain as well as by analogy to the other B7 family members. Each Ig domain of extracellular domain includes one disulfide bond formed between intradomain cystein residues, as is typical for this fold and may be important for structure-function. In SEQ ID NOS: 2, 5, 9 and 12 these

cysteines are located at residues 56 and 130 for the IgV domain, and 168 and 225 for the IgC domain. In addition, there is one predicted N-linked glycosylation site in the IgV domain and six glycosylation sites in the IgC domain, which are conserved between mouse and human B7-H4 sequences.

In one embodiment, the first fusion partner is a fragment of B7-H4. As used herein, a fragment of B7-H4 refers to any subset of the polypeptide that is at least one amino acid shorter than full length protein. Useful fragments are those that retain the ability to bind to their natural receptor or receptors. A B7-H4 polypeptide that is a fragment of full-length B7-H4 typically has at least 20 percent, 30 percent, 40 percent, 50 percent, 60 percent, 70 percent, 80 percent, 90 percent, 95 percent, 98 percent, 99 percent, 100 percent, or even more than 100 percent of the ability to bind its natural receptor(s) as compared to full-length B7-H4.

Fragments of B7-H4 polypeptides include soluble fragments. Soluble B7-H4 polypeptide fragments are fragments of B7-H4 polypeptides that may be shed, secreted or otherwise extracted from the producing cells. Soluble fragments of B7-H4 polypeptides include some or all of the extracellular domain of the receptor polypeptide, and lack some or all of the intracellular and/or transmembrane domains. In one embodiment, B7-H4 polypeptide fragments include the entire extracellular domain of the B7-H4 polypeptide. In other embodiments, the soluble fragments of B7-H4 polypeptides include fragments of the extracellular domain that retain B7-H4 biological activity. The extracellular domain can include 1, 2, 3, 4, or 5 contiguous amino acids from the transmembrane domain, and/or 1, 2, 3, 4, or 5 contiguous amino acids from the signal sequence. Alternatively, the extracellular domain can have 1, 2, 3, 4, 5 or more amino acids removed from the C-terminus, N-terminus, or both. In some embodiments the extracellular domain is only the IgV domain , or the region between the conserved cysteines of the IgV domain located at residues 56 and 130 of the full-length protein.

Generally, the B7-H4 polypeptides or fragments thereof are expressed from nucleic acids that include sequences that encode a signal sequence. The signal sequence is generally cleaved from the immature

polypeptide to produce the mature polypeptide lacking the signal sequence. SEQ ID NOs: 4, 7, 11, 14, 16, 18 and 20 each lack a signal peptide. The signal sequence of B7-H4 can be replaced by the signal sequence of another polypeptide using standard molecule biology techniques to affect the expression levels, secretion, solubility, or other property of the polypeptide. The signal sequence that is used to replace the B7-H4 signal sequence can be any known in the art. SEQ ID NOs: 2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19 each contain a signal peptide.

In a preferred embodiment, the fusion protein includes the extracellular domain of B7-H4, or a fragment thereof fused to an Ig Fc region. Recombinant B7-H4-Ig fusion proteins can be prepared by fusing the coding region of the extracellular domain of B7-H4 or a fragment thereof to the Fc region of human IgG1 or mouse IgG2a, as described previously (Chapoval, et al., *Methods Mol. Med.*, 45:247-255 (2000)).

a. Murine B7-DC extracellular domain fusion partners

In one embodiment, the first fusion partner of the fusion protein includes the extracellular domain of murine B7-H4 or a fragment thereof. The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct    60
ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg   120
accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct   180
gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggtc   240
cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc   300
acagcagtgt ttgctgatca ggtggtagtt ggcaatgctt ccctgagact gaaaaacgtg   360
cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat   420
gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat   480
gccagttcag agagtttacg ctgcgaggct cctcggtggt tcccccagcc cacagtggcc   540
tgggcatctc aagtcgacca aggagccaat ttctcagaag tctccaacac cagctttgag   600
ttgaactctg agaatgtgac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac   660
aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagtg   720
acagattcag aggtcaaaag gcgaagtcag ctgcagttgc tgaactctgg g             771
```

(SEQ ID NO:21),

```
atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga    60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt   120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta   180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac   240
```

gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt 300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac 360
acatgctata tccggtcctc taagggcaag gggaacgcta atctcgagta caaaacaggc 420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc 480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc 540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg 600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa 660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg 720
agtcaactgc aactcttgaa tagcggc 747

(SEQ ID NO:22) or

atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga 60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt 120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta 180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac 240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt 300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac 360
acatgctata tccggacctc taagggcaag gggaacgcta atctcgagta caaaacaggc 420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc 480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc 540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg 600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa 660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg 720
agtcaactgc aactcttgaa tagcggc 747

(SEQ ID NO:23).

In another embodiment, the first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the murine amino acid sequence:

MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV 60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY 120
TCYIRSSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR 240
SQLQLLNSG 249

(SEQ ID NO:24),

MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV 60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY 120
TCYIRTSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR 240
SQLQLLNSG 249

(SEQ ID NO:25),

MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP 60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV 120

```
QLTDAGTYTC YIRSSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSG                                                   257
```

(SEQ ID NO:26), or

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRTSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSG                                                   257
```

(SEQ ID NO:27).

The signal sequence is removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture. SEQ ID NO:28 provides the murine amino acid sequence of SEQ ID NO:24 and SEQ ID NO:26 without the signal sequence:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG               230
```

(SEQ ID NO:28).

SEQ ID NO:29 provides the murine amino acid sequence of SEQ ID NO:25 and SEQ ID NO:27 without the signal sequence:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK     60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT    120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG               230
```

(SEQ ID NO:29)

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of murine B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:2 or SEQ ID NO:5 to the cysteine at position 130 of SEQ ID NO:2 or SEQ ID NO:5. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the following nucleotide sequence encoding an exemplary IgV domain:

```
ggattcggca taagcggcaa gcacttcatc acagttacaa cgtttacaag tgcggggaac     60
```

```
attggggaag atggaacatt gtcatgtaca tttgagccag atatcaaact caatggaata   120
gtaattcagt ggcttaagga gggcatcaag ggcctggtcc acgaatttaa ggaggggaaa   180
gacgatctgt ctcagcagca cgagatgttc aggggcagaa ccgccgtctt cgcagaccag   240
gttgtggtag gcaacgccag tttgcggctg aaaaacgtgc agctgactga cgccggcacc   300
tacacatgct atatccggtc ctctaagggc aaggggaacg ctaatctcga gtacaaaaca   360
ggcgcctttt ctatgccaga gatcaac                                      387
```

(SEQ ID NO:30) or

```
ggattcggca taagcggcaa gcacttcatc acagttacaa cgtttacaag tgcgggaac    60
attggggaag atggaacatt gtcatgtaca tttgagccag atatcaaact caatggaata   120
gtaattcagt ggcttaagga gggcatcaag ggcctggtcc acgaatttaa ggaggggaaa   180
gacgatctgt ctcagcagca cgagatgttc aggggcagaa ccgccgtctt cgcagaccag   240
gttgtggtag gcaacgccag tttgcggctg aaaaacgtgc agctgactga cgccggcacc   300
tacacatgct atatccggac ctctaagggc aaggggaacg ctaatctcga gtacaaaaca   360
ggcgcctttt ctatgccaga gatcaac                                      387
```

(SEQ ID NO:31).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the murine amino acid sequence:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK    60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT   120
GAFSMPEIN                                                           129
```

(SEQ ID NO:32), or

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK    60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT   120
GAFSMPEIN                                                           129
```

(SEQ ID NO:33).

b. Human extracellular domain fusion partners

In another embodiment, the first fusion partner of the fusion protein includes the extracellular domain of human B7-H4 or a fragment thereof. The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to:

```
atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct    60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact   120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct   180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc   240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg   300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg   360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat   420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat   480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc   540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag   600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac   660
```

aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatcaaaag gcggagt 747

(SEQ ID NO:34),

atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct 60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact 120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct 180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc 240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg 300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg 360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat 420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat 480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc 540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag 600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac 660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatc 735

(SEQ ID NO:35),

atggcttccc tggggcagat cctcttctgg agcataatta gcatcatcat tattctggct 60
ggagcaattg cactcatcat tggctttggt atttcaggga gacactccat cacagtcact 120
actgtcgcct cagctgggaa cattggggag gatggaatcc tgagctgcac ttttgaacct 180
gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc 240
catgagttca aagaaggcaa agatgagctg tcggagcagg atgaaatgtt cagaggccgg 300
acagcagtgt ttgctgatca agtgatagtt ggcaatgcct ctttgcggct gaaaaacgtg 360
caactcacag atgctggcac ctacaaatgt tatatcatca cttctaaagg caaggggaat 420
gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat 480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc 540
tgggcatccc aagttgacca gggagccaac ttctcggaag tctccaatac cagctttgag 600
ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac 660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg 720
acagaatcgg agatcaaaag gcggagtcac ctacagctgc taaactcaaa ggcttct 777

(SEQ ID NO:36),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatccagtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg 720
tct 723

(SEQ ID NO:37),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatccagtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat c 711

(SEQ ID NO:38),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatccagtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg 720
tctcacctac agctgctaaa ctcaaaggct tct 753

(SEQ ID NO:39),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatcctgtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat 360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc 420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt 480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg 540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg 600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa 660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg 720
tct 723

(SEQ ID NO:40),

atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc 60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata 120
ggtgaggatg gcatcctgtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg 180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat 240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta 300

```
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat    360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc    420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt    480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg    540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg    600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa    660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat c             711
```

(SEQ ID NO:41), or

```
atggaatgga gctgggtatt tctgtttttc ctgtcagtaa cgactggcgt ccattcaggc     60
ttcggcatca gtggacggca cagtatcaca gtgaccaccg tcgcctccgc tggcaatata    120
ggtgaggatg gcatcctgtc ctgtaccttt gagccggaca tcaaactgtc tgacatagtg    180
atacaatggc tgaaggaggg ggtgctcggt ctggtacatg agtttaagga agggaaggat    240
gaactgtccg agcaggatga gatgttccgg gggaggaccg ctgtgttcgc cgatcaggta    300
atcgtcggaa atgcaagtct cagattgaaa aatgtgcaac tgactgatgc tggcacgtat    360
aaatgctaca ttatcacaag taagggcaaa ggaaatgcta accttgagta taaaacaggc    420
gcattctcaa tgcccgaggt caatgtcgac tataatgcca gcagtgaaac attgcgctgt    480
gaagctcccc gctggttccc ccagccaacc gtggtctggg cctctcaggt tgatcagggg    540
gctaactttt ccgaggtgag caacaccagc ttcgaactca actctgagaa tgtgaccatg    600
aaagttgtgt ctgtcctgta taatgtaaca atcaacaaca cttattcatg catgattgaa    660
aacgacatcg ccaaggcaac aggtgatatt aaggtaactg aatccgagat caaacggcgg    720
tctcacctac agctgctaaa ctcaaaggct tct                                 753
```

(SEQ ID NO:42).

In another embodiment, the first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the human amino acid sequence:

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV     60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY    120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR    240
S                                                                    241
```

(SEQ ID NO:43)

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV     60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY    120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEI       237
```

(SEQ ID NO:44),

```
MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV     60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY    120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR    240
SHLQLLNSKA S                                                         251
```

(SEQ ID NO:45),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
S 241

(SEQ ID NO:46),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEI 237

(SEQ ID NO:47),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKA S 251

(SEQ ID NO:48),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRS 249

(SEQ ID NO:49),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEI 245

(SEQ ID NO:50),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKAS 259

(SEQ ID NO:51),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRS 249

(SEQ ID NO:52),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180

```
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV   240
TESEI                                                               245
```

(SEQ ID NO:53), or

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP    60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV   120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV   180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV   240
TESEIKRRSH LQLLNSKAS                                                259
```

(SEQ ID NO:54).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture. SEQ ID NO:55 provides the human amino acid sequence of SEQ ID NO:43 and SEQ ID NO:49 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK    60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT   120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                      222
```

(SEQ ID NO:55).

SEQ ID NO:56 provides the human amino acid sequence of SEQ ID NO:46 and SEQ ID NO:52 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK    60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT   120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                      222
```

(SEQ ID NO:56).

SEQ ID NO:57 provides the human amino acid sequence of SEQ ID NO:44 and SEQ ID NO:50 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK    60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT   120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                           218
```

(SEQ ID NO:57).

SEQ ID NO:58 provides the human amino acid sequence of SEQ ID NO:47 and SEQ ID NO:53 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK    60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT   120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                           218
```

(SEQ ID NO:58).

SEQ ID NO:59 provides the human amino acid sequence of SEQ ID NO:45 and SEQ ID NO:51 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:59).

SEQ ID NO:60 provides the human amino acid sequence of SEQ ID NO:48 and SEQ ID NO:54 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:60).

In other embodiments the final alanine and serine residues are removed from SEQ ID NOS: 45, 48, 51, 54, 59, and 60.

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of human B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:9 or SEQ ID NO:12 to the cysteine at position 130 of SEQ ID NO:9 or SEQ ID NO:12. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion partner can be encoded by a nucleotide sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the following nucleotide sequence encoding an exemplary IgV domain:

```
ggcttcggca tcagtggacg gcacagtatc acagtgacca ccgtcgcctc cgctggcaat     60
ataggtgagg atggcatcca gtcctgtacc tttgagccgg acatcaaact gtctgacata    120
gtgatacaat ggctgaagga gggggtgctc ggtctggtac atgagtttaa ggaagggaag    180
gatgaactgt ccgagcagga tgagatgttc cgggggagga ccgctgtgtt cgccgatcag    240
gtaatcgtcg gaaatgcaag tctcagattg aaaaatgtgc aactgactga tgctggcacg    300
tataaatgct acattatcac aagtaagggc aaaggaaatg ctaaccttga gtataaaaca    360
ggcgcattct caatgcccga ggtcaat                                        387
```

(SEQ ID NO:61) or

```
ggcttcggca tcagtggacg gcacagtatc acagtgacca ccgtcgcctc cgctggcaat     60
ataggtgagg atggcatcct gtcctgtacc tttgagccgg acatcaaact gtctgacata    120
gtgatacaat ggctgaagga gggggtgctc ggtctggtac atgagtttaa ggaagggaag    180
gatgaactgt ccgagcagga tgagatgttc cgggggagga ccgctgtgtt cgccgatcag    240
gtaatcgtcg gaaatgcaag tctcagattg aaaaatgtgc aactgactga tgctggcacg    300
```

tataaatgct acattatcac aagtaagggc aaaggaaatg ctaaccttga gtataaaaca 360
ggcgcattct caatgcccga ggtcaat 387

(SEQ ID NO:62).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the human amino acid sequence:

GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVN 129

(SEQ ID NO:63), or

GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK 60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT 120
GAFSMPEVN 129

(SEQ ID NO:64).

c. Non-human primate extracellular domain fusion partners

In another embodiment, the first fusion partner of the fusion protein includes the extracellular domain of non-human primate B7-H4 or a fragment thereof. Exemplary non-human primates include, but are not limited to, chimapanzee (*Pan troglodytes*), rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the chimapanzee (*Pan troglodytes*) amino acid sequence:

MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL 60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD 120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ 180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE 240
IKRRS 245

(SEQ ID NO:65),

MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL 60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD 120
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ 180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE 240
I 241

(SEQ ID NO:66), or

MKPLTSRIIS IIIILAGAIA LIIGFGISGR HSITVTTVAS AGNIGEDGIL SCTFEPDIKL 60
SDIVIQWLKE GVLGLVHEFK EGKDELSEQD EMFRGRTAVF ADQVIVGNAS LRLKNVQLTD 120

```
AGTYKCYIIT SKGKGNANLE YKTGAFSMPE VNVDYNASSE TLRCEAPRWF PQPTVVWASQ    180
IDQGANFSEV SNTSFELNSE NVTMKVVSVL YNATINNTYS CMIENDIAKA TGDIKVTESE    240
IKRRSHLQLL NSKAS                                                     255
```

(SEQ ID NO:67).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture.

SEQ ID NO:68 provides the chimapanzee amino acid sequence of SEQ ID NO:65 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:68).

SEQ ID NO:69 provides the chimapanzee amino acid sequence of SEQ ID NO:66 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEI                            218
```

(SEQ ID NO:69).

SEQ ID NO:70 provides the chimapanzee amino acid sequence of SEQ ID NO:67 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQIDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNA TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:70).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the rhesus monkey (*Macaca mulatta*) amino acid sequence:

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRS                                                            249
```

(SEQ ID NO:71),

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEI                                                                245
```

(SEQ ID NO:72), or

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKAS                                                 259
```

(SEQ ID NO:73).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture.

SEQ ID NO:74 provides the rhesus monkey amino acid sequence of SEQ ID NO:71 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:74).

SEQ ID NO:75 provides the rhesus monkey amino acid sequence of SEQ ID NO:72 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                            218
```

(SEQ ID NO:75).

SEQ ID NO:76 provides the rhesus monkey amino acid sequence of SEQ ID NO:73 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS            232
```

(SEQ ID NO:76).

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the cynomolgus monkey (*Macaca fascicularis*) amino acid sequence:

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRS                                                            249
```

(SEQ ID NO:77),

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEI                                                                245
```

(SEQ ID NO:78), or

```
MASLGQILFW SIISIIFILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP     60
DIKLSDIVIQ WLKEGVIGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSH LQLLNSKAS                                                 259
```

(SEQ ID NO:79).

The signal sequence will be removed in the mature protein. Additionally, signal peptides from other polypeptides or organisms can be used to enhance the secretion of the fusion protein from a host during manufacture.

SEQ ID NO:80 provides the cynomolgus monkey amino acid sequence of SEQ ID NO:77 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RS                       222
```

(SEQ ID NO:80).

SEQ ID NO:81 provides the cynomolgus monkey amino acid sequence of SEQ ID NO:78 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEI                            218
```

(SEQ ID NO:81).

SEQ ID NO:82 provides the cynomolgus monkey amino acid sequence of SEQ ID NO:79 without the signal sequence:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK AS             232
```

(SEQ ID NO:82).

In other embodiments the final alanine and serine residues are removed from SEQ ID NOS:67, 70, 73, 76, 79, and 82.

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of chimpanzee B7-H4. In another embodiment, the IgV domain includes at least from the cysteine at position 52 of SEQ ID NO:15 to the cysteine at position 126 of SEQ ID NO:15. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion partner can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the following chimapnzee amino acid sequence of the following exemplary IgV domain:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:83).

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of rhesus monkey B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:17 to the cysteine at position 130 of SEQ ID NO:17. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion protein can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the rhesus monkey amino acid sequence of the following exemplary IgV domain:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:84).

In another embodiment, the first fusion partner of the fusion protein includes the IgV domain of cynomolgus monkey B7-H4. In one embodiment, the IgV domain includes at least from the cysteine at position 56 of SEQ ID NO:19 to the cysteine at position 130 of SEQ ID NO:19. In another embodiment, the IgV domain contains a fragment of at least 25 or 50 amino acids of the polypeptide defined by this amino acid range.

The first fusion protein can have at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to the cynomolgus monkey amino acid sequence of the following exemplary IgV domain:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVI GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVN                                                            129
```

(SEQ ID NO:85).

d. B7-H4 extracellular domain fragments

The B7-H4 extracellular domain can contain one or more amino acids from the signal peptide or the putative transmembrane domain of B7-H4. During secretion, the number of amino acids of the signal peptide that are cleaved can vary depending on the expression system and the host. Additionally, fragments of B7-H4 extracellular domain missing one or more amino acids from the C-terminus or the N-terminus that retain the ability to bind to the B7-H4 receptor can be used as a fusion partner for the disclosed fusion proteins.

For example, suitable fragments of murine B7-H4 that can be used as a first fusion partner include, but are not limited to, the following:

32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251, 32-250, 32-249,
31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251, 31-250, 31-249,
30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251, 30-250, 30-249,
29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251, 29-250, 29-249,
28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251, 28-250, 28-249,
27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251, 27-250, 27-249,
26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251, 26-250, 26-249,
25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251, 25-250, 25-249,
24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251, 24-250, 24-249,

of SEQ ID NO:26 or SEQ ID NO:27, or

24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241,
23-249, 23-248, 23-247, 23-246, 23-245, 23-244, 23-243, 23-242, 23-241,
22-249, 22-248, 22-247, 22-246, 22-245, 22-244, 22-243, 22-242, 22-241,
21-249, 21-248, 21-247, 21-246, 21-245, 21-244, 21-243, 21-242, 21-241,
20-249, 20-248, 20-247, 20-246, 20-245, 20-244, 20-243, 20-242, 20-241,
19-249, 19-248, 19-247, 19-246, 19-245, 19-244, 19-243, 19-242, 19-241,
18-249, 18-248, 18-247, 18-246, 18-245, 18-244, 18-243, 18-242, 18-241,
17-249, 17-248, 17-247, 17-246, 17-245, 17-244, 17-243, 17-242, 17-241,
16-249, 16-248, 16-247, 16-246, 16-245, 16-244, 16-243, 16-242, 16-241,
of SEQ ID NO:24 or SEQ ID NO:25.

Additional suitable fragments of murine B7-H4 include, but are not limited to, the following:

28-257, 28-258, 28-259, 28-260, 28-261, 28-262, 28-263,
29-257, 29-258, 29-259, 29-260, 29-261, 29-262, 29-263,
30-257, 30-258, 30-259, 30-260, 30-261, 30-262, 30-263,
31-257, 31-258, 31-259, 31-260, 31-261, 31-262, 31-263,
32-257, 32-258, 32-259, 32-260, 32-261, 32-262, 32-263,

of SEQ ID NO:2 or SEQ ID NO:5, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of murine B7-H4 include, but are not limited to, fragments containing at least 25, 20, 75, 100 or 125 amino acids of the IgV domain as set forth in SEQ ID NO:32 or SEQ ID NO:33. Exemplary fragments include, but are not limited to:

16-144, 16-145, 16-146, 16-147, 16-148, 16-149, 16-150, 16-151, 16-152,
17-144, 17-145, 17-146, 17-147, 17-148, 17-149, 17-150, 17-151, 17-152,
18-144, 18-145, 18-146, 18-147, 18-148, 18-149, 18-150, 18-151, 18-152,
19-144, 19-145, 19-146, 19-147, 19-148, 19-149, 19-150, 19-151, 19-152,
20-144, 20-145, 20-146, 20-147, 20-148, 20-149, 20-150, 20-151, 20-152,
21-144, 21-145, 21-146, 21-147, 21-148, 21-149, 21-150, 21-151, 21-152,
22-144, 22-145, 22-146, 22-147, 22-148, 22-149, 22-150, 22-151, 22-152,

23-144, 23-145, 23-146, 23-147, 23-148, 23-149, 23-150, 23-151, 23-152,
24-144, 24-145, 24-146, 24-147, 24-148, 24-149, 24-150, 24-151, 24-152,
of SEQ ID NO:24 or SEQ ID NO:25, or
24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160,
25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160,
26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160,
27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160,
28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160,
29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160,
30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160,
31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160,
32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160,

of SEQ ID NO:26 or SEQ ID NO:27, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Exemplary suitable fragments of human B7-H4 that can be used as a first fusion partner include, but are not limited to, the following:
32-249, 32-248, 32-247, 32-246, 32-245, 32-244, 32-243, 32-242, 32-241,
31-249, 31-248, 31-247, 31-246, 31-245, 31-244, 31-243, 31-242, 31-241,
30-249, 30-248, 30-247, 30-246, 30-245, 30-244, 30-243, 30-242, 30-241,
29-249, 29-248, 29-247, 29-246, 29-245, 29-244, 29-243, 29-242, 29-241,
28-249, 28-248, 28-247, 28-246, 28-245, 28-244, 28-243, 28-242, 28-241,
27-249, 27-248, 27-247, 27-246, 27-245, 27-244, 27-243, 27-242, 27-241,
26-249, 26-248, 26-247, 26-246, 26-245, 26-244, 26-243, 26-242, 26-241,
25-249, 25-248, 25-247, 25-246, 25-245, 25-244, 25-243, 25-242, 25-241,
24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241,
of SEQ ID NO:49, or SEQ ID NO:52, or
32-245, 32-244, 32-243, 32-242, 32-241, 32-240, 32-239, 32-238, 32-237,
31-245, 31-244, 31-243, 31-242, 31-241, 31-240, 31-239, 31-238, 31-237,
30-245, 30-244, 30-243, 30-242, 30-241, 30-240, 30-239, 30-238, 30-237,
29-245, 29-244, 29-243, 29-242, 29-241, 29-240, 29-239, 29-238, 29-237,

28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237,
27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237,
26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237,
25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238, 25-237,
24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237,
of SEQ ID NO:50 or SEQ ID NO:53, or
32-259, 32-258, 32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251,
31-259, 31-258, 31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251,
30-259, 30-258, 30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251,
29-259, 29-258, 29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251,
28-259, 28-258, 28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251,
27-259, 27-258, 27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251,
26-259, 26-258, 26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251,
25-259, 25-258, 25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251,
24-259, 24-258, 24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251,
of SEQ ID NO:51 or SEQ ID NO:54, or
24-241, 24-240, 24-239, 24-238, 24-237, 24-236, 24-235, 24-234, 24-233,
23-241, 23-240, 23-239, 23-238, 23-237, 23-236, 23-235, 23-234, 23-233,
22-241, 22-240, 22-239, 22-238, 22-237, 22-236, 22-235, 22-234, 22-233,
21-241, 21-240, 21-239, 21-238, 21-237, 21-236, 21-235, 21-234, 21-233,
20-241, 20-240, 20-239, 20-238, 20-237, 20-236, 20-235, 20-234, 20-233,
19-241, 19-240, 19-239, 19-238, 19-237, 19-236, 19-235, 19-234, 19-233,
18-241, 18-240, 18-239, 18-238, 18-237, 18-236, 18-235, 18-234, 18-233,
17-241, 17-240, 17-239, 17-238, 17-237, 17-236, 17-235, 17-234, 17-233,
16-241, 16-240, 16-239, 16-238, 16-237, 16-236, 16-235, 16-234, 16-233,
of SEQ ID NO:43 or SEQ ID NO:46, or
24-237, 24-236, 24-235, 24-234, 24-233, 24-232, 24-231, 24-230, 24-229,
23-237, 23-236, 23-235, 23-234, 23-233, 23-232, 23-231, 23-230, 23-229,
22-237, 22-236, 22-235, 22-234, 22-233, 22-232, 22-231, 22-230, 22-229,
21-237, 21-236, 21-235, 21-234, 21-233, 21-232, 21-231, 21-230, 21-229,
20-237, 20-236, 20-235, 20-234, 20-233, 20-232, 20-231, 20-230, 20-229,
19-237, 19-236, 19-235, 19-234, 19-233, 19-232, 19-231, 19-230, 19-229,
18-237, 18-236, 18-235, 18-234, 18-233, 18-232, 18-231, 18-230, 18-229,

17-237, 17-236, 17-235, 17-234, 17-233, 17-232, 17-231, 17-230, 17-229, 16-237, 16-236, 16-235, 16-234, 16-233, 16-232, 16-231, 16-230, 16-229, of SEQ ID NO:44 or SEQ ID NO:47, or

24-251, 24-250, 24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 23-251, 23-250, 23-249, 23-248, 23-247, 23-246, 23-245, 23-244, 23-243, 22-251, 22-250, 22-249, 22-248, 22-247, 22-246, 22-245, 22-244, 22-243, 21-251, 21-250, 21-249, 21-248, 21-247, 21-246, 21-245, 21-244, 21-243, 20-251, 20-250, 20-249, 20-248, 20-247, 20-246, 20-245, 20-244, 20-243, 19-251, 19-250, 19-249, 19-248, 19-247, 19-246, 19-245, 19-244, 19-243, 18-251, 18-250, 18-249, 18-248, 18-247, 18-246, 18-245, 18-244, 18-243, 17-251, 17-250, 17-249, 17-248, 17-247, 17-246, 17-245, 17-244, 17-243, 16-251, 16-250, 16-249, 16-248, 16-247, 16-246, 16-245, 16-244, 16-243, of SEQ ID NO:45 or SEQ ID NO:48.

Additional suitable fragments of human B7-H4 include, but are not limited to, the following:

27-249, 27-250, 27-251, 27-252, 27-253, 27-254, 27-255, 27-256, 27-257, 27-259, 27-260,

28-249, 28-250, 28-251, 28-252, 28-253, 28-254, 28-255, 28-256, 28-257, 28-259, 28-260,

29-249, 29-250, 29-251, 29-252, 29-253, 29-254, 29-255, 29-256, 29-257, 29-259, 29-260,

30-249, 30-250, 30-251, 30-252, 30-253, 30-254, 30-255, 30-256, 30-257, 30-259, 30-260,

31-249, 31-250, 31-251, 31-252, 31-253, 31-254, 31-255, 31-256, 31-257, 31-259, 31-260,

32-249, 32-250, 32-251, 32-252, 32-253, 32-254, 32-255, 32-256, 32-257, 32-259, 32-260

of SEQ ID NO:9 or SEQ ID NO:12, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of human B7-H4 include, but are not limited to, fragments containing at least 25, 20, 75, 100 or 125 amino acids of the IgV domain as set forth in SEQ ID NO:63 or SEQ ID NO:64. Exemplary fragments include, but are not limited to:

16-144, 16-145, 16-146, 16-147, 16-148, 16-149, 16-150, 16-151, 16-152,
17-144, 17-145, 17-146, 17-147, 17-148, 17-149, 17-150, 17-151, 17-152,
18-144, 18-145, 18-146, 18-147, 18-148, 18-149, 18-150, 18-151, 18-152,
19-144, 19-145, 19-146, 19-147, 19-148, 19-149, 19-150, 19-151, 19-152,
20-144, 20-145, 20-146, 20-147, 20-148, 20-149, 20-150, 20-151, 20-152,
21-144, 21-145, 21-146, 21-147, 21-148, 21-149, 21-150, 21-151, 21-152,
22-144, 22-145, 22-146, 22-147, 22-148, 22-149, 22-150, 22-151, 22-152,
23-144, 23-145, 23-146, 23-147, 23-148, 23-149, 23-150, 23-151, 23-152,
24-144, 24-145, 24-146, 24-147, 24-148, 24-149, 24-150, 24-151, 24-152,
of any of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, or SEQ ID NO:48, or

24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160,
25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160,
26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160,
27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160,
28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160,
29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160,
30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160,
31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160,
32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160,

of any of SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, or SEQ ID NO:54, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Exemplary suitable fragments of non-human primate B7-H4 that can be used as a first fusion partner include, but are not limited to, the following:

32-249, 32-248, 32-247, 32-246, 32-245, 32-244, 32-243, 32-242, 32-241,

31-249, 31-248, 31-247, 31-246, 31-245, 31-244, 31-243, 31-242, 31-241,
30-249, 30-248, 30-247, 30-246, 30-245, 30-244, 30-243, 30-242, 30-241,
29-249, 29-248, 29-247, 29-246, 29-245, 29-244, 29-243, 29-242, 29-241,
28-249, 28-248, 28-247, 28-246, 28-245, 28-244, 28-243, 28-242, 28-241,
27-249, 27-248, 27-247, 27-246, 27-245, 27-244, 27-243, 27-242, 27-241,
26-249, 26-248, 26-247, 26-246, 26-245, 26-244, 26-243, 26-242, 26-241,
25-249, 25-248, 25-247, 25-246, 25-245, 25-244, 25-243, 25-242, 25-241,
24-249, 24-248, 24-247, 24-246, 24-245, 24-244, 24-243, 24-242, 24-241,
of SEQ ID NO:71, or SEQ ID NO:77, or

32-245, 32-244, 32-243, 32-242, 32-241, 32-240, 32-239, 32-238, 32-237,
31-245, 31-244, 31-243, 31-242, 31-241, 31-240, 31-239, 31-238, 31-237,
30-245, 30-244, 30-243, 30-242, 30-241, 30-240, 30-239, 30-238, 30-237,
29-245, 29-244, 29-243, 29-242, 29-241, 29-240, 29-239, 29-238, 29-237,
28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237,
27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237,
26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237,
25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238, 25-237,
24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237,
of SEQ ID NO:72 or SEQ ID NO:78, or

32-259, 32-258, 32-257, 32-256, 32-255, 32-254, 32-253, 32-252, 32-251,
31-259, 31-258, 31-257, 31-256, 31-255, 31-254, 31-253, 31-252, 31-251,
30-259, 30-258, 30-257, 30-256, 30-255, 30-254, 30-253, 30-252, 30-251,
29-259, 29-258, 29-257, 29-256, 29-255, 29-254, 29-253, 29-252, 29-251,
28-259, 28-258, 28-257, 28-256, 28-255, 28-254, 28-253, 28-252, 28-251,
27-259, 27-258, 27-257, 27-256, 27-255, 27-254, 27-253, 27-252, 27-251,
26-259, 26-258, 26-257, 26-256, 26-255, 26-254, 26-253, 26-252, 26-251,
25-259, 25-258, 25-257, 25-256, 25-255, 25-254, 25-253, 25-252, 25-251,
24-259, 24-258, 24-257, 24-256, 24-255, 24-254, 24-253, 24-252, 24-251,
of SEQ ID NO:73 or SEQ ID NO:79, or

28-245, 28-244, 28-243, 28-242, 28-241, 28-240, 28-239, 28-238, 28-237,
27-245, 27-244, 27-243, 27-242, 27-241, 27-240, 27-239, 27-238, 27-237,
26-245, 26-244, 26-243, 26-242, 26-241, 26-240, 26-239, 26-238, 26-237,
25-245, 25-244, 25-243, 25-242, 25-241, 25-240, 25-239, 25-238, 25-237,

24-245, 24-244, 24-243, 24-242, 24-241, 24-240, 24-239, 24-238, 24-237,
23-245, 23-244, 23-243, 23-242, 23-241, 23-240, 23-239, 23-238, 23-237,
22-245, 22-244, 22-243, 22-242, 22-241, 22-240, 22-239, 22-238, 22-237,
21-245, 21-244, 21-243, 21-242, 21-241, 21-240, 21-239, 21-238, 21-237,
20-245, 20-244, 20-243, 20-242, 20-241, 20-240, 20-239, 20-238, 20-237,
of SEQ ID NO:65, or

28-241, 28-240, 28-239, 28-238, 28-237, 28-236, 28-235, 28-234, 28-233,
27-241, 27-240, 27-239, 27-238, 27-237, 27-236, 27-235, 27-234, 27-233,
26-241, 26-240, 26-239, 26-238, 26-237, 26-236, 26-235, 26-234, 26-233,
25-241, 25-240, 25-239, 25-238, 25-237, 25-236, 25-235, 25-234, 25-233,
24-241, 24-240, 24-239, 24-238, 24-237, 24-236, 24-235, 24-234, 24-233,
23-241, 23-240, 23-239, 23-238, 23-237, 23-236, 23-235, 23-234, 23-233,
22-241, 22-240, 22-239, 22-238, 22-237, 22-236, 22-235, 22-234, 22-233,
21-241, 21-240, 21-239, 21-238, 21-237, 21-236, 21-235, 21-234, 21-233,
20-241, 20-240, 20-239, 20-238, 20-237, 20-236, 20-235, 20-234, 20-233,
of SEQ ID NO:66, or

28-255, 28-254, 28-253, 28-252, 28-251, 28-250, 28-249, 28-248, 28-247,
27-255, 27-254, 27-253, 27-252, 27-251, 27-250, 27-249, 27-248, 27-247,
26-255, 26-254, 26-253, 26-252, 26-251, 26-250, 26-249, 26-248, 26-247,
25-255, 25-254, 25-253, 25-252, 25-251, 25-250, 25-249, 25-248, 25-247,
24-255, 24-254, 24-253, 24-252, 24-251, 24-250, 24-249, 24-248, 24-247,
23-255, 23-254, 23-253, 23-252, 23-251, 23-250, 23-249, 23-248, 23-247,
22-255, 22-254, 22-253, 22-252, 22-251, 22-250, 22-249, 22-248, 22-247,
21-255, 21-254, 21-253, 21-252, 21-251, 21-250, 21-249, 21-248, 21-247,
20-255, 20-254, 20-253, 20-252, 20-251, 20-250, 20-249, 20-248, 20-247,
of SEQ ID NO:67.

Additional suitable fragments of non-human primate B7-H4 include, but are not limited to, the following:

27-249, 27-250, 27-251, 27-252, 27-253, 27-254, 27-255, 27-256, 27-257, 27-259, 27-260,

28-249, 28-250, 28-251, 28-252, 28-253, 28-254, 28-255, 28-256, 28-257, 28-259, 28-260,

29-249, 29-250, 29-251, 29-252, 29-253, 29-254, 29-255, 29-256, 29-257, 29-259, 29-260,

30-249, 30-250, 30-251, 30-252, 30-253, 30-254, 30-255, 30-256, 30-257, 30-259, 30-260,

31-249, 31-250, 31-251, 31-252, 31-253, 31-254, 31-255, 31-256, 31-257, 31-259, 31-260,

32-249, 32-250, 32-251, 32-252, 32-253, 32-254, 32-255, 32-256, 32-257, 32-259, 32-260

of SEQ ID NO:17 or SEQ ID NO:19, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of non-human primate B7-H4 include, but are not limited to, the following:

23-245, 23-246, 23-247, 23-248, 23-249, 23-250, 23-251, 23-252, 23-253, 23-254, 23-255,

24-245, 24-246, 24-247, 24-248, 24-249, 24-250, 24-251, 24-252, 24-253, 24-254, 24-255,

25-245, 25-246, 25-247, 25-248, 25-249, 25-250, 25-251, 25-252, 25-253, 25-254, 25-255,

26-245, 26-246, 26-247, 26-248, 26-249, 26-250, 26-251, 26-252, 26-253, 26-254, 26-255,

27-245, 27-246, 27-247, 27-248, 27-249, 27-250, 27-251, 27-252, 27-253, 27-254, 27-255,

28-245, 28-246, 28-247, 28-248, 28-249, 28-250, 28-251, 28-252, 28-253, 28-254, 28-255

of SEQ ID NO:15, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

Additional suitable fragments of non-human primate B7-H4 include, but are not limited to, fragments containing at least 25, 20, 75, 100 or 125

amino acids of the IgV domain as set forth in SEQ ID NO:83, SEQ ID NO:84 or SEQ ID NO:85. Exemplary fragments include, but are not limited to:

20-148, 20-149, 20-150, 20-151, 20-152, 20-153, 20-154, 20-155, 20-156,
21-148, 21-149, 21-150, 21-151, 21-152, 21-153, 21-154, 21-155, 21-156,
22-148, 22-149, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 22-156,
23-148, 23-149, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 23-156,
24-148, 24-149, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 20-156,
25-148, 25-149, 25-150, 25-151, 25-152, 25-153, 25-154, 25-155, 25-156,
26-148, 26-149, 26-150, 26-151, 26-152, 26-153, 26-154, 26-155, 26-156,
27-148, 27-149, 27-150, 27-151, 27-152, 27-153, 27-154, 27-155, 27-156,
28-148, 28-149, 28-150, 28-151, 28-152, 28-153, 28-154, 28-155, 28-156,
of any of SEQ ID NO:65, SEQ ID NO:66, or SEQ ID NO:67, or
24-152, 24-153, 24-154, 24-155, 24-156, 24-157, 24-158, 24-159, 24-160,
25-152, 25-153, 25-154, 25-155, 25-156, 25-157, 25-158, 25-159, 25-160,
26-152, 26-153, 26-154, 26-155, 26-156, 26-157, 26-158, 26-159, 26-160,
27-152, 27-153, 27-154, 27-155, 27-156, 27-157, 27-158, 27-159, 27-160,
28-152, 28-153, 28-154, 28-155, 28-156, 28-157, 28-158, 28-159, 28-160,
29-152, 29-153, 29-154, 29-155, 29-156, 29-157, 29-158, 29-159, 29-160,
30-152, 30-153, 30-154, 30-155, 30-156, 30-157, 30-158, 30-159, 30-160,
31-152, 31-153, 31-154, 31-155, 31-156, 31-157, 31-158, 31-159, 31-160,
32-152, 32-153, 32-154, 32-155, 32-156, 32-157, 32-158, 32-159, 32-160,
of any of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:77, SEQ ID NO:78, or SEQ ID NO:79, optionally with one to five amino acids of a signal peptide attached to the N-terminal end. The signal peptide may be any disclosed herein, including those contained within SEQ ID NOs:2, 3, 5, 6, 9, 10, 12, 13, 15, 17 and 19, or may be any signal peptide known in the art.

2. Variants of B7-H4 polypeptides

Useful variants include those that increase biological activity, as indicated by any of the assays described herein, or that increase half life or stability of the protein. The B7-H4 polypeptides and B7-H4 fragments, or fusions thereof having B7-H4 activity, can be engineered to increase

biological activity. In a preferred embodiment, the B7-H4 polypeptide or fusion protein has been modified with at least one amino acid substitution, deletion, or insertion that increases the binding of the molecule to an immune cell, for example a T cell, and transmits an inhibitory signal into the T cell.

Other preferred variants are those B7-H4 polypetpides that are engineered to selectively bind to one type of T cell versus other immune cells. For example, the B7-H4 polypeptide can be engineered to bind preferentially to Tregs, Th0, Th1, Th17, or Th22 cells. Preferential binding refers to binding that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or greater for one type of cell over another type of cell.

Still other variants of B7-H4 can be engineered to have reduced binding to immune cells relative to wildtype B7-H4. These variants can be used in combination with variants having stronger binding properties to modulate the immune response with a moderate impact.

Finally, variant B7-H4 polypeptides can be engineered to have an increased half-life relative to wildtype. These variants typically are modified to resist enzymatic degradation. Exemplary modifications include modified amino acid residues and modified peptide bonds that resist enzymatic degradation. Various modifications to achieve this are known in the art. For example, the juxtamembrane region of B7-H4 includes a dibasic motif, KRRS, which could potentially be recognized and cleaved, for example by a member of the proprotein convertase family of proteases.This motif (KRRS) can be removed to increase half life. The variants can be modified to adjust for effects of affinity for the receptor on the half life of B7-H4 polypeptides, fragments, or fusions thereof at serum and endosomal pH.

B. Second polypeptide

The B7-H4 polypeptide may be fused to a second polypeptide. The presence of the second polypeptide can alter the solubility, stability, affinity and/or valency of the B7-H4 fusion polypeptide. As used herein, "valency" refers to the number of binding sites available per molecule. In one embodiment the second polypeptide is a polypeptide from a different source or different protein.

In one embodiment, the second polypeptide contains one or more domains of an immunoglobulin heavy chain constant region, preferably having an amino acid sequence corresponding to the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain or to the hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain. SEQ ID NOS: 88 and 89 provide exemplary sequences for the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1.

In a preferred dimeric fusion protein, the dimer results from the covalent bonding of Cys residue in the hinge region of two of the Ig heavy chains that are the same Cys residues that are disulfide linked in dimerized normal Ig heavy chains. Such proteins are referred to as B7-H4-Ig.

In one embodiment, the immunoglobulin constant domain may contain one or more amino acid insertions, deletions or substitutions that enhance binding to specific cell types, increase the bioavailablity, or increase the stability of the B7-H4 polypeptides, fusion proteins, or fragments thereof. Suitable amino acid substitutions include conservative and non-conservative substitutions, as described above.

In another embodiment the second polypeptide may have a conjugation domain through which additional molecules can be bound to the B7-H4 fusion proteins. In one such embodiment, the conjugated molecule is capable of targeting the fusion protein to a particular organ or tissue. In another such embodiment the conjugated molecule is another immunomodulatory agent that can enhance or augment the effects of the B7-H4 fusion protein. In another embodiment the conjugated molecule is Polyethylene Glycol (PEG).

The Fc portion of the fusion protein may be varied by isotype or subclass, may be a chimeric or hybrid, and/or may be modified, for example to improve effector functions, control of half-life, tissue accessibility, augment biophysical characteristics such as stability, and improve efficiency of production (and less costly). Many modifications useful in construction of disclosed fusion proteins and methods for making them are known in the art, see for example Mueller, et al., *Mol. Immun.*, 34(6):441-452 (1997), Swann, et al., *Cur. Opin. Immun.*, 20:493-499 (2008), and Presta, *Cur. Opin. Immun.*

20:460-470 (2008). In some embodiments the Fc region is the native IgG1, IgG2, or IgG4 Fc region. In some embodiments the Fc region is a hybrid, for example a chimeric consisting of IgG2/IgG4 Fc constant regions. Modications to the Fc region include, but are not limited to, IgG4 modified to prevent binding to Fc gamma receptors and complement, IgG1 modified to improve binding to one or more Fc gamma receptors, IgG1 modified to minimize effector function (amino acid changes), IgG1 with altered/no glycan (typically by changing expression host), and IgG1 with altered pH-dependent binding to FcRn. The Fc region may include the entire hinge region, or less than the entire hinge region.

The therapeutic outcome in patients treated with rituximab (a chimeric mouse/human IgG1 monoclonal antibody against CD20) for non-Hodgkin's lymphoma or Waldenstrom's macroglobulinemia correlated with the individual's expression of allelic variants of Fcγ receptors with distinct intrinsic affinities for the Fc domain of human IgG1. In particular, patients with high affinity alleles of the low affinity activating Fc receptor CD16A (FcγRIIIA) showed higher response rates and, in the cases of non-Hodgkin's lymphoma, improved progression-free survival. In another embodiment, the Fc domain may contain one or more amino acid insertions, deletions or substitutions that reduce binding to the low affinity inhibitory Fc receptor CD32B (FcγRIIB) and retain wild-type levels of binding to or enhance binding to the low affinity activating Fc receptor CD16A (FcγRIIIA).

Another embodiment includes IgG2-4 hybrids and IgG4 mutants that have reduce binding to FcR which increase their half life. Representative IG2-4 hybrids and IgG4 mutants are described in Angal, S. et al., *Molecular Immunology*, 30(1):105-108 (1993); Mueller, J. et al., *Molecular Immonology*, 34(6): 441-452 (1997); and U.S. Patent No. 6,982,323 to Wang et al. In some embodiments the IgG1 and/or IgG2 domain is deleted for example, Angal et al. describe IgG1 and IgG2 having serine 241 replaced with a proline.

In a preferred embodiment, the Fc domain contains amino acid insertions, deletions or substitutions that enhance binding to CD16A. A

large number of substitutions in the Fc domain of human IgG1 that increase binding to CD16A and reduce binding to CD32B are known in the art and are described in Stavenhagen, et al., *Cancer Res.*, 57(18):8882-90 (2007). Exemplary variants of human IgG1 Fc domains with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R929P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc domain in any combination. In one embodiment, the human IgG1 Fc domain variant contains a F243L, R929P and Y300L substitution. In another embodiment, the human IgG1 Fc domain variant contains a F243L, R929P, Y300L, V305I and P296L substitution. In another embodiment, the human IgG1 Fc domain variant contains an N297Q substitution, as this mutation abolishes FcR binding.

C. Peptide or polypeptide linker domain

The disclosed B7-H4 fusion proteins optionally contain a peptide or polypeptide linker domain that separates the B7-H4 polypeptide from the second polypeptide.

1. Hinge region of antibodies

In one embodiment, the linker domain contains the hinge region of an immunoglobulin. In a preferred embodiment, the hinge region is derived from a human immunoglobulin. Suitable human immunoglobulins that the hinge can be derived from include IgG, IgD and IgA. In a preferred embodiment, the hinge region is derived from human IgG. Amino acid sequences of immunoglobulin hinge regions and other domains are well known in the art.

In one embodiment, B7-H4 fusion polypeptides contain the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
gagcctaagt catgtgacaa gacccatacg tgcccaccct gtcccgctcc agaactgctg     60
gggggaccta gcgttttctt gttcccccca aagcccaagg acaccctcat gatctcacgg    120
actcccgaag taacatgcgt agtagtcgac gtgagccacg aggatcctga agtgaagttt    180
aattggtacg tggacggagt cgaggtgcat aatgccaaaa ctaaacctcg ggaggagcag    240
tataacagta cctaccgcgt ggtatccgtc ttgacagtgc tccaccagga ctggctgaat    300
ggtaaggagt ataaatgcaa ggtcagcaac aaagctcttc ccgccccaat tgaaaagact    360
atcagcaagg ccaagggaca accccgcgag ccccaggttt acacccttcc accttcacga    420
```

gacgagctga ccaagaacca ggtgtctctg acttgtctgg tcaaaggttt ctatccttcc 480
gacatcgcag tggagtggga gtcaaacggg cagcctgaga ataactacaa gaccacaccc 540
ccagtgcttg atagcgatgg gagctttttc ctctacagta agctgactgt ggacaaatcc 600
cgctggcagc agggaaacgt tttctcttgt agcgtcatgc atgaggccct ccacaaccat 660
tatactcaga aaagcctgag tctgagtccc ggcaaa 696

(SEQ ID NO:86), or

gacaagaccc atacgtgccc accctgtccc gctccagaac tgctgggggg acctagcgtt 60
ttcttgttcc ccccaaagcc caaggacacc ctcatgatct cacggactcc cgaagtaaca 120
tgcgtagtag tcgacgtgag ccacgaggat cctgaagtga agtttaattg gtacgtggac 180
ggagtcgagg tgcataatgc caaaactaaa cctcgggagg agcagtataa cagtacctac 240
cgcgtggtat ccgtcttgac agtgctccac caggactggc tgaatggtaa ggagtataaa 300
tgcaaggtca gcaacaaagc tcttcccgcc ccaattgaaa agactatcag caaggccaag 360
ggacaacccc gcgagcccca ggtttacacc cttccacctt cacgagacga gctgaccaag 420
aaccaggtgt ctctgacttg tctggtcaaa ggtttctatc cttccgacat cgcagtggag 480
tgggagtcaa acgggcagcc tgagaataac tacaagacca cacccccagt gcttgatagc 540
gatgggagct ttttcctcta cagtaagctg actgtggaca aatcccgctg gcagcaggga 600
aacgttttct cttgtagcgt catgcatgag gccctccaca accattatac tcagaaaagc 660
ctgagtctga gtcccggcaa a 681

(SEQ ID NO:87).

The hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain encoded by SEQ ID NO:86 has the following amino acid sequence:

EPKSCDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 60
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 120
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP 180
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 232

(SEQ ID NO:88).

The hinge, CH2 and CH3 regions of a human immunoglobulin Cγ1 chain encoded by SEQ ID NO:87 has the following amino acid sequence:

DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD 60
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK 120
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 180
DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK 227

(SEQ ID NO:89).

The hinge can be further shortened to remove amino acids 1, 2, 3, 4, 5, or combinations thereof of SEQ ID NO:89. In one embodiment, amino acids 1 and 2 of SEQ ID NO:89 are deleted.

In another embodiment, the B7-H4 fusion polypeptides contain the hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
gagccaagag gtcctacgat caagccctgc ccgccttgta aatgcccagc tccaaatttg     60
ctgggtggac cgtcagtctt tatcttcccg ccaaagataa aggacgtctt gatgattagt    120
ctgagcccca tcgtgacatg cgttgtggtg gatgtttcag aggatgaccc cgacgtgcaa    180
atcagttggt tcgttaacaa cgtggaggtg cataccgctc aaacccagac ccacagagag    240
gattataaca gcaccctgcg ggtagtgtcc gccctgccga tccagcatca ggattggatg    300
agcgggaaag agttcaagtg taaggtaaac aacaaagatc tgccagcgcc gattgaacga    360
accattagca agccgaaagg gagcgtgcgc gcacctcagg tttacgtcct tcctccacca    420
gaagaggaga tgacgaaaaa gcaggtgacc ctgacatgca tggtaactga ctttatgcca    480
gaagatattt acgtggaatg gactaataac ggaaagacag agctcaatta caagaacact    540
gagcctgttc tggattctga tggcagctac tttatgtact ccaaattgag ggtcgagaag    600
aagaattggg tcgagagaaa cagttatagt tgctcagtgg tgcatgaggg cctccataat    660
catcacacca caaagtcctt cagccgaacg cccgggaaa                            699
```

(SEQ ID NO:90)

The hinge, C_H2 and C_H3 regions of a murine immunoglobulin Cγ2a chain encoded by SEQ ID NO:90 has the following amino acid sequence:

```
EPRGPTIKPC PPCKCPAPNL LGGPSVFIFP PKIKDVLMIS LSPIVTCVVV DVSEDDPDVQ     60
ISWFVNNVEV HTAQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLPAPIER    120
TISKPKGSVR APQVYVLPPP EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT    180
EPVLDSDGSY FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK           233
```

(SEQ ID NO:91)

In another embodiment, the linker domain contains a hinge region of an immunoglobulin as described above, and further includes one or more additional immunoglobulin domains.

2. Other peptide/polypeptide linker domains

Other suitable peptide/polypeptide linker domains include naturally occurring or non-naturally occurring peptides or polypeptides. Peptide linker sequences are at least 2 amino acids in length. Preferably the peptide or polypeptide domains are flexible peptides or polypeptides. A "flexible linker" herein refers to a peptide or polypeptide containing two or more amino acid residues joined by peptide bond(s) that provides increased rotational freedom for two polypeptides linked thereby than the two linked polypeptides would have in the absence of the flexible linker. Such rotational freedom allows two or more antigen binding sites joined by the flexible linker to each access target antigen(s) more efficiently. Exemplary flexible peptides/polypeptides include, but are not limited to, the amino acid sequences Gly-Ser, Gly-Ser-Gly-Ser (SEQ ID NO:92), Ala-Ser, Gly-Gly-Gly-Ser (SEQ ID NO:93), $(Gly_4\text{-}Ser)_3$ (SEQ ID NO:94) and $(Gly_4\text{-}Ser)_4$

(SEQ ID NO:95). Additional flexible peptide/polypeptide sequences are well known in the art.

D. Dimerization, multimerization and targeting domains

The fusion proteins disclosed herein optionally contain a dimerization or multimerization domain that functions to dimerize or multimerize two or more fusion proteins. The domain that functions to dimerize or multimerize the fusion proteins can either be a separate domain, or alternatively can be contained within one of the other domains (B7-H4 polypeptide, second polypeptide, or peptide/polypeptide linker domain) of the fusion protein.

1. Dimerization domains

A "dimerization domain" is formed by the association of at least two amino acid residues or of at least two peptides or polypeptides (which may have the same, or different, amino acid sequences). The peptides or polypeptides may interact with each other through covalent and/or non-covalent association(s). Preferred dimerization domains contain at least one cysteine that is capable of forming an intermolecular disulfide bond with a cysteine on the partner fusion protein. The dimerization domain can contain one or more cysteine residues such that disulfide bond(s) can form between the partner fusion proteins. In one embodiment, dimerization domains contain one, two or three to about ten cysteine residues. In a preferred embodiment, the dimerization domain is the hinge region of an immunoglobulin.

Additional exemplary dimerization domain can be any known in the art and include, but not limited to, coiled coils, acid patches, zinc fingers, calcium hands, a C_H1-C_L pair, an "interface" with an engineered "knob" and/or "protruberance" as described in U.S. Patent No. 5,821,333, leucine zippers (e.g., from jun and/or fos) (U.S. Patent No. 5,932,448), SH2 (src homology 2), SH3 (src Homology 3) (Vidal, et al., *Biochemistry*, 43, 7336-44 ((2004)), phosphotyrosine binding (PTB) (Zhou, et al., *Nature*, 378:584-592 (1995)), WW (Sudol, *Prog. Biochys. Mol. Bio.*, 65:113-132 (1996)), PDZ (Kim, et al., *Nature*, 378: 85-88 (1995); Komau, et al., *Science*, 269:1737-1740 (1995)) 14-3-3, WD40 (Hu, et al., *J Biol Chem.*, 273, 33489-33494 (1998)) EH, Lim, an isoleucine zipper, a receptor dimer pair (e.g.,

interleukin-8 receptor (IL-8R); and integrin heterodimers such as LFA-1 and GPIIIb/IIIa), or the dimerization region(s) thereof, dimeric ligand polypeptides (e.g. nerve growth factor (NGF), neurotrophin-3 (NT-3), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, PDGF members, and brain-derived neurotrophic factor (BDNF) (Arakawa, et al., *J. Biol. Chem.*, 269(45): 27833-27839 (1994) and Radziejewski, et al., *Biochem.*, 32(48): 1350 (1993)) and can also be variants of these domains in which the affinity is altered. The polypeptide pairs can be identified by methods known in the art, including yeast two hybrid screens. Yeast two hybrid screens are described in U.S. Pat. Nos. 5,283,173 and 6,562,576. Affinities between a pair of interacting domains can be determined using methods known in the art, including as described in Katahira, et al., *J. Biol. Chem.*, 277, 9242-9246 (2002)). Alternatively, a library of peptide sequences can be screened for heterodimerization, for example, using the methods described in WO 01/00814. Useful methods for protein-protein interactions are also described in U.S. Patent No. 6,790,624.

2. Multimerization domains

A "multimerization domain" is a domain that causes three or more peptides or polypeptides to interact with each other through covalent and/or non-covalent association(s). Suitable multimerization domains include, but are not limited to, coiled-coil domains. A coiled-coil is a peptide sequence with a contiguous pattern of mainly hydrophobic residues spaced 3 and 4 residues apart, usually in a sequence of seven amino acids (heptad repeat) or eleven amino acids (undecad repeat), which assembles (folds) to form a multimeric bundle of helices. Coiled-coils with sequences including some irregular distribution of the 3 and 4 residues spacing are also contemplated. Hydrophobic residues are in particular the hydrophobic amino acids Val, Ile, Leu, Met, Tyr, Phe and Trp. "Mainly hydrophobic" means that at least 50% of the residues must be selected from the mentioned hydrophobic amino acids.

The coiled coil domain may be derived from laminin. In the extracellular space, the heterotrimeric coiled coil protein laminin plays an important role in the formation of basement membranes. Apparently, the

multifunctional oligomeric structure is required for laminin function. Coiled coil domains may also be derived from the thrombospondins in which three (TSP-1 and TSP-2) or five (TSP-3, TSP-4 and TSP-5) chains are connected, or from COMP (COMPcc) (Guo, et at., *EMBO J.*, 1998, 17: 5265-5272) which folds into a parallel five-stranded coiled coil (Malashkevich, et al., *Science*, 274: 761-765 (1996)).

Additional coiled-coil domains derived from other proteins, and other domains that mediate polypeptide multimerization are known in the art and are suitable for use in the disclosed fusion proteins.

In another embodiment, B7-H4 polypeptides, fusion proteins, or fragments thereof can be induced to form multimers by binding to a second multivalent polypeptide, such as an antibody. Antibodies suitable for use to multimerize B7-H4 polypeptides, fusion proteins, or fragments thereof include, but are not limited to, IgM antibodies and cross-linked, multivalent IgG, IgA, IgD, or IgE complexes.

3. Targeting Domains

The B7-H4 polypeptides and fusion proteins can contain a targeting domain to target the molecule to specific sites in the body. Preferred targeting domains target the molecule to areas of inflammation. Exemplary targeting domains are antibodies, or antigen binding fragments thereof that are specific for inflamed tissue or to a proinflammatory cytokine including but not limited to IL17, IL-4, IL-6, IL-12, IL-21, IL-22, and IL-23. In the case of neurological disorders such as Multiple Sclerosis, the targeting domain may target the molecule to the CNS or may bind to VCAM-1 on the vascular epithelium. Additional targeting domains can be peptide aptamers specific for a proinflammatory molecule. In other embodiments, the B7-H4 fusion protein can include a binding partner specific for a polypeptide displayed on the surface of an immune cell, for example a T cell. In still other embodiments, the targeting domain specifically targets activated immune cells. Preferred immune cells that are targeted include Th0, Th1, Th17 and Th22 T cells, other cells that secrete, or cause other cells to secrete inflammatory molecules including, but not limited to, IL-1β, TNF-α, TGF-

beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs, and Tregs. For example, a targeting domain for Tregs may bind specifically to CD25.

In another embodiment, the B7-H4 polypeptides, fragments, or fusions thereof can contain a targeting domain to target the molecule to an organ or tissue that is being transplanted. For example, the targeting domain can be an antibody, antigen binding fragment thereof, or another binding partner specific for a polypeptide displayed on the surface of cells specific to the type of organ or tissue being transplanted.

E. Exemplary fusion proteins

A representative murine B7-H4 fusion protein is encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
atggcttcct tggggcagat catcttttgg agtattatta acatcatcat catcctggct     60
ggggccatcg cactcatcat tggctttggc atttcaggca agcacttcat cacggtcacg    120
accttcacct cagctggaaa cattggagag gacgggaccc tgagctgcac ttttgaacct    180
gacatcaaac tcaacggcat cgtcatccag tggctgaaag aaggcatcaa aggtttggtc    240
cacgagttca aagaaggcaa agacgacctc tcacagcagc atgagatgtt cagaggccgc    300
acagcagtgt ttgctgatca ggtggtagtt ggcaatgctt ccctgagact gaaaaacgtg    360
cagctcacgg atgctggcac ctacacatgt tacatccgca cctcaaaagg caaagggaat    420
gcaaaccttg agtataagac cggagccttc agtatgccag agataaatgt ggactataat    480
gccagttcag agagtttacg ctgcgaggct cctcggtggt tcccccagcc cacagtggcc    540
tgggcatctc aagtcgacca aggagccaat ttctcagaag tctccaacac cagctttgag    600
ttgaactctg agaatgtgac catgaaggtc gtatctgtgc tctacaatgt cacaatcaac    660
aacacatact cctgtatgat tgaaaacgac attgccaaag ccaccgggga catcaaagtg    720
acagattcag aggtcaaaag gcgaagtcag ctgcagttgc tgaactctgg ggagccaaga    780
ggtcctacga tcaagccctg cccgccttgt aaatgcccag ctccaaattt gctgggtgga    840
ccgtcagtct ttatcttccc gccaaagata aaggacgtct tgatgattag tctgagcccc    900
atcgtgacat gcgttgtggt ggatgtttca gaggatgacc ccgacgtgca aatcagttgg    960
ttcgttaaca acgtggaggt gcataccgct caaacccaga cccacagaga ggattataac   1020
agcaccctgc gggtagtgtc cgccctgccg atccagcatc aggattggat gagcgggaaa   1080
gagttcaagt gtaaggtaaa caacaaagat ctgccagcgc cgattgaacg aaccattagc   1140
aagccgaaag ggagcgtgcg cgcacctcag gtttacgtcc ttcctccacc agaagaggag   1200
atgacgaaaa agcaggtgac cctgacatgc atggtaactg actttatgcc agaagatatt   1260
tacgtggaat ggactaataa cggaaagaca gagctcaatt acaagaacac tgagcctgtt   1320
ctggattctg atggcagcta ctttatgtac tccaaattga gggtcgagaa gaagaattgg   1380
gtcgagagaa acagttatag ttgctcagtg gtgcatgagg gcctccataa tcatcacacc   1440
acaaagtcct tcagccgaac gcccgggaaa                                    1470
```

(SEQ ID NO:96),

```
atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga     60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt    120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta    180
```

attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac 240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt 300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac 360
acatgctata tccggtcctc taagggcaag gggaacgcta atctcgagta caaaacaggc 420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc 480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc 540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg 600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa 660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg 720
agtcaactgc aactcttgaa tagcggcgag ccaagaggtc ctacgatcaa gccctgcccg 780
ccttgtaaat gcccagctcc aaatttgctg ggtggaccgt cagtctttat cttcccgcca 840
aagataaagg acgtcttgat gattagtctg agccccatcg tgacatgcgt tgtggtggat 900
gtttcagagg atgaccccga cgtgcaaatc agttggttcg ttaacaacgt ggaggtgcat 960
accgctcaaa cccagaccca cagagaggat tataacagca ccctgcgggt agtgtccgcc 1020
ctgccgatcc agcatcagga ttggatgagc gggaaagagt tcaagtgtaa ggtaaacaac 1080
aaagatctgc cagcgccgat tgaacgaacc attagcaagc cgaaagggag cgtgcgcgca 1140
cctcaggttt acgtccttcc tccaccagaa gaggagatga cgaaaaagca ggtgaccctg 1200
acatgcatgg taactgactt tatgccagaa gatatttacg tggaatggac taataacgga 1260
aagacagagc tcaattacaa gaacactgag cctgttctgg attctgatgg cagctacttt 1320
atgtactcca aattgagggt cgagaagaag aattgggtcg agagaaacag ttatagttgc 1380
tcagtggtgc atgagggcct ccataatcat cacaccacaa agtccttcag ccgaacgccc 1440
gggaaa 1446

(SEQ ID NO:97) or

atggagtggt catgggtttt tctgttcttt cttagcgtga ctacaggcgt ccattcagga 60
ttcggcataa gcggcaagca cttcatcaca gttacaacgt ttacaagtgc ggggaacatt 120
ggggaagatg gaacattgtc atgtacattt gagccagata tcaaactcaa tggaatagta 180
attcagtggc ttaaggaggg catcaagggc ctggtccacg aatttaagga ggggaaagac 240
gatctgtctc agcagcacga gatgttcagg ggcagaaccg ccgtcttcgc agaccaggtt 300
gtggtaggca acgccagttt gcggctgaaa aacgtgcagc tgactgacgc cggcacctac 360
acatgctata tccggacctc taagggcaag gggaacgcta atctcgagta caaaacaggc 420
gccttttcta tgccagagat caacgtggac tataacgcaa gctctgaaag tctgagatgc 480
gaggcgccaa ggtggttccc tcagcccacc gtcgcgtggg cttcccaggt ggatcaaggc 540
gccaactttt ctgaggtttc taacaccagc ttcgaactga acagcgaaaa tgtgacaatg 600
aaggtagtca gcgttctgta taacgtgacc atcaacaata cttactcctg tatgatagaa 660
aatgatatag ccaaggctac aggagatatt aaagtgacgg attcagaagt gaaaaggagg 720
agtcaactgc aactcttgaa tagcggcgag ccaagaggtc ctacgatcaa gccctgcccg 780
ccttgtaaat gcccagctcc aaatttgctg ggtggaccgt cagtctttat cttcccgcca 840
aagataaagg acgtcttgat gattagtctg agccccatcg tgacatgcgt tgtggtggat 900
gtttcagagg atgaccccga cgtgcaaatc agttggttcg ttaacaacgt ggaggtgcat 960
accgctcaaa cccagaccca cagagaggat tataacagca ccctgcgggt agtgtccgcc 1020
ctgccgatcc agcatcagga ttggatgagc gggaaagagt tcaagtgtaa ggtaaacaac 1080
aaagatctgc cagcgccgat tgaacgaacc attagcaagc cgaaagggag cgtgcgcgca 1140
cctcaggttt acgtccttcc tccaccagaa gaggagatga cgaaaaagca ggtgaccctg 1200
acatgcatgg taactgactt tatgccagaa gatatttacg tggaatggac taataacgga 1260
aagacagagc tcaattacaa gaacactgag cctgttctgg attctgatgg cagctacttt 1320
atgtactcca aattgagggt cgagaagaag aattgggtcg agagaaacag ttatagttgc 1380

```
tcagtggtgc atgagggcct ccataatcat cacaccacaa agtccttcag ccgaacgccc   1440
gggaaa                                                              1446
```

(SEQ ID NO:98)

In another embodiment, a representative murine B7-H4 fusion protein has at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRSSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGEPR GPTIKPCPPC KCPAPNLLGG PSVFIFPPKI KDVLMISLSP    300
IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMSGK    360
EFKCKVNNKD LPAPIERTIS KPKGSVRAPQ VYVLPPPEEE MTKKQVTLTC MVTDFMPEDI    420
YVEWTNNGKT ELNYKNTEPV LDSDGSYFMY SKLRVEKKNW VERNSYSCSV VHEGLHNHHT    480
TKSFSRTPGK                                                           490
```

(SEQ ID NO:99),

```
MASLGQIIFW SIINIIIILA GAIALIIGFG ISGKHFITVT TFTSAGNIGE DGTLSCTFEP     60
DIKLNGIVIQ WLKEGIKGLV HEFKEGKDDL SQQHEMFRGR TAVFADQVVV GNASLRLKNV    120
QLTDAGTYTC YIRTSKGKGN ANLEYKTGAF SMPEINVDYN ASSESLRCEA PRWFPQPTVA    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TDSEVKRRSQ LQLLNSGEPR GPTIKPCPPC KCPAPNLLGG PSVFIFPPKI KDVLMISLSP    300
IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMSGK    360
EFKCKVNNKD LPAPIERTIS KPKGSVRAPQ VYVLPPPEEE MTKKQVTLTC MVTDFMPEDI    420
YVEWTNNGKT ELNYKNTEPV LDSDGSYFMY SKLRVEKKNW VERNSYSCSV VHEGLHNHHT    480
TKSFSRTPGK                                                           490
```

(SEQ ID NO:100),

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRSSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGE PRGPTIKPCP PCKCPAPNLL GGPSVFIFPP KIKDVLMISL SPIVTCVVVD    300
VSEDDPDVQI SWFVNNVEVH TAQTQTHRED YNSTLRVVSA LPIQHQDWMS GKEFKCKVNN    360
KDLPAPIERT ISKPKGSVRA PQVYVLPPPE EEMTKKQVTL TCMVTDFMPE DIYVEWTNNG    420
KTELNYKNTE PVLDSDGSYF MYSKLRVEKK NWVERNSYSC SVVHEGLHNH HTTKSFSRTP    480
GK                                                                   482
```

(SEQ ID NO:101) or

```
MEWSWVFLFF LSVTTGVHSG FGISGKHFIT VTTFTSAGNI GEDGTLSCTF EPDIKLNGIV     60
IQWLKEGIKG LVHEFKEGKD DLSQQHEMFR GRTAVFADQV VVGNASLRLK NVQLTDAGTY    120
TCYIRTSKGK GNANLEYKTG AFSMPEINVD YNASSESLRC EAPRWFPQPT VAWASQVDQG    180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTDSEVKRR    240
SQLQLLNSGE PRGPTIKPCP PCKCPAPNLL GGPSVFIFPP KIKDVLMISL SPIVTCVVVD    300
VSEDDPDVQI SWFVNNVEVH TAQTQTHRED YNSTLRVVSA LPIQHQDWMS GKEFKCKVNN    360
```

```
KDLPAPIERT ISKPKGSVRA PQVYVLPPPE EEMTKKQVTL TCMVTDFMPE DIYVEWTNNG   420
KTELNYKNTE PVLDSDGSYF MYSKLRVEKK NWVERNSYSC SVVHEGLHNH HTTKSFSRTP   480
GK                                                                  482
```

(SEQ ID NO:102).

The amino acid sequence of the murine B7-H4 fusion protein of SEQ ID NO:99 and SEQ ID NO:101 without the signal sequence is:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK    60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRSSKG KGNANLEYKT   120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG EPRGPTIKPC   240
PPCKCPAPNL LGGPSVFIFP PKIKDVLMIS LSPIVTCVVV DVSEDDPDVQ ISWFVNNVEV   300
HTAQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLPAPIER TISKPKGSVR   360
APQVYVLPPP EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT EPVLDSDGSY   420
FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK                     463
```

(SEQ ID NO:103).

The amino acid sequence of the murine B7-H4 fusion protein of SEQ ID NO:100 and SEQ ID NO:102 without the signal sequence is:

```
GFGISGKHFI TVTTFTSAGN IGEDGTLSCT FEPDIKLNGI VIQWLKEGIK GLVHEFKEGK    60
DDLSQQHEMF RGRTAVFADQ VVVGNASLRL KNVQLTDAGT YTCYIRTSKG KGNANLEYKT   120
GAFSMPEINV DYNASSESLR CEAPRWFPQP TVAWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTDSEVKR RSQLQLLNSG EPRGPTIKPC   240
PPCKCPAPNL LGGPSVFIFP PKIKDVLMIS LSPIVTCVVV DVSEDDPDVQ ISWFVNNVEV   300
HTAQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLPAPIER TISKPKGSVR   360
APQVYVLPPP EEEMTKKQVT LTCMVTDFMP EDIYVEWTNN GKTELNYKNT EPVLDSDGSY   420
FMYSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK                     463
```

(SEQ ID NO:104).

A representative human B7-H4 fusion protein is encoded by a nucleic acid having at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

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gacatcaaac tttctgatat cgtgatacaa tggctgaagg aaggtgtttt aggcttggtc   240
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gctaaccttg agtataaaac tggagccttc agcatgccgg aagtgaatgt ggactataat   480
gccagctcag agaccttgcg gtgtgaggct ccccgatggt tcccccagcc cacagtggtc   540
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ctgaactctg agaatgtgac catgaaggtt gtgtctgtgc tctacaatgt tacgatcaac   660
aacacatact cctgtatgat tgaaaatgac attgccaaag caacagggga tatcaaagtg   720
acagaatcgg agatcaaaag gcggagtgag cctaagtcat gtgacaagac ccatacgtgc   780
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gactggctga atggtaagga gtataaatgc aaggtcagca acaaagctct tcccgcccca   1080
attgaaaaga ctatcagcaa ggccaaggga caaccccgcg agccccaggt ttacaccctt   1140
ccaccttcac gagacgagct gaccaagaac caggtgtctc tgacttgtct ggtcaaaggt   1200
ttctatcctt ccgacatcgc agtggagtgg gagtcaaacg ggcagcctga gaataactac   1260
aagaccacac ccccagtgct tgatagcgat gggagctttt tcctctacag taagctgact   1320
gtggacaaat cccgctggca gcagggaaac gttttctctt gtagcgtcat gcatgaggcc   1380
ctccacaacc attatactca gaaaagcctg agtctgagtc ccggcaaa                1428
```

(SEQ ID NO:113).

In another embodiment, a representative human B7-H4 fusion protein has at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

```
MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP     60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV    120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV    180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV    240
TESEIKRRSE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV    300
SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK    360
ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ    420
PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG    480
K                                                                     481
```

(SEQ ID NO:114).

In another embodiment, a representative human B7-H4 fusion protein has at least 80%, 85%, 90%, 95%, 99% or 100% sequence identity to:

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY 420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 476

(SEQ ID NO:115),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 300
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 360
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP 420
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 472

(SEQ ID NO:116),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGIQSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV 300
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV 360
SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES 420
NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL 480
SPGK 484

(SEQ ID NO:117),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV 300
SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK 360
ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ 420
PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG 480
K 481

(SEQ ID NO:118),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY 420

KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 476

(SEQ ID NO:119),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 300
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 360
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP 420
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 472

(SEQ ID NO:120),

MASLGQILFW SIISIIIILA GAIALIIGFG ISGRHSITVT TVASAGNIGE DGILSCTFEP 60
DIKLSDIVIQ WLKEGVLGLV HEFKEGKDEL SEQDEMFRGR TAVFADQVIV GNASLRLKNV 120
QLTDAGTYKC YIITSKGKGN ANLEYKTGAF SMPEVNVDYN ASSETLRCEA PRWFPQPTVV 180
WASQVDQGAN FSEVSNTSFE LNSENVTMKV VSVLYNVTIN NTYSCMIEND IAKATGDIKV 240
TESEIKRRSH LQLLNSKDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV 300
VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV 360
SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES 420
NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL 480
SPGK 484

(SEQ ID NO:121),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK 300
FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK 360
TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT 420
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 473

(SEQ ID NO:122),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 300
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA 360
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 420
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK 468

(SEQ ID NO:123),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIDKT 240
HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE 300
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP 360

REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS 420
FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK 464

(SEQ ID NO:124),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGIQSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY 420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK 476

(SEQ ID NO:125),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK 300
FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK 360
TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT 420
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 473

(SEQ ID NO:126),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 300
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA 360
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 420
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK 468

(SEQ ID NO:127),

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIDKT 240
HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE 300
VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP 360
REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS 420
FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK 464

(SEQ ID NO:128), or

MEWSWVFLFF LSVTTGVHSG FGISGRHSIT VTTVASAGNI GEDGILSCTF EPDIKLSDIV 60
IQWLKEGVLG LVHEFKEGKD ELSEQDEMFR GRTAVFADQV IVGNASLRLK NVQLTDAGTY 120
KCYIITSKGK GNANLEYKTG AFSMPEVNVD YNASSETLRC EAPRWFPQPT VVWASQVDQG 180
ANFSEVSNTS FELNSENVTM KVVSVLYNVT INNTYSCMIE NDIAKATGDI KVTESEIKRR 240
SHLQLLNSKD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP 300
EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP 360

```
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY    420
KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK       476
```

(SEQ ID NO:129).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:114 and SEQ ID NO:122 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSEPKSCDKT HTCPPCPAPE    240
LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE    300
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP    360
SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD    420
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK                                454
```

(SEQ ID NO:130).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:115 and SEQ ID NO:123 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSDKTHTCPP CPAPELLGGP    240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS    300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL    360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ    420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK                                      449
```

(SEQ ID NO:131).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:116 and SEQ ID NO:124 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIDK THTCPPCPAP ELLGGPSVFL    240
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV    300
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ    360
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV    420
FSCSVMHEAL HNHYTQKSLS LSPGK                                          445
```

(SEQ ID NO:132).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:117 and SEQ ID NO:125 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGIQSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK DKTHTCPPCP    240
```

```
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK    300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT    360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL    420
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK                            457
```

(SEQ ID NO:133).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:118 and SEQ ID NO:126 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSEPKSCDKT HTCPPCPAPE    240
LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE    300
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP    360
SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD    420
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK                               454
```

(SEQ ID NO:134).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:119 and SEQ ID NO:127 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSDKTHTCPP CPAPELLGGP    240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS    300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL    360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ    420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK                                     449
```

(SEQ ID NO:135).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:120 and SEQ ID NO:128 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT    180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIDK THTCPPCPAP ELLGGPSVFL    240
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV    300
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ    360
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV    420
FSCSVMHEAL HNHYTQKSLS LSPGK                                         445
```

(SEQ ID NO:136).

The amino acid sequence of the human B7-H4 fusion protein of SEQ ID NO:121 and SEQ ID NO:129 without the signal sequence is:

```
GFGISGRHSI TVTTVASAGN IGEDGILSCT FEPDIKLSDI VIQWLKEGVL GLVHEFKEGK     60
DELSEQDEMF RGRTAVFADQ VIVGNASLRL KNVQLTDAGT YKCYIITSKG KGNANLEYKT    120
```

```
GAFSMPEVNV DYNASSETLR CEAPRWFPQP TVVWASQVDQ GANFSEVSNT SFELNSENVT   180
MKVVSVLYNV TINNTYSCMI ENDIAKATGD IKVTESEIKR RSHLQLLNSK DKTHTCPPCP   240
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK   300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT   360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL   420
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK                           457
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(SEQ ID NO:137).

The aforementioned exemplary fusion proteins can incorporate any combination of the variants described herein. In another embodiment the terminal lysine of the aforementioned exemplary fusion proteins is deleted.

The disclosed fusion proteins can be isolated using standard molecular biology techniques. For example, an expression vector containing a DNA sequence encoding a B7-H4-Ig fusion protein is transfected into 293 cells by calcium phosphate precipitation and cultured in serum-free DMEM. The supernatant is collected at 72 h and the fusion protein is purified by Protein G, or preferably Protein A SEPHAROSE® columns (Pharmacia, Uppsala, Sweden).

F. Fusion protein dimers and multimers

B7-H4 fusion polypeptides can be dimerized or multimerized. Dimerization or multimerization can occur between or among two or more fusion proteins through dimerization or multimerization domains, including those described above. Alternatively, dimerization or multimerization of fusion proteins can occur by chemical crosslinking. Fusion protein dimers can be homodimers or heterodimers. Fusion protein multimers can be homomultimers or heteromultimers.

Fusion protein dimers as disclosed herein are of formula II:

$$N\text{-}R_1\text{-}R_2\text{-}R_3\text{-}C$$
$$N\text{-}R_4\text{-}R_5\text{-}R_6\text{-}C$$

or, alternatively, are of formula III:

$$N\text{-}R_1\text{-}R_2\text{-}R_3\text{-}C$$
$$C\text{-}R_4\text{-}R_5\text{-}R_6\text{-}N$$

wherein the fusion proteins of the dimer provided by formula II are defined as being in a parallel orientation and the fusion proteins of the dimer provided by formula III are defined as being in an antiparallel orientation. Parallel and antiparallel dimers are also referred to as cis and trans dimers, respectively. "N" and "C" represent the N- and C-termini of the fusion protein, respectively. The fusion protein constituents "R_1", "R_2" and "R_3" are as defined above with respect to formula I. With respect to both formula II and formula III, "R_4" is a B7-H4 polypeptide or a second polypeptide, "R_5" is an optional peptide/polypeptide linker domain, and "R_6" is a B7-H4 polypeptide or a second polypeptide, wherein "R_6" is a B7-H4 polypeptide when "R_4" is a second polypeptide, and "R_6" is a second polypeptide when "R_4" is a B7-H4 polypeptide. In one embodiment, "R_1" is a B7-H4 polypeptide, "R_4" is also a B7-H4 polypeptide, and "R_3" and "R_6" are both second polypeptides.

Fusion protein dimers of formula II are defined as homodimers when "R_1" = "R_4", "R_2" = "R_5" and "R_3" = "R_6". Similarly, fusion protein dimers of formula III are defined as homodimers when "R_1" = "R_6", "R_2" = "R_5" and "R_3" = "R_4". Fusion protein dimers are defined as heterodimers when these conditions are not met for any reason. For example, heterodimers may contain domain orientations that meet these conditions (i.e., for a dimer according to formula II, "R_1" and "R_4" are both B7-H4 polypeptides, "R_2" and "R_5" are both peptide/polypeptide liker domains and "R_3" and "R_6" are both second polypeptides), however the species of one or more of these domains is not identical. For example, although "R_3" and "R_6" may both be B7-H4 polypeptides, one polypeptide may contain a wild-type B7-H4 amino acid sequence while the other polypeptide may be a variant B7-H4 polypeptide. An exemplary variant B7-H4 polypeptide is B7-H4 polypeptide that has been modified to have increased or decreased binding to a target cell, increased activity on immune cells, increased or decreased half life or stability. Dimers of fusion proteins that contain either a C_H1 or C_L region of an immunoglobulin as part of the polypeptide linker domain preferably form

heterodimers wherein one fusion protein of the dimer contains a C_H1 region and the other fusion protein of the dimer contains a C_L region.

Fusion proteins can also be used to form multimers. As with dimers, multimers may be parallel multimers, in which all fusion proteins of the multimer are aligned in the same orientation with respect to their N- and C-termini. Multimers may be antiparallel multimers, in which the fusion proteins of the multimer are alternatively aligned in opposite orientations with respect to their N- and C-termini. Multimers (parallel or antiparallel) can be either homomultimers or heteromultimers.

G. Peptide and polypeptide modifications

The fusion proteins may be modified by chemical moieties that may be present in polypeptides in a normal cellular environment, for example, phosphorylation, methylation, amidation, sulfation, acylation, glycosylation, sumoylation and ubiquitylation. Fusion proteins may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotopes and fluorescent compounds.

The fusion proteins may also be modified by chemical moieties that are not normally added to polypeptides in a cellular environment. For example, the disclosed fusion proteins may also be modified by covalent attachment of polymer chains, including, but not limited to, polyethylene glycol polymer (PEG) chains (i.e. pegylation). Conjugation of macromolecules to PEG has emerged recently as an effective strategy to alter the pharmacokinetic (PK) profiles of a variety of drugs, and thereby to improve their therapeutic potential. PEG conjugation increases retention of drugs in the circulation by protecting against enzymatic digestion, slowing filtration by the kidneys and reducing the generation of neutralizing antibodies. In addition, PEG conjugates can be used to allow multimerization of the fusion proteins.

Modifications may be introduced into the molecule by reacting targeted amino acid residues of the polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Another modification is cyclization of the protein.

Examples of chemical derivatives of the polypeptides include lysinyl and amino terminal residues derivatized with succinic or other carboxylic acid anhydrides. Derivatization with a cyclic carboxylic anhydride has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; *O*-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate. Carboxyl side groups, aspartyl or glutamyl, may be selectively modified by reaction with carbodiimides (R—N=C=N--R') such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl)carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues can be converted to asparaginyl and glutaminyl residues by reaction with ammonia. Fusion proteins may also include one or more D-amino acids that are substituted for one or more L-amino acids.

H. Modified Binding Properties

Binding properties of the B7-H4 polypeptides, fragments and fusions thereof (collectively referred to as B7-H4 polypeptides) are relevant to the dose and dose regimen to be administered. In one embodiment the disclosed B7-H4 polypeptides have binding properties to at least one receptor on a T cell that demonstrate a higher term, or higher percentage, of occupancy of receptor molecules on immune cells relative to other ligands of the receptor molecules. In other embodiments, the disclosed B7-H4 polypeptides have reduced binding affinity to a receptor on T cells relative to wildtype B7-H4, allowing the protein to dissociate in a period of less than three months, two months, one month, three weeks, two weeks, one week, or a few days after administration.

In some embodiments the B7-H4 polypeptides, or fragments, or fusions thereof have a relatively high affinity for its receptor, and may therefore have a relatively slow off rate. In other embodiments, the B7-H4 polypeptides are administered intermittently over a period of days, weeks or months to dampen immune responses which are allowed to recover prior to the next administration, which may serve to reduce the immune response

without completely turning the immune response off and may avoid long term side effects.

III. Isolated nucleic acid molecules

Isolated nucleic acid sequences encoding B7-H4 polypeptides, fragments and fusions thereof are disclosed herein. As used herein, "isolated nucleic acid" refers to a nucleic acid that is separated from other nucleic acid molecules that are present in a mammalian genome, including nucleic acids that normally flank one or both sides of the nucleic acid in a mammalian genome (e.g., nucleic acids that encode non-B7-H4 proteins). The term "isolated" as used herein with respect to nucleic acids also includes the combination with any non-naturally-occurring nucleic acid sequence, since such non-naturally-occurring sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome.

An isolated nucleic acid can be, for example, a DNA molecule, provided one of the nucleic acid sequences normally found immediately flanking that DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a DNA molecule that exists as a separate molecule independent of other sequences (e.g., a chemically synthesized nucleic acid, or a cDNA or genomic DNA fragment produced by PCR or restriction endonuclease treatment), as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, lentivirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include an engineered nucleic acid such as a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid. A nucleic acid existing among hundreds to millions of other nucleic acids within, for example, a cDNA library or a genomic library, or a gel slice containing a genomic DNA restriction digest, is not to be considered an isolated nucleic acid.

Nucleic acids encoding B7-H4 fusion polypeptides may be optimized for expression in the expression host of choice. Codons may be substituted with alternative codons encoding the same amino acid to account for differences in codon usage between the mammal from which the B7-H4

nucleic acid sequence is derived and the expression host. In this manner, the nucleic acids may be synthesized using expression host-preferred codons.

Nucleic acids can be in sense or antisense orientation, or can be complementary to a reference sequence encoding a B7-H4 polypeptide. Nucleic acids can be DNA, RNA, or nucleic acid analogs. Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone. Such modification can improve, for example, stability, hybridization, or solubility of the nucleic acid. Modifications at the base moiety can include deoxyuridine for deoxythymidine, and 5-methyl-2'-deoxycytidine or 5-bromo-2'-deoxycytidine for deoxycytidine. Modifications of the sugar moiety can include modification of the 2' hydroxyl of the ribose sugar to form 2'-O-methyl or 2'-O-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. See, for example, Summerton and Weller (1997) *Antisense Nucleic Acid Drug Dev.* 7:187-195; and Hyrup *et al.* (1996) *Bioorgan. Med. Chem.* 4:5-23. In addition, the deoxyphosphate backbone can be replaced with, for example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone.

Nucleic acids encoding polypeptides can be administered to subjects in need thereof. Nucleic delivery involves introduction of "foreign" nucleic acids into a cell and ultimately, into a live animal. Compositions and methods for delivering nucleic acids to a subject are known in the art (see Understanding Gene Therapy, Lemoine, N.R., ed., BIOS Scientific Publishers, Oxford, 2008).

IV. Vectors and host cells

Vectors encoding B7-H4 polypeptides, fragments and fusions thereof are also provided. Nucleic acids, such as those described above, can be inserted into vectors for expression in cells. As used herein, a "vector" is a replicon, such as a plasmid, phage, virus or cosmid, into which another DNA

segment may be inserted so as to bring about the replication of the inserted segment. Vectors can be expression vectors. An "expression vector" is a vector that includes one or more expression control sequences, and an "expression control sequence" is a DNA sequence that controls and regulates the transcription and/or translation of another DNA sequence.

Nucleic acids in vectors can be operably linked to one or more expression control sequences. As used herein, "operably linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest. Examples of expression control sequences include promoters, enhancers, and transcription terminating regions. A promoter is an expression control sequence composed of a region of a DNA molecule, typically within 100 nucleotides upstream of the point at which transcription starts (generally near the initiation site for RNA polymerase II). To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the polypeptide between one and about fifty nucleotides downstream of the promoter. Enhancers provide expression specificity in terms of time, location, and level. Unlike promoters, enhancers can function when located at various distances from the transcription site. An enhancer also can be located downstream from the transcription initiation site. A coding sequence is "operably linked" and "under the control" of expression control sequences in a cell when RNA polymerase is able to transcribe the coding sequence into mRNA, which then can be translated into the protein encoded by the coding sequence.

Suitable expression vectors include, without limitation, plasmids and viral vectors derived from, for example, bacteriophage, baculoviruses, tobacco mosaic virus, herpes viruses, cytomegalo virus, retroviruses, vaccinia viruses, adenoviruses, and adeno-associated viruses. Numerous vectors and expression systems are commercially available from such corporations as Novagen (Madison, WI), Clontech (Palo Alto, CA), Stratagene (La Jolla, CA), and Invitrogen Life Technologies (Carlsbad, CA).

An expression vector can include a tag sequence. Tag sequences, are typically expressed as a fusion with the encoded polypeptide. Such tags can

be inserted anywhere within the polypeptide including at either the carboxyl or amino terminus. Examples of useful tags include, but are not limited to, green fluorescent protein (GFP), glutathione S-transferase (GST), polyhistidine, c-myc, hemagglutinin, Flag™ tag (Kodak, New Haven, CT), maltose E binding protein and protein A. In one embodiment, a nucleic acid molecule encoding a B7-H4 fusion polypeptide is present in a vector containing nucleic acids that encode one or more domains of an Ig heavy chain constant region, preferably having an amino acid sequence corresponding to the hinge, C_H2 and C_H3 regions of a human immunoglobulin Cγ1 chain.

Vectors containing nucleic acids to be expressed can be transferred into host cells. The term "host cell" is intended to include prokaryotic and eukaryotic cells into which a recombinant expression vector can be introduced. As used herein, "transformed" and "transfected" encompass the introduction of a nucleic acid molecule (e.g., a vector) into a cell by one of a number of techniques. Although not limited to a particular technique, a number of these techniques are well established within the art. Prokaryotic cells can be transformed with nucleic acids by, for example, electroporation or calcium chloride mediated transformation. Nucleic acids can be transfected into mammalian cells by techniques including, for example, calcium phosphate co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, or microinjection. Host cells (e.g., a prokaryotic cell or a eukaryotic cell such as a CHO cell) can be used to, for example, produce the B7-H4 fusion polypeptides described herein.

The vectors described can be used to express B7-H4 in cells, for example, cells for transplantation such as islet cells. An exemplary vector includes, but is not limited to, an adenoviral vector. One approach includes nucleic acid transfer into primary cells in culture followed by autologous transplantation of the *ex vivo* transformed cells into the host, either systemically or into a particular organ or tissue. *Ex vivo* methods can include, for example, the steps of harvesting cells from a subject, culturing the cells, transducing them with an expression vector, and maintaining the cells under conditions suitable for expression of the encoded polypeptides.

These methods are known in the art of molecular biology. The transduction step can be accomplished by any standard means used for *ex vivo* gene therapy, including, for example, calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced then can be selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells then can be lethally irradiated (if desired) and injected or implanted into the subject. In one embodiment, expression vectors containing nucleic acids encoding fusion proteins are transfected into cells that are administered to a subject in need thereof.

In vivo nucleic acid therapy can be accomplished by direct transfer of a functionally active DNA into mammalian somatic tissue or organ *in vivo*. For example, nucleic acids encoding polypeptides disclosed herein can be administered directly to lymphoid tissues or tumors. Alternatively, lymphoid tissue specific targeting can be achieved using lymphoid tissue-specific transcriptional regulatory elements (TREs) such as a B lymphocyte-, T lymphocyte-, or dendritic cell-specific TRE. Lymphoid tissue specific TREs are known in the art.

Nucleic acids may also be administered *in vivo* by viral means. Nucleic acid molecules encoding fusion proteins may be packaged into retrovirus vectors using packaging cell lines that produce replication-defective retroviruses, as is well-known in the art. Other virus vectors may also be used, including recombinant adenoviruses and vaccinia virus, which can be rendered non-replicating. In addition to naked DNA or RNA, or viral vectors, engineered bacteria may be used as vectors.

Nucleic acids may also be delivered by other carriers, including liposomes, polymeric micro- and nanoparticles and polycations such as asialoglycoprotein/polylysine.

In addition to virus- and carrier-mediated gene transfer *in vivo*, physical means well-known in the art can be used for direct transfer of DNA, including administration of plasmid DNA and particle-bombardment mediated gene transfer.

V. Pharmaceutical compositions

Pharmaceutical compositions including fusion polypeptides disclosed herein are provided. Pharmaceutical compositions containing peptides or polypeptides may be for administration by parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), transdermal (either passively or using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

In some *in vivo* approaches, the compositions disclosed herein are administered to a subject in a therapeutically effective amount. As used herein the term "effective amount" or "therapeutically effective amount" means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of the disorder being treated or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease, and the treatment being effected.

For the polypeptide compositions disclosed herein and nucleic acids encoding the same, as further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age, and general health of the recipient, will be able to ascertain proper dosing. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment desired. Generally dosage levels of 0.001 to 10 mg/kg of body weight daily are administered to mammals. Generally, for intravenous injection or infusion, dosage may be lower.

In certain embodiments, the polypeptide compositions are administered locally, for example by injection directly into a site to be treated. Typcially, the injection causes an increased localized concentration of the polypeptide compositions which is greater than that which can be achieved by systemic administration. In the case of organ transplants, the

protein may be administered locally to a site near the transplanted organ. The polypeptide compositions can be combined with a matrix as described above to assist in creating a increased localized concentration of the polypeptide compositions by reducing the passive diffusion of the polypeptides out of the site to be treated.

1. Formulations for parenteral administration

In a preferred embodiment, compositions disclosed herein, including those containing peptides and polypeptides, are administered in an aqueous solution, by parenteral injection. The formulation may also be in the form of a suspension or emulsion. In general, pharmaceutical compositions are provided including effective amounts of a peptide or polypeptide, and optionally include pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents sterile water, buffered saline of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; and optionally, additives such as detergents and solubilizing agents (e.g., TWEEN 20, TWEEN 80 also referred to as polysorbate 20 or 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), and preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. The formulations may be lyophilized and redissolved/resuspended immediately before use. The formulation may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions.

2. Formulations for topical administration

Fusion proteins disclosed herein can be applied topically. Topical administration does not work well for most peptide formulations, although it can be effective especially if applied to the lungs, nasal, oral (sublingual, buccal), vaginal, or rectal mucosa.

Compositions can be delivered to the lungs while inhaling and traverse across the lung epithelial lining to the blood stream when delivered

either as an aerosol or spray dried particles having an aerodynamic diameter of less than about 5 microns.

A wide range of mechanical devices designed for pulmonary delivery of therapeutic products can be used, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices are the Ultravent nebulizer (Mallinckrodt Inc., St. Louis, Mo.); the Acorn II nebulizer (Marquest Medical Products, Englewood, Colo.); the Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, N.C.); and the Spinhaler powder inhaler (Fisons Corp., Bedford, Mass.). Nektar, Alkermes and Mannkind all have inhalable insulin powder preparations approved or in clinical trials where the technology could be applied to the formulations described herein.

Formulations for administration to the mucosa will typically be spray dried drug particles, which may be incorporated into a tablet, gel, capsule, suspension or emulsion. Standard pharmaceutical excipients are available from any formulator. Oral formulations may be in the form of chewing gum, gel strips, tablets or lozenges.

Transdermal formulations may also be prepared. These will typically be ointments, lotions, sprays, or patches, all of which can be prepared using standard technology. Transdermal formulations will require the inclusion of penetration enhancers.

3. Controlled delivery polymeric matrices

Fusion proteins disclosed herein may also be administered in controlled release formulations. Controlled release polymeric devices can be made for long term release systemically following implantation of a polymeric device (rod, cylinder, film, disk) or injection (microparticles). The matrix can be in the form of microparticles such as microspheres, where peptides are dispersed within a solid polymeric matrix or microcapsules, where the core is of a different material than the polymeric shell, and the peptide is dispersed or suspended in the core, which may be liquid or solid in nature. Unless specifically defined herein, microparticles, microspheres, and microcapsules are used interchangeably. Alternatively, the polymer may be

cast as a thin slab or film, ranging from nanometers to four centimeters, a powder produced by grinding or other standard techniques, or even a gel such as a hydrogel.

Either non-biodegradable or biodegradable matrices can be used for delivery of fusion polypeptides or nucleic acids encoding the fusion polypeptides, although biodegradable matrices are preferred. These may be natural or synthetic polymers, although synthetic polymers are preferred due to the better characterization of degradation and release profiles. The polymer is selected based on the period over which release is desired. In some cases linear release may be most useful, although in others a pulse release or "bulk release" may provide more effective results. The polymer may be in the form of a hydrogel (typically in absorbing up to about 90% by weight of water), and can optionally be crosslinked with multivalent ions or polymers.

The matrices can be formed by solvent evaporation, spray drying, solvent extraction and other methods known to those skilled in the art. Bioerodible microspheres can be prepared using any of the methods developed for making microspheres for drug delivery, for example, as described by Mathiowitz and Langer, *J. Controlled Release*, 5:13-22 (1987); Mathiowitz, et al., *Reactive Polymers*, 6:275-283 (1987); and Mathiowitz, et al., *J. Appl. Polymer Sci.*, 35:755-774 (1988).

The devices can be formulated for local release to treat the area of implantation or injection – which will typically deliver a dosage that is much less than the dosage for treatment of an entire body – or systemic delivery. These can be implanted or injected subcutaneously, into the muscle, fat, or swallowed.

In another embodiment, B7-H4 polypeptides or fragments, or fusions thereof are administered with transplanted cells encapsulated within a matrix to allow release of the B7-H4 polypeptides or fragments, or fusions thereof over a period of time in the area of transplantation. The matrix can be a polymeric matrix made using any polymer suitable for cell encapsulation. Exemplary polymeric materials suitable for encapsulating cells include, but are not limited to alginate, agarose, hyaluronic acid, collagen, synthetic

monomers, albumin, fibrinogen, fibronectin, vitronectin, laminin, dextran, dextran sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, chitin, chitosan, heparan, heparan sulfate, or a combination thereof. For example, for treatment of diabetes, B7-H4 polypeptides or fragments, or fusions thereof can be encapsulated with pancreatic islet cells within a polymeric matrix. Ecapsulation of pancreatic islet cells is described, for example, in Barnett, et al., *Nature Medicine*, 13(8):986-91 (2007)).

VI. Methods of manufacture

A. Methods for producing fusion proteins

Isolated fusion proteins can be obtained by, for example, chemical synthesis or by recombinant production in a host cell. To recombinantly produce a fusion protein, a nucleic acid containing a nucleotide sequence encoding the fusion protein can be used to transform, transduce, or transfect a bacterial or eukaryotic host cell (e.g., an insect, yeast, or mammalian cell). In general, nucleic acid constructs include a regulatory sequence operably linked to a nucleotide sequence encoding the fusion protein. Regulatory sequences (also referred to herein as expression control sequences) typically do not encode a gene product, but instead affect the expression of the nucleic acid sequences to which they are operably linked.

Useful prokaryotic and eukaryotic systems for expressing and producing polypeptides are well known in the art include, for example, *Escherichia coli* strains such as BL-21, and cultured mammalian cells such as CHO cells.

In eukaryotic host cells, a number of viral-based expression systems can be utilized to express fusion proteins. Viral based expression systems are well known in the art and include, but are not limited to, baculoviral, SV40, retroviral, or vaccinia based viral vectors.

Mammalian cell lines that stably express variant fusion proteins can be produced using expression vectors with appropriate control elements and a selectable marker. For example, the eukaryotic expression vectors pCR3.1 (Invitrogen Life Technologies) and p91023(B) (see Wong *et al.* (1985) *Science* 228:810-815) are suitable for expression of variant costimulatory polypeptides in, for example, Chinese hamster ovary (CHO) cells, COS-1

cells, human embryonic kidney 293 cells, NIH3T3 cells, BHK21 cells, MDCK cells, and human vascular endothelial cells (HUVEC). Additional suitable expression systems include the GS Gene Expression System™ available through Lonza Group Ltd.

Following introduction of an expression vector by electroporation, lipofection, calcium phosphate, or calcium chloride co-precipitation, DEAE dextran, or other suitable transfection method, stable cell lines can be selected (e.g., by metabolic selection, or antibiotic resistance to G418, kanamycin, or hygromycin or by metabolic selection using the Glutamine Synthetase-NS0 system). The transfected cells can be cultured such that the polypeptide of interest is expressed, and the polypeptide can be recovered from, for example, the cell culture supernatant or from lysed cells. Alternatively, a fusion protein can be produced by (a) ligating amplified sequences into a mammalian expression vector such as pcDNA3 (Invitrogen Life Technologies), and (b) transcribing and translating *in vitro* using wheat germ extract or rabbit reticulocyte lysate.

Fusion proteins can be isolated using, for example, chromatographic methods such as affinity chromatography, ion exhange chromatography, hydrophobic interaction chromatography, DEAE ion exchange, gel filtration, and hydroxylapatite chromatography. In some embodiments, fusion proteins can be engineered to contain an additional domain containing amino acid sequence that allows the polypeptides to be captured onto an affinity matrix. For example, an Fc-fusion polypeptide in a cell culture supernatant or a cytoplasmic extract can be isolated using a protein A column. In addition, a tag such as c-myc, hemagglutinin, polyhistidine, or Flag™ (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide, including at either the carboxyl or amino terminus. Other fusions that can be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase. Immunoaffinity chromatography also can be used to purify costimulatory polypeptides. Fusion proteins can additionally be engineered to contain a secretory signal (if there is not a secretory signal already present) that causes the fusion

protein to be secreted by the cells in which it is produced. The secreted fusion proteins can then conveniently be isolated from the cell media.

B. Methods for producing isolated nucleic acid molecules

Isolated nucleic acid molecules can be produced by standard techniques, including, without limitation, common molecular cloning and chemical nucleic acid synthesis techniques. For example, polymerase chain reaction (PCR) techniques can be used to obtain an isolated nucleic acid encoding a variant costimulatory polypeptide. PCR is a technique in which target nucleic acids are enzymatically amplified. Typically, sequence information from the ends of the region of interest or beyond can be employed to design oligonucleotide primers that are identical in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic DNA or total cellular RNA. Primers typically are 14 to 40 nucleotides in length, but can range from 10 nucleotides to hundreds of nucleotides in length. General PCR techniques are described, for example in PCR Primer: A Laboratory Manual, ed. by Dieffenbach and Dveksler, Cold Spring Harbor Laboratory Press, 1995. When using RNA as a source of template, reverse transcriptase can be used to synthesize a complementary DNA (cDNA) strand. Ligase chain reaction, strand displacement amplification, self-sustained sequence replication or nucleic acid sequence-based amplification also can be used to obtain isolated nucleic acids. See, for example, Lewis (1992) *Genetic Engineering News* 12:1; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878; and Weiss (1991) *Science* 254:1292-1293.

Isolated nucleic acids can be chemically synthesized, either as a single nucleic acid molecule or as a series of oligonucleotides (e.g., using phosphoramidite technology for automated DNA synthesis in the 3' to 5' direction). For example, one or more pairs of long oligonucleotides (e.g., >100 nucleotides) can be synthesized that contain the desired sequence, with each pair containing a short segment of complementarity (e.g., about 15 nucleotides) such that a duplex is formed when the oligonucleotide pair is

annealed. DNA polymerase can be used to extend the oligonucleotides, resulting in a single, double-stranded nucleic acid molecule per oligonucleotide pair, which then can be ligated into a vector. Isolated nucleic acids can also obtained by mutagenesis. Fusion protein-encoding nucleic acids can be mutated using standard techniques, including oligonucleotide-directed mutagenesis and/or site-directed mutagenesis through PCR. See, Short Protocols in Molecular Biology. Chapter 8, Green Publishing Associates and John Wiley & Sons, edited by Ausubel *et al,* 1992. Examples of amino acid positions that can be modified include those described herein.

VII. Methods of Use

The B7-H4 polypeptides or fragments, or fusions thereof disclosed herein are useful as therapeutic agents. One embodiment provides a method for inhibiting or reducing transplant rejection in a host by administering to the host an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof. Immune cells, preferably T cells, can be contacted *in vivo* or *ex vivo* with B7-H4 polypeptides to decrease or inhibit immune responses including, but not limited to transplant rejection. The T cells contacted with B7-H4 fusion polypeptides can be any cell which express the T cell receptor, including α/β and γ/δ T cell receptors. T-cells include all cells which express CD3, including T-cell subsets which also express CD4 and CD8. T-cells include both naive and memory cells and effector cells such as CTL. T-cells also include regulatory cells such as Th1, Tc1, Th2, Tc2, Th3, Th17, Th22, Treg, and Tr1 cells. For example the compositions can be used to modulate Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, inflammatory molecules, including, but not limited to, IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs. T-cells also include NKT-cells and similar unique classes of the T-cell lineage. The compositions can also be used to increase or promote the activity of Tregs, increase the production of cytokines such as IL-10 from Tregs, increase the differentiation of Tregs, increase the number of Tregs, or increase the survival of Tregs.

Other immune cells that can be treated with the disclosed B7-H4 polypeptides, fusion proteins, or fragments thereof include T cell precursors, antigen presenting cells, B cells, or combinations thereof. The B7-H4 compositions can be used to modulate the production of antibodies by B cells by contacting the B cells with an effective amount of the B7-H4 composution to inhibit or reduce the antibody production by the B cell relative to a control. The B7-H4 compositions can also modulate the production of cytokines by the B cells.

A. Methods of treating transplant rejection

A preferred embodiment provides methods for reducing or inhibiting transplant rejection in a subject, preferably a human subject. Transplant rejection can be inhibited or reduced in a subject by administering an effective amount of B7-H4 polypeptides or fragments, or fusions thereof to inhibit or reduce the biological activity of an immune cell or to reduce the amounts of proinflammatory cytokines or other molecules associated with or that promote inflammation at a site of transplant. Exemplary proinflammatory molecules include, but are not limited to IL-1β, TNF-α, TGF-beta, IFN-γ, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

Th1 and Th17 are exemplary T cells that can be targeted for inhibition by B7-H4 polypeptides, fusion proteins or fragments thereof to inhibit or reduce inflammation. The B7-H4 fusion proteins are useful for treating inflammation by any or all of the following: inhibiting or reducing differentiation of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules; inhibiting or reducing activity of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules; inhibiting or reducing the Th1 and/or Th17 pathways; inhibiting or reducing cytokine production and/or secretion by Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules; inhibiting or reducing proliferation of Th1, Th17, Th22, and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules.

Additionally, B7-H4 polypeptides, fusion proteins or fragments thereof can cause Tregs to have an enhanced suppressive effect on the Th1,

Th17, Th22 and/or other cells that secrete, or cause other cells to secrete, inflammatory molecules to reduce the level of IFN-γ and/or IL-17 produced. B7-H4 polypeptides, fusion proteins or fragments thereof can also act directly on Tregs to promote or enhance production of IL-10 to suppress the Th1 and/or Th17 pathways, or to increase the number of Tregs.

B7-H4 polypeptides, fusion proteins or fragments thereof act at multiple points in multiple pathways. For example, they can inhibit the development of naïve T cells into either Th1 or Th17 cells. Alternatively, they can interact with Th1 cells or Th17 cells, or both to inhibit or reduce the production of proinflammatory molecules. Additionally, they can target Tregs to cause an enhanced suppressive effect on the Th1 and/or Th17 pathways to reduce the level of INF-γ and/or IL-17 produced. B7-H4 compositions can also act directly on Tregs to promote or enhance production of IL-10 to suppress the Th1 and /or Th17 pathway. Additionally they can work by enhancing recruitment or expansion (or both) of Treg cells in the region of engrafted tissue or at peripheral sites.

1. Inhibition of the Th1 Pathway

a. Inhibition of Th1 Development

One method for inhibiting or reducing transplant rejection includes administering an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof to inhibit or block naïve T cells from developing into Th1 cells in a subject in need thereof by an amount effective to inhibit or reduce transplant rejection relative to a control. It has been discovered that transplant rejection can be inhibited or reduced by blocking naïve T cells from differentiating into Th1 cells by administering B7-H4 polypeptides or fragments, or fusions thereof or variants thereof. In one embodiment, the B7-H4 polypeptide or fragment, or fusion thereof increases the suppressive ability of Tregs on naïve T cells to inhibit or reduce naïve T cells from differentiating into Th1 cells and thereby reduce the number of Th1 cells in a subject. Alternatively, the B7-H4 polypeptide or fragment, or fusion thereof inhibits or reduces proliferation of Th1 cells. By restricting the number of Th1 cells that can develop in the subject, the amount of proinflammatory molecules being produced such as INF-γ can be reduced or contained. INF-γ

stimulates the production or release of other proinflammatory molecules including IL-1β, TNF-α, and MMPs. Thus, by controlling the number of Th1 cells in a subject, the levels of these other proinflammatory molecules can be controlled, thereby reducing transplant rejection.

b. Inhibition of Proinflammatory molecules

Another embodiment provides a method of inhibiting or reducing transplant rejection in a subject by administering to the subject an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof to inhibit or reduce production of proinflammatory molecules by Th1 cells by an amount effective to inhibit or reduce transplant rejection in the subject. Exemplary proinflammatory molecules produced by Th1 cells includes IFN-γ. In this embodiment the B7-H4 polypeptide or fragment, or fusion thereof can interact directly with the Th1 cell and inhibit or reduce IFN-γ production by the Th1 cells. In this embodiment, the amount of proinflammatory molecules are regulated rather than the population of Th1 cells.

2. Inhibition of the Th17 Pathway

a. Inhibition of Th17 Development

Transplant rejection can also be inhibited or reduced in a subject by administering an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof to inhibit or block naïve T cells from developing into Th17 cells by an amount effective to inhibit or reduce transplant rejection in the subject relative to a control. In one embodiment, the B7-H4 polypeptide or fragment, or fusion thereof increases the suppressive activity of Tregs on the differentiation of naïve T cells into Th17 cells by an amount sufficient to reduce the number of Th17 cells in a subject. Alternatively, the B7-H4 polypeptide or fragment, or fusion thereof inhibits or reduces proliferation of Th17 cells. By reducing the population of Th17 cells in a subject, the amount of IL-17 being produced can be reduced, as well as IL-22 and IL-21. IL-17 is a proinflammatory molecule that causes increases in other proinflammatory molecules such as IL-1β, TNF-α, and MMPs. Thus, by reducing the amount of IL-17 these other proinflammatory molecules can be reduced thereby reducing or inhibiting transplant rejection.

b. Inhibition of IL-17 Production

Still another embodiment provides a method for inhibiting or reducing transplant rejection in a subject by administering an effective amount of B7-H4 polypeptide or fragment, or fusion thereof, to inhibit production of IL-17 by Th17 cells, as well as IL-22 and IL-21 by an amount effective to inhibit or reduce transplant rejection relative to a control. In this embodiment, the B7-H4 polypeptide or fusion protein can act directly on Th17 cells, for example by binding to Th17 cells resulting in inhibition of IL-17 (or IL-22 and IL-21) production by those Th17 cells. As noted above, inhibition or reduction of IL-17 (and IL-22 or IL-21) leads to the reduction of other proinflammatory molecules, thereby reducing or inhibitng transplant rejection.

3. Inhibiting Th1 and Th17 Pathways

The disclosed B7-H4 polypeptide or fragment, or fusion thereof can be used to inhibit both the Th1 and Th17 pathways simultaneously. Using one anti-inflammatory agent to inhibit two separate pathways provides more robust inhibition or reduction of the immune response thereby reducing or inhibiting transplant rejection. Inhibition of the Th1 and Th17 pathways inhibits or reduces the recruitment of neutrophils and may thereby reduce one or more symptoms of inflammation. In one emvbodiment, inhibition of the Th1 and Th17 pathways reduce or inhibit the proliferation of neutrophils and or macrophages.

4. Tregs

Inflammation can also be treated by administering a B7-H4 polypeptide or fragment, or fusion thereof to a subject in an amount effective to target IL-10 producing Tregs to enhance the suppressive activity on the Th1 and/or Th17 pathways. In this embodiment the disclosed B7-H4 polypeptides or fragments, or fusions thereof cause an increased suppressive effect on IL-17 production relative to Tregs alone thereby inhibiting or reducing transplant rejection relative to a control.

Another embodiment provides a method for inhibiting or reducing transplant rejection by administering an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof to increase production of IL-10 by Tregs. Increased production of IL-10 results in the descreased production of

IL-17 by Th17 cells and deceased production of IFN- α by Th1 cells. In this embodiment, the B7-H4 polypeptides or fragments, or fusions thereof can interact directly with Tregs to increase IL-10 production by the Tregs and thereby inhibit or reduce transplant rejection in a subject relative to a control.

Additionally the B7-H4 polypeptides or fragments, or fusions thereof can work by enhancing recruitment or expansion (or both) of Treg cells in the region of engrafted tissue. Thus, another embodiment provides a method for inhibiting or reducing transplant rejection in a subject by adminstering to the subject an effective amount of a B7-H4-Ig fusion protein to enhance recruitment of Treg cells in the region of the engrafted tissue. Increased infiltration of Treg cells into the engrafted tissue can increase the level of IL-10 locally and result in the descreased production of IL-17 by Th17 cells and deceased production of IFN- α by Th1 cells at the site of transplantation. This can lead to prolonged survival and decreased rejection of the transplant.

Still another embodiment provides a method for inhibiting or reducing transplant rejection in a subject by administering an effective amount of B7-H4 polypeptides or fragments, or fusions thereof to inhibit or interfere with the Th1 pathway, Th17 pathway and to enhace the suppressive effect on the Th17 pathway by Tregs by an amount effective to inhibit or reduce transplant rejection in the subject relative to a control.

The B7-H4 polypeptides, fusion proteins thereof and fragments thereof can also be administered to a subject in an amount effective to increase Treg cell populations or numbers.

IL-10 and TGF-β production by Tregs can be increased relative to a control by contacting the Tregs with an effective amount of B7-H4 polypeptides or fragments, or fusions thereof having B7-H4 activity. The increase can occur *in vitro* or *in vivo*.

5. Soluble B7-H4

Soluble B7-H4 (sH4) acts as a decoy molecule that binds to the B7-H4 receptor and may block endogenous B7-H4 from binding to its receptor. sH4 does not deliver an inhibitory signal like cell-surface B7-H4. B7-H4 inhibits cell cycle progression of T cells in the presence of antigen stimulation. B7-H4 can inhibit innate immunity by suppressing proliferation

of neutrophil progenitors. It is believed that elevated levels of sH4 block the inhibitory effect of endogenous B7-H4.

Therefore, transplant rejection can be inhibited or reduced by interfering with the biological activity of sH4 *in vivo*, for example, by administering to an individual in need thereof an effective amount of an agent that inhibits or decreases the ability of sH4 to bind to the B7-H4 receptor, or augments the activity of the endogenous inhibitory B7-H4 molecules. Interference of sH4 biological activity can be accomplished by administering B7-H4 fusion polypeptides disclosed herein.

Administration is not limited to the treatment of existing conditions, diseases or disorders (i.e. an existing inflammatory or autoimmune disease or disorder) but can also be used to prevent or lower the risk of developing such diseases in an individual, i.e., for prophylactic use. Thus, the disclosed composition can be administered prior to transplant, during transplant, and after transplant.

6. Transplants

The transplanted material can be cells, tissues, organs, limbs, digits or a portion of the body, preferably the human body. The transplants are typically allogenic or xenogenic. The disclosed B7-H4 polypeptides, fusion proteins, or fragments thereof are administered to a subject in an effective amount to reduce or inhibit transplant rejection. B7-H4 polypeptides, fusion proteins, or fragments thereof can be administered systemically or locally by any acceptable route of administration. In some embodiments, B7-H4 polypeptides or fragments, or fusions thereof are administered to a site of transplantation prior to, at the time of, or following transplantation. In one embodiment, the B7-H4 polypeptides or fragments, or fusions thereof are administered to a site of transplantation parenterally, such as by subcutaneous injection.

In other embodiments, or B7-H4 polypeptides, fusion proteins, or fragments thereof are administered directly to cells, tissue or organ to be transplanted *ex vivo*. In one embodiment, the transplant material is contacted with B7-H4 polypeptides, fusion proteins thereof, and fragments thereof prior to transplantation, after transplantion, or both.

In other embodiments, B7-H4 polypeptides or fragments, or fusions thereof are administered to immune tissues or organs, such as lymph nodes or the spleen.

The transplant material can be modified prior to transplant. For example, the transplant material can be genetically modified to express a protein that aids in the inhibition or reduction of transplant rejection. In a preferred embodiment, the transplant material is genetically modified to express B7-H4 polypeptides or fragments, or fusions thereof in an amount effective to inhibit or reduce transplant rejection in a transplant recipient.

The transplant material can be treated with enzymes or other materials that remove cell surface proteins, carbohydrates, or lipids that are known or suspected in being involved with immune responses such as transplant rejection.

B7-H4 acts at multiple points in the inflammatory pathway and at a higher level whereby it acts as a master regulator to control to influence the expression and/or activity of effectory cytokines such as TNF-α. Therefore, the B7-H4 compositions described herein are particularly useful for treating patients that do not respond to TNF-α blockers such as Enbrel, Remicade, Cimzia and Humira. In addition, because of its activity as a master regulator in the inflammatory pathway, the B7-H4 compositions disclosed are particularly useful for treating chronic transplant rejection.

a. Cells

Populations of any types of cells can be transplanted into a subject. The cells can be homogenous or heterogenous. Heterogeneous means the cell population contains more than one type of cell. Exemplary cells include progenitor cells such as stem cells and pluripotent cells which can be harvested from a donor and transplanted into a subject. The cells are optionally treated prior to transplantation as mention above. Such treatment includes transfecting the cells *ex vivo* with a nucleic acid construct enabling the cells to express B7-H4 polypeptides or fragments, or fusions thereof *in vitro* and *in vivo*. Methods for transfecting cells are well known in the art.

Ex vivo methods of nucleic acid delivery can include, for example, the steps of harvesting cells from a subject, culturing the cells, transducing

them with an expression vector, and maintaining the cells under conditions suitable for expression of the encoded polypeptides. These methods are known in the art of molecular biology. An exemplary nucleic acid vector includes but is not limited to an adenoviral vector. The transduction step can be accomplished by any standard means used for *ex vivo* gene therapy, including, for example, calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced then can be selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells then can be lethally irradiated (if desired) and injected or implanted into the subject. Other exemplary cells that can be transplanted include, but are not limited to, islet cells, hematopoietic cells, muscle cells, cardiac cells, neural cells, embryonic stem cells, adult stem cells, T cells, lymphocytes, dermal cells, mesoderm, endoderm, and ectoderm cells.

b. Tissues

Any tissue can be used as a transplant. Exemplary tissues include skin, adipose tissue, cardiovascular tissue such as veins, arteries, capularies, valves; neural tissue, bone marrow, pulmonary tissue, ocular tissue such as corneas and lens, cartilage, bone, and mucosal tissue. The tissue can be modified as discussed above.

c. Organs

Exemplary organs that can be used for transplant include, but are not limited to kidney, liver, heart, spleen, bladder, lung, stomach, eye, tongue, pancreas, intestine, etc. The organ to be transplanted can also be modified prior to transplantation as discussed above.

One embodiment provides a method of inhibiting or reducing chronic transplant rejection in a subject by administering an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof to inhibit or reduce chronic transplant rejection relative to a control. The effects of B7-H4 polypeptides, fusion proteins thereof, and fragments thereof on Treg cell are particularly important in this context.

B. Graft-versus-host disease (GVHD)

The disclosed B7-H4 polypeptides or fragments, or fusions thereof can also be used to treat graft-versus-host disease (GVHD) by administering an effective amount of of B7-H4 polypeptide, fusion proteins thereof, or fragments thereof to alleviate one or more symptoms associated with GVHD. GVHD is a major complication associated with allogeneic hematopoietic stem cell transplantation in which functional immune cells in the transplanted marrow recognize the recipient as "foreign" and mount an immunologic attack. It can also take place in a blood transfusion under certain circumstances. Symptoms of GVD include skin rash or change in skin color or texture, diarrhea, nausea, abnormal liver function, yellowing of the skin, increased susceptibility to infection, dry, irritated eyes, and sensitive or dry mouth.

D. Diabetes

The B7-H4 polypetpides and fusion proteins thereof can also be used to treat diabetes. The method includes transplanting insulin producing cells in a subject and adiminstering to the subject an effective amount of a B7-H4 polypeptide or fragment, or fusion thereof to reduce or inhibit transplant rejection. Preferably the insulin producing cells are beta cells or islet cells. In certain embodiments, the insulin producing cells are recombinant cells engineered to produce insulin. The insulin producing cells may also be genetically modified to produce B7-H4 polypeptides or fragments, or fusions thereof, as described herein.

The insulin producing cells can be encapsulated within a matrix, such as a polymeric matrix, using suitable polymers, including, but not limited to alginate, agarose, hyaluronic acid, collagen, synthetic monomers, albumin, fibrinogen, fibronectin, vitronectin, laminin, dextran, dextran sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, chitin, chitosan, heparan, heparan sulfate, or a combination thereof.

E. Combination Therapy

B7-H4 polypeptides or fragments, or fusions thereof can be used alone or in combination with additional therapeutic agents. The additional therapeutic agents include, but are not limited to, immunosuppressive agents (e.g., antibodies against other lymphocyte surface markers (e.g., CD40,

alpha-4 integrin) or against cytokines), other fusion proteins (e.g., CTLA-4-Ig (Orencia®), TNFR-Ig (Enbrel®)), TNF-α blockers such as Enbrel, Remicade, Cimzia and Humira, cyclophosphamide (CTX) (i.e. Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune™), methotrexate (MTX) (i.e. Rheumatrex®, Trexall®), belimumab (i.e. Benlysta®), or other immunosuppressive drugs (e.g., cyclosporin A, FK506-like compounds, rapamycin compounds, or steroids), anti-proliferatives, cytotoxic agents, or other compounds that may assist in immunosuppression.

In a preferred embodiment, the additional therapeutic agent functions to inhibit or reduce T cell activation and cytokine production through a separate pathway. In one such embodiment, the additional therapeutic agent is a CTLA-4 fusion protein, such as CTLA-4 Ig (abatacept). CTLA-4 Ig fusion proteins compete with the co-stimulatory receptor, CD28, on T cells for binding to CD80/CD86 (B7-1/B7-2) on antigen presenting cells, and thus function to inhibit T cell activation. In a preferred embodiment, the additional therapeutic agent is a CTLA-4-Ig fusion protein known as belatacept. Belatacept contains two amino acid substuitutions (L104E and A29Y) that markedly increase its avidity to CD86 *in vivo*. In another embodiment, the additional therapeutic agent is Maxy-4.

In another embodiment, the second therapeutic agent preferentially treats chronic transplant rejection or GvHD, whereby the treatment regimen effectively targets both acute and chronic transplant rejection or GvHD. In a preferred embodiment the second therapeutic is a TNF-α blocker.

In another embodiment, the second therapeutic agent increases the amount of adenosine in the serum, see, for example, WO 08/147482. In a preferred embodiment, the second therapeutic is CD73-Ig, recombinant CD73, or another agent (e.g. a cytokine or monoclonal antibody or small moelcule) that increases the expression of CD73, see for example WO 04/084933. In another embodiment the second therapeutic agent is Interferon-beta.

In a preferred embodiment, the compositions are used in combination or succession with compounds that increase Treg activity or production. Exemplary Treg enhancing agents include but are not limited to

glucocorticoid fluticasone, salmeteroal, antibodies to IL-12, IFN-γ, and IL-4; vitamin D3, and dexamethasone, and combinations thereof. Antibodies to other proinflammatory molecules can also be used in combination or alternation with the disclosed B7-H4 polyypeptides, fusion proteins, or fragments theref. Preferred antibodies bind to IL-6, IL-23, IL-22 or IL-21.

As used herein the term "rapamycin compound" includes the neutral tricyclic compound rapamycin, rapamycin derivatives, rapamycin analogs, and other macrolide compounds which are thought to have the same mechanism of action as rapamycin (e.g., inhibition of cytokine function). The language "rapamycin compounds" includes compounds with structural similarity to rapamycin, e.g., compounds with a similar macrocyclic structure, which have been modified to enhance their therapeutic effectiveness. Exemplary Rapamycin compounds are known in the art (See, e.g. WO95122972, WO 95116691, WO 95104738, U.S. Pat. No. 6,015,809; 5,989,591; U.S. Pat. No. 5,567,709; 5,559,112; 5,530,006; 5,484,790; 5,385,908; 5,202,332; 5,162,333; 5,780,462; 5,120,727).

The language "FK506-like compounds" includes FK506, and FK506 derivatives and analogs, e.g., compounds with structural similarity to FK506, e.g., compounds with a similar macrocyclic structure which have been modified to enhance their therapeutic effectiveness. Examples of FK506-like compounds include, for example, those described in WO 00101385. Preferably, the language "rapamycin compound" as used herein does not include FK506-like compounds.

Other suitable therapeutics include, but are not limited to, anti-inflammatory agents. The anti-inflammatory agent can be non-steroidal, steroidal, or a combination thereof. One embodiment provides oral compositions containing about 1% (w/w) to about 5% (w/w), typically about 2.5 % (w/w) or an anti-inflammatory agent. Representative examples of non-steroidal anti-inflammatory agents include, without limitation, oxicams, such as piroxicam, isoxicam, tenoxicam, sudoxicam; salicylates, such as aspirin, disalcid, benorylate, trilisate, safapryn, solprin, diflunisal, and fendosal; acetic acid derivatives, such as diclofenac, fenclofenac, indomethacin, sulindac, tolmetin, isoxepac, furofenac, tiopinac, zidometacin,

acematacin, fentiazac, zomepirac, clindanac, oxepinac, felbinac, and ketorolac; fenamates, such as mefenamic, meclofenamic, flufenamic, niflumic, and tolfenamic acids; propionic acid derivatives, such as ibuprofen, naproxen, benoxaprofen, flurbiprofen, ketoprofen, fenoprofen, fenbufen, indopropfen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, and tiaprofenic; pyrazoles, such as phenylbutazone, oxyphenbutazone, feprazone, azapropazone, and trimethazone. Mixtures of these non-steroidal anti-inflammatory agents may also be employed.

Representative examples of steroidal anti-inflammatory drugs include, without limitation, corticosteroids such as hydrocortisone, hydroxyl-triamcinolone, alpha-methyl dexamethasone, dexamethasone-phosphate, beclomethasone dipropionates, clobetasol valerate, desonide, desoxymethasone, desoxycorticosterone acetate, dexamethasone, dichlorisone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, fludrocortisone, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylesters, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone, fludrocortisone, diflurosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, chlorprednisone acetate, clocortelone, clescinolone, dichlorisone, diflurprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone valerate, hydrocortisone cyclopentylpropionate, hydrocortamate, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate, triamcinolone, and mixtures thereof.

In another embodiment, the additional therapeutic agents includes compositions that inhibit or interfere with sH4 activity, to treat inflammatory disorders in subjects. In one embodiment, B7-H4 fusion polypeptides are administered to a subject for the treatment of an inflammatory disease wherein the subject has little or non-detectable amounts of sH4. In another

embodiment, B7-H4 fusion polypeptides are administered to treat one or more symptoms of an inflammatory disease in subjects having elevated levels of sH4. Elevated levels of sH4 can be determined by comparing levels of sH4 is subjects known to have an inflammatory disorder with levels of sH4 in subjects that do not have an inflammatory disorder.

F. Selection of Candidates for Transplantation

Detection of sH4 and B7-H4 can be used to evaluate candidates for transplantation. Elevated levels of sH4 may block the inhibitory effect of endogenous B7-H4, therefore it is believed that levels of sH4 in serum may be predictive of a patient's likelihood of rejecting transplanted material. As B7-H4 is a negative regulator of T cell responses, levels of B7-H4 expressed at the cell surface may also be predictive of a patient's likelihood of rejecting transplanted material. Levels of sH4 present in a biological sample from the individual can be determined prior to transplantation. The amounts of sH4 that correlate with transplantation rejection, including different levels of severity, can be predetermined by quantifying sH4 in patients who have had successful transplantations and those who have had unsuccessful transplantations. The levels of sH4 present in the biological sample of the transplantation candidate can be compared to the predetermined reference levels of sH4 present in biological samples from other individuals, and used to predict the candidate's likelihood of a successful transplantation. For example if 75% of patients having about "x" level of sH4 have experienced rejection of a transplant, than a patient having about "x" level of sH4 may have a 75% chance of rejecting a transplantation. Other factors that may influence a patient's likelihood of rejecting a transplant are known in the art and may be considered when determining a patient's chance of rejecting a transplantation. For example donor age, recipient diabetes, sex of the recipient, chronic GVHD, and T cell levels may also be considered.

The candidate's likelihood of a successful transplantation can be used to select patients for transplantation. For example, patients whose level of sH4 corresponds with a 0%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% likelihood of rejection may be a good candidate for transplantation, while a paient whose level of sH4 corresponds with a 80%, 90%, or 100% likelihood

of rejection may not be a good candidate for transplantation. The levels of sH4 in biological samples of the candidate for transplantation can additionally or alternatively be compared to amounts of sH4 indicative of different stages of an inflammatory response or autoimmune disease.

Alternatively, the amount of sH4 can be correlated to levels of neutrophils. Neutrophils are thought to contribute to early allograph rejection (Healy, et al., *Eur J Cardiothorac Surg,* 29:760-766 (2006)). Therefore, in certain individuals, elevated levels of neutrophils may be predictive of transplantation rejection, particularly acute rejection. Thus, sH4 levels in an individual can be correlated to neutrophil levels. Levels of sH4 that correspond to specific levels of neutrophils can be predetermined by assaying the levels of sH4 in subjects and assaying the levels of neutrophils in the subjects. Once the reference levels are determined, a biological sample from a subject can be assayed for sH4 levels. The resulting sH4 levels are then compared to the predetermined sH4 levels correlated to specific levels of neutrophils. The resulting sH4 levels are matched to the predetermined levels to determine the neutrophils levels in the subject. The number of neutrophils in a healthy individual ranges from about 15,000 to 20,000 cells/μl.

The amount of sH4 in a sample can be determined using conventional techniques such as enzyme-linked immunosorbent assays, mass spectrometry, spectrophotometry, or a combination thereof.

Methods for detecting the presence and/or measuring a level of sH4 in a biological sample, may include use of an sH4-specific antibody or an anti-B7-H4 antibody. Preferably the antibody recognizes an epitope on any one of the polypeptides encoded by SEQ ID NOs:2-7, 9-20, 24-33, 42-80, The methods generally include:

a) contacting the sample with an antibody specific for sH4; and

b) detecting binding between the antibody and molecules of the sample.

Detection of specific binding of the sH4-specific antibody, when compared to a suitable control, is an indication that sH4 is present in the sample. Suitable controls include a sample known not to contain sH4, and a

sample contacted with an antibody not specific for sH4, e.g., an anti-idiotype antibody.

A variety of methods to detect specific antibody-antigen interactions are known in the art and can be used in the method, including, but not limited to, standard immunohistological methods, immunoprecipitation, an enzyme immunoassay, and a radioimmunoassay. In general, the sH4-specific antibody will be detectably labeled, either directly or indirectly. Direct labels include radioisotopes; enzymes whose products are detectable (e.g., luciferase, β-galactosidase, and the like); fluorescent labels (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, and the like); fluorescence emitting metals, e.g., ^{152}Eu, or others of the lanthanide series, attached to the antibody through metal chelating groups such as EDTA; chemiluminescent compounds, e.g., luminol, isoluminol, acridinium salts, and the like; bioluminescent compounds, e.g., luciferin, aequorin (green fluorescent protein), and the like. The antibody may be attached (coupled) to an insoluble support, such as a polystyrene plate or a bead. Indirect labels include second antibodies specific for sH4-specific antibodies, wherein the second antibody is labeled as described above; and optionally contain members of specific binding pairs, e.g., biotin-avidin. The biological sample may be brought into contact with and immobilized on a solid support or carrier, such as nitrocellulose, that is capable of immobilizing cells, cell particles, or soluble proteins. The support may then be washed with suitable buffers, followed by contacting with a detectably-labeled sH4-specific antibody.

Still other embodiments provide methods for detecting the presence and/or measuring a level of sH4 in a biological sample. The methods generally include:

a) contacting the sample with an sH4 ligand, for example a B7-H4 receptor or fragment thereof that binds sH4; and

b) detecting binding between the B7-H4 receptor and molecules of the sample.

Detection of specific binding of the B7-H4 receptor is an indication that sH4 polypeptides are present in the sample.

Methods for detecting binding between a B7-H4 receptor and sH4 are known in the art and include immunoprecipitation of B7-H4 receptor-ligand complexes using an antibody specific to the B7-H4 receptor, as long as the antibody does not disrupt B7-H4 receptor sH4 binding. Alternatively, the B7-H4 receptor used may be a fusion protein which provides for specific immunoprecipitation of the fusion partner, an enzymatic detection, a fluorescent signal (e.g., a green fluorescent protein). The B7-H4 receptor can be labeled with any detectable label, as described above. The B7-H4 receptor can be attached, directly or through a linker, to an insoluble support (e.g., polystyrene beads, magnetic beads, and the like), thereby providing a means for separating sH4/receptor complexes from the biological sample, and subsequently detecting the presence of and/or measuring the amount (level) of sH4.

Examples

Example 1: Effect of B7-H4-Ig in allogeneic islet graft transplantation:

An allogeneic transplant model of C57BL/6 (B6) mice as recipients and BALB/c mice as islet donors were used to study the *in vivo* effects of B7-H4-Ig in prolonging islet graft survival of transplant recipients. Female B6 mouse recipients (n=4) were rendered diabetic by i.p administration of streptozotocin (STZ, 200 mg/kg), a widely used drug that is specifically toxic to islet cells and results in a permanent state of hyperglycemia. After STZ injection, the mice were tested for blood glucose level (BGL) by Glucometer Elite and signs of diabetes. Two to three days after STZ administration, B6 mice demonstrating signs of diabetes were used as transplantation recipients. Islet cells for allogeneic transplantation were isolated from pancreata of 8- to 10-week-old BALB/c donor mice by collagenase digestion (donor pancreata was perfused *in bile situ* through the common duct with collagenase), followed by separation on a discontinuous Ficoll gradient and purified by handpicking under a stereo microscope. Then, groups of 500 islets were transplanted under the left kidney capsule of each recipient. The transplant recipients were then treated with i.p. injections of B7-H4.Ig (250 μg) on Day 0. This was followed by injection of the same

amount of reagents, respectively, on Days 2, 4, 6, and 8. BGL was measured every 2 days. Clinical recurrence of diabetes was defined as a random BGL reading of >250 mg/dl for three consecutive days, and survival of islet cells is determined by the ability of mice to maintain a BGL reading of less than 250 mg/dl. To determine the effects of B7-H4.Ig injection on islet transplantation in B6 mice with diabetes, graft-survival curves of the B7-H4-Ig treatment and no-treatment groups were compared by Kaplan–Meier analysis. Figure 1 illustrates diabetes disease induction in the recipient B6 mice and allogeneic islet transplant together with B7-H4-Ig administration. Figure 2 shows the results of this experiment that injection of B7-H4-Ig slowed the transplant rejection and prolonged graft survival in allogeneic islet transplant–recipient B6 mice.

Example 2: B7-H4-Ig increase survivability in an allogenic transplant mouse model of GvHD

Transplantation and Treatment Protocol

Recipient Balb/C mice were lethally irradiated with a dose of 8.4 Gy and were reconstituted within 4 hours with a single intravenous inoculum of either 5 x 10^6 allogeneic bone marrow (BM) cells from C57B/6 + spleen cells (1.5 x10^7) or 5 x 10^6 syngeneic bone marrow cells. To avoid bias from cage-related effects, animals in different groups were randomized between cages. Four groups of mice were given included. Group I was a syngeneic BM transplant that BM cells from Balb/C mice were transferred to Balb/C mice and served as a negative control. Group II was an allogenic bone marrow transplant in which control IgG (0.5 mg) was administered on Day 0, 1, 7 and 14. Group III was an allogenic bone marrow transplant in which B7-H4-Ig (0.5 mg) was administed on Day 0, 1, 7, and 14. Group IV was allogenic bone marrow transplants treated with phosphate buffered saline (PBS) on Day 0, 1, 7, and 14. There were 4 mice in the negative, syngeneic BM transplantation group and 8 mice each in the other 3 groups. Figure 3 is a schematic diagram of an experimental design for the graft versus host disease assay.

Results

Figure 4A shows that allogenic BM transplanted mice treated with B7-H4-Ig had significantly reduced diarrhea clinic score compared to allogenic BM transplanted mice treated with control IgG on Day 7 post transplantation. Figure 4B shows that allogenic BM transplanted mice treated with B7-H4-Ig showed reduced weight loss compared to allogenic transplanted mice treated with control IgG. Figure 5 shows that allogenic BM transplanted mice treated with B7-H4-Ig transiently survived longer compared to controls. Taken together the results show with optimized dose regimen B7-H4-Ig potentially can be usuful for allogenic BM transplantation.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A method for inhibiting or reducing transplant rejection in a subject comprising administering an effective amount of a B7-H4 protein to the subject to inhibit or reduce the differentiation of, proliferation of, activity of, or cytokine production or secretion by an immune cell selected from the group consisting of Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, inflammatory molecules, by an amount effective to inhibit or reduce transplant rejection relative to a control.

2. The method of claim 1 wherein the B7-H4 protein inhibits development of naïve T cells into Th17, Th1 or Th22 cells.

3. The method of claim 4 wherein the B7-H4 protein inhibits production of INFγ by immune cells in the subject.

4. The method of claim 6 wherein the B7-H4 protein inhibits production of IL-17 or IL-22 by immune cells in the subject.

5. A method for inhibiting or reducing transplant rejection in a subject comprising administering to the subject an effective amount of a B7-H4 protein to inhibit or reduce differentiation of, proliferation of, activity of, and/or cytokine production and/or secretion by two or more types of immune cells selected from the group consisting of Th1, Th17, Th22, or other cells that secrete, or cause other cells to secrete, inflammatory molecules, by an amount effective to inhibit or reduce transplant rejection relative to a control.

6. A method for inhibiting or reducing transplant rejection in a subject comprising administering to the subject an effective amount of a B7-H4 protein to enhance the suppressive effect of Tregs on the Th17 pathway in an amount effective to inhibit or reduce transplant rejection.

7. A method for inhibiting or reducing transplant rejection in a subject comprising administering to the subject an effective amount of a B7-H4 protein to promote or enhance IL-10 production by Tregs by an amount effective to inhibit or reduce transplant rejection in the subject relative to a control.

8. A method for inhibiting or reducing transplant rejection in a subject comprising administering to the subject an effective amount of a B7-H4 protein to increase of the number of Tregs by an amount effective to

inhibit or reduce transplant rejection in the subject relative to a control or to enhance recruitment or expansion of Treg cells in the region of engrafted tissue.

9. A method for inhibiting or reducing transplant rejection in a subject comprising administering to the subject an effective amount of a B7-H4 protein to inhibit the Th1 and Th17 pathways and to work with Tregs to enhance their suppressive effect on the Th17 pathway or to promote or enhance IL-10 secretion by Tregs by an amount effective to inhibit or reduce transplant rejection in the subject relative to a control.

10. A method for reducing cytokine production in a subject comprising administering to the subject an effective amount of a B7-H4 polypeptide, B7-H4 fusion protein, B7-H4 agonistic antibody, or fragments thereof to inhibit the production of one or more cytokines in the subject.

11. The method of any one of claims 1-10 further comprising administering a second therapeutic agent.

12. The method of claim 11 wherein the second therapeutic agent is selected from the group consisting of glucocorticoid fluticasone, salmeteroal; antibodies to IL-12, IL-6, IFN-γ, IL-23, IL-22 , IL-21 and IL-4; vitamin D3, CTLA-4-Ig, belatacept, dexamethasone, and combinations thereof.

13. A method of inhibiting or reducing transplant rejection in a subject comprising adiministering to the subject an effective amount of a B7-H4 polypeptide, B7-H4 fusion protein, a B7-H4 agonistic antibody, or fragments thereof to inhibit differentiation of Th0 cells into one or more of Th1 cells, Th17 cells or Th22 cells by an amount effective to inhibit or reduce transplant rejection in the subject relative to a control.

14. The method of any one of claims 1-13 wherein the transplant is an allogeneic transplant.

15. The method of any one of claims 1-14, wherein the transplant is a skin graft, a tissue transplant, an organ transplant, or a bone marrow transplant.

16. The method of any of claims 1-15 wherein the B7-H4 fusion protein is administered directly to cells, tissue or organs to be transplanted prior to transplantation, after transplantation, or both.

17. The method of any of claims 1-15 wherein the B7-H4 fusion protein is administered directly to an immune organ.

18. A method for treating one or more symptoms of graft versus host disease (GVHD) in a subject comprising administering to a subject who has received or will receive a transplant an effective amount of B7-H4 polypeptide, fusion proteins thereof, or fragments thereof to allievate one or more symptoms of GVHD.

19. A method for treating diabetes comprising transplanting insulin producing cells into a subject in need thereof and administering to the subject an effective amount of B7-H4 polypeptide, fusion proteins thereof, or fragments thereof to allievate one or more symptoms associated with diabetes.

20. A method for inhibiting or reducing chronic transplant rejection in a subject comprising administering an effective amount of a B7-H4 fusion protein to the subject to inhibit or reduce chronic rejection of a transplant relative to a control.

21. A method for predicting a patient's likelihood of rejecting a transplantation comprising

(a) determining the level of sH4 in the patient;

(b) comparing the level of sH4 in the patient to the level of sH4 in one or more other patients who have had a transplantation to determine the frequency of transplantation rejection in the other patients having the level of sH4 determined in (a).

22. A method of selecting patients for transplantation comprising

(a) the method of the claim 21;

(b) selecting patients with 30% or less likelihood of rejection a transplant.

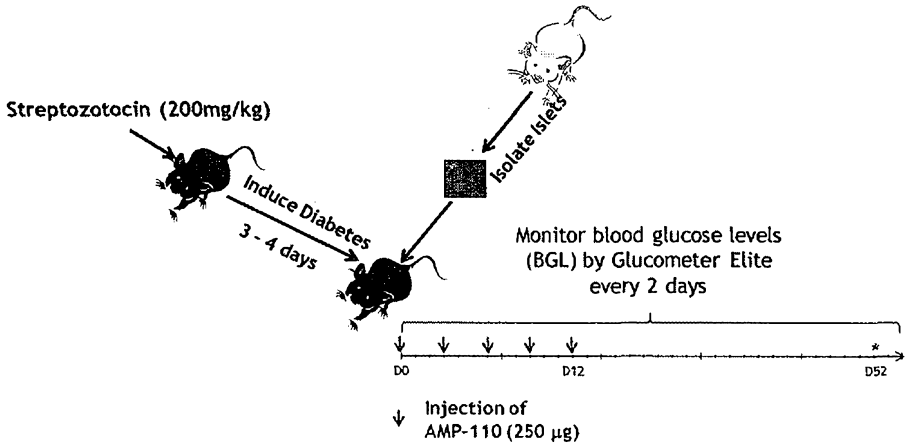


FIGURE 1

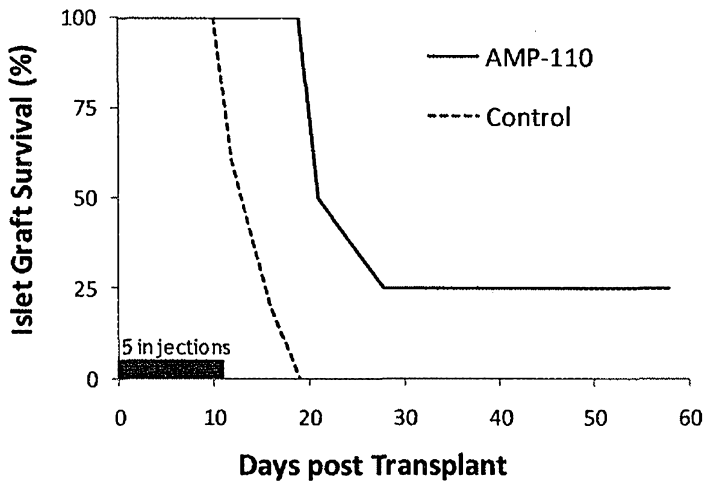
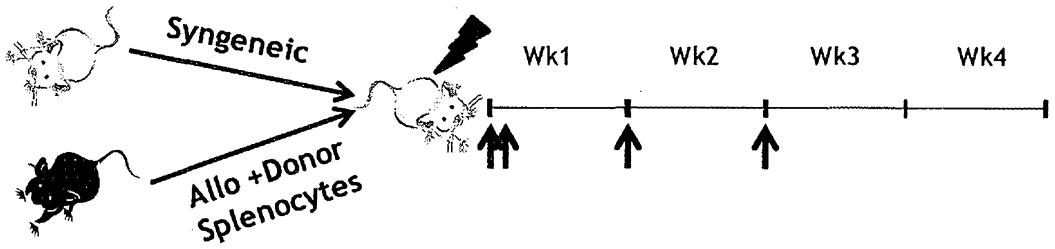


FIGURE 2



4 groups:

- Syngeneic BM transplants
- Allo BM transplants, control IgG, 0.5 mg, Day 0, 1, 7 and 14
- Allo BM transplants, B7-H4-Ig, 0.5 mg, Day 0, 1, 7 and 14
- Allo BM transplants, PBS, Day 0, 1, 7 and 14

FIGURE 3

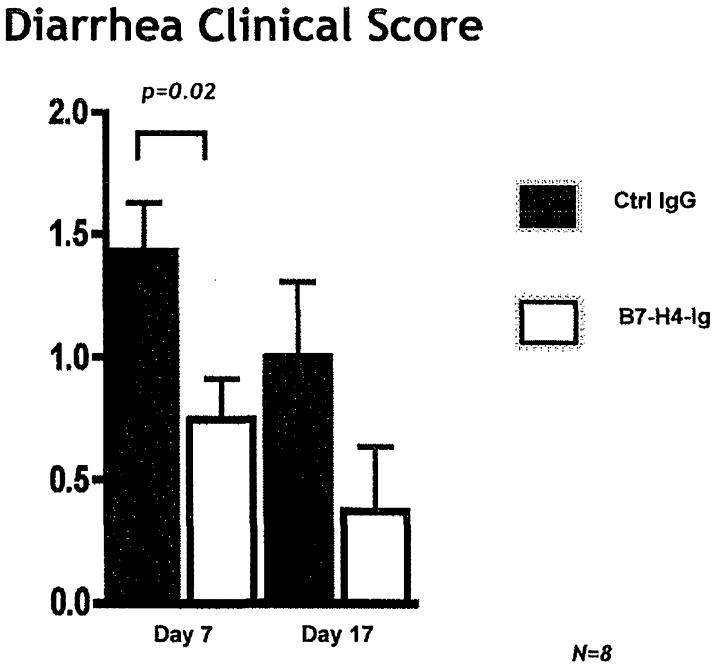


FIGURE 4A

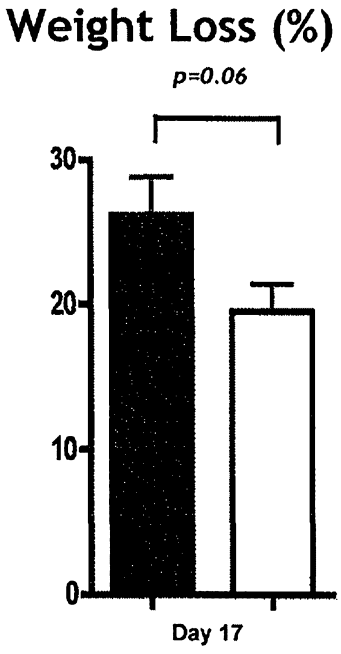


FIGURE 4B

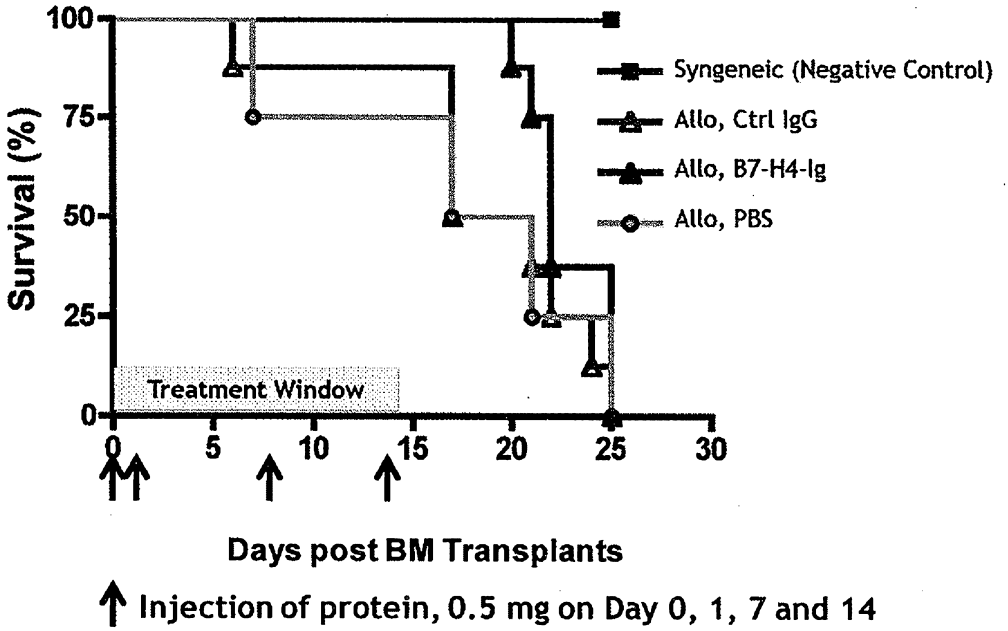

100
75
50
25
0
Survival (%)
Treatment Window
0
5
10
15
20
25
30
Syngeneic (Negative Control)
Allo, Ctrl IgG
Allo, B7-H4-Ig
Allo, PBS
Days post BM Transplants
Injection of protein, 0.5 mg on Day 0, 1, 7 and 14

FIGURE 5