COMPOSITIONS MONOVALENT FOR CD40L BINDING AND METHODS OF USE

BACKGROUND OF THE INVENTION

CD40 is a 50 kD cell surface glycoprotein molecule expressed on the surface of mature and immature B cells, macrophages, follicular dendritic cells, thymic epithelium, normal basal epithelium, and some tumor-derived cell lines. The CD40 molecule is a member of the TNF receptor family, and has important signaling functions leading to a variety of downstream effects in various cell types. Early studies showed that cross-linking of CD40 on the B cell surface with an antibody resulted in B cell proliferation and activation. Antibody cross linking of CD40 in the presence of IL-4 induces proliferation and class switching *in vitro*, B cell aggregation via LFA-1 (Gordon et al., 1988, J. Immunol. 140: 1425), and serine/threonine and tyrosine phosphorylation of a number of intracellular substrates (Gordon et al., 1988, supra; Uckun et al., 1991, J. Biol. Chem. 266:17478). Anti-CD40 monoclonal antibodies also prime B cells to proliferate in response to agents such as PMA (Gordon et al., 1987, Eur. J. Immunol. 17: 1535) and anti-CD20 antibody (Clark & Ledbetter, 1986, Proc. Natl. Acad. Sci. U.S.A. 83: 4494).

The receptor homology of CD40 and the antibody cross-linking studies showing a central role for CD40 in B cell activation prompted the search for a natural ligand. A mutant of the Jurkat T cell line was found to constitutively activate human B cells to secrete immunoglobulin (Yellin et al., 1991, J. Immunol. 147: 3389-3395). A monoclonal antibody, termed 5c8, was raised which specifically reacted with the mutant line, but not with the parental Jurkat cell line. The 5c8 antibody immunoprecipitated a 30 kD (more accurately, 29.3 kD, 261 amino acids) cell surface polypeptide and was found to specifically inhibit the B cell helper function of the mutant cell line. (Lederman et al., 1992, J. Exp. Med., 175: 1091-1101; Lederman et al., 1992, J. Immunol. 149: 3817-3826; Lederman et al., 1993, Curr. Opin. Immunol. 5: 439-444;). The 30 kD polypeptide ligand of the 5c8 antibody was termed T-BAM, for T-B-cell Activating Molecule. A second line of studies used molecular cloning

techniques to identify polypeptides that specifically bind the CD40 molecule. cDNA clones for a specific ligand of CD40 were identified in a CD40 binding assay and alternately termed CD40 Ligand (CD40L), gp39, CD154, or TRAP (Graf et al., 1992, Eur. J. Immunol. 22: 3191-3194; Armitage et al., 1992, Nature 357: 80-82; and Aruffo et al., 1993, Cell 72: 291-300). Subsequently, the CD40L clone was found to have the same structure as T-BAM (Covey et al., 1994, Mol. Immunol. 31: 471-484). Human CD40L protein shows 82.8% and 77.4% identity at the nucleic acid and amino acid levels, respectively, to a similar protein isolated from murine EL4 thymoma cells. Both of these proteins are ligands for CD40 cell surface antigen expressed on resting B cells. CD40L has also been described as IMD3, a protein involved in hyper-IgM immunodeficiency syndrome.

The human gene encoding CD40L maps to chromosome Xq26.3-q27. The gene contains five exons. Deletions, point mutations and frameshift mutations clustering within a limited region of the CD40L extracellular domain have been found to be the basis of a rare X-linked immunodeficiency syndrome (Hyper-IgM immunodeficiency syndrome, HIGM1) characterized by recurrent bacterial infections, very low or absent IgG, IgA and IgE, and normal to increased IgM and IgD serum levels. Causally-related mutations have been found to consist of clustered deletions arising by splice-donor mutations with exon skipping, splice-acceptor mutations with utilization of a cryptic splice site, and deletion/insertion events with the creation of a new splice site.

CD40L is expressed on activated, but not resting CD4+ T cells, and was found to play a particularly important role in the humoral immune response, being linked to B cell proliferation, antibody and cytokine production, and cell viability. *In vivo*, deletion or mutation of CD40L leads to severe immunodeficiency, both in mice and in humans, characterized by hypogammaglobulinemia and T cell deficits in cell-mediated immunity (Chess, C., 2001, in Therapeutic Immunology, 2nd edition, Austen, K.F., Burakoff, S., Rosen, F. and Strom, T., eds., Blackwell Sciences, pp. 441-456). Human CD4+ T cells infected by HIV1, which causes severe dysfunction of cellular immunity, but paradoxically results in intense polyclonal activation of B

cells, do not express CD40L. Gene and cell surface expression of the CD40L by activated T cells has been shown to be depressed in a subgroup of patients with common variable immunodeficiency (CVI). Thus, inefficient signaling via CD40 may be responsible, at least in part, for the failure of B cell differentiation in these patients.

The functional consequences of CD40L binding to CD40 include, for example, a) rescuing B cells from apoptosis induced by Fas or cross-linking of IgM, b) induction of the co-stimulator molecules CD80 (B7-1) and CD86 (B7-2) which interact with CD28 and CD152 (CTLA-4) on the surface of activated T cells; c) increased expression of other cell surface activation molecules including CD23, CD54, CD95 and lymphotoxin-a; and d) inducing immunoglobulin class switching (see Chess, supra, and references 25, 44, and 47-60 cited therein). CD40L binding to CD40 also augments the antigen-presenting functions of dendritic cells, inducing maintenance of high levels of MHC class II antigens and upregulation of accessory molecules including CD58 (LFA-3). CD40L induces cytokine production and tumoricidal activity in peripheral blood monocytes. CD40L also co-stimulates the proliferation of activated T cells, and the co-stimulation is accompanied by the production of IFN-γ, TNF-α and IL2. The expression of CD40L on murine T-helper cells and CD4+ T cells is inhibited by IFN-γ, and is inhibited on T-helper-type 2 cells by TGF-β.

CD40L upregulates the expression of CD54 by cultured Hodgkin and Reed-Sternberg cells. The increased CD54 surface expression is accompanied by increased shedding of surface-bound CD54.

CD40L has also been suggested to be important in the induction of tolerance – CD80 and CD86, which are upregulated by CD40L, interact with CD28 to provide essential co-stimulation of T cells, in concert with T cell receptor activation, that results in full activation of T cells. In the absence of CD80 and CD86-triggered activation of CD28, anergy or tolerance occurs as a consequence of antigen triggering (Linsley & Ledbetter, 1993, Ann. Rev. Immunol. 11: 191-212; Jenkins et al., 1993,

Curr Opin. Immunol. 5: 361-367; and Boussiotis et al., 1996, Immunol. Rev. 153: 5-26).

The CD40L/CD40 pathway has been implicated in the *in vivo* priming of CD8+ cytotoxic T lymphocytes (CTSs) by CD4+ T cells. As noted, CD40L expressed on the surface of activated CD4+ T cells interacts with CD40 expressed on dendritic cells, inducing the dendritic cells to express more MHC, and signaling through CD40 can replace the requirement for CD4+ T-helper cells in priming CD8+ CTL responses. Blockade of CD40L inhibits CTL priming, emphasizing the vital role of CD40L/CD40 interactions in CTL priming by helper T cells (Ridge et al., 1998, Nature 393: 474-478; Schoenberger et al., 1998, Nature 393: 480-483; Bennett et al., 1998, Nature 393: 478-480).

CD40L can also mediate functional interactions of CD4+ T cells with other cells that express CD40, such as fibroblasts, synovial cells and endothelial cells (Yellin et al., 1995, J. Leuko. Biol. 58: 209-216; Yellin et al., 1995, J. Exp. Med. 182: 1857-1864). CD40L induces the expression of CD54 (ICAM-1) and CD106 (VCAM-1) by fibroblasts, as well as increasing fibroblast IL-6, collagenase and collagen production and inducing fibroblast proliferation. Thus, CD40L/CD40 interactions may be involved in the induction of fibrosis associated with autoimmunity and immune responses.

CD40L interaction with CD40 induces endothelial cells to express CD62E (E-selectin), ICAM-1 and VCAM-1. The upregulation of these adhesion molecules may be involved in the binding of inflammatory cells to vascular endothelium and the subsequent migration of the inflammatory cells to sites of inflammation. CD40L blockade retards the migration of leukocytes through endothelial cell barriers. In animal models of autoimmunity, antibodies to CD40L interfere with the accumulation of inflammatory cells at the site of inflammation.

CD40/CD40L interactions have been implicated in diseases having an immune or autoimmune connection. Animal models of immune-related disease in which the CD40L/CD40 pathway has been demonstrated to play a role in the

pathology include, for example, murine models of systemic lupus erythematosis (Lupus or SLE; see, e.g., Kalled et al., 1998, J. Immunol. 160: 2158-2165), arthritis (collagen-induced arthritis, see, e.g., Durie et al., 1993, Science 261: 1328-1330), multiple sclerosis (experimental autoimmune encephalomyelitis, EAE; see, e.g., Howard et al., 1999, J. Clin . Invest. 103: 281-290), autoimmune thyroiditis (experimental autoimmune thyroiditis, EAT; see, e.g., Caryanniotis et al., 1997, Immunology 90: 421-426), colitis (hapten-induced colitis; see, e.g., Stuber et al., 1996, J. Exp. Med. 183: 693-698), atherosclerosis and coronary artery disease (see, e.g., Mach et al., 1998, Nature 394: 200-203), and allograft rejection (see, e.g., Parker et al., 1995, Proc. Natl. Acad. Sci. U.S.A. 92: 9560-9564; Kirk et al., 1997, Proc. Natl. Acad. Sci. U.S.A. 94: 8789-8794; Larsen et al., 1996, Nature 381: 434-438 and Blazar et al., 1997, J. Immunol. 158: 29-39).

CD40L antibody trials for treatment of human immune-related diseases include studies in patients with Lupus (see, e.g., Huang et al., 2002, Arthritis Rheum. 46: 1554-1562). A phase I trial demonstrated that anti-CD40L humanized monoclonal antibody (IDEC-131) is safe and well tolerated by patients with Lupus (Davis et al., 2001, J. Rheumatol. 28: 95-101). A phase II study with the IDEC-131 antibody showed improvement in clinical symptoms, but efficacy of the drug over placebo controls was not demonstrated (Kalunian et al., 2002, Arthritis Rheum. 46: 3251-3258). In a phase II study with BG9588 anti-CD40L antibody, clinical efficacy was demonstrated, but the study was terminated due to the occurrence of thromboembolic events (Boumpas et al., 2003, Arthritis Rheum. 48: 719-727).

U.S. Patent Nos. 5,474,771 (Lederman et al.) and 5,876,950 (Siadak et al.) disclose murine monoclonal antibodies specific for different epitopes of human gp39. WO95/06666 (Noelle & Foy) discloses murine anti-gp39 antibodies.

U.S. Patent No. 6,328,964 (Noelle & Claassen) discloses methods for the treatment of multiple sclerosis using gp39-specific antibodies.

U.S. Patent No. 5,747,037 (Noelle et al.), and EP0721469B1 (Ledbetter et al.) and its U.S. counterpart U.S. 5,869,049 disclose anti-human monoclonal (mouse)

antibodies specific for gp39. U.S. Patent No. 5,876,718 (Noelle et al.) discloses methods of inducing T cell non-responsiveness to transplanted tissues and of treating graft-versus-host disease with anti-gp39 monoclonal (mouse) antibodies. EP0742721B1 (Noelle et al.) discloses methods of inhibiting a humoral immune response to a thymus-dependent antigen that use anti-gp39 monoclonal (mouse) antibodies. U.S. Patent No. 6,375,950 describes methods for inducing T cell unresponsiveness to donor tissue or organs in a transplant recipient through use of anti-gp39 monoclonal (murine) antibodies.

EP1005372B1 (De Boer et al.) describes methods for the selective killing of autoreactive CD40L+ T cells using anti-CD40L monoclonal (mouse) antibody-toxin fusion proteins.

U.S. Patent No. 6,340,459 (Yellin et al.) describes the use of murine anti gp39 monoclonal antibody 5c8 for the treatment or prevention of reperfusion injury.

EP0831906B1 (Claassen et al.) describes methods for the treatment of T cell-mediated tissue destruction in autoimmune diseases such as multiple sclerosis using anti-gp39 monoclonal (mouse) antibodies. Antibodies used in therapeutic approaches in the prior art have been divalent antibodies of murine origin.

A number of smaller antigen binding fragments of naturally occurring antibodies have been identified following protease digestion. These include, for example, the "Fab fragment" (V_L-C_L-C_H1-V_H), "Fab' fragment" (a Fab with the heavy chain hinge region) and "F(ab')$_2$ fragment" (a dimer of Fab' fragments joined by the heavy chain hinge region). Recombinant methods have been used to generate even smaller antigen-binding fragments, referred to as "single chain Fv" (variable fragment) or "scFv," consisting of V_L and V_H joined by a synthetic peptide linker.

While the antigen binding unit of a naturally-occurring antibody (e.g., in humans and most other mammals) is generally known to be comprised of a pair of V regions (V_L/V_H), camelid species express a large proportion of fully functional, highly specific antibodies that are devoid of light chain sequences. The camelid heavy chain antibodies are found as homodimers of a single heavy chain, dimerized via their

constant regions. The variable domains of these camelid heavy chain antibodies are referred to as V_HH domains and retain the ability, when isolated as fragments of the V_H chain, to bind antigen with high specificity ((Hamers-Casterman et al., 1993, Nature 363: 446-448; Gahroudi et al., 1997, FEBS Lett. 414: 521-526). Antigen binding single V_H domains have also been identified from, for example, a library of murine V_H genes amplified from genomic DNA from the spleens of immunized mice and expressed in *E. coli* (Ward et al., 1989, Nature 341: 544-546). Ward et al. named the isolated single V_H domains "dAbs," for "domain antibodies." The term "dAb" will refer herein to an antibody single variable domain (V_H or V_L) polypeptide that specifically binds antigen. A "dAb" binds antigen independently of other V domains; however, as the term is used herein, a "dAb" can be present in a homo- or heteromultimer with other V_H or V_L domains where the other domains are not required for antigen binding by the dAb, i.e., where the dAb binds antigen independently of the additional V_H or V_L domains.

Antibody single variable domains, for example, V_HH, are the smallest antigen-binding antibody unit known. For use in therapy, human antibodies are preferred, primarily because they are not as likely to provoke an immune response when administered to a patient. As noted above, isolated non-camelid V_H domains tend to be relatively insoluble and are often poorly expressed. Comparisons of camelid V_{HH} with the V_H domains of human antibodies reveals several key differences in the framework regions of the camelid V_{HH} domain corresponding to the V_H/V_L interface of the human V_H domains. Mutation of these residues of human V_H3 to more closely resemble the V_{HH} sequence (specifically Gly 44→Glu, Leu 45→Arg and Trp 47→Gly) has been performed to produce "camelized" human V_H domains that retain antigen binding activity (Davies & Riechmann, 1994, FEBS Lett. 339: 285-290) yet have improved expression and solubility. (Variable domain amino acid numbering used herein is consistent with the Kabat numbering convention (Kabat et al., 1991, Sequences of Immunological Interest, 5th ed. U.S. Dept. Health & Human Services, Washington, D.C.)) WO 03/035694 (Muyldermans) reports that the Trp 103→Arg mutation improves the solubility of non-camelid V_H domains. Davies & Riechmann (1995, Biotechnology N.Y. 13: 475-479) also report production of a phage-displayed

repertoire of camelized human V_H domains and selection of clones that bind hapten with affinities in the range of 100-400 nM, but clones selected for binding to protein antigen had weaker affinities.

While many antibodies and their derivatives are useful for diagnosis and therapy, the ideal pharmacokinetics of antibodies are often not achieved for a particular application. In order to provide improvement in the pharmacokinetics of antibody molecules, the present invention provides single domain variable region polypeptides that are linked to polymers which provide increased stability and half-life. The attachment of polymer molecules (e.g., polyethylene glycol; PEG) to proteins is well established and has been shown to modulate the pharmacokinetic properties of the modified proteins. For example, PEG modification of proteins has been shown to alter the *in vivo* circulating half-life, antigenicity, solubility, and resistance to proteolysis of the protein (Abuchowski et al., *J. Biol. Chem.* 1977, 252:3578; Nucci et al., *Adv. Drug Delivery Reviews* 1991, 6:133; Francis et al., *Pharmaceutical Biotechnology* Vol. 3 (Borchardt, R. T. ed.); and Stability of Protein Pharmaceuticals: *in vivo* Pathways of Degradation and Strategies for Protein Stabilization 1991 pp235-263, Plenum, NY).

Both site-specific and random PEGylation of protein molecules is known in the art (See, for example, Zalipsky and Lee, Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications 1992, pp 347-370, Plenum, NY; Goodson and Katre, 1990, *Bio/Technology,* 8:343; Hershfield et al., 1991, *PNAS* 88:7185). More specifically, random PEGylation of antibody molecules has been described at lysine residues and thiolated derivatives (Ling and Mattiasson, 1983, *Immunol. Methods* 59: 327; Wilkinson et al., 1987, *Immunol. Letters*, 15: 17; Kitamura et al., 1991, *Cancer Res.* 51:4310; Delgado et al., 1996 *Br. J. Cancer*, 73: 175; Pedley et al., 1994, *Br. J. Cancer*, 70:1126).

SUMMARY OF THE INVENTION

The invention relates to antibody polypeptides that monovalently bind CD40L. Because of the clear importance of CD40L in the production of antibodies, the CD40/CD40L interaction and pathways present important targets for the

development of therapeutic approaches for the treatment of diseases and disorders that involve inappropriate or excessive antibody responses, such as autoimmune diseases. Antibody polypeptides that are monovalent for binding of CD40L can inhibit CD40L activity, including binding and activation of CD40 on the B cell surface and downstream effects, while avoiding potential undesirable effects that can occur with antibodies capable of divalent or multivalent binding of CD40L. Monovalent anti-CD40L antibody polypeptides can also be applied to any of a number of uses for which standard divalent antibodies are also used, e.g., *in vivo* imaging and diagnosis.

In one aspect, the antibody polypeptide consists of or comprises a single immunoglobulin variable domain that specifically binds and antagonizes the activity of CD40L, preferably without substantially agonizing CD40 and/or CD40L activity. In another aspect, because human antibodies will avoid the generation of an immune response to the antibodies when administered to human subjects for the treatment or prevention of disease, the antibody polypeptide is a human antibody polypeptide that monovalently binds CD40L, preferably without substantially agonizing CD40 and/or CD40L activity.

In summary then, in one embodiment, the invention provides an antibody polypeptide, preferably a human antibody polypeptide, that is monovalent for binding to CD40L (gp39).

In one embodiment, the human antibody polypeptide dissociates from human CD40L with a K_d in the range of 50 nM to 20 pM, inclusive, as measured by surface plasmon resonance. For example, the K_d for human CD40L can be 25 nM to 20 pM, 10 nM to 20 pM, 5 nm to 20 pM, 1 nM to 20 pM, 0.5 nM to 20 pM, 0.1 nM to 20 pM, 0.1 nM to 50 nM, 75 pM to 20 pM or even 50 pM to 20 pM.

Unless otherwise stated, all ranges described herein are inclusive of the specific endpoints.

In another embodiment, the antibody polypeptide inhibits the binding of CD40L to CD40.

In another embodiment, the binding of the antibody polypeptide to CD40L does not substantially agonize CD40 and/or CD40L activity.

In another embodiment, the human antibody polypeptide inhibits the binding of CD40 to CD40L, and does not substantially agonize signaling by CD40.

In another embodiment, the binding of the antibody polypeptide to CD40L does not substantially induce JNK phosphorylation in Jurkat T-cells.

In another embodiment, the binding of the antibody polypeptide to CD40L does not substantially induce IFN-γ secretion by Jurkat T-cells co-stimulated with anti-CD3 antibody.

In another embodiment, the presence of the antibody polypeptide in a standard platelet aggregation assay does not result in aggregation of more than 25% over the aggregation observed in a negative control assay performed without the addition of antibody.

In another embodiment, the human antibody polypeptide comprises a single immunoglobulin variable domain that binds CD40L. In a preferred embodiment, the single immunoglobulin variable domain is a V_H or a V_L domain.

In another embodiment, the antibody polypeptide is selected from the group consisting of a dAb, a FAb, an scFv, an Fv, or a disulfide-bonded Fv.

In another embodiment, the human antibody polypeptide is PEG-linked. In one embodiment, the PEG is covalently linked to the human antibody polypeptide. In one preferred embodiment, the PEG-linked human antibody polypeptide has a hydrodynamic size of at least 24 kD. In another preferred embodiment, the PEG is linked to the antibody polypeptide at a cysteine or lysine residue. In another preferred embodiment, the total PEG size is from 20 to 60 kD, inclusive. In another preferred

embodiment, the PEG-linked human antibody polypeptide has a hydrodynamic size of at least 200 kD.

In one embodiment, the antibody polypeptide has an increased in vivo half-life relative to the same antibody polypeptide composition lacking polyethylene glycol.

In another embodiment, the tα-half life of the antibody polypeptide composition is increased by 10% or more. In another embodiment, the tα-half life of the antibody polypeptide composition is increased by 50% or more. In another embodiment, the tα-half life of the antibody polypeptide composition is increased by 2X or more. In another embodiment, the tα-half life of the antibody polypeptide composition is increased by 5X or more, e.g., 10X, 15X, 20X, 25X, 30X, 40X, or more. In another embodiment, the tα-half life of the antibody polypeptide composition is increased by 50X or more.

In another embodiment, the PEG-linked antibody polypeptide has a tα half-life of 0.25 to 6 hours, inclusive. In another embodiment, the tα half-life is in the range of 30 minutes to 12 hours, inclusive. In another embodiment, the tα-half life of the antibody polypeptide composition is in the range of 1 to 6 hours.

In another embodiment, the tβ-half life of the antibody polypeptide composition is increased by 10% or more. In another embodiment, the tβ-half life of the antibody polypeptide composition is increased by 50% or more. In another embodiment, the tβ-half life of the antibody polypeptide composition is increased by 2X or more. In another embodiment, the tβ-half life of the antibody polypeptide composition is increased by 5X or more, e.g., 10X, 15X, 20X, 25X, 30X, 40X, .or more. In another embodiment, the tβ-half life of the antibody polypeptide composition is increased by 50X or more.

In another embodiment, the antibody polypeptide composition has a tβ half-life of 1 to 170 hours, inclusive. In another embodiment, the tβ-half life is in the

range of 12 to 48 hours, inclusive. In another embodiment, the tβ-half life is in the range of 12 to 26 hours, inclusive.

In addition, or alternatively to the above criteria, the present invention provides a dAb containing composition comprising a ligand according to the invention having an AUC value (area under the curve) in the range of 1 mg.min/ml or more. In one embodiment, the lower end of the range is 5, 10, 15, 20, 30, 100, 200 or 300 mg.min/ml. In addition, or alternatively, a ligand or composition according to the invention has an AUC in the range of up to 600 mg.min/ml. In one embodiment, the upper end of the range is 500, 400, 300, 200, 150, 100, 75 or 50 mg.min/ml. Advantageously a ligand according to the invention will have an AUC in the range selected from the group consisting of the following: 15 to 150 mg.min/ml, 15 to 100 mg.min/ml, 15 to 75 mg.min/ml, and 15 to 50 mg.min/ml.

In another embodiment, the antibody polypeptides described herein can be linked to human serum albumin (HSA), which also has the effect of increasing the *in vivo* half life of the molecule. The human serum albumin coding sequences can be obtained by PCR using primers derived from the cDNA sequence available at GenBank Accession No. NM000477. Such coding sequences can be fused to the coding sequence for a monovalent anti-CD40L antibody polypeptide as described herein, and the fusion can be expressed by one of skill in the art.

In another embodiment, the tα-half life of the HSA-linked human antibody polypeptide composition is increased by 10% or more.

In another embodiment, the tα-half life of the HSA-linked human antibody polypeptide composition is in the range of 0.25 hours to 6 hours.

In another embodiment, the tβ-half life of the HSA-linked human antibody polypeptide composition is increased by 10% or more.

In another embodiment, the tβ-half life of the HSA-linked human antibody polypeptide composition is in the range of 12 to 48 hours.

In another embodiment, the human antibody polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 7-82 and 246-360.

In another embodiment, the human antibody polypeptide inhibits binding of CD40L to CD40 with an IC_{50} in the range of 20 pM to 1.5 μM, inclusive; IC_{50} for inhibition of CD40L binding to CD40 in any embodiment described herein is preferably measured as described herein in Example 6. The IC_{50} can preferably be in the range of 20 pM to 1 μM, 20 pM to 900 nM, 20 pM to 800 nM, 20 pM to 700 nM, 20 pM to 600 nM, 20 pM to 500 nM, 20 pM to 400 nM, 20 pM to 300 nM, 20 pM to 200 nM, 20 pM to 100 nM, or 20 pM to 50 nM. Further acceptable or preferred ranges include, for example, 50 pM to 1 μM, 100 pM to 500 nM, 125 pM to 250 nM, 150 pM to 200 nM, 150 pM to 100 nM and 200 pM to 50 nM.

In another embodiment, the antibody polypeptide is fused to a second antibody polypeptide which binds a ligand other than CD40L. In a preferred embodiment, the antibody polypeptide which binds a ligand other than CD40L binds a ligand selected from the group consisting of HSA, TNFα, IL-1, IL-2, IL-4, IL-6, IL-8, IL-12, IL-18, IFN-γ, CD2, CD4, CD8, CTLA4, LFA1, LFA3, VLA4, CD80 (B7-1), CD28, CD86 (B7-2), and CTLA-4.

In another embodiment, the human antibody polypeptide is free of an Fc domain. The limits of an Fc domain are set out in Kabat et al. (1991, Sequences of Immunological Interest, 5th ed. U.S. Dept. Health & Human Services, Washington, D.C.; incorporated herein by reference). In the alternative, an Fc domain consists of the CH2-CH3 regions, optionally including a hinge region linked to the CH2. In a preferred embodiment, the human antibody polypeptide does not mediate platelet aggregation in a standard platelet aggregation assay.

The invention further encompasses a human antibody polypeptide which has an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs 7-82 and 246-360, which antibody polypeptide specifically and monovalently binds CD40L.

The invention further encompasses an antigen-binding polypeptide, the polypeptide comprising a single immunoglobulin variable domain which specifically and monovalently binds CD40L. Recited differently, the invention further encompasses a polypeptide comprising a moiety which specifically binds CD40L, which moiety consists of a single immunoglobulin variable domain.

In one embodiment, the polypeptide consists of a human single immunoglobulin variable domain.

In another embodiment, the polypeptide has a Kd for human CD40L in the range of 50 nM to 20 pM, inclusive, as determined by surface plasmon resonance. For example, the Kd for human CD40L can be 25 nM to 20 pM, 10 nM to 20 pM, 5 nm to 20 pM, 1 nM to 20 pM, 0.5 nM to 20 pM, 0.1 nM to 20 pM, 75 pM to 20 pM or even 50 pM to 20 pM.

In another embodiment, the polypeptide inhibits the binding of CD40L to CD40.

In another embodiment, the polypeptide inhibits the binding of CD40 to CD40L and has an IC_{50} in the range of 20 pM to 1.5 μM, inclusive. For example, the IC_{50} can be in the range of 20 pM to 1 μM, 20 pM to 900 nM, 20 pM to 800 nM, 20 pM to 700 nM, 20 pM to 600 nM, 20 pM to 500 nM, 20 pM to 400 nM, 20 pM to 300 nM, 20 pM to 200 nM, 20 pM to 100 nM, or 20 pM to 50 nM. Further acceptable or preferred ranges include, for example, 50 pM to 1 μM, 100 pM to 500 nM, 125 pM to 250 nM, 150 pM to 200 nM, 150 pM to 100 nM and 200 pM to 50 nM.

In another embodiment, the binding of the polypeptide to CD40L does not substantially agonize CD40 and/or CD40L activity.

In another embodiment, the binding of the polypeptide to CD40L does not substantially induce JNK phosphorylation in Jurkat T-cells.

In another embodiment, the binding of the polypeptide to CD40L does not substantially induce IFN-γ secretion by Jurkat T-cells co-stimulated with anti-CD3 antibody.

In another embodiment, the presence of the antibody polypeptide in a standard platelet aggregation assay does not result in aggregation more than 25% over the aggregation observed in a negative control assay lacking antibody polypeptide.

In another embodiment, the single immunoglobulin variable domain is a human single immunoglobulin variable domain.

In another embodiment, the single immunoglobulin variable domain is a V_H or a V_L domain.

In one embodiment, the polypeptide is PEG-linked. In one embodiment, the PEG is covalently linked. In one preferred embodiment, the PEG-linked antigen-binding polypeptide has a hydrodynamic size of at least 24 kD. In another preferred embodiment, the PEG is linked to the antigen-binding polypeptide at a cysteine or lysine residue. In another preferred embodiment, the total PEG size is from 20 to 60 kD, inclusive. In another preferred embodiment, the PEG-linked antigen-binding polypeptide has a hydrodynamic size of at least 200 kD.

In another embodiment, the PEG-linked polypeptide has an increased in vivo half-life relative to the same polypeptide composition lacking linked polyethylene glycol. In another embodiment, the tα-half life of the polypeptide composition is increased by 10% or more. In another embodiment, the tα-half life of the polypeptide composition is increased by 50% or more. In another embodiment, the tα-half life of the polypeptide composition is increased by 2X or more. In another embodiment, the tα-half life of the polypeptide composition is increased by 5X or more, e.g., 10X, 15X, 20X, 25X, 30X, 40X, or more. In another embodiment, the tα-half life of the polypeptide composition is increased by 50X or more.

In another embodiment, the PEG-linked antibody polypeptide has a tα half-life of 0.25 to 6 hours, inclusive. In another embodiment, the tα half-life is in the range of 30 minutes to 12 hours, inclusive. In another embodiment, the tα-half life of the polypeptide composition is in the range of 1 to 6 hours.

In another embodiment, the tβ-half life of the polypeptide composition is increased by 10% or more. In another embodiment, the tβ-half life of the polypeptide composition is increased by 50% or more. In another embodiment, the tβ-half life of the polypeptide composition is increased by 2X or more. In another embodiment, the tβ-half life of the polypeptide composition is increased by 5X or more, e.g., 10X, 15X, 20X, 25X, 30X, 40X, .or more. In another embodiment, the tβ-half life of the polypeptide composition is increased by 50X or more.

In another embodiment, the antibody polypeptide composition has a tβ half-life of 1 to 170 hours, inclusive. In another embodiment, the tβ-half life is in the range of 12 to 48 hours, inclusive. In another embodiment, the tβ-half life is in the range of 12 to 26 hours, inclusive.

In another embodiment, the composition has an AUC value (area under the curve) in the range of 1 mg.min/ml or more. In one embodiment, the lower end of the range is 5, 10, 15, 20, 30, 100, 200 or 300 mg.min/ml. In addition, or alternatively, a ligand or composition according to the invention has an AUC in the range of up to 600 mg.min/ml. In one embodiment, the upper end of the range is 500, 400, 300, 200, 150, 100, 75 or 50 mg.min/ml. Advantageously a ligand according to the invention will have an AUC in the range selected from the group consisting of the following: 15 to 150 mg.min/ml, 15 to 100 mg.min/ml, 15 to 75 mg.min/ml, and 15 to 50 mg.min/ml.

In another embodiment, the antibody polypeptide is linked to human serum albumin (HSA). In another embodiment, the antibody polypeptide has an increased in vivo half-life relative to the same polypeptide composition lacking linked HSA. In another embodiment, the antibody polypeptide has a tα-half life that is increased by 10% or more relative to a molecule lacking linked HSA. In another embodiment, the

tα-half life of the polypeptide composition is in the range of 0.25 minutes to 6 hours. In another embodiment, the tβ-half life of the polypeptide composition is increased by 10% or more. In another embodiment, the tβ-half life is in the range of 12 to 48 hours.

In another embodiment, the antigen-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 7-82 and 246-360.

In another embodiment, the antigen-binding polypeptide is free of an Fc domain.

In another aspect, the invention encompasses an immunoglobulin variable domain polypeptide which has an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs 7-82 and 246-360, which polypeptide specifically and monovalently binds CD40L.

In one embodiment, the immunoglobulin variable domain polypeptide antagonizes the binding of CD40L to CD40.

In another embodiment, the immunoglobulin variable domain polypeptide inhibits the binding of CD40 to CD40L and has an IC_{50} in the range of 20 pM to 1.5 μM, inclusive. For example, the IC_{50} can be in the range of 20 pM to 1 μM, 20 pM to 900 nM, 20 pM to 800 nM, 20 pM to 700 nM, 20 pM to 600 nM, 20 pM to 500 nM, 20 pM to 400 nM, 20 pM to 300 nM, 20 pM to 200 nM, 20 pM to 100 nM, or 20 pM to 50 nM. Further acceptable or preferred ranges include, for example, 50 pM to 1 μM, 100 pM to 500 nM, 125 pM to 250 nM, 150 pM to 200 nM, 150 pM to 100 nM and 200 pM to 50 nM.

In another embodiment, the immunoglobulin variable domain polypeptide inhibits the interaction of CD40 with CD40L, but does not substantially agonize intracellular signaling by CD40. In a preferred embodiment, the binding of the polypeptide to CD40L does not substantially induce JNK phosphorylation in Jurkat T-cells. In another preferred embodiment, the binding of the polypeptide to CD40L

does not substantially induce IFN-γ secretion by Jurkat T-cells co-stimulated with anti-CD3 antibody. In another preferred embodiment, the binding of the antibody polypeptide to CD40L does not substantially induce platelet aggregation in a platelet aggregation assay.

In another embodiment, the antigen-binding polypeptide further comprises a second antibody polypeptide which binds a ligand other than CD40L. In a preferred embodiment, the second antibody polypeptide binds a ligand selected from the group consisting of HSA, TNFα, IL-1, IL-2, IL-4, IL-6, IL-8, IL-12, IL-18, IFN-γ, CD2, CD4, CD8, CTLA4, LFA1, LFA3 and VLA4.

In one embodiment, the invention relates to an antibody polypeptide comprising an immunoglobulin variable domain which specifically and monovalently binds CD40L (e.g., an anti-CD40L dAb, FAb, an scFv, an Fv, or a disulfide-bonded Fv), and which comprises one or more framework regions comprising an amino acid sequence that is the same as the amino acid sequence of a corresponding framework region encoded by a human germline antibody gene segment, or the amino acid sequence of one or more of said framework regions collectively comprises up to 5 amino acid differences relative to the amino acid sequence of said corresponding framework region encoded by a human germline antibody gene segment.

In one embodiment, the amino acid sequences of FW1, FW2, FW3 and FW4 of the anti-CD40L variable domain or dAb are the same as the amino acid sequences of corresponding framework regions encoded by a human germline antibody gene segment, or the amino acid sequences of FW1, FW2, FW3 and FW4 collectively contain up to 10 amino acid differences relative to the amino acid sequences of corresponding framework regions encoded by said human germline antibody gene segment. In a further embodiment, the amino acid sequences of FW1, FW2 and FW3 of the anti-CD40L variable domain or dAb are the same as the amino acid sequences of corresponding framework regions encoded by human germline antibody gene segments.

In a further embodiment of the foregoing, the human germline antibody gene segment can be selected from the group consisting of DP47, DP45, DP48 and DPK9.

The invention further encompasses a method of antagonizing the binding of CD40 to CD40L in an individual, the method comprising administering a monovalent anti-CD40L antibody polypeptide as described herein to the individual, wherein the polypeptide antagonizes the binding of CD40 to CD40L in the individual.

The invention further encompasses a method of antagonizing an activity of CD40 or CD40L in an individual, the method comprising administering a monovalent anti-CD40L antibody polypeptide as described herein to the individual, wherein the polypeptide antagonizes an activity of CD40 or CD40L or both.

The invention further encompasses a composition comprising an extended release formulation comprising a monovalent anti-CD40L antibody polypeptide, preferably, but not limited to, a polypeptide comprising a single immunoglobulin variable domain that binds CD40L. In one embodiment, the single immunoglobulin variable domain is a non-human mammalian single immunoglobulin variable domain. In another embodiment, the single immunoglobulin variable domain is a human single immunoglobulin variable domain.

The invention further encompasses a method of treating or preventing a disease or disorder mediated by CD40L in an individual in need of such treatment, the method comprising administering to the individual a therapeutically effective amount of a composition comprising a monovalent anti-CD40L antibody polypeptide, preferably a composition comprising a single human immunoglobulin variable domain that binds CD40L. In one embodiment, the disease or disorder is an autoimmune disease or disorder.

The invention further encompasses a method of treating or preventing a symptom of systemic lupus erythematosus (SLE) in an individual, the method comprising administering a monovalent anti-CD40L antibody polypeptide to said individual in an amount effective to treat or prevent a symptom of SLE. The

invention further encompasses a method of reducing or alleviating a symptom of a disease such as systemic lupus erythematosis, multiple sclerosis, rheumatoid arthritis, allograft rejection, xenograft rejection, and Diabetes, including insulin-dependent Type I Diabetes.

The invention further encompasses an antibody polypeptide that is monovalent for binding to CD40L, wherein the antibody polypeptide comprises a universal framework.

In one embodiment, the universal framework comprises a VH framework selected from the group consisting of DP47, DP45 and DP38, and/or the VL framework is DPK9.

In another embodiment, the antibody polypeptide comprises a generic ligand binding site. In another embodiment, the generic ligand binding site binds a generic ligand selected from the group consisting of protein A, protein L and protein G.

In another embodiment, the antibody polypeptide comprises a variable domain having one or more framework regions comprising an amino acid sequence that is the same as the amino acid sequence of a corresponding framework region encoded by a human germline antibody gene segment, or the amino acid sequences of one or more of the framework regions collectively comprises up to 5 amino acid differences relative to the amino acid sequence of the corresponding framework region encoded by a human germline antibody gene segment.

In another embodiment, the antibody polypeptide comprises a variable domain, wherein the amino acid sequences of FW1, FW2, FW3 and FW4 are the same as the amino acid sequences of corresponding framework regions encoded by a human germline antibody gene segment, or the antibody sequences of FW1, FW2, FW3 and FW4 collectively contain up to 10 amino acid differences relative to the amino acid sequences of corresponding framework regions encoded by the human germline antibody gene segment.

In another embodiment, the antibody polypeptide comprises an antibody variable domain comprising FW1, FW2 and FW3 regions, and the amino acid sequence of said FW1, FW2 and FW3 are the same as the amino acid sequences of corresponding framework regions encoded by human germline antibody gene segments. In another embodiment, the human germline antibody gene segment is selected from the group consisting of DP47, DP45, DP48 and DPK9.

The invention includes an antibody single variable domain polypeptide that binds to CD40L, wherein the polypeptide has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a sequence that is at least 80% homologous to the sequence of DOM8-24. In one embodiment, the antibody single variable domain polypeptide differs form the amino acid sequence of DOM8-24 at 25 or fewer amino acid positions, 20 or fewer amino acid positions, 15 or fewer amino acid positions, 10 or fewer amino acid positions, 5 or fewer amino acid positions, 2 or fewer amino acid positions, or as few as one amino acid position. In a further embodiment, the antibody single variable domain polypeptide is at least 80% homologous to the sequence of DOM8-24, for example, at least 85% homologous, at least 90% homologous, at least 95% homologous, and up to and including 96%, 97%, 98%, or 99% homologous.

The invention includes an antibody single variable domain polypeptide that binds to CD40L, wherein the polypeptide has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24, or has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24, or has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

The invention also includes an antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the

amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 .

The invention also includes an antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24 .

The invention also includes an antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24 .

The invention also includes an antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

In one embodiment, the antibody single variable domain polypeptide that binds to CD40L, if not identical in sequence to that of DOM8-24, differs form the amino acid sequence of DOM8-24 at 25 or fewer amino acid positions, 20 or fewer amino acid positions, 15 or fewer amino acid positions, 10 or fewer amino acid

positions, 5 or fewer amino acid positions, 2 or fewer amino acid positions, or as few as one amino acid position.

The invention also includes a CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24.

The invention also includes a CD40L antagonist having a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24.

The invention also includes a CD40L antagonist having a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

The invention also includes a CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24.

The invention also includes a CD40L antagonist having a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

The invention also includes a CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

The invention also includes a CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

In one embodiment the CD40L antagonist inhibits the binding of CD40 to CD40L, and/or inhibits an activity of CD40 and/or CD40L, and/or results in no more than 25% platelet aggregation in a platelet aggregation assay. In one embodiment, the antagonist results in platelet aggregation of 25% or less, 20% or less, 15% or less, 10% or less, 5% or less, and as little as zero platelet aggregation.

The invention also includes a dual specific ligand comprising a first immunoglobulin single variable domain having a binding specificity to a first antigen and a second single variable domain having a binding activity to a second antigen, wherein the first antigen is CD40L and binding of the second single variable domain to the second antigen acts to increase the half-life of the ligand *in vivo*. In one embodiment, the dual specific ligand is a four chain IgG immunoglobulin.

In one embodiment, the four chain IgG comprises two dual specific ligands, said dual specific ligands being different in their variable domains.

The invention also includes a dual specific ligand comprising an anti-human CD40L dAb and an anti-SA dAb.

In one embodiment, the dAbs are Camelid V_{HH} domains.

In one embodiment of the dual specific ligand, either (i) the first and second immunoglobulin variable domains are heavy chain variable domains; or (ii) the first and the second immunoglobulin variable domains are light chain variable domains.

In one embodiment, the ligand is provided as an IgG immunoglobulin comprising four heavy chain single variable domains or four light chain single variable domains. The heavy chain can comprise Camelid VHH domains.

In a further embodiment of the dual specific ligand, the first and second domains bind independently, such that the dual specific ligand may simultaneously bind both the first and second antigens.

In one embodiment of the dual specific ligand, the first single variable domain has a dissociation constant (K_d) of $1x10^{-8}$ M or less for human CD40L, and a K_{off} rate constant of $1x10^{-3}$ s^{-1} or less, as determined by surface plasmon resonance.

In one embodiment of the dual specific ligand, the second single variable domain is specific for serum albumin (SA) and has a dissociation constant (K_d) of 1nM to 500μm for SA, as determined by surface plasmon resonance.

In a further embodiment, the second domain binds SA in a standard ligand binding assay with an IC50 of 1nM to 500μM. The second single variable domain may be specific for SA, and comprise the amino acid sequence of MSA-16 or a sequence that is at least 80% homologous thereto. Alternatively, the second single variable domain may be specific for SA, and comprise the amino acid sequence of MSA-26 or a sequence that is at last 80% homologous thereto.

In one embodiment of the dual specific ligand, the anti-CD40L variable domain or dAb comprises a universal framework. The anti-CD40L variable domain or dAb may also comprise a V_H framework selected from the group consisting of DP47, DP45 and DP38; or a V_L framework which is DPK9. In a further embodiment, the dual specific ligand or dAb can comprise a binding site for a generic ligand.

In one embodiment, the generic ligand binding site is selected from the group consisting of protein A, protein L and protein G binding site.

In one embodiment of the dual specific ligand, the anti-CD40L variable domain or dAb comprises one or more framework regions comprising an amino acid sequence that is the same as the amino acid sequence of a corresponding framework region encoded by a human germline antibody gene segment, or the amino acid sequence of one or more of said framework regions collectively comprises up to 5 amino acid differences relative to the amino acid sequence of said corresponding framework region encoded by a human germline antibody gene segment.

In one embodiment, the amino acid sequences of FW1, FW2, FW3 and FW4 of the anti-CD40L variable domain or dAb are the same as the amino acid sequences of corresponding framework regions encoded by a human germline antibody gene segment, or the amino acid sequences of FW1, FW2, FW3 and FW4 collectively contain up to 10 amino acid differences relative to the amino acid sequences of corresponding framework regions encoded by said human germline antibody gene segment.

In one embodiment, the amino acid sequences of said FW1, FW2 and FW3 of the anti-CD40L variable domain or dAb are the same as the amino acid sequences of corresponding framework regions encoded by human germline antibody gene segments. The human germline antibody gene segments are preferably selected from the group consisting of DP47, DP45, DP48 and DPK9.

The invention also includes a method for producing a dual specific ligand as described herein, comprising a first immunoglobulin single variable domain having a binding specificity for CD40L and a second single immunoglobulin single variable domain having a binding specificity for a protein which increases the half-life of the ligand *in vivo*, the method comprising the steps of:selecting a first variable domain by its ability to bind CD40L; selecting a second variable domain by its ability to bind to said protein; combining the variable domains; and selecting the ligand by its ability to bind to CD40L and said protein.

In one embodiment, the first variable domain is selected for binding to CD40L in absence of a complementary variable domain.

The invention also includes nucleic acid encoding a dual specific ligand described herein. The nucleic acid may comprise the nucleic acid sequence of MSA-16 or a sequence that is at least 80% homologous thereto, or alternatively may comprise, the nucleic acid sequence of MSA-26 or a sequence that is at least 70% homologous thereto. The nucleic acid may be incorporated into a vector, which may be incorporated into a host cell.

The invention also includes a pharmaceutical composition comprising a dual specific ligand as described herein and a pharmaceutically acceptable excipient, carrier or diluent.

The invention also includes a dAb monomer specific for CD40L, which monomer has a dissociation constant (K_d) of $1x10^{-8}$ M or less for human CD40L, and a K_{off} rate constant of $1x10^{-3}$ s^{-1} or less, as determined by surface plasmon resonance.

In one embodiment, the dAb monomer specific for CD40L has a dissociation constant (K_d) of $1x10^{-7}$ M or less, as determined by surface plasmon resonance.

In one embodiment, the dAb monomer has binding specificity to CD40L with a dissociation constant (K_d) of $1x10^{-8}$ M or less, as determined by surface plasmon resonance.

In one embodiment, the dAb monomer has binding specificity to CD40L with a dissociation constant (K_d) of 50nM to 20pM, as determined by surface plasmon resonance.

In one embodiment, the monomer inhibits binding of CD40 to CD40L with an IC_{50} of 50nM or less.

In a further embodiment, the dAb monomer has binding specificity to CD40L with a K_{off} rate constant of $1x10^{-3}$ s^{-1} or less, $1x10^{-4}$ s^{-1} or less, $1x10^{-5}$ s^{-1} or less, or $1x10^{-6}$ s^{-1} or less,as determined by surface plasmon resonance.

In one embodiment, the dAb monomer neutralizes CD40L in a standard assay with an ND50 of 50nM or less.

In invention also includes a dual specific ligand comprising first and second heavy chain single variable domains, or first and second light chain single variable domains, wherein the first variable domain is an anti-CD40L dAb monomer.

In one embodiment, the second variable domain has binding specificity for an antigen other than CD40L.

In a further embodiment, the second variable domain has binding specificity for an antigen selected from the group consisting of EPO receptor, ApoE, Apo-SAA, BDNF, Cardiotrophin-1, EGF, EGF receptor, ENa-78, Eotaxin, Eotaxin-2, Exodus-2, EpoR, FGF-acidic, FGF-basic, fibroblast growth factor-10, FLT3 ligand, Fractalkine (CX3C), GDNF, G-CSF, GM-CSF, GF-bl, insulin, IFN-g, IGF-I, IGF-II, IL-1a, Il-1b, IL-2, Il-3, IL-4, IL-5, IL-6, IL-7, IL-8 (72 a.a.), IL-8 (77 a.a.), IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18 (IGIF), Inhibin a, Inhibin b, IP-10, keratinocyte

growth factor-2 (KGF-2), KGF, Leptin, LIF, Lymphotactin, Mullerian inhibitory substance, monocyte colony inhibitory factor, monocyte attractant protein, M-CSF, MDC (67 a.a.), MDC (69 a.a.), MCP-1 (MCAF), MCP-2, MCP-3, MCP-4, MDC (67 a.a.), MIG, MIP-1a, MIP-1b, MIP-3a, MIP-3b, MIP-4, myeloid progenitor inhibitor factor-1 (MPIF-1), NAP-2, Neurturin, Nerve growth factor, b-NGF, NT-3, NT-4, Oncostatin M, PDGF-AA, PDGF-AB, PDGF-BB, PF-4, RANTES, SDF1a, DFG1b SCF, SCGF, stem cell factor, (SCF), TARC, TGF-a, TGF-b, TGF-b2, TGF-b3, tumour necrosis factor (TNF), TNF-a, TNF-b, TNF receptor I, TNF receptor II, TNIL-1, TPO, VEGF, VEGF receptor 1, VEGF receptor 2, VEGF receptor-3, GCP-2, GRO/MGSA, GRO-b, GRO-g, HCC1, 1-309, HER 1, HER 2, HER 3, HER 4, TACE recognition site, TNF BP-1, TNF BP-II, and an antigen disclosed in Annex 2 or 3.

The invention also includes an antibody polypeptide that antagonizes or inhibits the binding of DOM8-24 to CD40L, or an antibody polypeptide that binds to the same epitope of CD40L bound by DOM8-24.

The invention also includes a dual specific ligand comprising a first immunoglobulin single variable domain having a binding specificity to a first antigen and a second single variable domain having a binding activity to a second antigen, wherein the first antigen is CD40L and the second single variable domain is an Antigen Presenting Cell surface antigen or a T cell surface antigen. The Antigen Presenting Cell surface antigen can be selected from one of the group consisting of dendritic cell surface antigens, activated macrophage surface antigens, activated B cell surface antigens, co-stimulatory signal pathway surface antigens, and MHC.

In one embodiment, the MHC is class II, and the class II can be alpha or beta.

The Antigen Presenting Cell surface antigen may be selected from the group consisting of CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, CD3, CD70, Inducible costimulatory molecule ligand (ICOSL), OX40L, CD80, CD86, HVEM (Herpes Virus Entry Mediator), and LIGHT, but is preferably one of CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, or CD3.

The surface antigen is preferably a B7 gene surface antigen such as B7-2 or B7-1.

Definitions:

As used herein, the term "human" when applied to an antibody polypeptide or to an immunoglobulin variable domain means that the polypeptide has a sequence derived from a human immunoglobulin. A sequence is "derived from" a human immunoglobulin coding sequence when the sequence is either: a) isolated from a human individual or from cells or a cell line from a human individual; b) isolated from a library of cloned human antibody gene sequences (or a library of human antibody V domain sequences); or c) when a cloned human antibody gene sequence (or a cloned human V region sequence (including, e.g., a germline V gene segment)) was used to generate one or more diversified sequences that were then selected for binding to a desired target antigen. The term "human" as applied herein to an antibody polypeptide or to an immunoglobulin variable domain does *not* encompass an immunoglobulin from another species, e.g., mouse, camel, etc., that has been "humanized" through grafting of human constant region sequences onto an antibody polypeptide (i.e., replacing non-human constant regions with human constant regions) or through grafting of human V region framework sequences onto an immunoglobulin variable domain from a non-human mammal (i.e., replacing non-human framework regions of a V domain with human framework regions).

At a minimum, a human variable domain has at least 85% amino acid similarity (including, for example, 87%, 90%, 93%, 95%, 97%, 99% or higher similarity) to a naturally-occurring human immunoglobulin variable domain sequence.

As used herein, the term "domain" refers to a folded protein structure which retains its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain.

By "single immunoglobulin variable domain" is meant a folded polypeptide domain which comprises a sequence characteristic of immunoglobulin variable domains and which specifically binds an antigen (e.g., dissociation constant of 500 nM or less). A "single immunoglobulin variable domain" therefore includes complete antibody variable domains as well as modified variable domains, for example in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain a dissociation constant of 500 nM or less (e.g., 450 nM or less, 400 nM or less, 350 nM or less, 300 nM or less, 250 nM or less, 200 nM or less, 150 nM or less, 100 nM or less) and the target antigen specificity of the full-length domain. Where necessary or in case of any doubt, the numbering convention and boundaries set forth by Kabat et al. (1991, *supra*) are applicable to immunoglobulin variable and constant domains referred to herein.

An antibody single variable domain polypeptide, as used herein refers to a mammalian single immunoglobulin variable domain polypeptide, preferably human, but also includes rodent (for example, as disclosed in WO00/29004, the contents of which are incorporated herein in their entirety) or camelid V_{HH} dAbs. Camelid dAbs are antibody single variable domain polypeptides which are derived from species including camel, llama, alpaca, dromedary, and guanaco, and comprise heavy chain antibodies naturally devoid of light chain: V_{HH}. V_{HH} molecules are about 10x smaller than IgG molecules, and as single polypeptides, they are very stable, resisting extreme pH and temperature conditions. Moreover, camelid antibody single variable domain polypeptides are resistant to the action of proteases. Camelid antibodies are described in, for example, U.S. Pat. Nos. 5,759,808; 5,800,988; 5,840,526; 5,874,541; 6,005,079; and 6,015,695, the contents of each of which are incorporated herein in their entirety. Camelid V_{HH} antibody single variable domain polypeptides useful according to the invention include a class of camelid antibody single variable domain polypeptides having human-like sequences, wherein the class is characterized in that the V_{HH} domains carry an amino acid from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, methionine,

serine, threonine, asparagine, or glutamine at position 45, such as for example L45, and further comprise a tryptophan at position 103 according to the Kabat numbering. Humanized camelid V_{HH} polypeptides are taught, for example in WO04/041862, the teachings of which are incorporated herein in their entirety. It will be understood by one of skill in the art that naturally occurring camelid antibody single variable domain polypeptides may be modified according to the teachings of WO04/041862 (e.g., amino acid substitutions at positions 45 and 103) to generate humanized camelid V_{HH} polypeptides. Also included in the present invention are antibody single variable domain polypeptides which are nurse shark V_{HH}. Nurse shark dAbs are antibody single variable domain polypeptides derived from the nurse shark, that comprise heavy chain antibodies naturally devoid of light chain: V_{HH}. Nurse Shark V_{HH} dAbs are described, for example, in Greenberg et al. (Nature 374 pp168-173 1995) and U.S. 20050043519.

The phrase "single immunoglobulin variable domain polypeptide" encompasses not only an isolated single immunoglobulin variable domain polypeptide, but also larger polypeptides that comprise a monomer of a single immunoglobulin variable domain polypeptide sequence. A "domain antibody" or "dAb" is equivalent to a "single immunoglobulin variable domain polypeptide" as the term is used herein. With regard to a single immunoglobulin variable domain polypeptide, the binding to antigen, e.g., CD40L, is mediated by the single immunoglobulin V domain without a requirement for a complementary V domain.

According to the invention, the terms "antibody single variable domain polypeptide", "antibody single variable domain", "single antibody variable domain", and "single immunoglobulin variable domain" are understood to be equivalent.

As used herein, the phrase "sequence characteristic of immunoglobulin variable domains" refers to an amino acid sequence that is homologous, over 20 or more, 25 or more, 30 or more, 35 or more, 40 or more, 45 or more, or even 50 or more contiguous amino acids, to a sequence comprised by an immunoglobulin variable domain sequence.

Sequences similar or homologous (e.g., at least about 70% sequence identity) to the sequences disclosed herein are also part of the invention. In some embodiments, the sequence identity at the amino acid level can be about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. At the nucleic acid level, the sequence identity can be about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. Alternatively, substantial identity exists when the nucleic acid segments will hybridize under selective hybridization conditions (e.g., very high stringency hybridization conditions), to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

As used herein, the terms "homology" or "similarity" refer to the degree with which two nucleotide or amino acid sequences structurally resemble each other. As used herein, sequence "similarity" is a measure of the degree to which amino acid sequences share similar amino acid residues at corresponding positions in an alignment of the sequences. Amino acids are similar to each other where their side chains are similar. Specifically, "similarity" encompasses amino acids that are conservative substitutes for each other. A "conservative" substitution is any substitution that has a positive score in the blosum62 substitution matrix (Hentikoff and Hentikoff, 1992, Proc. Natl. Acad. Sci. USA 89: 10915-10919). By the statement "sequence A is n% similar to sequence B" is meant that n% of the positions of an optimal global alignment between sequences A and B consists of identical amino acids or conservative substitutions. Optimal global alignments can be performed using the following parameters in the Needleman-Wunsch alignment algorithm:

For polypeptides:

Substitution matrix: blosum62.

Gap scoring function: -A -B*LG, where A=11 (the gap penalty), B=1 (the gap length penalty) and LG is the length of the gap.

For nucleotide sequences:

Substitution matrix: 10 for matches, 0 for mismatches.

Gap scoring function: -A -B*LG where A=50 (the gap penalty), B=3 (the gap length penalty) and LG is the length of the gap.

Typical conservative substitutions are among Met, Val, Leu and Ile; among Ser and Thr; among the residues Asp, Glu and Asn; among the residues Gln, Lys and Arg; or aromatic residues Phe and Tyr.

As used herein, two sequences are "homologous" or "similar" to each other where they have at least 70%, 80%, or 85% sequence similarity to each other, including, e.g., 90%, 95%, 97%, 99% or even 100% sequence similarity, when aligned using either the Needleman-Wunsch algorithm or the "BLAST 2 sequences" algorithm described by Tatusova & Madden, 1999, FEMS Microbiol Lett. 174:247-250. Where amino acid sequences are aligned using the "BLAST 2 sequences algorithm," the Blosum 62 matrix is the default matrix.

As used herein, the terms "inhibit," "inhibits" and "inhibited" refer to a decrease in a given measurable activity (e.g., binding activity) by at least 10% relative to a reference. Where inhibition is desired, such inhibition is preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more, up to and including 100%, i.e., complete inhibition or absence of the given activity. One way that inhibition of CD40L binding to CD40 is measured is as described in Example 6 herein. As used herein, the term "substantially inhibits" refers to a decrease in a given measurable activity (e.g., the binding of CD40L to CD40) by at least 50% relative to a reference. For example, "substantially inhibits" refers to a decrease in a given measurable activity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% and up to and including 100% relative to a reference. As used herein, "inhibits the binding", with reference to the binding of an antibody polypeptide binding to CD40L, or CD40 binding to CD40L, refers to a decrease in binding by at least 10% relative to a reference. "Inhibits the binding" preferably refers to a decrease in binding of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more, up to and including 100%.

As used herein, the terms "activate," "activates" and "activated" refer to an increase in a given measurable activity by at least 5% relative to a reference, for example, at least 10%, 25%, 50%, 75% or even 100%.

As used herein, the term "antagonist" refers to an agent that inhibits at least one activity mediated by CD40L, inhibits the binding of CD40 to CD40L, and/or results in no more than 25% platelet activation and/or aggregation in a platelet aggregation assay or platelet activation assay as described herein, and preferably results in 25% or less platelet activation and/or aggregation, 20% or less, 15% or less, 10% or less, 5% or less, and as little as zero platelet activation and/or aggregation. An activity is "antagonized" if the activity (i.e., CD40L mediated activity, binding of CD40 or CD40L, or platelet activation and/or aggregation) is reduced by at least 10%, and preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97% or even 100% (i.e., no activity) in the presence, relative to the absence of an antagonist. An antagonist as the term is used herein preferably comprises a single immunoglobulin variable domain that binds monovalently to CD40L.

As used herein, the term "agonist" refers to an agent that activates at least one activity mediated by CD40L, either alone or when combined with another co-stimulus, relative to a reference. An activity is "agonized" if the activity is increased by at least 10%, e.g., 50%, in the presence, relative to the absence of an agonist.

As used herein, the term "epitope" refers to a unit of structure conventionally bound by an immunoglobulin V_H/V_L pair. Epitopes define the minimum binding site for an antibody, and thus represent the target of specificity of an antibody. In the case of a single immunoglobulin variable domain, an epitope represents the unit of structure bound by a single variable domain in isolation. That is, the binding site is provided by one, single immunoglobulin variable domain.

As used herein, the term "extended release" or the equivalent terms "controlled release" or "slow release" refer to drug formulations that release active drug, such as a polypeptide drug, over a period of time following administration to an individual. Extended release of polypeptide drugs, which can occur over a range of desired times, e.g., minutes, hours, days, weeks or longer, depending upon the drug formulation, is in contrast to standard formulations in which substantially the entire dosage unit is available for immediate absorption or immediate distribution via the

bloodstream. Preferred extended release formulations result in a level of circulating drug from a single administration that is sustained, for example, for 8 hours or more, 12 hours or more, 24 hours or more, 36 hours or more, 48 hours or more, 60 hours or more, 72 hours or more 84 hours or more, 96 hours or more, or even, for example, for 1 week or 2 weeks or more, for example, 1 month or more.

As used herein, a "CD40L activity" is an activity involving or resulting from the binding of CD40L to CD40, and includes, but is not limited to binding to CD40 (assayed, for example, according to the method described in Example 6), activation of Jun-N-terminal Kinase (JNK), the induction of T cells to produce and secrete cytokines including, for example, IL-10, IFN-γ and TNF-α, and the mediation of platelet activation and/or aggregation. Assays for these activities are provided herein below.

As used herein, the term "does not substantially agonize" means that a given agent, e.g., an anti-CD40L antibody polypeptide, does not activate one or more of the CD40L activities including Jun-N-terminal kinase activation (phosphorylation) in Jurkat T cells and induction of IFN-γ production or secretion in anti-CD3-stimulated Jurkat T cells, as the term "activate" is defined herein. As used herein, "does not substantially agonize" means that the agent does not activate more than 20% of the activity which is activated by CD40 binding to CD40L, preferably, the agent does not activate more than 10%, 8%, 5%, 3%, or more than 2% or less, including zero activation, of the activity which is activated by CD40 binding to CD40L.

As used herein, the term "antibody polypeptide" refers to a polypeptide which either is an antibody or is a part of an antibody, modified or unmodified, which retains the ability to specifically bind antigen. Thus, the term antibody polypeptide includes an antigen-binding heavy chain, light chain, heavy chain-light chain dimer, Fab fragment, F(ab')2 fragment, dAb, or an Fv fragment, including a single chain Fv (scFv). The phrase "antibody polypeptide" is intended to encompass recombinant fusion polypeptides that comprise an antibody polypeptide sequence that retains the ability to specifically bind antigen in the context of the fusion.

As used herein, the term "monovalent" means that a given antibody polypeptide or single immunoglobulin variable domain polypeptide can bind only a single molecule of its target. Naturally-occurring antibodies are generally divalent, in that they have two functional antigen-binding arms, each comprising a VH and a VL domain. Where steric hindrance is not an issue, a divalent antibody can bind two separate molecules of the same antigen. In contrast, a "monovalent" antibody has the capacity to bind only one such antigen molecule. As the term is used herein, a "monovalent" antibody can also comprise more than one antigen binding site, e.g., two antigen binding sites, but the binding sites must be for different antigens, such that the antibody can only bind one molecule of CD40L at a time. The antigen-binding domain of a monovalent antibody can comprise a V_H and a V_L domain, but preferably comprises only a single immunoglobulin variable domain, i.e., a V_H or a V_L domain, that has the capacity to bind CD40L without the need for a corresponding V_L or V_H domain, respectively. A monovalent antibody lacks the capacity to cross link molecules of a single antigen.

As used herein, the term "standard platelet aggregation assay" means the assay described in the section herein below, entitled "Platelet Aggregation Assay."

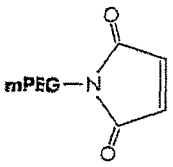
As used herein, the terms "V_H domain" and "V_L domain" refer to immunoglobulin variable regions as defined by Kabat et al.(supra), which is incorporated herein by reference.

As used herein, "linked" refers to the attachment of a polymer moiety, such as PEG to an amino acid residue of an antibody polypeptide. Attachment of a PEG polymer to an amino acid residue of an antibody polypeptide, e.g., an anti-CD40L dAb, is referred to as "PEGylation" and may be achieved using several PEG attachment moieties including, but not limited to N-hydroxylsuccinimide (NHS) active ester, succinimidyl propionate (SPA), maleimide (MAL), vinyl sulfone (VS), or thiol. A PEG polymer, or other polymer, can be linked to an antibody polypeptide at either a predetermined position, or may be randomly linked to the an antibody polypeptide molecule. It is preferred, however, that the PEG polymer be linked to an

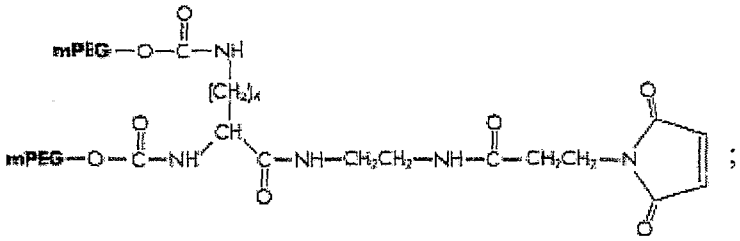
antibody polypeptide at a predetermined position. A PEG polymer may be linked to any residue in the an antibody polypeptide, however, it is preferable that the polymer is linked to either a lysine or cysteine, which is either naturally occurring in the antibody polypeptide, or which has been engineered into the antibody polypeptide, for example, by mutagenesis of a naturally occurring residue in the antibody polypeptide to either a cysteine or lysine. PEG-linkage can also be mediated through a peptide linker attached to an antibody polypeptide. That is, the PEG moiety can be attached to a peptide linker fused to an antibody polypeptide, where the linker provides the site, e.g., a free cysteine or lysine, for PEG attachment. As used herein, "linked" can also refer to the association of two or more antibody polypeptides, e.g., dAb monomers, to form a dimer, trimer, tetramer, or other multimer. Antibody polypeptide monomers can be linked to form a multimer by several methods known in the art, including, but not limited to, expression of the antibody polypeptide monomers as a fusion protein, linkage of two or more monomers via a peptide linker between monomers, or by chemically joining monomers after translation, either to each other directly, or through a linker by disulfide bonds, or by linkage to a di-, tri- or multivalent linking moiety (e.g., a multi-arm PEG). While dAb multimers are specifically contemplated herein, e.g., in the context of dual- or multi-specific antibody polypeptide constructs, it is emphasized that for any given antibody polypeptide construct, the construct should only be able to bind one molecule of CD40L, i.e., the constructs can have only one CD40L-binding element, and cannot cross link CD40L.

As used herein, "polymer" refers to a macromolecule made up of repeating monomeric units, and can refer to a synthetic or naturally occurring polymer such as an optionally substituted straight or branched chain polyalkylene, polyalkenylene, or polyoxyalkylene polymer or a branched or unbranched polysaccharide. A "polymer" as used herein, specifically refers to an optionally substituted or branched chain poly(ethylene glycol), poly(propylene glycol), or poly(vinyl alcohol) and derivatives thereof.

As used herein, "PEG" or "PEG polymer" refers to polyethylene glycol, and more specifically can refer to a derivitized form of PEG, including, but not limited to N-hydroxylsuccinimide (NHS) active esters of PEG such as succinimidyl propionate, benzotriazole active esters, PEG derivatized with maleimide, vinyl sulfones, or thiol groups. Particular PEG formulations can include PEG-O-$CH_2CH_2CH_2$-CO_2-NHS; PEG-O-CH_2-NHS; PEG-O-CH_2CH_2-CO_2-NHS; PEG-S-CH_2CH_2-CO-NHS; PEG-O_2CNH-CH(R)-CO_2-NHS; PEG-NHCO-CH_2CH_2-CO-NHS; and PEG-O-CH_2-CO_2-NHS; where R is $(CH_2)_4$)$NHCO_2$(mPEG). PEG polymers useful in the invention may be linear molecules, or may be branched wherein multiple PEG moieties are present in a single polymer. Some particularly preferred PEG conformations that are useful in the invention include, but are not limited to the following:

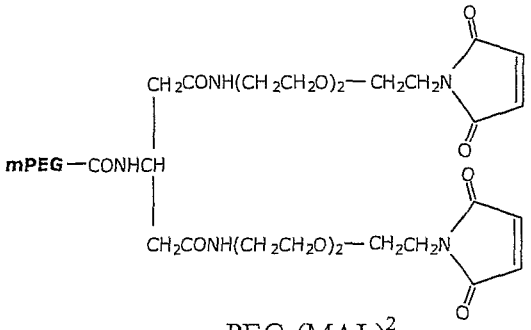


;

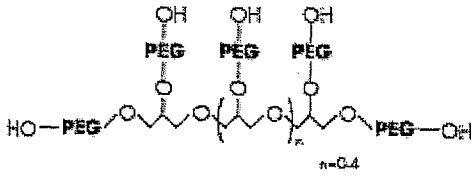


;

mPEG-MAL mPEG2-MAL

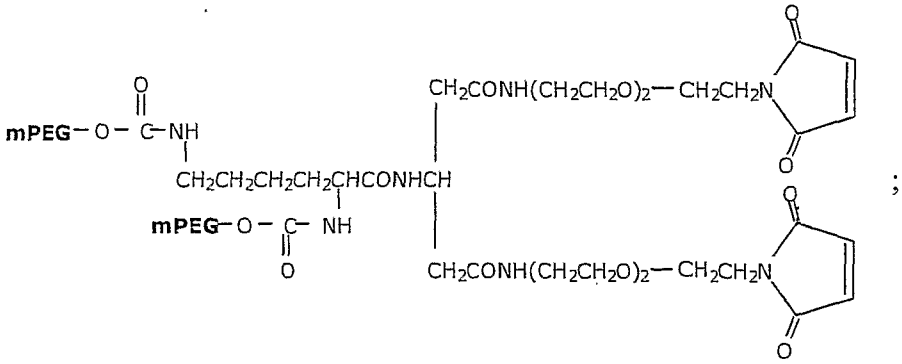


;



;

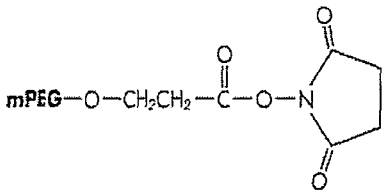
mPEG-(MAL)^2 multi-arm PEG

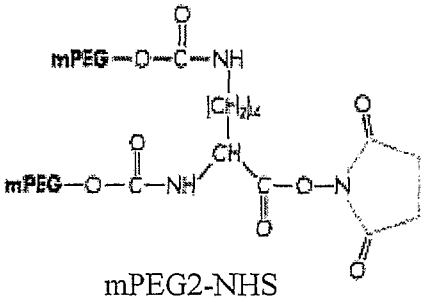


;

mPEG2-(MAL)^2

5





mPEG-SPA mPEG2-NHS

As used herein, a "sulfhydryl-selective reagent" is a reagent which is useful for the attachment of a PEG polymer to a thiol-containing amino acid. Thiol groups on the amino acid residue cysteine are particularly useful for interaction with a sulfhydryl-selective reagent. Sulfhydryl-selective reagents which are useful for such attachment include, but are not limited to maleimide, vinyl sulfone, and thiol. The use of sulfhydryl-selective reagents for coupling to cysteine residues is known in the art and may be adapted as needed according to the present invention (See Eg., Zalipsky, 1995, *Bioconjug. Chem.* 6:150; Greenwald et al., 2000, *Crit. Rev. Ther. Drug Carrier Syst.* 17:101; Herman et al., 1994, *Macromol. Chem. Phys.* 195:203).

The attachment of PEG or another agent, e.g., HSA, to an antibody polypeptide or to a single immunoglobulin variable domain polypeptide as described herein will preferably not impair the ability of the polypeptide to specifically bind CD40L. That is, the PEG-linked antibody polypeptide or single immunoglobulin variable domain polypeptide will retain its binding activity relative to a non-PEG-linked counterpart. As used herein, "retains activity" refers to a level of activity of a PEG-linked antibody polypeptide which is at least 10% of the level of activity of a non-PEG-linked antibody polypeptide, preferably at least 20%, 30%, 40%, 50%, 60%, 70%, 80% and up to 90%, preferably up to 95%, 98%, and up to 100% of the activity of a non-PEG-linked antibody polypeptide comprising the same antigen-binding domain or domains. More specifically, the activity of a PEG-linked antibody polypeptide compared to a non-PEG linked antibody variable domain should be determined on an antibody polypeptide molar basis; that is equivalent numbers of moles of each of the PEG-linked and non-PEG-linked antibody polypeptides should be used in each trial. In determining whether a particular PEG-linked antibody polypeptide "retains activity", it is preferred that the activity of a PEG-linked antibody polypeptide be compared with the activity of the same antibody polypeptide in the absence of PEG.

As used herein, the term "*in vivo* half-life" refers to the time taken for the serum concentration of a ligand (e.g., a single immunoglobulin variable domain) to reduce by 50%, *in vivo*, for example due to degradation of the ligand and/or clearance

or sequestration of the ligand by natural mechanisms. The anti CD40L antibody polypeptides or single immunoglobulin variable domain polypeptides described herein can be stabilized *in vivo* and their half-life increased by binding to molecules, such as PEG, which resist degradation and/or clearance or sequestration. The half-life of an antibody polypeptide is increased if its functional activity persists, *in vivo*, for a longer period than a similar antibody polypeptide which is not linked to a PEG polymer. Typically, the half life of a PEGylated antibody polypeptide is increased by 10%, 20%, 30%, 40%, 50% or more relative to a non-PEGylated antibody polypeptide. Increases in the range of 2x, 3x, 4x, 5x, 10x, 20x, 30x, 40x, 50x or more of the half life are possible. Alternatively, or in addition, increases in the range of up to 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 150x of the half life are possible. According to the invention, a PEG-linked antibody single variable domain has a half-life of between 0.25 and 170 hours, preferably between 1 and 100 hours, more preferably between 30 and 100 hours, and still more preferably between 50 and 100 hours, and up to 170, 180, 190, and 200 hours or more.

As used herein, "resistant to degradation" or "resists degradation" with respect to a PEG or other polymer-linked antibody polypeptide monomer or multimer means that the PEG- or other polymer-linked antibody polypeptide monomer or multimer is degraded by no more than 10% when exposed to pepsin at pH 2.0 for 30 minutes and preferably not degraded at all.

As used herein, "hydrodynamic size" refers to the apparent size of a molecule (e.g., a protein molecule) based on the diffusion of the molecule through an aqueous solution. The diffusion, or motion of a protein through solution can be processed to derive an apparent size of the protein, where the size is given by the "Stokes radius" or "hydrodynamic radius" of the protein particle. The "hydrodynamic size" of a protein depends on both mass and shape (conformation), such that two proteins having the same molecular mass may have differing hydrodynamic sizes based on the overall conformation of the protein. Hydrodynamic size is measured, for example, by size exclusion chromatography. The hydrodynamic size of a PEG-linked antibody polypeptide, e.g., a single immunoglobulin variable domain (including antibody

variable domain multimers as described herein), can be in the range of 24 kDa to 500 kDa; 30 to 500 kDa; 40 to 500 kDa; 50 to 500 kDa; 100 to 500 kDa; 150 to 500 kDa; 200 to 500 kDa; 250 to 500 kDa; 300 to 500 kDa; 350 to 500 kDa; 400 to 500 kDa and 450 to 500 kDa. Preferably the hydrodynamic size of a PEGylated antibody polypeptide of the invention is 30 to 40 kDa; 70 to 80 kDa or 200 to 300 kDa. Where a single immunoglobulin variable domain polypeptide is desired for use in imaging applications, the polypeptide should have a hydrodynamic size of between 50 and 100 kDa. Alternatively, where a single immunoglobulin variable domain polypeptide is desired for therapeutic applications, the polypeptide preparation should have a hydrodynamic size of greater than 200 kDa.

As used herein, the term "IC_{50}" refers to the concentration of an inhibitor necessary to inhibit a given activity by 50%. IC_{50} is determined by assaying a given activity, e.g., binding of CD40L to CD40, in the presence of varying amounts of the inhibitor (e.g., monovalent anti-CD40L antibody polypeptide), and plotting the inhibitor concentration versus the activity being targeted. Binding of CD40L to CD40 is measured herein by the method described in Example 6. Alternatively, SPR can be used.

As used herein, the term "fused to an antibody polypeptide" means that a polypeptide is fused to a given antibody through use of recombinant DNA techniques. Thus, an antibody "fused to" another polypeptide, e.g., to another antibody of different binding specificity, does not exist in nature and is generated through recombinant means. The term "fused to an antibody polypeptide" also encompasses the linkage of a polypeptide to a given antibody polypeptide through, for example, disulfide or other chemical linkages, where the fused polypeptide is not naturally found fused to the antibody polypeptide. Recombinant and chemical methods of fusing a polypeptide to another polypeptide, e.g., to an antibody, are well known in the art.

As used herein, the term "Fc domain" refers to the constant region antibody sequences comprising CH2 and CH3 constant domains as delimited according to

Kabat et al., *supra*. The Fc portion of the heavy chain polypeptide has the ability to self-associate, a function which facilitates the formation of divalent antibodies. The term "lacks an Fc domain" means that a given antibody polypeptide lacks at least the portion of an immunoglobulin Fc domain (as such domains are defined according to Kabat et al., *supra*) sufficient to mediate the dimerization of Fc-containing antibody polypeptides. Dimerization of Fc-containing antibody polypeptides is measured, for example, by chromatographic methods or by surface plasmon resonance. An antibody polypeptide lacking an Fc domain avoids Fc-platelet interactions and therefore avoids induction of platelet aggregation.

As used herein "treat", "reduce", "prevent", or "alleviate" as it relates to a symptom of disease refer to a decrease of the a symptom by at least 10% based on a a clinically measurable parameter, or by at least one point on a clinically-accepted scale of disease or symptom severity. As used herein, the term "symptom of systemic lupus erythematosus" refers to any of the clinically relevant symptoms of SLE known to those of skill in the art. Non-limiting examples include the accumulation of IgG autoantibodies (e.g., against nuclear antigens such as chromatin, snRNPs (especially U1, Sm, Ro/SSA and La/SSB), phospholipids and cell surface molecules), hemolytic anemia, thrombocytopenia, leukopenia, glomerulonephritis, vasculitis, arthritis, and serositis). A reduction in such a symptom is a reduction by at least 10% in a clinically measurable parameter, or by at least one point on a clinically-accepted scale of disease severity.

As used herein, the phrase "specifically binds" refers to the binding of an antigen by an immunoglobulin variable domain with a dissociation constant (K_d) of 1 μM or lower as measured by surface plasmon resonance analysis using, for example, a BIAcore™ surface plasmon resonance system and BIAcore™ kinetic evaluation software (e.g., version 2.1). The affinity or K_d for a specific binding interaction is preferably about 500 nM or lower, more preferably about 300 nM or lower.

As used herein, a "generic ligand" is a ligand that binds a substantial proportion of functional members in a given repertoire, e.g., in a phage display

library. Thus, the same generic ligand can bind many members of the repertoire regardless of their target ligand specificities. In general, the presence of a functional generic ligand binding site indicates that the repertoire member is expressed and folded correctly. Thus, binding of the generic ligand to its binding site provides a method for preselecting functional polypeptides from a repertoire of polypeptides. Generic ligands include, for example, Protein A, Protein G and Protein L.

As used herein, the term "universal framework" refers to a single antibody framework sequence corresponding to the regions of an antibody conserved in sequence as defined by Kabat (supra) or corresponding to the human germline immunoglobulin repertoire or structure as defined by Chothia and Lesk, (1987) J. Mol. Biol. 196:910-917. The invention provides for the use of a single framework, or a set of such frameworks, which has been found to permit the derivation of virtually any binding specificity though variation in the hypervariable regions alone.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows gel analysis of the quality of biotin-labeled CD40L used in the screening procedures described herein. (a) 1 μg of non-biotinylated-CD40L (Lane 1) and 0.3 μg of biotin-CD40L (Lane 2) were analysed on SDS-PAGE and detected by Simply Blue Safe-Stain. (b) 0.1 μg of biotin-CD40L (Lane 1) and 0.02 μg of biotin-CD40L (Lane 2) were detected by Western-blot probing with 1:5000 Streptavidin-HRP.

Figure 2 shows a graphical representation of a dose response receptor binding assay (RBA) readout, analysing the inhibition of CD40L binding to CD40-Fc by dAbs DOM-10, -20, -27, -30, -31, -62, -77, titrated from 1 μM down to 10 pM. dAbs DOM-20, -30, and -31 are the most potent with IC_{50} values of approximately 8 nM.

Figure 3 shows a graphical representation of a dose response receptor binding assay readout, analysing the inhibition of CD40L binding to CD40-Fc by dAbs DOM-

4 and DOM-5, titrated from 1 μM down to 500 pM. The IC_{50} values for dAbs DOM-5 and DOM-4 are approximately 3 nM and 100 nM respectively.

Figure 4 shows a graphical representation of a dose response receptor binding assay readout, analysing the inhibition of CD40L binding to CD40-Fc by dAb DOM-24, titrated from 100 nM down to 0.5 pM. The data were curve-fitted using GraphPad Prism software.

Figure 5 shows the sequence of the V_H framework based on germline sequence DP47 – JH4b (SEQ ID NO: 1, amino acid sequence; SEQ ID NO: 2, nucleotide sequence – both sense and antisense strands are shown – SEQ ID NO: 2 is the top strand (sense) and SEQ ID NO: 476 is the lower strand (anti-sense)). HCDRs 1-3 are indicated by underlining.

Figure 6 shows the sequence of the V_κ framework based on germline sequence DPK9 - J_K1 (SEQ ID NO: 3, amino acid sequence; SEQ ID NO: 4, nucleotide sequence – both sense and antisense strands are shown – SEQ ID NO: 4 is the top strand (sense) and SEQ ID NO: 477 is the lower strand (anti-sense)). LCDRs 1-3 are indicated by underlining.

Figure 7 shows a schematic representation of the CD40L binding assay used herein, e.g., in Example 6.

Figure 8 shows various GAS1 secretion signal peptide coding sequences.

GAS wt: The natural occurring sequence in yeast. GAS E.Coli: The nucleotide sequence according to optimal E. Coli codon usage (Wada et al. 1992 NAR 20 p 2111). GAS leader AT: AT rich nucleotide sequence. All nucleotide sequences encode the same amino acid sequence. Yellow (light grey in greyscale) indicates nucleotides that are similar for all sequences. Blue (dark grey in greyscale) indicates nucleotides that are similar to the wt sequence. White (white in greyscale) indicates nucleotides that are different from the wt sequence.

Figure 9 shows the results of a receptor binding assay which demonstrates the affinity of PEGylated DOM8-24cys with either 30K PEG MAL or 40K PEG2-MAL.

Figure 10 shows the results of an assay to assess the simultaneous binding of a dual specific dimer to HSA and CD40L (shaded bars). Binding to control BSA antigen is also shown (solid bars).

Figure 11 shows the results of an assay to assess the simultaneous binding of a dual specific Fab to HSA and CD40L (shaded bars). Binding to control skimmed mild powder antigen is also shown (solid bars).

Figure 12 shows the results of FACS analysis of the inhibitory effect of monomeric DOM-24 (grey dotted line). Control stimulated cells ae shown as the solid black line and a control dAb is shown as the grey solid line.

Figure 13 shows the results of FACS analysis of the inhibitory effect of the Vk dAb DOM-116 (dotted line). Control stimulated cells are shown as the solid black line and a control dAb is shown as the grey solid line.

DETAILED DESCRIPTION

The invention provides antibody polypeptides that are monovalent for binding to CD40L. Monovalency for CD40L binding removes the possibility for cross-linking that occurs with prior art antibodies, and which plays a role in undesirable side effects observed with anti-CD40L monoclonal antibodies. Further, while not wishing to be limited to any specific mechanism or theory, because antibody polypeptides monovalent for CD40L cannot cross link CD40L, the possibility is eliminated that cross-linked CD40L may in turn cross-link cell surface CD40 and result in agonism of CD40 signaling activity. Thus, in a preferred aspect, the anti-CD40L antibodies disclosed herein not only inhibit or antagonize the binding of CD40L to CD40, they do not substantially agonize CD40 and/or CD40L activity.

In one aspect, the antibodies monovalent for CD40L binding are human antibody polypeptides. Human antibody polypeptides can be administered to human patients while largely avoiding the anti-antibody immune response often provoked by the administration of antibodies from other species, e.g., mouse. While murine antibodies can be "humanized" by grafting human constant domains onto the murine antigen-binding domains, human antibodies as disclosed herein are produced without the need for laborious and time-consuming genetic manipulation of a murine antibody sequence.

Monovalent antibody polypeptides:

The heavy and light polypeptide chains of antibodies comprise variable (V) regions that directly participate in antigen interactions, and constant (C) regions that

provide structural support and function in non-antigen-specific interactions with immune effectors. The antigen binding domain of a conventional antibody is comprised of two separate domains: a heavy chain variable domain (V_H) and a light chain variable domain (V_L: which can be either V_K or $V\lambda$). The antigen binding site itself is formed by six polypeptide loops: three from the V_H domain (H1, H2 and H3) and three from the V_L domain (L1, L2 and L3). *In vivo*, a diverse primary repertoire of V genes that encode the V_H and V_L domains is produced by the combinatorial rearrangement of gene segments. C regions include the light chain C regions (referred to as C_L regions) and the heavy chain C regions (referred to as C_H1, C_H2 and C_H3 regions). A naturally-occurring antibody generally comprises two antigen binding domains and is therefore divalent.

A number of smaller antigen binding fragments of naturally occurring antibodies have been identified following protease digestion. These include, for example, the "Fab fragment" (V_L-C_L-C_H1-V_H), "Fab' fragment" (a Fab with the heavy chain hinge region), and "$F(ab')_2$ fragment" (a dimer of Fab' fragments joined by the heavy chain hinge region). Recombinant methods have been used to generate such fragments and to generate even smaller antigen-binding fragments, e.g., those referred to as "single chain Fv" (variable fragment) or "scFv," consisting of V_L and V_H joined by a synthetic peptide linker (V_L-linker-V_H). Fab fragments, Fab' fragments and scFv fragments are monovalent for antigen binding, as they each comprise only one antigen binding domain comprising one V_H/V_L dimer. Even smaller monovalent antibody fragments are the "domain antibodies," or "dAbs," which comprise only a single immunoglobulin variable domain, e.g., V_H or V_L, that alone specifically binds antigen, i.e., without the need for a complementary V_L or V_H domain, respectively.

The term "dAb" will refer herein to a single immunoglobulin variable domain (V_H or V_L) polypeptide that specifically binds antigen. A "dAb" binds antigen independently of other V domains; however, a "dAb" can be present in a homo- or heteromultimer with other V_H or V_L domains where the other domains are not required for antigen binding by the dAb, i.e., where the dAb binds antigen

independently of the additional V_H or V_L domains. The preparation of single immunoglobulin variable domains is described and exemplified herein below.

Monovalent antibody polypeptides can be generated in several different ways. For example, the nucleic acid sequence encoding heavy and light chains of an antibody known to bind CD40L can be manipulated to generate a number of different antibody polypeptides that are monovalent for CD40L binding. Thus, given the sequences encoding the heavy and light chain polypeptides that constitute an antibody and standard molecular cloning methodologies, one can generate monovalent antigen-binding polypeptide constructs such as Fab fragments, scFv, dAbs, or even bispecific antibodies (i.e., antibodies that comprise two different antigen-binding moieties and can therefore bind two separate antigens, preferably simultaneously) that are monovalent for CD40L.

Thus, one means of generating monovalent antibody polypeptides specific for CD40L is to amplify and express the V_H and V_L regions of the heavy chain and light chain gene sequences isolated, for example, from a hybridoma (e.g., a mouse hybridoma) that expresses anti-CD40L monoclonal antibody. The boundaries of V_H and V_L domains are set out by Kabat et al. (1991, supra). The information regarding the boundaries of the V_H and V_L domains of heavy and light chain genes is used to design PCR primers that amplify the V domain from a heavy or light chain coding sequence encoding an antibody known to bind CD40L. The amplified V domains are inserted into a suitable expression vector, e.g., pHEN-1 (Hoogenboom et al., 1991, Nucleic Acids Res. 19: 4133-4137) and expressed, e.g., as a fusion of the V_H and V_L in an scFv or other suitable monovalent format. The resulting polypeptide is then screened for high affinity monovalent binding to CD40L. For all aspects of the present invention, screening for binding is performed as known in the art or as described herein below.

Alternatively, library screening methods can be used to identify monovalent CD40L-specific binding proteins. Phage display technology (see, e.g., Smith, 1985, Science 228: 1315; Scott & Smith, 1990, Science 249: 386; McCafferty et al., 1990,

Nature 348: 552) provides an approach for the selection of antibody polypeptides which bind a desired target from among large, diverse repertoires of antibody polypeptides. These phage-antibody libraries can be grouped into two categories: natural libraries which use rearranged V genes harvested from human B cells (Marks et al., 1991, J. Mol. Biol., 222: 581; Vaughan et al., 1996, Nature Biotech., 14: 309) or synthetic libraries whereby germline V gene segments or other antibody polypeptide coding sequences are 'rearranged' in vitro (Hoogenboom & Winter, 1992, J. Mol. Biol., 227: 381; Nissim et al., 1994, EMBO J., 13: 692; Griffiths et al., 1994, EMBO J., 13: 3245; De Kruif et al., 1995, J. Mol. Biol., 248: 97) or where synthetic CDRs are incorporated into a single rearranged V gene (Barbas et al., 1992. Proc. Natl. Acad. Sci. USA, 89: 4457). Methods involving genetic display packages (e.g., phage display, polysome display) are well-suited for the selection of monovalent CD40L-specific antibody constructs because they generally express only monovalent fragments, rather than whole, divalent antibodies, on the display packages. Methods for the preparation of phage display libraries displaying various antibody fragments are described in the preceding references. Such methods are also described, for example, in U.S. Patent No. 6,696,245, which is incorporated herein by reference. The methods described in the '245 patent generally involve the randomization of selected regions of immunoglobulin gene coding regions, in particular V_H and V_L coding regions, while leaving other regions non-randomized (see below). The '245 patent also describes the generation of scFv constructs comprising individually randomized V_H and V_L domains.

The V_H gene is produced by the recombination of three gene segments, V_H, D and J_H. In humans, there are approximately 51 functional V_H segments (Cook and Tomlinson (1995) Immunol Today 16: 237), 25 functional D segments (Corbett et al. (1997) J. Mol. Biol. 268: 69) and 6 functional J_H segments (Ravetch et al. (1981) Cell 27: 583), depending on the haplotype. The V_H segment encodes the region of the polypeptide chain which forms the first and second antigen binding loops of the V_H domain (H1 and H2), while the V_H, D and J_H segments combine to form the third antigen binding loop of the V_H domain (H3).

The V_L gene is produced by the recombination of only two gene segments, V_L and J_L. In humans, there are approximately 40 functional V_K segments (Schäble and Zachau (1993) Biol. Chem. Hoppe-Seyler 374: 1001), 31 functional $V\lambda$ segments (Williams et al. (1996) J. Mol. Biol. 264: 220; Kawasaki et al. (1997) Genome Res. 7: 250), 5 functional J_K segments (Hieter et al. (1982) J. Biol. Chem. 257: 1516) and 4 functional $J\lambda$ segments (Vasicek and Leder (1990) J. Exp. Med. 172: 609), depending on the haplotype. The V_L segment encodes the region of the polypeptide chain which forms the first and second antigen binding loops of the V_L domain (L1 and L2), while the V_L and J_L segments combine to form the third antigen binding loop of the V_L domain (L3). Antibodies selected from this primary repertoire are believed to be sufficiently diverse to bind almost all antigens with at least moderate affinity. High affinity antibodies are produced *in vivo* by "affinity maturation" of the rearranged genes, in which point mutations are generated and selected by the immune system on the basis of improved binding.

Analysis of the structures and sequences of antibodies has shown that five of the six antigen binding loops (H1, H2, L1, L2, L3) possess a limited number of main-chain conformations or canonical structures (Chothia and Lesk (1987) J. Mol. Biol. 196: 901; Chothia et al. (1989) Nature 342: 877). The main-chain conformations are determined by (i) the length of the antigen binding loop, and (ii) particular residues, or types of residue, at certain key positions in the antigen binding loop and the antibody framework. Analysis of the loop lengths and key residues has enabled the prediction of the main-chain conformations of H1, H2, L1, L2 and L3 encoded by the majority of human antibody sequences (Chothia et al. (1992) J. Mol. Biol. 227: 799; Tomlinson et al. (1995) EMBO J. 14: 4628; Williams et al. (1996) J. Mol. Biol. 264: 220). Although the H3 region is much more diverse in terms of sequence, length and structure (due to the use of D segments), it also forms a limited number of main-chain conformations for short loop lengths which depend on the length and the presence of particular residues, or types of residue, at key positions in the loop and the antibody framework (Martin et al. (1996) J. Mol. Biol. 263: 800; Shirai et al. (1996) FEBS Letters 399: 1.

While, in one approach, diversity can be added to synthetic repertoires at any site in the CDRs of the various antigen-binding loops, this approach results in a greater proportion of V domains that do not properly fold and therefore contribute to a lower proportion of molecules with the potential to bind antigen. An understanding of the residues contributing to the main chain conformation of the antigen-binding loops permits the identification of specific residues to diversify in a synthetic repertoire of V_H or V_L domains. That is, diversity is best introduced in residues that are not essential to maintaining the main chain conformation. As an example, for the diversification of loop L2, the conventional approach would be to diversify all the residues in the corresponding CDR (CDR2) as defined by Kabat et al. (1991, supra), some seven residues. However, for L2, it is known that positions 50 and 53 are diverse in naturally occurring antibodies and are observed to make contact with the antigen. The preferred approach would be to diversify only those two residues in this loop. This represents a significant improvement in terms of the functional diversity required to create a range of antigen binding specificities.

Immunoglobulin polypeptide libraries can advantageously be designed to be based on predetermined variable domain main chain conformation. Such libraries may be constructed as described in International Patent Application WO 99/20749, the contents of which are incorporated herein by reference. Thus, in one aspect, an antibody polypeptide comprises the amino acid sequence of a given human germline V region gene segment, e.g., V_H germline gene segment DP-47, or V_κ germline gene segment DPK9. Such variable region polypeptides can be used for the production of scFvs or Fabs, e.g., an scFv or Fab comprising (i) an antibody heavy chain variable domain (V_H), or antigen binding fragment thereof, which comprises the amino acid sequence of germline V_H segment DP-47 and (ii) an antibody light chain variable domain (V_L), or antigen binding fragment thereof, which comprises the amino acid sequence of germline V_κ segment DPK9. Diversification of sequences within the context of the selected heavy and light chain germline gene segments, e.g., DP-47, DPK 9, DP45, DP38, etc. can generate a repertoire of diverse immunoglobulin coding sequences. One approach to diversification is described below in the context of generating a library of diversified dAb or scFv sequences. These variable region

polypeptides can also be expressed as dAbs and screened for high affinity binding to CD40L. The repertoire can be cloned into or generated in a vector suitable for phage display, e.g., a lambda or filamentous bacteriophage display vector and is then screened for binding to a given target antigen, e.g., CD40L.

Preparation of Human Single Immunoglobulin Variable Domain Polypeptides:

A single immunoglobulin variable domain is a folded polypeptide domain which comprises sequences characteristic of immunoglobulin variable domains and which specifically binds an antigen (e.g., dissociation constant of 500 nM or less), and which binds antigen as a single variable domain; that is, there is one binding site provided by a single immunoglobulin variable domain without any complementary variable domain. A single immunoglobulin variable domain therefore includes complete antibody variable domains as well as modified variable domains, for example in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain a dissociation constant of 500 nM or less (e.g., 450 nM or less, 400 nM or less, 350 nM or less, 300 nM or less, 250 nM or less, 200 nM or less, 150 nM or less, 100 nM or less) and the target antigen specificity of the full-length domain. Preferably an antibody single variable domain useful in the invention is selected from the group of V_H and V_L, including V_{kappa} and V_{lambda}. The single immunoglobulin variable domains of use herein are preferably "human" as that term is defined herein.

Preparation of Single Immunoglobulin Variable Domains:

Single immunoglobulin variable domains are prepared in a number of ways. For each of these approaches, well-known methods of preparing (e.g., amplifying, mutating, etc.) and manipulating nucleic acid sequences are applicable.

One means of preparing single immunoglobulin variable domains is to amplify and express the V_H or V_L region of a heavy chain or light chain gene for a

cloned antibody known to bind the desired antigen. That is, the V_H or V_L domain of a known anti-CD40L antibody coding region can be amplified and expressed as a single domain (or as a fusion of a single domain) and evaluated for binding to CD40L. The boundaries of V_H and V_L domains are set out by Kabat et al. (1991, supra). The information regarding the boundaries of the V_H and V_L domains of heavy and light chain genes is used to design PCR primers that amplify the V domain from a cloned heavy or light chain coding sequence encoding an antibody known to bind CD40L. The amplified V domain is inserted into a suitable expression vector, e.g., pHEN-1 (Hoogenboom et al., 1991, Nucleic Acids Res. 19: 4133-4137) and expressed, either alone or as a fusion with another polypeptide sequence.

In a preferred approach, a repertoire of V_H or V_L domains, preferably human V_H or V_L domains, is screened by, for example, phage display, panning against the desired antigen. Methods for the construction of bacteriophage display libraries and lambda phage expression libraries are well known in the art, and taught, for example, by: McCafferty et al., 1990, Nature 348: 552; Kang et al., 1991, Proc. Natl. Acad. Sci. U.S.A., 88: 4363; Clackson et al., 1991, Nature 352: 624; Lowman et al., 1991, Biochemistry 30: 10832; Burton et al., 1991, Proc. Natl. Acad. Sci U.S.A. 88: 10134; Hoogenboom et al., 1991, Nucleic Acids Res. 19: 4133; Chang et al.,1991, J. Immunol. 147: 3610; Breitling et al., 1991, Gene 104: 147; Marks et al., 1991, J. Mol. Biol. 222: 581; Barbas et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89: 4457; Hawkins and Winter (1992) J. Immunol., 22: 867; Marks et al. (1992) J. Biol. Chem., 267: 16007; and Lerner et al. (1992) Science, 258: 1313. Fab phage display libraries are taught, for example, by U.S. 5,922,545. scFv phage libraries are taught, for example, by Huston et al., 1988, Proc. Natl. Acad. Sci U.S.A. 85: 5879-5883; Chaudhary et al., 1990, Proc. Natl. Acad. Sci U.S.A. 87: 1066-1070; McCafferty et al., 1990, supra; Clackson et al., 1991, supra; Marks et al., 1991, supra; Chiswell et al., 1992, Trends Biotech. 10: 80; and Marks et al., 1992, supra. Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described. Refinements of phage display approaches are also known, for example as described in WO96/06213 and WO92/01047 (Medical Research Council et al.) and WO97/08320 (Morphosys, supra).

The repertoire of V_H or V_L domains can be a naturally-occurring repertoire of immunoglobulin sequences or a synthetic repertoire. A naturally-occurring repertoire is one prepared, for example, from immunoglobulin-expressing cells harvested from one or more individuals. Such repertoires can be "naïve," i.e., prepared, for example, from human fetal or newborn immunoglobulin-expressing cells, or rearranged, i.e., prepared from, for example, adult human B cells. Natural repertoires are described, for example, by Marks et al., 1991, J. Mol. Biol. 222: 581 and Vaughan et al., 1996, Nature Biotech. 14: 309. If desired, clones identified from a natural repertoire, or any repertoire, for that matter, that bind the target antigen are then subjected to mutagenesis and further screening in order to produce and select variants with improved binding characteristics.

Synthetic repertoires of single immunoglobulin variable domains are prepared by artificially introducing diversity into a cloned V domain. Synthetic repertoires are described, for example, by Hoogenboom & Winter, 1992, J. Mol. Biol. 227: 381; Barbas et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89: 4457; Nissim et al., 1994, EMBO J. 13: 692; Griffiths et al., 1994, EMBO J. 13: 3245; DeKriuf et al., 1995, J. Mol. Biol. 248: 97; and WO 99/20749.

In one aspect, synthetic variable domain repertoires are prepared in V_H or $V\kappa$ backgrounds, based on artificially diversified germline V_H or $V\kappa$ sequences. For example, the V_H domain repertoire can be based on cloned germline V_H gene segments V3-23/DP47 (Tomlinson et al., 1992, J. Mol. Biol. 227: 7768) and JH4b. The V_κ domain repertoire can be based, for example, on germline V_κ gene segments O2/O12/DPK9 (Cox et al., 1994, Eur. J. Immunol. 24: 827) and $J_\kappa 1$. Diversity is introduced into these or other gene segments by, for example, PCR mutagenesis. Diversity can be randomly introduced, for example, by error prone PCR (Hawkins, et al., 1992, J. Mol. Biol. 226: 889) or chemical mutagenesis. As discussed above, however it is preferred that the introduction of diversity is targeted to particular residues. It is further preferred that the desired residues are targeted by introduction of the codon NNK using mutagenic primers (using the IUPAC nomenclature, where N = G, A, T or C, and K = G or T), which encodes all amino acids and the TAG stop

codon. Other codons which achieve similar ends are also of use, including the NNN codon (which leads to the production of the additional stop codons TGA and TAA), DVT codon ((A/G/T) (A/G/C)T), DVC codon ((A/G/T)(A/G/C)C), and DVY codon ((A/G/T)(A/G/C)(C/T). The DVT codon encodes 22% serine and 11% tyrosine, asgpargine, glycine, alanine, aspartate, threonine and cysteine, which most closely mimics the distribution of amino acid residues for the antigen binding sites of natural human antibodies. Repertoires are made using PCR primers having the selected degenerate codon or codons at each site to be diversified. PCR mutagenesis is well known in the art.

In one aspect, diversity is introduced into the sequence of human germline V_H gene segments V3-23/DP47 (Tomlinson et al., 1992, J. Mol. Biol. 227: 7768) and JH4b using the NNK codon at sites H30, H31, H33, H35, H50, H52, H52a, H53, H55, H56, H58, H95, H97 and H98, corresponding to diversity in CDRs 1, 2 and 3, with the numbering as used in U.S. 6,696,245.

In another aspect, diversity is also introduced into the sequence of human germline V_H gene segments V3-23/DP47 and JH4b, for example, using the NNK codon at sites H30, H31, H33, H35, H50, H52, H52a, H53, H55, H56, H58, H95, H97, H98, H99, H100, H100a and H100b, corresponding to diversity in CDRs 1, 2 and 3,with the numbering as used in U.S. 6,696,245.

In another aspect, diversity is introduced into the sequence of human germline V_κ gene segments O2/O12/DPK9 and $J_\kappa 1$, for example, using the NNK codon at sites L30, L31, L32, L34, L50, L53, L91, L92, L93, L94 and L96, corresponding to diversity in CDRs 1, 2 and 3,with the numbering as used in U.S. 6,696,245.

Diversified repertoires are cloned into phage display vectors as known in the art and as described, for example, in WO 99/20749. In general, the nucleic acid molecules and vector constructs required for the performance of the present invention are available in the art and are constructed and manipulated as set forth in standard laboratory manuals, such as Sambrook et al. (1989). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, USA.

The manipulation of nucleic acids in the present invention is typically carried out in recombinant vectors. As used herein, "vector" refers to a discrete element that is used to introduce heterologous DNA into cells for the expression and/or replication thereof. Methods by which to select or construct and, subsequently, use such vectors are well known to one of skill in the art. Numerous vectors are publicly available, including bacterial plasmids, bacteriophage, artificial chromosomes and episomal vectors. Such vectors may be used for simple cloning and mutagenesis; alternatively, as is typical of vectors in which repertoire (or pre-repertoire) members of the invention are carried, a gene expression vector is employed. A vector of use according to the invention is selected to accommodate a polypeptide coding sequence of a desired size, typically from 0.25 kilobase (kb) to 40 kb in length. A suitable host cell is transformed with the vector after in vitro cloning manipulations. Each vector contains various functional components, which generally include a cloning (or "polylinker") site, an origin of replication and at least one selectable marker gene. If a given vector is an expression vector, it additionally possesses one or more of the following: enhancer element, promoter, transcription termination and signal sequences, each positioned in the vicinity of the cloning site, such that they are operatively linked to the gene encoding a polypeptide repertoire member according to the invention.

Both cloning and expression vectors generally contain nucleic acid sequences that enable the vector to replicate in one or more selected host cells. Typically in cloning vectors, this sequence is one that enables the vector to replicate independently of the host chromosomal DNA and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 micron plasmid origin is suitable for yeast, and various viral origins (e.g. SV 40, adenovirus) are useful for cloning vectors in mammalian cells. Generally, the origin of replication is not needed for mammalian expression vectors unless these are used in mammalian cells able to replicate high levels of DNA, such as COS cells.

Advantageously, a cloning or expression vector also contains a selection gene also referred to as selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will therefore not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics and other toxins, e.g. ampicillin, neomycin, methotrexate or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients not available in the growth media.

Because the replication of vectors according to the present invention is most conveniently performed in E. coli, an E. coli-selectable marker, for example, the β-lactamase gene that confers resistance to the antibiotic ampicillin, is of use. These can be obtained from E. coli plasmids, such as pBR322 or a pUC plasmid such as pUC18 or pUC19.

Expression vectors usually contain a promoter that is recognized by the host organism and is operably linked to the coding sequence of interest. Such a promoter may be inducible or constitutive. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

Promoters suitable for use with prokaryotic hosts include, for example, the β-lactamase and lactose promoter systems, alkaline phosphatase, the tryptophan (trp) promoter system and hybrid promoters such as the tac promoter. Promoters for use in bacterial systems will also generally contain a Shine-Dalgarno sequence operably linked to the coding sequence.

In libraries or repertoires as described herein, the preferred vectors are expression vectors that enable the expression of a nucleotide sequence corresponding to a polypeptide library member. Thus, selection is performed by separate propagation and expression of a single clone expressing the polypeptide library

member or by use of any selection display system. As described above, a preferred selection display system uses bacteriophage display. Thus, phage or phagemid vectors can be used. Preferred vectors are phagemid vectors, which have an E. coli origin of replication (for double stranded replication) and also a phage origin of replication (for production of single-stranded DNA). The manipulation and expression of such vectors is well known in the art (Hoogenboom and Winter (1992) supra; Nissim et al. (1994) supra). Briefly, the vector contains a β-lactamase or other selectable marker gene to confer selectivity on the phagemid, and a lac promoter upstream of a expression cassette that consists (N to C terminal) of a pelB leader sequence (which directs the expressed polypeptide to the periplasmic space), a multiple cloning site (for cloning the nucleotide version of the library member), optionally, one or more peptide tags (for detection), optionally, one or more TAG stop codons and the phage protein pIII. In one embodiment, the vector encodes, rather than the pelB leader sequence, a eukaryotic GAS1 leader sequence which serves to direct the secretion of the fusion polypeptide to the periplasmic space in E. coli or to the medium in eukaryotic cell systems. Using various suppressor and non-suppressor strains of E. coli and with the addition of glucose, iso-propyl thio-β-D-galactoside (IPTG) or a helper phage, such as VCS M13, the vector is able to replicate as a plasmid with no expression, produce large quantities of the polypeptide library member only, or produce phage, some of which contain at least one copy of the polypeptide-pIII fusion on their surface.

An example of a preferred vector is the pHEN1 phagemid vector (Hoogenboom et al., 1991, Nucl. Acids Res. 19: 4133-4137; sequence is available, e.g., as SEQ ID NO: 7 in WO 03/031611), in which the production of pIII fusion protein is under the control of the LacZ promoter, which is inhibited in the presence of glucose and induced with IPTG. When grown in suppressor strains of E. coli, e.g., TG1, the gene III fusion protein is produced and packaged into phage, while growth in non-suppressor strains, e.g., HB2151, permits the secretion of soluble fusion protein into the bacterial periplasm and into the culture medium. Because the expression of gene III prevents later infection with helper phage, the bacteria

harboring the phagemid vectors are propagated in the presence of glucose before infection with VCSM13 helper phage for phage rescue.

Construction of vectors according to the invention employs conventional ligation techniques. Isolated vectors or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the required vector. If desired, sequence analysis to confirm that the correct sequences are present in the constructed vector is performed using standard methods. Suitable methods for constructing expression vectors, preparing in vitro transcripts, introducing DNA into host cells, and performing analyses for assessing expression and function are known to those skilled in the art. The presence of a gene sequence in a sample is detected, or its amplification and/or expression quantified by conventional methods, such as Southern or Northern analysis, Western blotting, dot blotting of DNA, RNA or protein, in situ hybridization, immunocytochemistry or sequence analysis of nucleic acid or protein molecules. Those skilled in the art will readily envisage how these methods may be modified, if desired.

Screening Single Immunoglobulin Variable Domains for Antigen Binding:

Following expression of a repertoire of single immunoglobulin variable domains on the surface of phage, selection is performed by contacting the phage repertoire with immobilized target antigen (e.g., CD40L and/or an epitope bound by DOM8-24), washing to remove unbound phage, and propagation of the bound phage, the whole process frequently referred to as "panning." This process is applicable to the screening of single immunoglobulin variable domains as well as other antibody fragments that can be expressed on a display library, e.g., scFv, Fab, etc. Alternatively, phage are pre-selected for the expression of properly folded member variants by panning against an immobilized generic ligand (e.g., protein A or protein L) that is only bound by folded members. This has the advantage of reducing the proportion of non-functional members, thereby increasing the proportion of members likely to bind a target antigen. Pre-selection with generic ligands is taught in WO

99/20749. The screening of phage antibody libraries is generally described, for example, by Harrison et al., 1996, Meth. Enzymol. 267: 83-109.

Screening is commonly performed using purified antigen immobilized on a solid support, for example, plastic tubes or wells, or on a chromatography matrix, for example Sepharose™ (Pharmacia). Screening or selection can also be performed on complex antigens, such as the surface of cells (Marks et al., 1993, BioTechnology 11: 1145; de Kruif et al., 1995, Proc. Natl. Acad. Sci. U.S.A. 92: 3938). Another alternative involves selection by binding biotinylated antigen in solution, followed by capture on streptavidin-coated beads.

In a preferred aspect, panning is performed by immobilizing antigen (generic or specific) on tubes or wells in a plate, e.g., Nunc MAXISORP™ immunotube 8 well strips. Wells are coated with 150 µl of antigen (100 µg/ml in PBS) and incubated overnight. The wells are then washed 3 times with PBS and blocked with 400 µl PBS-2% skim milk (2%MPBS) at 37°C for 2 hr. The wells are rinsed 3 times with PBS and phage are added in 2%MPBS. The mixture is incubated at room temperature for 90 minutes and the liquid, containing unbound phage, is removed. Wells are rinsed 10 times with PBS-0.1% tween 20, and then 10 times with PBS to remove detergent. Bound phage are eluted by adding 200 µl of freshly prepared 100 mM triethylamine, mixing well and incubating for 10 min at room temperature. Eluted phage are transferred to a tube containing 100 µl of 1M Tris-HCl, pH 7.4 and vortexed to neutralize the triethylamine. Exponentially-growing E. coli host cells (e.g., TG1) are infected with, for example, 150 ml of the eluted phage by incubating for 30 min at 37°C. Infected cells are spun down, resuspended in fresh medium and plated in top agarose. Phage plaques are eluted or picked into fresh cultures of host cells to propagate for analysis or for further rounds of selection. One or more rounds of plaque purification are performed if necessary to ensure pure populations of selected phage. Other screening approaches are described by Harrison et al., 1996, supra.

Following identification of phage expressing a single immunoglobulin variable domain that binds a desired target, if a phagemid vector such as pHEN1 has been used, the variable domain fusion proteins are easily produced in soluble form by infecting non-suppressor strains of bacteria, e.g., HB2151 that permit the secretion of soluble gene III fusion protein. If a GAS1 secretion signal peptide is encoded by the vector, the fusion polypeptide can be secreted by eukaryotic (e.g., yeast or mammalian) or prokaryotic (e.g., *E. coli*) cells. Alternatively, the V domain sequence can be sub-cloned into an appropriate expression vector to produce soluble protein according to methods known in the art.

Purification and Concentration of Single Immunoglobulin Variable Domains:

Single immunoglobulin variable domain polypeptides or other monovalent antibody polypeptides secreted into the periplasmic space or into the medium of bacteria are harvested and purified according to known methods (Harrison et al., 1996, supra). Skerra & Pluckthun (1988, Science 240: 1038) and Breitling et al. (1991, Gene 104: 147) describe the harvest of antibody polypeptides from the periplasm, and Better et al. (1988, Science 240: 1041) describes harvest from the culture supernatant. For some antibody polypeptides, purification can also be achieved by binding to generic ligands, such as protein A or Protein L. Alternatively, the variable domains can be expressed with a peptide tag, e.g., the Myc, HA or 6X-His tags, which facilitates purification by affinity chromatography.

If necessary, monovalent anti-CD40L antibody polypeptides are concentrated by any of several methods well known in the art, including, for example, ultrafiltration, diafiltration and tangential flow filtration. The process of ultrafiltration uses semi-permeable membranes and pressure to separate molecular species on the basis of size and shape. The pressure is provided by gas pressure or by centrifugation. Commercial ultrafiltration products are widely available, e.g., from Millipore (Bedford, MA; examples include the Centricon™ and Microcon™ concentrators) and Vivascience (Hannover, Germany; examples include the Vivaspin™ concentrators). By selection of a molecular weight cutoff smaller than the target polypeptide (usually 1/3 to 1/6 the molecular weight of the target

polypeptide, although differences of as little as 10 kD can be used successfully), the polypeptide is retained when solvent and smaller solutes pass through the membrane. Thus, a molecular weight cutoff of about 5 kD is useful for concentration of anti-CD40L single immunoglobulin variable domain polypeptides described herein.

Diafiltration, which uses ultrafiltration membranes with a "washing" process, is used where it is desired to remove or exchange the salt or buffer in a polypeptide preparation. The polypeptide is concentrated by the passage of solvent and small solutes through the membrane, and remaining salts or buffer are removed by dilution of the retained polypeptide with a new buffer or salt solution or water, as desired, accompanied by continued ultrafiltration. In continuous diafiltration, new buffer is added at the same rate that filtrate passes through the membrane. A diafiltration volume is the volume of polypeptide solution prior to the start of diafiltration – using continuous diafiltration, greater than 99.5% of a fully permeable solute can be removed by washing through six diafiltration volumes with the new buffer. Alternatively, the process can be performed in a discontinuous manner, wherein the sample is repeatedly diluted and then filtered back to its original volume to remove or exchange salt or buffer and ultimately concentrate the polypeptide. Equipment for diafiltration and detailed methodologies for its use are available, for example, from Pall Life Sciences (Ann Arbor, MI) and Sartorius AG/Vivascience (Hannover, Germany).

Tangential flow filtration (TFF), also known as "cross-flow filtration," also uses ultrafiltration membrane. Fluid containing the target polypeptide is pumped tangentially along the surface of the membrane. The pressure causes a portion of the fluid to pass through the membrane while the target polypeptide is retained above the filter. In contrast to standard ultrafiltration, however, the retained molecules do not accumulate on the surface of the membrane, but are carried along by the tangential flow. The solution that does not pass through the filter (containing the target polypeptide) can be repeatedly circulated across the membrane to achieve the desired degree of concentration. Equipment for TFF and detailed methodologies for its use are available, for example, from Millipore (e.g., the ProFlux M12^{TM} Benchtop TFF

system and the Pellicon™ systems), Pall Life Sciences (e.g., the Minim™ Tangential Flow Filtration system).

Protein concentration is measured in a number of ways that are well known in the art. These include, for example, amino acid analysis, absorbance at 280 nm, the "Bradford" and "Lowry" methods, and SDS-PAGE. The most accurate method is total hydrolysis followed by amino acid analysis by HPLC, concentration is then determined then comparison with the known sequence of the single immunoglobulin variable domain polypeptide. While this method is the most accurate, it is expensive and time-consuming. Protein determination by measurement of UV absorbance at 280 nm faster and much less expensive, yet relatively accurate and is preferred as a compromise over amino acid analysis. Absorbance at 280 nm was used to determine protein concentrations reported in the Examples described herein.

"Bradford" and "Lowry" protein assays (Bradford, 1976, Anal. Biochem. 72: 248-254; Lowry et al.,1951, J. Biol. Chem. 193: 265-275) compare sample protein concentration to a standard curve most often based on bovine serum albumin (BSA). These methods are less accurate, tending to undersetimate the concentration of single immunoglobulin variable domains. Their accuracy could be improved, however, by using a V_H or V_κ single domain polypeptide as a standard.

An additional protein assay method is the bicinchoninic acid assay described in U.S. Patent No. 4,839,295 (incorporated herein by reference) and marketed by Pierce Biotechnology (Rockford, IL) as the "BCA Protein Assay" (e.g., Pierce Catalog No. 23227).

The SDS-PAGE method uses gel electrophoresis and Coomassie Blue staining in comparison to known concentration standards, e.g., known amounts of a single immunoglobulin variable domain polypeptide. Quantitation can be done by eye or by densitometry.

Single human immunoglobulin variable domain antigen-binding polypeptides described herein retain solubility at high concentration (e.g., at least 4.8 mg (~400

μM) in aqueous solution (e.g., PBS), and preferably at least 5 mg/ml (~417 μM), 10 mg/ml (~833 μM), 20 mg/ml (~1.7 mM), 25 mg/ml (~2.1 mM), 30 mg/ml (~2.5 mM), 35 mg/ml (~2.9 mM), 40 mg/ml (~3.3 mM), 45 mg/ml (~3.75 mM), 50 mg/ml (~4.2 mM), 55 mg/ml (~4.6 mM), 60 mg/ml (~5.0 mM), 65 mg/ml (~5.4 mM), 70 mg/ml (~5.8 mM), 75 mg/ml (~6.3 mM), 100 mg/ml (~8.33 mM), 150 mg/ml (~12.5 mM), 200 mg/ml (~16.7 mM), 240 mg/ml (~20 mM) or higher). One structural feature that promotes high solubility is the relatively small size of the single immunoglobulin variable domain polypeptides. A full length conventional four chain antibody, e.g., IgG is about 150 kD in size. In contrast, single immunoglobulin variable domains, which all have a general structure comprising 4 framework (FW) regions and 3 CDRs, have a size of approximately 12 kD, or less than 1/10 the size of a conventional antibody. Similarly, single immunoglobulin variable domains are approximately ½ the size of an scFv molecule (~26 kD), and approximately 1/5 the size of a Fab molecule (~60 kD). It is preferred that the size of a single immunoglobulin variable domain-containing structure disclosed herein is 100 kD or less, including structures of, for example, about 90 kD or less, 80 kD or less, 70 kD or less, 60 kD or less, 50 kD or less, 40 kD or less, 30 kD or less, 20 kD or less, down to and including about 12 kD, or a single immunoglobulin variable domain in isolation.

The solubility of a polypeptide is primarily determined by the interactions of the amino acid side chains with the surrounding solvent. Hydrophobic side chains tend to be localized internally as a polypeptide folds, away from the solvent-interacting surfaces of the polypeptide. Conversely, hydrophilic residues tend to be localized at the solvent-interacting surfaces of a polypeptide. Generally, polypeptides having a primary sequence that permits the molecule to fold to expose more hydrophilic residues to the aqueous environment are more soluble than one that folds to expose fewer hydrophilic residues to the surface. Thus, the arrangement and number of hydrophobic and hydrophilic residues is an important determinant of solubility. Other parameters that determine polypeptide solubility include solvent pH, temperature, and ionic strength. In a common practice, the solubility of polypeptides can be maintained or enhanced by the addition of glycerol (e.g., ~10% v/v) to the solution.

As discussed above, specific amino acid residues have been identified in conserved residues of human V_H domains that vary in the V_H domains of camelid species, which are generally more soluble than human V_H domains. These include, for example, Gly 44 (Glu in camelids), Leu 45 (Arg in camelids) and Trp 47 (Gly in camelids). Amino acid residue 103 of V_H is also implicated in solubility, with mutation from Trp to Arg tending to confer increased V_H solubility.

In preferred aspects of the invention, single immunoglobulin variable domain polypeptides are based on the DP47 germline V_H gene segment or the DPK9 germline V_κ gene segment. Thus, these germline gene segments are capable, particularly when diversified at selected structural locations described herein, of producing specific binding single immunoglobulin variable domain polypeptides that are highly soluble. In particular, the four framework regions, which are preferably not diversified, can contribute to the high solubility of the resulting proteins.

It is expected that a single human immunoglobulin variable domain that is highly homologous to one having a known high solubility will also tend to be highly soluble. Thus, as one means of prediction or recognition that a given single immunoglobulin variable domain would have the high solubility recited herein, one can compare the sequence of a single immunoglobulin variable domain polypeptide to one or more single immunoglobulin variable domain polypeptides having known solubility. Thus, when a single immunoglobulin variable domain polypeptide is identified that has high binding affinity but unknown solubility, comparison of its amino acid sequence with that of one or more (preferably more) human single immunoglobulin variable domain polypeptides known to have high solubility (e.g., a dAb sequence disclosed herein) can permit prediction of its solubility. While it is not an absolute predictor, where there is a high degree of similarity to a known highly soluble sequence, e.g., 90-95% or greater similarity, and particularly where there is a high degree of similarity with respect to hydrophilic amino acid residues, or residues likely to be exposed at the solvent interface, it is more likely that a newly identified binding polypeptide will have solubility similar to that of the known highly soluble sequence.

Molecular modeling software can also be used to predict the solubility of a polypeptide sequence relative to that of a polypeptide of known solubility. For example, the substitution or addition of a hydrophobic residue at the solvent-exposed surface, relative to a molecule of known solubility that has a less hydrophobic or even hydrophilic residue exposed in that position is expected to decrease the relative solubility of the polypeptide. Similarly, the substitution or addition of a more hydrophilic residue at such a location is expected to increase the relative solubility. That is, a change in the net number of hydrophilic or hydrophobic residues located at the surface of the molecule (or the overall hydrophobic or hydrophilic nature of the surface-exposed residues) relative to a single immunoglobulin variable domain polypeptide structure with known solubility can predict the relative solubility of a single immunoglobulin variable domain polypeptide.

Alternatively, or in conjunction with such prediction, one can determine limits of a single immunoglobulin variable domain polypeptide's solubility by simply concentrating the polypeptide.

Affinity Determination:

Isolated single immunoglobulin variable domain- and antibody polypeptide-containing polypeptides as described herein preferably have affinities (dissociation constant, K_d, = K_{off}/K_{on}) of at least 500 nM or less, and preferably at least 400 nM-50 pM, 300 nM-50 pM, 200 nM – 50 pM, and more preferably at least 100 nM – 50 pM, 75 nM – 50 pM, 50 nM – 50 pM, 25 nM – 50 pM, 10 nM – 50 pM, 5 nM – 50 pM, 1 nM – 50 pM, 950 pM – 50 pM, 900 pM – 50 pM, 850 pM – 50 pM, 800 pM – 50 pM, 750 pM – 50 pM, 700 pM – 50 pM, 650 pM – 50 pM, 600 pM – 50 pM, 550 pM – 50 pM, 500 pM – 50 pM, 450 pM – 50 pM, 400 pM – 50 pM, 350 pM – 50 pM, 300 pM – 50 pM, 250 pM – 50 pM, 200 pM – 50 pM, 150 pM – 50 pM, 100 pM – 50 pM, 90 pM – 50 pM, 80 pM – 50 pM, 70 pM – 50 pM, 60 pM – 50 pM, or even as low as 50 pM.

The antigen-binding affinity of an antibody polypeptide, e.g., a single immunoglobulin variable domain polypeptide or other monovalent antibody polypeptide, can be conveniently measured by SPR using the BIAcore system

(Pharmacia Biosensor, Piscataway, N.J.). In this method, antigen is coupled to the BIAcore chip at known concentrations, and variable domain polypeptides are introduced. Specific binding between the variable domain polypeptide and the immobilized antigen results in increased protein concentration on the chip matrix and a change in the SPR signal. Changes in SPR signal are recorded as resonance units (RU) and displayed with respect to time along the Y axis of a sensorgram. Baseline signal is taken with solvent alone (e.g., PBS) passing over the chip. The net difference between baseline signal and signal after completion of antibody polypeptide injection represents the binding value of a given sample. To determine the off rate (K_{off}), on rate (K_{on}) and dissociation rate (K_d) constants, BIAcore kinetic evaluation software (e.g., version 2.1) is used.

Thus, SPR can be used to monitor antagonism of CD40L binding to CD40 by a monovalent anti-CD40L antibody preparation by measuring the displacement or inhibition of binding of CD40L to CD40 caused the monovalent antibody preparation. SPR can also be used to monitor the dimerization, or preferably, the lack of dimerization, occurring via Fc region in antibody preparations as described herein.

High affinity is dependent upon the complementarity between a surface of the antigen and the CDRs of the antibody or antibody fragment. Complementarity is determined by the type and strength of the molecular interactions possible between portions of the target and the CDR, for example, the potential ionic interactions, van der Waals attractions, hydrogen bonding or other interactions that can occur. CDR3 tends to contribute more to antigen binding interactions than CDRs 1 and 2, probably due to its generally larger size, which provides more opportunity for favorable surface interactions. (See, e.g., Padlan et al., 1994, Mol. Immunol. 31: 169-217; Chothia & Lesk, 1987, J. Mol. Biol. 196: 904-917; and Chothia et al., 1985, J. Mol. Biol. 186: 651-663.) High affinity indicates antibody polypeptide/antigen pairings that have a high degree of complementarity, which is directly related to the structures of the variable domain and the target.

In one aspect, a monovalent anti-CD40L antibody polypeptide, e.g., a single immunoglobulin variable domain polypeptide, is linked to another antibody polypeptide to form a heterodimer in which each individual antibody polypeptide is capable of binding a different cognate antigen. Fusing antibody polypeptides, such as single immunoglobulin variable domains, as heterodimers, wherein each monomer binds a different target antigen, can produce a dual-specific ligand capable, for example, of bridging the respective target antigens. Such dual specific ligands may be used to target cytokines and other molecules which cooperate synergistically in therapeutic situations in the body of an organism. Thus, there is provided a method for synergising the activity of two or more cytokines, comprising administering a dual specific antibody heterodimer capable of binding to the two or more cytokines.

Non-limiting examples of second targets for anti-CD40L dual specific antibody polypeptides include the following: TNF-α; IL-1; IL-2; IL-4; IL-6; IL-8; IL-12; IL-18; IFN-γ; CD2; CD4; CD8; CTLA4; LFA1; LFA3, VLA4, CD80, B7-1, CD28, CD86, B7-2, and CTLA-4. In particular, second targets useful according to the invention include CD80, B7-1, CD28, CD86, B7-2, and CTLA-4. These targets are thought to be involved in a co-stimulatory pathway critical for T-cell activation (termed, co-stimulatory signal pathway antigens). This pathway includes activation of the molecule CD28 on the surface of T cells. This molecule can receive a costimulatory signal delivered by a ligand on B cells or other APCs. Ligands for CD28 include members of the B7 family of B lymphocyte activation antigens, such as B7-1 and/or B7-2 (Freedman, A. S. et al. (1987) J. Immunol. 137, 3260-3267; Freeman, G. J. et al. (1989) J. Immunol. 143, 2714-2722; Freeman, G. J. et al. (1991) J. Exp. Med. 174, 625-631; Freeman, G. J. et al. (1993) Science 262, 909-911; Azuma, M. et al. (1993) Nature 366, 76-79; Freeman, G. J. et al. (1993) J. Exp. Med. 178, 2185-2192). B7-1 and B7-2 are also ligands for another molecule, CTLA4, present on the surface of activated T cells. Accordingly, the present invention contemplates that members of the CD28 signalling pathway may be useful second targets for the dual-specific format anti-CD40L antibody polypeptides.

Homologous sequences:

The invention encompasses anti-CD40L antibody polypeptides, e.g., CD40L-binding single immunoglobulin variable domain clones, and clones with substantial sequence similarity or homology to them that also bind target antigen with high affinity. As used herein, "substantial" sequence similarity or homology is at least 85% similarity or homology.

Calculations of "homology" or "sequence identity" between two sequences (the terms are equivalent and used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

As used herein, sequence "similarity" is a measure of the degree to which amino acid sequences share similar amino acid residues at corresponding positions in an alignment of the sequences. Amino acids are similar to each other where their side chains are similar. Specifically, "similarity" encompasses amino acids that are conservative substitutes for each other. A "conservative" substitution is any substitution that has a positive score in the blosum62 substitution matrix (Hentikoff and Hentikoff, 1992, Proc. Natl. Acad. Sci. USA 89: 10915-10919). By the statement

"sequence A is n% similar to sequence B" is meant that n% of the positions of an optimal global alignment between sequences A and B consists of identical amino acids or conservative substitutions. Optimal global alignments can be performed using the following parameters in the Needleman-Wunsch alignment algorithm:

For polypeptides:

Substitution matrix: blosum62.

Gap scoring function: -A -B*LG, where A=11 (the gap penalty), B=1 (the gap length penalty) and LG is the length of the gap.

For nucleotide sequences:

Substitution matrix: 10 for matches, 0 for mismatches.

Gap scoring function: -A -B*LG where A=50 (the gap penalty), B=3 (the gap length penalty) and LG is the length of the gap.

Typical conservative substitutions are among Met, Val, Leu and Ile; among Ser and Thr; among the residues Asp, Glu and Asn; among the residues Gln, Lys and Arg; or aromatic residues Phe and Tyr. In calculating the degree (most often as a percentage) of similarity between two polypeptide sequences, one considers the number of positions at which identity or similarity is observed between corresponding amino acid residues in the two polypeptide sequences in relation to the entire lengths of the two molecules being compared.

Alternatively, the BLAST (Basic Local Alignment Search Tool) algorithm is employed for sequence alignment, with parameters set to default values. The BLAST algorithm "BLAST 2 Sequences" is available at the world wide web site ("www") of the National Center for Biotechnology Information (".ncbi"), of the National Library of Medicine (".nlm") of the National Institutes of Health ("nih") of the U.S. government (".gov"), in the "/blast/" directory, sub-directories "bl2seq/bl2.html." This algorithm aligns two sequences for comparison and is described by Tatusova & Madden, 1999, FEMS Microbiol Lett. 174:247-250.

An additional measure of homology or similarity is the ability to hybridize under highly stringent hybridization conditions. Thus, a first sequence encoding a single immunoglobulin variable domain polypeptide is substantially similar to a

second coding sequence if the first sequence hybridizes to the second sequence (or its complement) under highly stringent hybridization conditions (such as those described by Sambrook et al., Molecular Cloning, Laboratory Manuel, Cold Spring, Harbor Laboratory press, New York). "Highly stringent hybridization conditions" refer to hybridization in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. "Very highly stringent hybridization conditions" refer to hybridization in 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C.

Assays for CD40L Activities:

It is preferred that a monovalent anti-CD40L antibody polypeptides as described herein bind to CD40L yet do not substantially agonize CD40 signaling. Activation of the CD40L/CD40 pathway manifests a number of different outcomes that can be measured in order to assess the effect of a given monovalent anti-CD40L antibody polypeptide on the activity of the pathway. However, for the assessment of the antagonist or agonist function of monovalent anti-CD40L antibody polypeptides described herein, at least one of the following CD40L assays can be used:

1) Activation of Jun-N-Terminal Kinase (JNK):

Stimulation of T-lymphocytes via CD40L induces strong activation of JNK. The ability of a monovalent anti-CD40L antibody polypeptide to activate this signaling pathway is measured as follows. Human leukemic Jurkat cells are stimulated with a positive control agonistic anti-CD40L antibody (2 μg/ml monoclonal anti-human or anti-mouse gp39/CD40L antibody (Pharmingen, San Diego, CA, USA) or isotype matched hamster or mouse immunoglobulins (Dianova, Hamburg, Germany)), monovalent anti-CD40L antibody polypeptide, or a negative control irrelevant antibody as described by Brenner et al., 1997, FEBS Lett. 417: 301-306, which is incorporated herein by reference. The cells are lysed and the extract assayed for phosphorylated JNK via colorimetric assay (e.g., Titerzyme™ colorimetric (EIA) phospho-JNK1/2 immunoassay kit, by Assay Designs Inc.,

Catalog # 900-106). An increase in phospho-JNK (e.g., by 5% or more) for anti-CD40L-stimulated cells over non-stimulated cells indicates agonism of CD40L activity by the antibody polypeptide.

2. Induction of Cytokine Secretion:

Co-stimulation of T cells with anti-CD3 Ab and CD40L has been shown to upregulate the production of IL-10, IFN-γ and TNF-α by those cells. The ability of a monovalent anti-CD40L antibody polypeptide to activate this signaling pathway is measured as follows. Human leukemic Jurkat T cells (or freshly isolated CD4+ T cells) are plated into 96 well plates containing immobilized anti-CD3 antibody. The cells are then cultured for 72 hours in the presence of a positive control agonistic anti-CD40L antibody, CD40L, monovalent anti-CD40L antibody polypeptide, or a negative control irrelevant antibody, as described by Blair et al., 2000, J. Exp. Med. 191: 651-660. IFN-γ (or IL-10 of TNF-α) is then quantitated in the supernatant by sandwich ELISA using an IFN-g standard to generate a standard curve from which all other unknowns can be calculated. An increase in IFN-g (e.g., by 5% or more) for anti-CD40L-stimulated cells over non-stimulated cells indicates agonism by the antibody polypeptide.

3. Platelet Aggregation Assay:

Divalent anti-CD40L antibodies tend to cause platelet aggregation, which is likely associated with the thromboembolic events observed in clinical trials of divalent anti-CD40L antibodies in the prior art. Monovalent anti-CD40L antibody polypeptides as described herein preferably do not substantially mediate or agonize CD40L-mediated platelet aggregation. With regard tothis aspect, the "standard platelet aggregation assay" is as follows:

Platelets are prepared at $2.5x10^5$/ml and left stirring in a 500-Ca lumi-aggregometer (or its equivalent, e.g., a Platelet Aggregation Profiler (BioData, Horsham, PA)). Platelets are partially activated by the addition of a dilution series of 0.1-10 μM ADP (the 10μM ADP induces aggregation, and is used as a positive

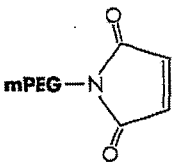
control - lower concentrations activate platelets but do not induce aggregation). CD40L mediated platelet aggregation is stimulated by addition of either anti-CD40L monoclonal antibodies (i.e., divalent monoclonal antibodies, available from, e.g., Pharmingen, San Diego, CA, USA) or soluble CD40/Fc fusion protein (available from R&D Systems). The reaction is allowed to proceed for between 3 and 5 minutes. Stimulation of platelet aggregation above the mininimal aggregation/activation achieved with ADP alone is plotted against stimulating anti-CD40L or CD40/Fc concentration. The percentage of platelet aggregation is measured by the change in light transmittance following addition of antibody polypeptide being tested or positive control peptide. A value greater than that observed for negative control lacking antibody and amounting to 25% or more of the positive control value (divalent anti-CD40L or CD40/Fc fusion) is considered to be indicative of induction of platelet aggregation.

Other methods to assess platelet aggregation and/or activation, including events which precede aggregation, or which are downstream from platelet aggregation, include assays which determine various indicators of platelet activation, and are known in the art. For example, platelet activation (and, thus, CD40/CD40L activity) can be determined by assaying for CD62P expression in platelets (e.g., using anti-CD26P antibodies and flow cytometry), assaying for monocyte-platelet-conjugate formation, assaying for platelet closure time under high shear conditions (e.g., using a PFA-100, Dade Behring, Newark, DE), and assaying for platelet dense granule release. Methods for performing such assays are known in the art and can be found, for example, in Langer et al., 2005 Thromb. Haemost. 93: 1137-46.

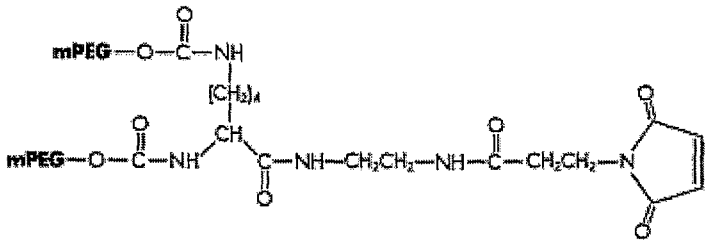
PEGylation of monovalent anti-CD40L antibody polypeptides

The present invention provides PEGylated monovalent anti-CD40L antibody polypeptides which have increased half-life and preferably also resistance to degradation without a loss in activity (e.g., binding affinity) relative to non-PEGylated antibody polypeptides.

Monovalent anti-CD40L antibody polypeptides according to this aspect can be coupled, using methods known in the art to polymer molecules (preferably PEG) useful for achieving the increased half-life and degradation resistance properties encompassed by the present invention. Polymer moieties which can be utilized in the invention can be synthetic or naturally occurring and include, but are not limited to straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymers, or a branched or unbranched polysaccharide such as a homo- or heteropolysaccharide. Preferred examples of synthetic polymers which may be used in the invention include straight or branched chain poly(ethylene glycol) (PEG), poly(propylene glycol), or poly(vinyl alcohol) and derivatives or substituted forms thereof. Particularly preferred substituted polymers useful in the invention include substituted PEG, including methoxy(polyethylene glycol). Naturally occurring polymer moieties which may be used according to the invention in addition to or in place of PEG include lactose, amylose, dextran, or glycogen, as well as derivatives thereof which would be recognized by one of skill in the art. Derivatized forms of polymer molecules of the invention include, for example, derivatives which have additional moieties or reactive groups present therein to permit interaction with amino acid residues of the dAb polypeptides described herein. Such derivatives include N-hydroxylsuccinimide (NHS) active esters, succinimidyl propionate polymers, and sulfhydryl-selective reactive agents such as maleimide, vinyl sulfone, and thiol. Particularly preferred derivatized polymers include, but are not limited to PEG polymers having the formulae: PEG-O-$CH_2CH_2CH_2$-CO_2-NHS; PEG-O-CH_2-NHS; PEG-O-CH_2CH_2-CO_2-NHS; PEG-S-CH_2CH_2-CO-NHS; PEG-O_2CNH-CH(R)-CO_2-NHS; PEG-NHCO-CH_2CH_2-CO-NHS; and PEG-O-CH_2-CO_2-NHS; where R is $(CH_2)_4$)NHCO_2(mPEG). PEG polymers useful in the invention may be linear molecules, or may be branched wherein multiple PEG moieties are present in a single polymer. Some particularly preferred PEG derivatives which are useful in the invention include, but are not limited to the following:



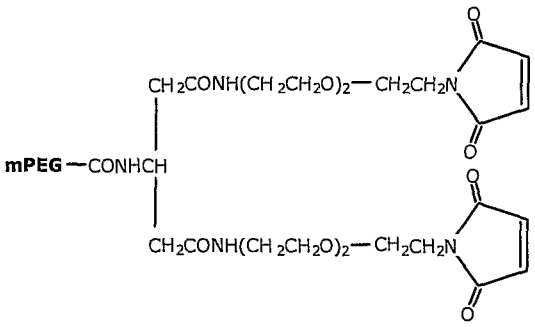
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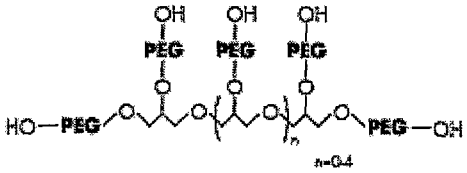
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mPEG-MAL

mPEG2-MAL



;

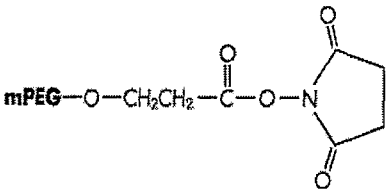


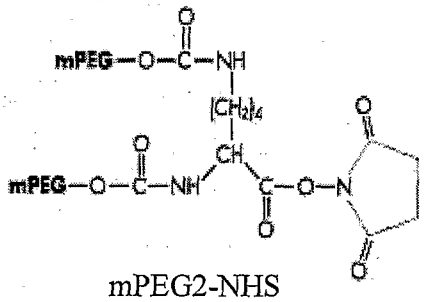
mPEG-(MAL)^2

multi-arm PEG

;

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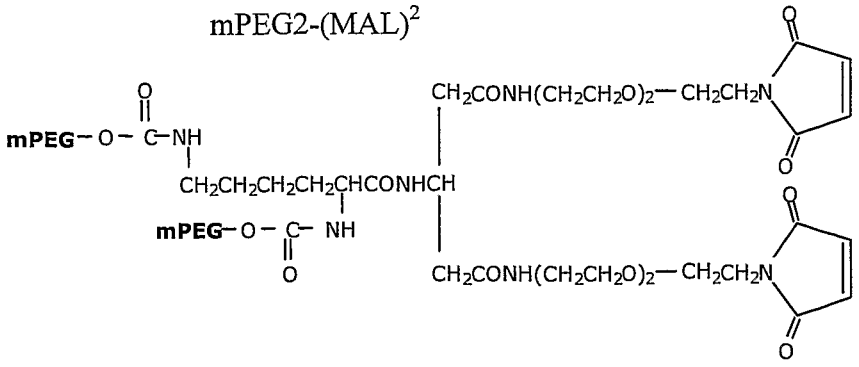


mPEG-SPA

mPEG2-NHS

;

mPEG2-(MAL)^2



; and

The reactive group (e.g., MAL, NHS, SPA, VS, or Thiol) may be attached directly to the PEG polymer or may be attached to PEG via a linker molecule.

The size of polymers useful in the invention can be in the range of between 500 Da to 60 kDa, for example, between 1000 Da and 60 kDa, 10 kDa and 60 kDa, 20 kDa and 60 kDa, 30 kDa and 60 kDa, 40 kDa and 60 kDa, and up to between 50 kDa and 60 kDa. The polymers used in the invention, particularly PEG, can be straight chain polymers or can possess a branched conformation. Depending on the combination of molecular weight and conformation, the polymer molecules useful in the invention, when attached to a monovalent anti-CD40L antibody polypeptide, will yield a molecule having an average hydrodynamic size of between 24 and 500 kDa. The hydrodynamic size of a polymer molecule used herein refers to the apparent size of a molecule (e.g., a protein molecule) based on the diffusion of the molecule through an aqueous solution. The diffusion, or motion of a protein through solution can be processed to derive an apparent size of the protein, where the size is given by the Stokes radius or hydrodynamic radius of the protein particle. The "hydrodynamic size" of a protein depends on both mass and shape (conformation), such that two proteins having the same molecular mass may have differing hydrodynamic sizes based on the overall conformation of the protein. The hydrodynamic size of a PEG-linked monovalent anti-CD40L antibody polypeptide, e.g., an anti-CD40L single

immunoglobulin variable domain as described herein, can be in the range of 24 kDa to 500 kDa; 30 to 500 kDa; 40 to 500 kDa; 50 to 500 kDa; 100 to 500 kDa; 150 to 500 kDa; 200 to 500 kDa; 250 to 500 kDa; 300 to 500 kDa; 350 to 500 kDa; 400 to 500 kDa and 450 to 500 kDa. Preferably the hydrodynamic size of a PEGylated antibody polypeptide as described herein is 30 to 40 kDa; 70 to 80 kDa or 200 to 300 kDa. The size of a polymer molecule attached to a monovalent anti-CD40L antibody polypeptide may thus be varied depending upon the desired application. For example, where the PEGylated antibody polypeptide is intended to leave the circulation and enter into peripheral tissues, it is desirable to keep the size of the attached polymer low to facilitate extravazation from the blood stream. Alternatively, where it is desired to have the PEGylated antibody polypeptide remain in the circulation for a longer period of time, a higher molecular weight polymer can be used (e.g., a 30 to 60 kDa polymer).

The polymer (PEG) molecules useful in the invention can be attached to antibody polypeptides using methods that are well known in the art. The first step in the attachment of PEG or other polymer moieties to an antibody polypeptide is the substitution of the hydroxyl end-groups of the PEG polymer by electrophile-containing functional groups. Particularly, PEG polymers are attached to either cysteine or lysine residues present in the antibody polypeptide. The cysteine and lysine residues can be naturally occurring, or can be engineered into the antibody polypeptide molecule. For example, cysteine residues can be recombinantly engineered at the C-terminus of antibody polypeptides, or residues at specific solvent accessible locations in the antibody polypeptide can be substituted with cysteine or lysine. In a preferred embodiment, a PEG moiety is attached to a cysteine residue which is present in the hinge region at the C-terminus of an antibody polypeptide.

In a further preferred embodiment a PEG moiety or other polymer is attached to a cysteine or lysine residue which is either naturally occurring at or engineered into the N-terminus of antibody single variable domain polypeptide of the invention. In a still further embodiment, a PEG moiety or other polymer is attached to an antibody single variable domain according to the invention at a cysteine or lysine residue

(either naturally occurring or engineered) which is at least 2 residues away from (e.g., internal to) the C- and/or N-terminus of the antibody single variable domain polypeptide.

In one embodiment, the PEG polymer(s) is attached to one or more cysteine or lysine residues present in a framework region (FWs) and one or more heterologous CDRs of a single immunoglobulin variable domain. CDRs and framework regions (e.g., CDR1-CDR3 and FW1-FW4) are those regions of an immunoglobulin variable domain as defined in the Kabat database of Sequences of Proteins of Immunological Interest (Kabat et al., 1991, supra). In a preferred embodiment, a PEG polymer is linked to a cystine or lysine residue in the V_H framework segment DP47, or the V_k framework segment DPK9. Cysteine and/or lysine residues of DP47 which may be linked to PEG according to the invention include the cysteine at positions 22, or 96 and the lysine at positions 43, 65, 76, or 98 of SEQ ID NO: 1 (Figure 5). Cysteine and/or lysine residues of DPK9 which may be linked to PEG according to the invention include the cysteine residues at positions 23, or 88 and the lysine residues at positions 39, 42, 45, 103, or 107 of SEQ ID NO: 3 (Figure 6). In addition, specific cysteine or lysine residues may be linked to PEG in the V_H canonical framework region DP38, or DP45.

In addition, specific solvent accessible sites in the antibody molecule which are not naturally occurring cysteine or lysine residues may be mutated to a cysteine or lysine for attachment of a PEG polymer. Solvent accessible residues in any given antibody, e.g., a dAb, can be determined using methods known in the art such as analysis of the crystal structure of the antibody polypeptide. For example, using the solved crystal structure of the V_H dAb HEL4 (SEQ ID NO: 3; a dAb that binds hen egg lysozyme), the residues Gln-13, Pro-14, Gly-15, Pro-41, Gly-42, Lys-43, Asp-62, Lys-65, Arg-87, Ala-88, Glu-89, Gln-112, Leu-115, Thr-117, Ser-119, and Ser-120 have been identified as being solvent accessible, and according to the present invention would be attractive candidates for mutation to cysteine or lysine residues for the attachment of a PEG polymer. In addition, using the solved crystal structure of the V_k dummy dAb (SEQ ID NO: 4), the residues Val-15, Pro-40, Gly-41, Ser-56,

Gly-57, Ser-60, Pro-80, Glu-81, Gln-100, Lys-107, and Arg-108 have been identified as being solvent accessible, and according to the present invention would be attractive candidates for mutation to cysteine or lysine residues for the attachment of a PEG polymer. In one embodiment of the invention, a PEG polymer is linked to multiple solvent accessible cysteine or lysine residues, or to solvent accessible residues which have been mutated to a cysteine or lysine residue. Alternatively, only one solvent accessible residue is linked to PEG, either where the particular antibody polypeptide only possesses one solvent accessible cysteine or lysine (or residue modified to a cysteine or lysine) or where a particular solvent accessible residue is selected from among several such residues for PEGylation.

Primary amino acid sequence of HEL4 (SEQ ID NO: 5).

1 EVQLLESGGG LVQPGGSLRL SCAASGFRIS DEDMGWVRQA PGKGLEWVSS

51 IYGPSGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCASAL

101 EPLSEPLGFW GQGTLVTVSS

Primary amino acid sequence of V_k dummy (SEQ ID NO: 6).

1 DIQMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYA

51 ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYSTPNTFGQ

101 GTKVEIKR

Several PEG attachment schemes which are useful in the invention are provided by the company Nektar (SanCarlos, CA). For example, where attachment of PEG or other polymer to a lysine residue is desired, active esters of PEG polymers which have been derivatized with N-hydroxylsuccinimide, such as succinimidyl propionate may be used. Where attachment to a cysteine residue is intended, PEG polymers which have been derivatized with sulfhydryl-selective reagents such as maleimide, vinyl sulfone, or thiols may be used. Other examples of specific

embodiments of PEG derivatives which may be used according to the invention to generate PEGylated antibodies can be found in the Nektar Catalog (available on the world wide web at nektar.com). In addition, several derivitized forms of PEG may be used according to the invention to facilitate attachment of the PEG polymer to an antibody polypeptide. PEG derivatives useful in the invention include, but are not limited to PEG-succinimidyl succinate, urethane linked PEG, PEG phenylcarbonate, PEG succinimidyl carbonate, PEG-carboxymethyl azide, dimethylmaleic anhydride PEG, PEG dithiocarbonate derivatives, PEG-tresylates (2,2,2-trifluoroethanesolfonates), mPEG imidoesters, and other as described in Zalipsky and Lee, (1992) ("Use of functionalized poly(ethylene glycol)s for modification of peptides" in Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, J. Milton Harris, Ed., Plenum Press, NY).

In one embodiment, the invention provides an anti-CD40L antibody single variable domain composition comprising an antibody single variable domain and PEG polymer wherein the ratio of PEG polymer to antibody single variable domain is a molar ratio of at least 0.25:1. In a further embodiment, the molar ratio of PEG polymer to antibody single variable domain is 0.33:1 or greater. In a still further embodiment the molar ratio of PEG polymer to antibody single variable domain is 0.5:1 or greater.

Dual-specific Ligands

The invention also provides dual-specific ligands comprising immunoglobulin single variable domains which each have different specificities; that is, the first and the second epitopes bound by the dual-specific ligand are preferably different. As used herein a "dual-specific ligand" refers to a ligand comprising a first immunoglobulin single variable domain and a second immunoglobulin single variable domain as herein defined, wherein the variable regions are capable of binding to two different antigens or two epitopes on the same antigen which are not normally bound by a monospecific immunoglobulin. For example, the two epitopes may be on the same hapten, but are not the same epitope or sufficiently adjacent to be bound by a monospecific ligand. The dual specific ligands according to the invention are

composed of variable domains which have different specificities, and do not contain mutually complementary variable domain pairs which have the same specificity. Dual-specific ligands may be, or be part of, polypeptides, proteins or nucleic acids, which may be naturally occurring or synthetic. In this respect, the ligand of the invention may bind an epiotpe or antigen and act as an antagonist or agonist (eg, EPO receptor agonist). The epitope binding domains of the ligand in one embodiment have the same epitope specificity, and may for example simultaneously bind their epitope when multiple copies of the epitope are present on the same antigen. In another embodiment, these epitopes are provided on different antigens such that the ligand can bind the epitopes and bridge the antigens. One skilled in the art will appreciate that the choice of epitopes and antigens is large and varied. They may be for instance human or animal proteins, cytokines, cytokine receptors, enzymes co-factors for enzymes or DNA binding proteins. Suitable cytokines and growth factors include but are not limited to: ApoE, Apo-SAA, BDNF, Cardiotrophin-1, EGF, EGF receptor, ENA-78, Eotaxin, Eotaxin-2, Exodus-2, EpoR, FGF-acidic, FGF-basic, fibroblast growth factor-10, FLT3 ligand, Fractalkine (CX3C), GDNF, G-CSF, GM-CSF, GF-β1, insulin, IFN-γ, IGF-I, IGF-II, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (72 a.a.), IL-8 (77 a.a.), IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18 (IGIF), Inhibin α, Inhibin β, IP-10, keratinocyte growth factor-2 (KGF-2), KGF, Leptin, LIF, Lymphotactin, Mullerian inhibitory substance, monocyte colony inhibitory factor, monocyte attractant protein, M-CSF, MDC (67 a.a.), MDC (69 a.a.), MCP-1 (MCAF), MCP-2, MCP-3, MCP-4, MDC (67 a.a.), MDC (69 a.a.), MIG, MIP-1α, MIP-1β, MIP-3α, MIP-3β, MIP-4, myeloid progenitor inhibitor factor-1 (MPIF-1), NAP-2, Neurturin, Nerve growth factor, β-NGF, NT-3, NT-4, Oncostatin M, PDGF-AA, PDGF-AB, PDGF-BB, PF-4, RANTES, SDF1α, SDF1β, SCF, SCGF, stem cell factor (SCF), TARC, TGF-α, TGF-β, TGF-β2, TGF-β3, tumour necrosis factor (TNF), TNF-α, TNF-β, TNF receptor I, TNF receptor II, TNIL-1, TPO, VEGF, VEGF receptor 1, VEGF receptor 2, VEGF receptor 3, GCP-2, GRO/MGSA, GRO-β, GRO-γ, HCC1, 1-309, HER 1, HER 2, HER 3, HER 4, TACE recognition site, TNF BP-I and TNF BP-II, as well as any target disclosed in Annex 2 or Annex 3 hereto, whether in combination as set forth in the Annexes, in a different combination or

individually. Cytokine receptors include receptors for the foregoing cytokines, e.g. IL-1 R1; IL-6R; IL-10R; IL-18R, as well as receptors for cytokines set forth in Annex 2 or Annex 3 and also receptors disclosed in Annex 2 and 3. It will be appreciated that this list is by no means exhaustive. Where the multispecific ligand binds to two epitopes (on the same or different antigens), the antigen(s) may be selected from this list.

In one embodiment of the second configuration of the invention, the variable domains are derived from an antibody directed against the first and/or second antigen or epitope. In a preferred embodiment the variable domains are derived from a repertoire of single variable antibody domains. In one example, the repertoire is a repertoire that is not created in an animal or a synthetic repertoire. In another example, the single variable domains are not isolated (at least in part) by animal immunisation. Thus, the single domains can be isolated from a naïve library.

In another aspect, the invention provides a multi-specific ligand comprising a first epitope binding domain having a first epitope binding specificity and a non-complementary second epitope binding domain having a second epitope binding specificity. The first and second binding specificities may be the same or different.

In a further aspect, the present invention provides a closed conformation multi-specific ligand comprising a first epitope binding domain having a first epitope binding specificity and a non-complementary second epitope binding domain having a second epitope binding specificity wherein the first and second binding specificities are capable of competing for epitope binding such that the closed conformation multi-specific ligand cannot bind both epitopes simultaneously.

In a still further aspect, the invention provides open conformation ligands comprising non-complementary binding domains, wherein the domains are specific for a different epitope on the same target. Such ligands bind to targets with increased avidity. Similarly, the invention provides multivalent ligands comprising non-complementary binding domains specific for the same epitope and directed to targets which comprise multiple copies of said epitope.

In a similar aspect, ligands according to the invention can be configured to bind individual epitopes with low affinity, such that binding to individual epitopes is not therapeutically significant; but the increased avidity resulting from binding to two epitopes provides a therapeutic benefit. In a particular example, epitopes may be targeted which are present individually on normal cell types, but present together only on abnormal or diseased cells, such as tumour cells. In such a situation, only the abnormal or diseased cells are effectively targeted by the bispecific ligands according to the invention.

Ligand specific for multiple copies of the same epitope, or adjacent epitopes, on the same target (known as chelating dAbs) may also be trimeric or polymeric (tertrameric or more) ligands comprising three, four or more non-complementary binding domains. For example, ligands may be constructed comprising three or four V_H domains or V_L domains.

Moreover, ligands are provided which bind to multisubunit targets, wherein each binding domain is specific for a subunit of said target. The ligand may be dimeric, trimeric or polymeric.

The invention also includes a dual specific ligand comprising a first immunoglobulin single variable domain having a binding specificity to a first antigen and a second single variable domain having a binding activity to a second antigen, wherein the first antigen is CD40L and the second single variable domain is an Antigen Presenting Cell surface antigen or a T cell surface antigen. The Antigen Presenting Cell (APC) surface antigen can be selected from one of the group consisting of dendritic cell surface antigens, activated macrophage surface antigens, activated B cell surface antigens, co-stimulatory signal pathway surface antigens, and MHC, such as MHC II alpha or beta.

The (APC) surface antigen may be selected from the group consisting of CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, CD3, CD70, Inducible costimulatory molecule ligand (ICOSL), OX40L, CD80, CD86, HVEM (Herpes Virus Entry Mediator), and LIGHT, but is preferably one of

CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, or CD3.

The surface antigen is preferably a B7 gene surface antigen such as B7-2 or B7-1.

Dendritic cell surface antigens are known in the art and can include but are not limited to ICAM-1, ICAM-2, LFA-1, LFA-3, DEC205, MHC class I, MHC class II, B7-1, and B7-2. Activated macrophage surface antigens include, but are not limited to, TNF receptor, CD40, MHC class I and II, and B7 molecules. Activated B-cell surface antigens are known in the art (e.g., including but not limited to CD20 and CD86)and further described above (see, for example, Janeway et al., 1999, Immunobiology, Garland Publishing NY, NY).

Preferably, the multi-specific ligands according to the above aspects of the invention are obtainable by the method comprising the steps of:

a) selecting a first epitope binding domain by its ability to bind to a first epitope,

b) selecting a second epitope binding domain by its ability to bind to a second epitope,

c) combining the epitope binding domains; and

d) selecting the closed conformation multispecific ligand by its ability to bind to said first second epitope and said second epitope..

Advantageously the first epitope binding domain and the second epitope binding domains are non-complementary immunoglobulin variable domains, as herein defined. That is either V_H-V_H or V_L-V_L variable domains.

Chelating dAbs in particular may be prepared according to a preferred aspect of the invention, namely the use of anchor dAbs, in which a library of dimeric, trimeric or multimeric dAbs is constructed using a vector which comprises a constant dAb upstream or downstream of a linker sequence, with a repertoire of second, third

and further dAbs being inserted on the other side of the linker. In alternative methodologies, the use of linkers may be avoided, for example by the use of non-covalent bonding or natural affinity between binding domains such as V_H and V_K. The invention accordingly provides a method for preparing a multimeric ligand comprising the steps of:

(a) providing a vector comprising a nucleic acid sequence encoding a single binding domain specific for a first epitope on a target;

(b) providing a vector encoding a repertoire comprising second binding domains specific for a second epitope on said target, which epitope can be the same or different to the first epitope, said second epitope being adjacent to said first epitope; and

(c) expressing said first and second binding domains; and

(d) isolating those combinations of first and second binding domains which combine together to produce a target-binding dimer.

The first and second epitopes are adjacent such that a multimeric ligand is capable of binding to both epitopes simultaneously. This provides the ligand with the advantages of increased avidity if binding. Where the epitopes are the same, the increased avidity is obtained by the presence of multiple copies of the epitope on the target, allowing at least two copies to be simultaneously bound in order to obtain the increased avidity effect.

In an alternative embodiment of the above aspect of the second configuration of the invention, at least one epitope binding domain comprises a non-immunoglobulin 'protein scaffold' or 'protein skeleton' as herein defined. Suitable non-immunoglobulin protein scaffolds include but are not limited to any of those selected from the group consisting of: SpA, fibronectin, GroEL and other chaperones, lipocallin, CCTLA4 and affibodies, as set forth above.

According to the above aspect of the second configuration of the invention, advantageously, the epitope binding domains are attached to a 'protein skeleton'. Advantageously, a protein skeleton according to the invention is an immunoglobulin skeleton.

According to the present invention, the term 'immunoglobulin skeleton' refers to a protein which comprises at least one immunoglobulin fold and which acts as a nucleus for one or more epitope binding domains, as defined herein.

Preferred immunoglobulin skeletons as herein defined includes any one or more of those selected from the following: an immunoglobulin molecule comprising at least (i) the CL (kappa or lambda subclass) domain of an antibody; or (ii) the CH1 domain of an antibody heavy chain; an immunoglobulin molecule comprising the CH1 and CH2 domains of an antibody heavy chain; an immunoglobulin molecule comprising the CH1, CH2 and CH3 domains of an antibody heavy chain; or any of the subset (ii) in conjunction with the CL (kappa or lambda subclass) domain of an antibody. A hinge region domain may also be included. Such combinations of domains may, for example, mimic natural antibodies, such as IgG or IgM, or fragments thereof, such as Fv, scFv, Fab or $F(ab')_2$ molecules. Those skilled in the art will be aware that this list is not intended to be exhaustive.

Linking of the skeleton to the epitope binding domains, as herein defined may be achieved at the polypeptide level, that is after expression of the nucleic acid encoding the skeleton and/or the epitope binding domains. Alternatively, the linking step may be performed at the nucleic acid level. Methods of linking a protein skeleton according to the present invention, to the one or more epitope binding domains include the use of protein chemistry and/or molecular biology techniques which will be familiar to those skilled in the art and are described herein.

Advantageously, the dual- or multispecific ligand may comprise a first domain capable of binding a target molecule, and a second domain capable of binding a molecule or group which extends the half-life of the ligand. For example, the molecule or group may be a bulky agent, such as HSA or a cell matrix protein. As

used herein, the phrase "molecule or group which extends the half-life of a ligand" refers to a molecule or chemical group which, when bound by a dual-specific ligand as described herein increases the in vivo half-life of such dual specific ligand when administered to an animal, relative to a ligand that does not bind that molecule or group. Examples of molecules or groups that extend the half-life of a ligand are described hereinbelow. In a preferred embodiment, the closed conformation multispecific ligand may be capable of binding the target molecule only on displacement of the half-life enhancing molecule or group. Thus, for example, a closed conformation multispecific ligand is maintained in circulation in the bloodstream of a subject by a bulky molecule such as HSA. When a target molecule is encountered, competition between the binding domains of the closed conformation multispecific ligand results in displacement of the HSA and binding of the target. Molecules which increase half-life are discussed in further detail above.

Ligands according to any aspect of the present invention, as well as dAb monomers useful in constructing such ligands, may advantageously dissociate from their cognate target(s) with a K_d of 300nM to 5pM (ie, 3 x 10^{-7} to 5 x 10^{-12}M), preferably 50nM to20pM, or 5nM to 200pM or 1nM to 100pM, 1 x 10^{-7} M or less, 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, 1 x 10^{-11} M or less; and/or a K_{off} rate constant of 5 x 10^{-1} to 1 x 10^{-7} S^{-1}, preferably 1 x 10^{-2} to 1 x 10^{-6} S^{-1}, or 5 x 10^{-3} to 1 x 10^{-5} S^{-1}, or 5 x 10^{-1} S^{-1} or less, or 1 x 10^{-2} S^{-1} or less, or 1 x 10^{-3} S^{-1} or less, or 1 x 10^{-4} S^{-1} or less, or 1 x 10^{-5} S^{-1} or less, or 1 x 10^{-6} S^{-1} or less as determined by surface plasmon resonance. The K_d rate constand is defined as K_{off}/K_{on}.

Furthermore, the invention provides a dAb monomer(or dual specific ligand comprising such a dAb) that binds to serum albumin (SA) with a K_d of 1nM to 500μM (ie, x 10^{-9} to 5 x 10^{-4}), preferably 100nM to 10μM. Preferably, for a dual specific ligand comprising a first anti-SA dAb and a second dAb to another target, the affinity (eg K_d and/or K_{off} as measured by surface plasmon resonance, eg using BiaCore) of the second dAb for its target is from 1 to 100000 times (preferably 100 to 100000, more preferably 1000 to 100000, or 10000 to 100000 times) the affinity of the first dAb for SA. For example, the first dAb binds SA with an affinity of

approximately 10μM, while the second dAb binds its target with an affinity of 100pM. Preferably, the serum albumin is human serum albumin (HSA).

In one embodiment, the first dAb (or a dAb monomer) binds SA (eg, HSA) with a K_d of approximately 50, preferably 70, and more preferably 100, 150 or 200 nM.

The invention moreover provides dimers, trimers and polymers of the aforementioned dAb monomers, in accordance with the foregoing aspect of the present invention.

Ligands according to the invention, including dAb monomers, dimers and trimers, can be linked to an antibody Fc region, comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding ligands linked as a single nucleotide sequence to an Fc region may be used to prepare such polypeptides. Alternatively, ligands according to the invention may be free of an Fc domain.

In a further aspect, the present invention provides one or more nucleic acid molecules encoding at least a dual- or multispecific ligand as herein defined. In one embodiment, the ligand is a closed conformation ligand. In another embodiment, it is an open conformation ligand. The multispecific ligand may be encoded on a single nucleic acid molecule; alternatively, each epitope binding domain may be encoded by a separate nucleic acid molecule. Where the ligand is encoded by a single nucleic acid molecule, the domains may be expressed as a fusion polypeptide, or may be separately expressed and subsequently linked together, for example using chemical linking agents. Ligands expressed from separate nucleic acids will be linked together by appropriate means.

The nucleic acid may further encode a signal sequence for export of the polypeptides from a host cell upon expression and may be fused with a surface component of a filamentous bacteriophage particle (or other component of a selection display system) upon expression. Leader sequences, which may be used in bacterial

expression and/or phage or phagemid display, include pelB, stII, ompA, phoA, bla and pelA.

In a further aspect of the second configuration of the invention the present invention provides a vector comprising nucleic acid according to the present invention.

In a yet further aspect, the present invention provides a host cell transfected with a vector according to the present invention.

Expression from such a vector may be configured to produce, for example on the surface of a bacteriophage particle, epitope binding domains for selection. This allows selection of displayed domains and thus selection of 'multispecific ligands' using the method of the present invention.

Combining single variable domains

Domains useful in the invention, once selected using methods exemplified above, may be combined by a variety of methods known in the art, including covalent and non-covalent methods.

Preferred methods include the use of polypeptide linkers, as described, for example, in connection with scFv molecules (Bird *et al.*, (1988) Science 242:423-426). Discussion of suitable linkers is provided in *Bird et al.* Science 242, 423-426; *Hudson et al* , Journal Immunol Methods 231 (1999) 177-189; *Hudson et al*, Proc Nat Acad Sci USA 85, 5879-5883. Linkers are preferably flexible, allowing the two single domains to interact. One linker example is a $(Gly_4\ Ser)_n$ linker, where n=1 to 8, eg, 2, 3, 4, 5 or 7. The linkers used in diabodies, which are less flexible, may also be employed (Holliger *et al.*, (1993) PNAS (USA) 90:6444-6448).

In one embodiment, the linker employed is not an immunoglobulin hinge region.

Variable domains may be combined using methods other than linkers. For example, the use of disulphide bridges, provided through naturally-occurring or

engineered cysteine residues, may be exploited to stabilise V_H-V_H, V_L-V_L or V_H-V_L dimers (Reiter *et al.*, (1994) Protein Eng. 7:697-704) or by remodelling the interface between the variable domains to improve the "fit" and thus the stability of interaction (Ridgeway *et al.*, (1996) Protein Eng. 7:617-621; Zhu *et al.*, (1997) Protein Science 6:781-788).

Other techniques for joining or stabilizing variable domains of immunoglobulins, and in particular antibody V_H domains, may be employed as appropriate.

In accordance with the present invention, dual specific ligands can be in "closed" conformations in solution. A "closed" configuration is that in which the two domains (for example V_H and V_L) are present in associated form, such as that of an associated V_H-V_L pair which forms an antibody binding site. For example, scFv may be in a closed conformation, depending on the arrangement of the linker used to link the V_H and V_L domains. If this is sufficiently flexible to allow the domains to associate, or rigidly holds them in the associated position, it is likely that the domains will adopt a closed conformation.

Similarly, V_H domain pairs and V_L domain pairs may exist in a closed conformation. Generally, this will be a function of close association of the domains, such as by a rigid linker, in the ligand molecule. Ligands in a closed conformation will be unable to bind both the molecule which increases the half-life of the ligand and a second target molecule. Thus, the ligand will typically only bind the second target molecule on dissociation from the molecule which increases the half-life of the ligand.

Moreover, the construction of V_H/V_H, V_L/V_L or V_H/V_L dimers without linkers provides for competition between the domains.

Ligands according to the invention may moreover be in an open conformation. In such a conformation, the ligands will be able to simultaneously bind both the molecule which increases the half-life of the ligand and the second target molecule.

Typically, variable domains in an open configuration are (in the case of V_H-V_L pairs) held far enough apart for the domains not to interact and form an antibody binding site and not to compete for binding to their respective epitopes. In the case of V_H/V_H or V_L/V_L dimers, the domains are not forced together by rigid linkers. Naturally, such domain pairings will not compete for antigen binding or form an antibody binding site.

Fab fragments and whole antibodies will exist primarily in the closed conformation, although it will be appreciated that open and closed dual specific ligands are likely to exist in a variety of equilibria under different circumstances. Binding of the ligand to a target is likely to shift the balance of the equilibrium towards the open configuration. Thus, certain ligands according to the invention can exist in two conformations in solution, one of which (the open form) can bind two antigens or epitopes independently, whilst the alternative conformation (the closed form) can only bind one antigen or epitope; antigens or epitopes thus compete for binding to the ligand in this conformation.

Although the open form of the dual specific ligand may thus exist in equilibrium with the closed form in solution, it is envisaged that the equilibrium will favor the closed form; moreover, the open form can be sequestered by target binding into a closed conformation. Preferably, therefore, certain dual specific ligands of the invention are present in an equilibrium between two (open and closed) conformations.

Dual specific ligands according to the invention may be modified in order to favor an open or closed conformation. For example, stabilisation of V_H-V_L interactions with disulphide bonds stabilises the closed conformation. Moreover, linkers used to join the domains, including V_H domain and V_L domain pairs, may be constructed such that the open from is favoured; for example, the linkers may sterically hinder the association of the domains, such as by incorporation of large amino acid residues in opportune locations, or the designing of a suitable rigid structure which will keep the domains physically spaced apart.

Characterisation of the dual-specific ligand.

The binding of the dual-specific ligand to its specific antigens or epitopes (e.g., CD40L and/or an epitope bound by DOM8-24) can be tested by methods which will be familiar to those skilled in the art and include ELISA. In a preferred embodiment of the invention binding is tested using monoclonal phage ELISA.

Phage ELISA may be performed according to any suitable procedure: an exemplary protocol is set forth below.

Populations of phage produced at each round of selection can be screened for binding by ELISA to the selected antigen or epitope, to identify "polyclonal" phage antibodies. Phage from single infected bacterial colonies from these populations can then be screened by ELISA to identify "monoclonal" phage antibodies. It is also desirable to screen soluble antibody fragments for binding to antigen or epitope, and this can also be undertaken by ELISA using reagents, for example, against a C- or N-terminal tag (see for example Winter *et al.* (1994) Ann. Rev. Immunology 12, 433-55 and references cited therein.

The diversity of the selected phage monoclonal antibodies may also be assessed by gel electrophoresis of PCR products (Marks *et al.* 1991, *supra*; Nissim *et al.* 1994 *supra*), probing (Tomlinson *et al.*, 1992) J. Mol. Biol. 227, 776) or by sequencing of the vector DNA.

Structure of 'Dual-specific ligands'.

As described above, an antibody is herein defined as an antibody (for example IgG, IgM, IgA, IgA, IgE) or fragment (Fab, Fv, disulphide linked Fv, scFv, diabody) which comprises at least one heavy and a light chain variable domain, at least two heavy chain variable domains or at least two light chain variable domains. It may be at least partly derived from any species naturally producing an antibody, or created by recombinant DNA technology; whether isolated from serum, B-cells, hybridomas, transfectomas, yeast or bacteria).

In a preferred embodiment of the invention the dual-specific ligand comprises at least one single heavy chain variable domain of an antibody and one single light chain variable domain of an antibody, or two single heavy or light chain variable domains. For example, the ligand may comprise a V_H/V_L pair, a pair of V_H domains or a pair of V_L domains.

The first and the second variable domains of such a ligand may be on the same polypeptide chain. Alternatively they may be on separate polypeptide chains. In the case that they are on the same polypeptide chain they may be linked by a linker, which is preferentially a peptide sequence, as described above.

The first and second variable domains may be covalently or non-covalently associated. In the case that they are covalently associated, the covalent bonds may be disulphide bonds.

In the case that the variable domains are selected from V-gene repertoires selected for instance using phage display technology as herein described, then these variable domains comprise a universal framework region, such that is they may be recognised by a specific generic ligand as herein defined. The use of universal frameworks, generic ligands and the like is described in WO99/20749.

Where V-gene repertoires are used variation in polypeptide sequence is preferably located within the structural loops of the variable domains. The polypeptide sequences of either variable domain may be altered by DNA shuffling or by mutation in order to enhance the interaction of each variable domain with its complementary pair. DNA shuffling is known in the art and taught, for example, by Stemmer, 1994, Nature 370: 389-391 and U.S. Patent No. 6,297,053, both of which are incorporated herein by reference. Other methods of mutagenesis are well known to those of skill in the art.

In one embodiment of the invention the 'dual-specific ligand' is a single chain Fv fragment. In an alternative embodiment of the invention, the 'dual-specific ligand' consists of a Fab format.

In a further aspect, the present invention provides nucleic acid encoding at least a 'dual-specific ligand' as herein defined.

One skilled in the art will appreciate that, depending on the aspect of the invention, both antigens or epitopes may bind simultaneously to the same antibody molecule. Alternatively, they may compete for binding to the same antibody molecule. For example, where both epitopes are bound simultaneously, both variable domains of a dual specific ligand are able to independently bind their target epitopes. Where the domains compete, the one variable domain is capable of binding its target, but not at the same time as the other variable domain binds its cognate target; or the first variable domain is capable of binding its target, but not at the same time as the second variable domain binds its cognate target.

The variable regions may be derived from antibodies directed against target antigens or epitopes. Alternatively they may be derived from a repertoire of single antibody domains such as those expressed on the surface of filamentous bacteriophage. Selection may be performed as described below.

In general, the nucleic acid molecules and vector constructs required for the performance of the present invention may be constructed and manipulated as set forth in standard laboratory manuals, such as Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, USA.

The manipulation of nucleic acids useful in the present invention is typically carried out in recombinant vectors.

Thus in a further aspect, the present invention provides a vector comprising nucleic acid encoding at least a 'dual-specific ligand' as herein defined.

As used herein, vector refers to a discrete element that is used to introduce heterologous DNA into cells for the expression and/or replication thereof. Methods by which to select or construct and, subsequently, use such vectors are well known to one of ordinary skill in the art. Numerous vectors are publicly available, including bacterial plasmids, bacteriophage, artificial chromosomes and episomal vectors. Such

vectors may be used for simple cloning and mutagenesis; alternatively gene expression vector is employed. A vector of use according to the invention may be selected to accommodate a polypeptide coding sequence of a desired size, typically from 0.25 kilobase (kb) to 40 kb or more in length A suitable host cell is transformed with the vector after *in vitro* cloning manipulations. Each vector contains various functional components, which generally include a cloning (or "polylinker") site, an origin of replication and at least one selectable marker gene. If given vector is an expression vector, it additionally possesses one or more of the following: enhancer element, promoter, transcription termination and signal sequences, each positioned in the vicinity of the cloning site, such that they are operatively linked to the gene encoding a ligand according to the invention.

Both cloning and expression vectors generally contain nucleic acid sequences that enable the vector to replicate in one or more selected host cells. Typically in cloning vectors, this sequence is one that enables the vector to replicate independently of the host chromosomal DNA and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 micron plasmid origin is suitable for yeast, and various viral origins (e.g. SV 40, adenovirus) are useful for cloning vectors in mammalian cells. Generally, the origin of replication is not needed for mammalian expression vectors unless these are used in mammalian cells able to replicate high levels of DNA, such as COS cells.

Advantageously, a cloning or expression vector may contain a selection gene also referred to as selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will therefore not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics and other toxins, e.g. ampicillin, neomycin, methotrexate or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients not available in the growth media.

Since the replication of vectors encoding a ligand according to the present invention is most conveniently performed in *E. coli*, an *E. coli*-selectable marker, for example, the β-lactamase gene that confers resistance to the antibiotic ampicillin, is of use. These can be obtained from *E. coli* plasmids, such as pBR322 or a pUC plasmid such as pUC18 or pUC19.

Expression vectors usually contain a promoter that is recognised by the host organism and is operably linked to the coding sequence of interest. Such a promoter may be inducible or constitutive. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

Promoters suitable for use with prokaryotic hosts include, for example, the β-lactamase and lactose promoter systems, alkaline phosphatase, the tryptophan (trp) promoter system and hybrid promoters such as the tac promoter. Promoters for use in bacterial systems will also generally contain a Shine-Delgarno sequence operably linked to the coding sequence.

The preferred vectors are expression vectors that enables the expression of a nucleotide sequence corresponding to a polypeptide library member. Thus, selection with the first and/or second antigen or epitope can be performed by separate propagation and expression of a single clone expressing the polypeptide library member or by use of any selection display system. As described above, the preferred selection display system is bacteriophage display. Thus, phage or phagemid vectors may be used, eg pIT1 or pIT2. Leader sequences useful in the invention include pelB, stII, ompA, phoA, bla and pelA. One example are phagemid vectors which have an *E. coli.* origin of replication (for double stranded replication) and also a phage origin of replication (for production of single-stranded DNA). The manipulation and expression of such vectors is well known in the art (Hoogenboom and Winter (1992) supra; Nissim *et al.* (1994) supra). Briefly, the vector contains a β-lactamase gene to

confer selectivity on the phagemid and a lac promoter upstream of a expression cassette that consists (N to C terminal) of a pelB leader sequence (which directs the expressed polypeptide to the periplasmic space), a multiple cloning site (for cloning the nucleotide version of the library member), optionally, one or more peptide tag (for detection), optionally, one or more TAG stop codon and the phage protein pIII. Thus, using various suppressor and non-suppressor strains of *E. coli* and with the addition of glucose, iso-propyl thio-β-D-galactoside (IPTG) or a helper phage, such as VCS M13, the vector is able to replicate as a plasmid with no expression, produce large quantities of the polypeptide library member only or produce phage, some of which contain at least one copy of the polypeptide-pIII fusion on their surface.

Construction of vectors encoding ligands according to the invention employs conventional ligation techniques. Isolated vectors or DNA fragments are cleaved, tailored, and religated in the form desired to generate the required vector. If desired, analysis to confirm that the correct sequences are present in the constructed vector can be performed in a known fashion. Suitable methods for constructing expression vectors, preparing *in vitro* transcripts, introducing DNA into host cells, and performing analyses for assessing expression and function are known to those skilled in the art. The presence of a gene sequence in a sample is detected, or its amplification and/or expression quantified by conventional methods, such as Southern or Northern analysis, Western blotting, dot blotting of DNA, RNA or protein, *in situ* hybridisation, immunocytochemistry or sequence analysis of nucleic acid or protein molecules. Those skilled in the art will readily envisage how these methods may be modified, if desired.

Structure of ligands

According to one aspect of the invention, two or more non-complementary epitope binding domains are linked so that they are in a closed conformation as herein defined. Advantageously, they may be further attached to a skeleton which may, as a alternative, or on addition to a linker described herein, facilitate the formation and/or maintenance of the closed conformation of the epitope binding sites with respect to

one another. Alternatively, the monomeric anti-CD40L antibody single variable domain polypeptides of the invention may be constructed using scaffold or skeleton frameworks as discussed herein.

(I) Skeletons

Skeletons may be based on immunoglobulin molecules or may be non-immunoglobulin in origin as set forth above. Preferred immunoglobulin skeletons as herein defined includes any one or more of those selected from the following: an immunoglobulin molecule comprising at least (i) the CL (kappa or lambda subclass) domain of an antibody; or (ii) the CH1 domain of an antibody heavy chain; an immunoglobulin molecule comprising the CH1 and CH2 domains of an antibody heavy chain; an immunoglobulin molecule comprising the CH1, CH2 and CH3 domains of an antibody heavy chain; or any of the subset (ii) in conjunction with the CL (kappa or lambda subclass) domain of an antibody. A hinge region domain may also be included.. Such combinations of domains may, for example, mimic natural antibodies, such as IgG or IgM, or fragments thereof, such as Fv, scFv, Fab or $F(ab')_2$ molecules. Those skilled in the art will be aware that this list is not intended to be exhaustive.

(II) Protein scaffolds

Each epitope binding domain comprises a protein scaffold and one or more CDRs which are involved in the specific interaction of the domain with one or more epitopes. Advantageously, an epitope binding domain according to the present invention comprises three CDRs. Suitable protein scaffolds, in addition to those based on immunoglobulin domains, may also be based on protein scaffolds or skeletons other than immunoglobulin domains. For example natural bacterial receptors such as SpA have been used as scaffolds for the grafting of CDRs to generate ligands which bind specifically to one or more epitopes. Details of this procedure are described in US 5,831,012. Other suitable scaffolds include those based on fibronectin and affibodies (Affibody, Bromma, Sweeden). Details of suitable procedures are described in WO 98/58965. Other suitable scaffolds include lipocallin

and CTLA4, as described in van den Beuken *et al.*, J. Mol. Biol. (2001) 310, 591-601, and scaffolds such as those described in WO0069907 (Medical Research Council), which are based for example on the ring structure of bacterial GroEL or other chaperone polypeptides. Other non-immunoglobulin based scaffolds which may be used according to the invention include those based on the LDL receptor class A, EGF domain monomers and mutimers, and scaffolds available from Biorexis (King of Prussia, PA) or Avidia (Mountain View, CA). Other non-immunoglobulin scaffolds which may be used are described, for example, in WO05/040229, WO04/044011, and US20050089932

Scaffolds for use in Constructing Ligands

i. Selection of the main-chain conformation

The members of the immunoglobulin superfamily all share a similar fold for their polypeptide chain. For example, although antibodies are highly diverse in terms of their primary sequence, comparison of sequences and crystallographic structures has revealed that, contrary to expectation, five of the six antigen binding loops of antibodies (H1, H2, L1, L2, L3) adopt a limited number of main-chain conformations, or canonical structures (Chothia and Lesk (1987) *J. Mol. Biol.*, 196: 901; Chothia *et al.* (1989) *Nature*, 342: 877). Analysis of loop lengths and key residues has therefore enabled prediction of the main-chain conformations of H1, H2, L1, L2 and L3 found in the majority of human antibodies (Chothia *et al.* (1992) *J. Mol. Biol.*, 227: 799; Tomlinson *et al.* (1995) *EMBO J.*, 14: 4628; Williams *et al.* (1996) *J. Mol. Biol.*, 264: 220). Although the H3 region is much more diverse in terms of sequence, length and structure (due to the use of D segments), it also forms a limited number of main-chain conformations for short loop lengths which depend on the length and the presence of particular residues, or types of residue, at key positions in the loop and the antibody framework (Martin *et al.* (1996) *J. Mol. Biol.*, 263: 800; Shirai *et al.* (1996) *FEBS Letters*, 399: 1).

The ligands of the present invention are advantageously selected and/or assembled from libraries of domains, such as libraries of V_H domains and/or libraries

of V_L domains. Moreover, the ligands of the invention may themselves be provided in the form of libraries. In one aspect of the present invention, libraries of ligands and/or domains are designed in which certain loop lengths and key residues have been chosen to ensure that the main-chain conformation of the members is known. Advantageously, these are real conformations of immunoglobulin superfamily molecules found in nature, to minimise the chances that they are non-functional, as discussed above. Germline V gene segments serve as one suitable basic framework for constructing antibody or T-cell receptor libraries; other sequences are also of use. Variations may occur at a low frequency, such that a small number of functional members may possess an altered main-chain conformation, which does not affect its function.

Canonical structure theory is also of use to assess the number of different main-chain conformations encoded by ligands, to predict the main-chain conformation based on ligand sequences and to chose residues for diversification which do not affect the canonical structure. It is known that, in the human V_κ domain, the L1 loop can adopt one of four canonical structures, the L2 loop has a single canonical structure and that 90% of human V_κ domains adopt one of four or five canonical structures for the L3 loop (Tomlinson *et al.* (1995) supra); thus, in the V_κ domain alone, different canonical structures can combine to create a range of different main-chain conformations. Given that the V_λ domain encodes a different range of canonical structures for the L1, L2 and L3 loops and that V_κ and V_λ domains can pair with any V_H domain which can encode several canonical structures for the H1 and H2 loops, the number of canonical structure combinations observed for these five loops is very large. This implies that the generation of diversity in the main-chain conformation may be essential for the production of a wide range of binding specificities. However, by constructing an antibody library based on a single known main-chain conformation it has been found, contrary to expectation, that diversity in the main-chain conformation is not required to generate sufficient diversity to target substantially all antigens. Even more surprisingly, the single main-chain conformation need not be a consensus structure - a single naturally occurring conformation can be

used as the basis for an entire library. Thus, in a preferred aspect, the ligands of the invention possess a single known main-chain conformation.

The single main-chain conformation that is chosen is preferably commonplace among molecules of the immunoglobulin superfamily type in question. A conformation is commonplace when a significant number of naturally occurring molecules are observed to adopt it. Accordingly, in a preferred aspect of the invention, the natural occurrence of the different main-chain conformations for each binding loop of an immunoglobulin domain are considered separately and then a naturally occurring variable domain is chosen which possesses the desired combination of main-chain conformations for the different loops. If none is available, the nearest equivalent may be chosen. It is preferable that the desired combination of main-chain conformations for the different loops is created by selecting germline gene segments which encode the desired main-chain conformations. It is more preferable, that the selected germline gene segments are frequently expressed in nature, and most preferable that they are the most frequently expressed of all natural germline gene segments.

In designing ligands or libraries thereof the incidence of the different main-chain conformations for each of the antigen binding loops may be considered separately. For H1, H2, L1, L2 and L3, a given conformation that is adopted by between 20% and 100% of the antigen binding loops of naturally occurring molecules is chosen. Typically, its observed incidence is above 35% (i.e. between 35% and 100%) and, ideally, above 50% or even above 65%. Since the vast majority of H3 loops do not have canonical structures, it is preferable to select a main-chain conformation which is commonplace among those loops which do display canonical structures. For each of the loops, the conformation which is observed most often in the natural repertoire is therefore selected. In human antibodies, the most popular canonical structures (CS) for each loop are as follows: H1 - CS 1 (79% of the expressed repertoire), H2 - CS 3 (46%), L1 - CS 2 of V_κ (39%), L2 - CS 1 (100%), L3 - CS 1 of V_κ (36%) (calculation assumes a κ:λ ratio of 70:30, Hood *et al.* (1967) *Cold Spring Harbor Symp. Quant. Biol.*, 48: 133). For H3 loops that have canonical

structures, a CDR3 length (Kabat *et al.* (1991) *Sequences of proteins of immunological interest*, U.S. Department of Health and Human Services) of seven residues with a salt-bridge from residue 94 to residue 101 appears to be the most common. There are at least 16 human antibody sequences in the EMBL data library with the required H3 length and key residues to form this conformation and at least two crystallographic structures in the protein data bank which can be used as a basis for antibody modelling (2cgr and 1tet). The most frequently expressed germline gene segments that this combination of canonical structures are the V_H segment 3-23 (DP-47), the J_H segment JH4b, the V_κ segment O2/O12 (DPK9) and the J_κ segment $J_\kappa 1$. V_H segments DP45 and DP38 are also suitable. These segments can therefore be used in combination as a basis to construct a library with the desired single main-chain conformation.

Alternatively, instead of choosing the single main-chain conformation based on the natural occurrence of the different main-chain conformations for each of the binding loops in isolation, the natural occurrence of combinations of main-chain conformations is used as the basis for choosing the single main-chain conformation. In the case of antibodies, for example, the natural occurrence of canonical structure combinations for any two, three, four, five or for all six of the antigen binding loops can be determined. Here, it is preferable that the chosen conformation is commonplace in naturally occurring antibodies and most preferable that it observed most frequently in the natural repertoire. Thus, in human antibodies, for example, when natural combinations of the five antigen binding loops, H1, H2, L1, L2 and L3, are considered, the most frequent combination of canonical structures is determined and then combined with the most popular conformation for the H3 loop, as a basis for choosing the single main-chain conformation.

ii. Diversification of the canonical sequence

Having selected several known main-chain conformations or, preferably a single known main-chain conformation, ligands according to the invention or libraries for use in the invention can be constructed by varying the binding site of the molecule

in order to generate a repertoire with structural and/or functional diversity. This means that variants are generated such that they possess sufficient diversity in their structure and/or in their function so that they are capable of providing a range of activities.

The desired diversity is typically generated by varying the selected molecule at one or more positions. The positions to be changed can be chosen at random or are preferably selected. The variation can then be achieved either by randomisation, during which the resident amino acid is replaced by any amino acid or analogue thereof, natural or synthetic, producing a very large number of variants or by replacing the resident amino acid with one or more of a defined subset of amino acids, producing a more limited number of variants.

Various methods have been reported for introducing such diversity. Error-prone PCR (Hawkins *et al.* (1992) *J. Mol. Biol.*, 226: 889), chemical mutagenesis (Deng *et al.* (1994) *J. Biol. Chem.*, 269: 9533) or bacterial mutator strains (Low *et al.* (1996) *J. Mol. Biol.*, 260: 359) can be used to introduce random mutations into the genes that encode the molecule. Methods for mutating selected positions are also well known in the art and include the use of mismatched oligonucleotides or degenerate oligonucleotides, with or without the use of PCR. For example, several synthetic antibody libraries have been created by targeting mutations to the antigen binding loops. The H3 region of a human tetanus toxoid-binding Fab has been randomised to create a range of new binding specificities (Barbas *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89: 4457). Random or semi-random H3 and L3 regions have been appended to germline V gene segments to produce large libraries with unmutated framework regions (Hoogenboom & Winter (1992) *J. Mol. Biol.*, 227: 381; Barbas *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89: 4457; Nissim *et al.* (1994) *EMBO J.*, 13: 692; Griffiths *et al.* (1994) *EMBO J.*, 13: 3245; De Kruif *et al.* (1995) *J. Mol. Biol.*, 248: 97). Such diversification has been extended to include some or all of the other antigen binding loops (Crameri *et al.* (1996) *Nature Med.*, 2: 100; Riechmann *et al.* (1995) *Bio/Technology*, 13: 475; Morphosys, WO97/08320, supra).

Since loop randomisation has the potential to create approximately more than 10^{15} structures for H3 alone and a similarly large number of variants for the other five loops, it is not feasible using current transformation technology or even by using cell free systems to produce a library representing all possible combinations. For example, in one of the largest libraries constructed to date, 6×10^{10} different antibodies, which is only a fraction of the potential diversity for a library of this design, were generated (Griffiths *et al.* (1994) supra).

In a preferred embodiment, only those residues which are directly involved in creating or modifying the desired function of the molecule are diversified. For many molecules, the function will be to bind a target and therefore diversity should be concentrated in the target binding site, while avoiding changing residues which are crucial to the overall packing of the molecule or to maintaining the chosen main-chain conformation.

Diversification of the canonical sequence as it applies to antibody domains

In the case of the ligands of the invention, the binding site for the target is most often the antigen binding site. Thus, in a highly preferred aspect, the invention provides libraries of or for the assembly of antibody ligands in which only those residues in the antigen binding site are varied. These residues are extremely diverse in the human antibody repertoire and are known to make contacts in high-resolution antibody/antigen complexes. For example, in L2 it is known that positions 50 and 53 are diverse in naturally occurring antibodies and are observed to make contact with the antigen. In contrast, the conventional approach would have been to diversify all the residues in the corresponding Complementarity Determining Region (CDR1) as defined by Kabat *et al.* (1991, supra), some seven residues compared to the two diversified in the library for use according to the invention. This represents a significant improvement in terms of the functional diversity required to create a range of antigen binding specificities.

In nature, antibody diversity is the result of two processes: somatic recombination of germline V, D and J gene segments to create a naive primary

repertoire (so called germline and junctional diversity) and somatic hypermutation of the resulting rearranged V genes. Analysis of human antibody sequences has shown that diversity in the primary repertoire is focused at the centre of the antigen binding site whereas somatic hypermutation spreads diversity to regions at the periphery of the antigen binding site that are highly conserved in the primary repertoire (see Tomlinson *et al.* (1996) *J. Mol. Biol.*, 256: 813). This complementarity has probably evolved as an efficient strategy for searching sequence space and, although apparently unique to antibodies, it can easily be applied to other polypeptide repertoires. The residues which are varied are a subset of those that form the binding site for the target. Different (including overlapping) subsets of residues in the target binding site are diversified at different stages during selection, if desired.

In the case of an antibody repertoire, an initial 'naive' repertoire is created where some, but not all, of the residues in the antigen binding site are diversified. As used herein in this context, the term "naive" refers to antibody molecules that have no pre-determined target. These molecules resemble those which are encoded by the immunoglobulin genes of an individual who has not undergone immune diversification, as is the case with fetal and newborn individuals, whose immune systems have not yet been challenged by a wide variety of antigenic stimuli. This repertoire is then selected against a range of antigens or epitopes. If required, further diversity can then be introduced outside the region diversified in the initial repertoire. This matured repertoire can be selected for modified function, specificity or affinity.

The invention provides two different naive repertoires of binding domains for the construction of ligands, or a naïve library of ligands, in which some or all of the residues in the antigen binding site are varied. The "primary" library mimics the natural primary repertoire, with diversity restricted to residues at the centre of the antigen binding site that are diverse in the germline V gene segments (germline diversity) or diversified during the recombination process (junctional diversity). Those residues which are diversified include, but are not limited to, H50, H52, H52a, H53, H55, H56, H58, H95, H96, H97, H98, L50, L53, L91, L92, L93, L94 and L96. In the "somatic" library, diversity is restricted to residues that are diversified during

the recombination process (junctional diversity) or are highly somatically mutated). Those residues which are diversified include, but are not limited to: H31, H33, H35, H95, H96, H97, H98, L30, L31, L32, L34 and L96. All the residues listed above as suitable for diversification in these libraries are known to make contacts in one or more antibody-antigen complexes. Since in both libraries, not all of the residues in the antigen binding site are varied, additional diversity is incorporated during selection by varying the remaining residues, if it is desired to do so. It shall be apparent to one skilled in the art that any subset of any of these residues (or additional residues which comprise the antigen binding site) can be used for the initial and/or subsequent diversification of the antigen binding site.

In the construction of libraries for use in the invention, diversification of chosen positions is typically achieved at the nucleic acid level, by altering the coding sequence which specifies the sequence of the polypeptide such that a number of possible amino acids (all 20 or a subset thereof) can be incorporated at that position. Using the IUPAC nomenclature, the most versatile codon is NNK, which encodes all amino acids as well as the TAG stop codon. The NNK codon is preferably used in order to introduce the required diversity. Other codons which achieve the same ends are also of use, including the NNN codon, which leads to the production of the additional stop codons TGA and TAA.

A feature of side-chain diversity in the antigen binding site of human antibodies is a pronounced bias which favours certain amino acid residues. If the amino acid composition of the ten most diverse positions in each of the V_H, V_κ and V_λ regions are summed, more than 76% of the side-chain diversity comes from only seven different residues, these being, serine (24%), tyrosine (14%), asparagine (11%), glycine (9%), alanine (7%), aspartate (6%) and threonine (6%). This bias towards hydrophilic residues and small residues which can provide main-chain flexibility probably reflects the evolution of surfaces which are predisposed to binding a wide range of antigens or epitopes and may help to explain the required promiscuity of antibodies in the primary repertoire.

Since it is preferable to mimic this distribution of amino acids, the distribution of amino acids at the positions to be varied preferably mimics that seen in the antigen binding site of antibodies. Such bias in the substitution of amino acids that permits selection of certain polypeptides (not just antibody polypeptides) against a range of target antigens is easily applied to any polypeptide repertoire. There are various methods for biasing the amino acid distribution at the position to be varied (including the use of tri-nucleotide mutagenesis, see WO97/08320), of which the preferred method, due to ease of synthesis, is the use of conventional degenerate codons. By comparing the amino acid profile encoded by all combinations of degenerate codons (with single, double, triple and quadruple degeneracy in equal ratios at each position) with the natural amino acid use it is possible to calculate the most representative codon. The codons (AGT)(AGC)T, (AGT)(AGC)C and (AGT)(AGC)(CT) - that is, DVT, DVC and DVY, respectively using IUPAC nomenclature - are those closest to the desired amino acid profile: they encode 22% serine and 11% tyrosine, asparagine, glycine, alanine, aspartate, threonine and cysteine. Preferably, therefore, libraries are constructed using either the DVT, DVC or DVY codon at each of the diversified positions.

Increased Half-life

In vivo, the PEGylated monovalent anti-CD40L antibodies as described herein confer a distinct advantage over non-PEGylated antibody polypeptides, in that the PEGylated antibody molecules will have a greatly prolonged in vivo half life. Without being bound to one particular theory, it is believed that the increased half-life of the molecules described herein is conferred by the increased hydrodynamic size of the antibody polypeptide resulting from the attachment of PEG polymer(s). More specifically, it is believed that two parameters play an important role in determining the serum half-life of PEGylated antibody polypeptides. The first criterion is the nature and size of the PEG attachment, i.e., if the polymer used is simply a linear chain or a branched/forked chain, wherein the branched/forked chain gives rise to a longer half-life. The second is the location of the PEG moiety or moieties on the antibody polypeptide in the final format and how many "free" unmodified PEG arms

the molecule has. The resulting hydrodynamic size of the PEGylated antibody polypeptide, as estimated, for example, by size exclusion chromatography, reflects the serum half-life of the molecule. Accordingly, the larger the hydrodynamic size of the PEGylated molecule, the greater the serum half life.

Increased half-life is useful in vivo applications of immunoglobulins, especially antibodies and most especially antibody fragments of small size. Such fragments (Fvs, Fabs, scFvs, dAbs) suffer from rapid clearance from the body; thus, while they are able to reach most parts of the body rapidly, and are quick to produce and easier to handle, their in vivo applications have been limited by their only brief persistence in vivo.

In one aspect, a monovalent anti-CD40L antibody polypeptide as described herein is stabilized in vivo by fusion with a moiety, such as PEG, that increases the hydrodynamic size of the antibody polypeptide. Methods for pharmacokinetic analysis and determination of half-life will be familiar to those skilled in the art. Details may be found in Kenneth et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al, Pharmacokinetc analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. ex edition (1982), which describes pharmacokinetic parameters such as t alpha and t beta half lives and area under the curve (AUC).

Typically, the half life of a PEGylated antibody polypeptide as described herein is increased by 10%, 20%, 30%, 40%, 50% or more relative to a non-PEGylated dAb (wherein the antibody polypeptide of the PEGylated antibody polypeptide and non-PEGylated antibody polypeptide are the same). Increases in the range of 2x, 3x, 4x, 5x, 7x, 10x, 20x, 30x, 40x, and up to 50x or more of the half life are possible. Alternatively, or in addition, increases in the range of up to 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 150x of the half life are possible.

Half lives (t½ alpha and t½ beta) and AUC can be determined from a curve of serum concentration of ligand against time. The WinNonlin analysis package

(available from Pharsight Corp., Mountain View, CA 94040, USA) can be used, for example, to model the curve. In a first phase (the alpha phase) the ligand is undergoing mainly distribution in the patient, with some elimination. A second phase (beta phase) is the terminal phase when the ligand has been distributed and the serum concentration is decreasing as the ligand is cleared from the patient. The tα half life is the half life of the first phase and the tβ half life is the half life of the second phase. "Half-life" as used herein, unless otherwise noted, refers to the overall half-life of an antibody single variable domain of the invention determined by non-compartment modeling (as contrasted with biphasic modeling, for example). Beta half-life is a measurement of the time it takes for the amount of dAb monomer or multimer to be cleared from the mammal to which it is administered. Thus, advantageously, the present invention provides a dAb-containing composition, e.g., a dAb-effector group composition, having a tα half-life in the range of 0.25 hours to 6 hours or more. In one embodiment, the lower end of the range is 30 minutes, 45 minutes, 1 hour, 1.3 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 10 hours, 11 hours or 12 hours. In addition or alternatively, a dAb containing composition will have a tα half-life in the range of up to and including 12 hours. In one embodiment, the upper end of the range is 11, 10, 9, 8, 7, 6, or 5 hours. An example of a suitable range is 1.3 to 6 hours, 2 to 5 hours or 3 to 4 hours.

Advantageously, the present invention provides a dAb containing composition comprising a ligand according to the invention having a tβ half-life in the range of 1-170 hours or more. In one embodiment, the lower end of the range is 2.5 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours or 12 hours. In addition, or alternatively, a dAb containing composition, e.g. a dAb-effector group composition has a tβ half-life in the range of up to and including 21 days. In one embodiment, the upper end of the range is 12 hours, 24 hours, 2 days, 3 days, 5 days, 10 days, 15 days, or 20 days. Advantageously a dAb containing composition according to the invention will have a tβ half-life in the range 2-100 hours, 4-80 hours, and 10-40 hours. In a further embodiment, it will be in the range 12-48 hours. In a further embodiment still, it will be in the range 12-26 hours. The present invention provides a dAb containing composition comprising a ligand according to

the invention having a half-life in the range of 1-170 hours or more. In one embodiment, the lower end of the range is 1.3 hours, 2.5 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours or 12 hours. In addition, or alternatively, a dAb containing composition, e.g. a dAb-effector group composition has a half-life in the range of up to and including 21 days. In one embodiment, the upper end of the range is 12 hours, 24 hours, 2 days, 3 days, 5 days, 10 days, 15 days, or 20 days.

In addition, or alternatively to the above criteria, the present invention provides a dAb containing composition comprising a ligand according to the invention having an AUC value (area under the curve) in the range of 1 mg.min/ml or more. In one embodiment, the lower end of the range is 5, 10, 15, 20, 30, 100, 200 or 300 mg.min/ml. In addition, or alternatively, a ligand or composition according to the invention has an AUC in the range of up to 600 mg.min/ml. In one embodiment, the upper end of the range is 500, 400, 300, 200, 150, 100, 75 or 50 mg.min/ml. Advantageously a ligand according to the invention will have an AUC in the range selected from the group consisting of the following: 15 to 150 mg.min/ml, 15 to 100 mg.min/ml, 15 to 75 mg.min/ml, and 15 to 50 mg.min/ml.

The ligands according to the invention, including, mono-, dual- and multi-specific, in one configuration thereof, are capable of binding to one or more molecules which can increase the half-life of the ligand *in vivo*. Typically, such molecules are polypeptides which occur naturally *in vivo* and which resist degradation or removal by endogenous mechanisms which remove unwanted material from the organism. For example, the molecule which increases the half-life of the organism may be selected from the following:

Proteins from the extracellular matrix; for example collagen, laminins, integrins and fibronectin. Collagens are the major proteins of the extracellular matrix. About 15 types of collagen molecules are currently known, found in different parts of the body, eg type I collagen (accounting for 90% of body collagen) found in bone, skin, tendon, ligaments, cornea, internal organs or type II collagen found in cartilage, invertebral disc, notochord, vitreous humour of the eye.

Proteins found in blood, including:

Plasma proteins such as fibrin, α-2 macroglobulin, serum albumin, fibrinogen A, fibrinogen B, serum amyloid protein A, heptaglobin, profilin, ubiquitin, uteroglobulin and β-2-microglobulin;

Enzymes and inhibitors such as plasminogen, lysozyme, cystatin C, alpha-1-antitrypsin and pancreatic trypsin inhibitor. Plasminogen is the inactive precursor of the trypsin-like serine protease plasmin. It is normally found circulating through the blood stream. When plasminogen becomes activated and is converted to plasmin, it unfolds a potent enzymatic domain that dissolves the fibrinogen fibers that entgangle the blood cells in a blood clot. This is called fibrinolysis.

Immune system proteins, such as IgE, IgG, IgM.

Transport proteins such as retinol binding protein, α-1 microglobulin.

Defensins such as beta-defensin 1, Neutrophil defensins 1,2 and 3.

Proteins found at the blood brain barrier or in neural tissues, such as melanocortin receptor, myelin, ascorbate transporter.

Transferrin receptor specific ligand-neuropharmaceutical agent fusion proteins (see US5977307); brain capillary endothelial cell receptor, transferrin, transferrin receptor, insulin, insulin-like growth factor 1 (IGF 1) receptor, insulin-like growth factor 2 (IGF 2) receptor, insulin receptor.

Proteins localised to the kidney, such as polycystin, type IV collagen, organic anion transporter K1, Heymann's antigen.

Proteins localised to the liver, for example alcohol dehydrogenase, G250.

Blood coagulation factor X

α1 antitrypsin

HNF 1α

Proteins localised to the lung, such as secretory component (binds IgA).

Proteins localised to the Heart, for example HSP 27. This is associated with dilated cardiomyopathy.

Proteins localised to the skin, for example keratin.

Bone specific proteins, such as bone morphogenic proteins (BMPs), which are a subset of the transforming growth factor β superfamily that demonstrate osteogenic activity. Examples include BMP-2, -4, -5, -6, -7 (also referred to as osteogenic protein (OP-1) and -8 (OP-2).

Tumour specific proteins, including human trophoblast antigen, herceptin receptor, oestrogen receptor, cathepsins eg cathepsin B (found in liver and spleen).

Disease-specific proteins, such as antigens expressed only on activated T-cells: including

LAG-3 (lymphocyte activation gene), osteoprotegerin ligand (OPGL) see *Nature* **402**, 304–309; 1999, OX40 (a member of the TNF receptor family, expressed on activated T cells and the only costimulatory T cell molecule known to be specifically up-regulated in human T cell leukaemia virus type-I (HTLV-I)-producing cells.) See *J Immunol. 2000 Jul 1;165(1):263-70*; Metalloproteases (associated with arthritis/cancers), including CG6512 Drosophila, human paraplegin, human FtsH, human AFG3L2, murine ftsH; angiogenic growth factors, including acidic fibroblast

growth factor (FGF-1), basic fibroblast growth factor (FGF-2), Vascular endothelial growth factor / vascular permeability factor (VEGF/VPF), transforming growth factor-a (TGF a), tumor necrosis factor-alpha (TNF-α), angiogenin, interleukin-3 (IL-3), interleukin-8 (IL-8), platelet-derived endothelial growth factor (PD-ECGF), placental growth factor (PlGF), midkine platelet-derived growth factor-BB (PDGF), fractalkine.

Stress proteins (heat shock proteins)

HSPs are normally found intracellularly. When they are found extracellularly, it is an indicator that a cell has died and spilled out its contents. This unprogrammed cell death (necrosis) only occurs when as a result of trauma, disease or injury and therefore *in vivo*, extracellular HSPs trigger a response from the immune system that will fight infection and disease. A dual specific which binds to extracellular HSP can be localised to a disease site.

Proteins involved in Fc transport

Brambell receptor (also known as FcRB):

This Fc receptor has two functions, both of which are potentially useful for delivery

The functions are

(1) The transport of IgG from mother to child across the placenta

(2) the protection of IgG from degradation thereby prolonging its serum half life of IgG. It is thought that the receptor recycles IgG from endosome.

See Holliger *et al*, Nat Biotechnol 1997 Jul;15(7):632-6.

Other proteins involved in Fc transport include the neonatal Fc receptor (FcRn) described in Gastinel et al., 1992, PNAS 89:638; and Roopenian et al., 2003 J. Immunol. 170:3528.

Ligands according to the invention may designed to be specific for the above targets without requiring any increase in or increasing half life *in vivo*. For example, ligands according to the invention can be specific for targets selected from the foregoing which are tissue-specific, thereby enabling tissue-specific targeting of the dual specific ligand, or a dAb monomer that binds a tissue-specific therapeutically relevant target, irrespective of any increase in half-life, although this may result. Moreover, where the ligand or dAb monomer targets kidney or liver, this may redirect the ligand or dAb monomer to an alternative clearance pathway *in vivo* (for example, the ligand may be directed away from liver clearance to kidney clearance).

Polypeptides useful for increasing half-life include, but are not limited to those shown in Annex I.

Increased Protease Stability

A further advantage of the present invention is that the PEGylated dAbs and dAb multimers described herein possess increased stability to the action of proteases. Depending on the assay conditions, dAbs are generally intrinsically stable to the action of proteases. In the presence of pepsin, however, many dAbs are totally degraded at pH 2 because the protein is unfolded under the acid conditions, thus making the protein more accessible to the protease enzyme. The present invention provides PEGylated dAb molecules, including dAb multimers, wherein it is believed that the PEG polymer provides protection of the polypeptide backbone due the physical coverage of the backbone by the PEG polymer, thereby preventing the protease from gaining access to the polypeptide backbone and cleaving it. In a preferred embodiment a PEGylated dAb having a higher hydrodynamic size (e.g., 200 to 500 kDa) is generated according to the invention, because the larger hydrodynamic size will confirm a greater level of protection from protease degradation than a PEGylated dAb having a lower hydrodynamic size. In one embodiment, a PEG- or other polymer-linked antibody single variable domain monomer or multimer is degraded by no more than 10% when exposed to one or more of pepsin, trypsin, elastase, chymotrypsin, or carboxypeptidase, wherein if the protease is pepsin then

exposure is carried out at pH 2.0 for 30 minutes, and if the protease is one or more of trypsin, elastase, chymotrypsin, or carboxypeptidase, then exposure is carried out at pH 8.0 for 30 minutes. In a preferred embodiment, a PEG- or other polymer-linked dAb monomer or multimer is degraded by no more than 10% when exposed to pepsin at pH 2.0 for 30 minutes, preferably no more than 5%, and preferably not degraded at all. In a further preferred embodiment, a PEG- or other polymer-linked dAb multimer (e.g., hetero- or homodimer, trimer, tetramer, octamer, etc.) of the invention is degraded by less than 5%, and is preferably not degraded at all in the presence of pepsin at pH 2.0 for 30 minutes. In a preferred embodiment, a PEG- or other polymer-linked dAb monomer or multimer is degraded by no more than 10% when exposed to trypsin, elastase, chymotrypsin, or carboxypeptidase at pH 8.0 for 30 minutes, preferably no more than 5%, and preferably not degraded at all. In a further preferred embodiment, a PEG- or other polymer-linked dAb multimer (e.g., hetero- or homodimer, trimer, tetramer, octamer, etc.) of the invention is degraded by less than 5%, and is preferably not degraded at all in the presence of trypsin, elastase, chymotrypsin, or carboxypeptidase at pH 8.0 for 30 minutes.

The relative ratios of protease:antibody single variable domain polypeptide may be altered according to the invention to achieve the desired level of degradation as described above. For example the ratio or protease to antibody single variable domain may be from about 1:30, to about 10:40, to about 20:50, to about 30:50, about 40:50, about 50:50, about 50:40, about 50:30, about 50:20, about 50:10, about 50:1, about 40:1, and about 30:1.

Accordingly, the present invention provides a method for decreasing the degradation of an antibody single variable domain comprising linking an antibody single variable domain monomer or multimer to a PEG polymer according to any of the embodiments described herein. According to this aspect of the invention, the antibody single variable domain is degraded by no more than 10% in the presence of pepsin at pH2.0 for 30 minutes. In particular, a PEG-linked dAb multimer is degraded by no more than 5%, and preferably not degraded at all in the presence of pepsin at pH 2.0 for 30 minutes. In an alternate embodiment, the antibody single variable domain is degraded by no more than 10% when exposed to trypsin, elastase,

chymotrypsin, or carboxypeptidase at pH 8.0 for 30 minutes, preferably no more than 5%, and preferably not degraded at all.

Degradation of PEG-linked dAb monomers and multimers according to the invention may be measured using methods which are well known to those of skill in the art. For example, following incubation of a PEG-linked dAb with pepsin at pH 2.0 for 30 minutes, or with trypsin, elastase, chymotrypsin, or carboxypeptidase at pH 8.0 for 30 minutes, the dAb samples may be analyzed by gel filtration, wherein degradation of the dAb monomer or multimer is evidenced by a gel band of a smaller molecular weight than an un-degraded (i.e., control dAb not treated with pepsin, trypsin, chymotrypsin, elastase, or carboxypeptidase) dAb. Molecular weight of the dAb bands on the gel may be determined by comparing the migration of the band with the migration of a molecular weight ladder (see Figure 5). Other methods of measuring protein degradation are known in the art and may be adapted to evaluate the PEG-linked dAb monomers and multimers of the present invention.

Pharmaceutical Compositions, Dosage and Administration

The antibody polypeptides of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a monovalent anti-CD40L antibody polypeptide and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The term "pharmaceutically acceptable carrier" excludes tissue culture medium comprising bovine or horse serum. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances include minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody polypeptide.

The compositions as described herein may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular).

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

The antibody polypeptides described herein can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. The polypeptide can also be administered by intramuscular or subcutaneous injection.

As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Single immunoglobulin variable domains and other relatively small monovalent antibody polypeptides are well suited for formulation as extended release preparations due, in part, to their small size – the number of moles per dose can be significantly higher than the dosage of, for example, full sized antibodies. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Additional methods applicable to the controlled or extended release of polypeptide agents such as the monovalent antibody polypeptides disclosed herein are described, for example, in U.S. Patent Nos. 6,306,406 and 6,346,274, as well as, for example, in U.S. Patent Application Nos. US20020182254 and US20020051808, all of which are incorporated herein by reference.

In certain embodiments, a monovalent anti-CD40L antibody polypeptide can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Additional active compounds can also be incorporated into the compositions. In certain embodiments, a monovalent anti-CD40L antibody polypeptide is coformulated with and/or coadministered with one or more additional therapeutic agents. For example, a monovalent anti-CD40L antibody polypeptide can be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules), or, for example, one or more cytokines. Such combination therapies may utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

The pharmaceutical compositions of the invention can include a "therapeutically effective amount" or a "prophylactically effective amount" of a monovalent anti-CD40L antibody polypeptide. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody polypeptide can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the monovalent anti-CD40L antibody polypeptide to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, because a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit

form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

A non-limiting range for a therapeutically or prophylactically effective amount of a monovalent anti-CD40L antibody polypeptide is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values can vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the administering clinician.

The efficacy of treatment with a monovalent anti-CD40L antibody polypeptide as described herein is judged by the skilled clinician on the basis of improvement in one or more symptoms or indicators of the disease state or disorder being treated. An improvement of at least 10% (increase or decrease, depending upon the indicator being measured) in one or more clinical indicators is considered "effective treatment," although greater improvements are preferred, such as 20%, 30%, 40%, 50%, 75%, 90%, or even 100%, or, depending upon the indicator being measured, more than 100% (e.g., two-fold, three-fold, ten-fold, etc., up to and including attainment of a disease-free state. Indicators can be physical measurements, e.g., enzyme, cytokine, growth factor or metabolite levels, rate of cell growth or cell death, or the presence or amount of abnormal cells. One can also measure, for example, differences in the amount of time between flare-ups of symptoms of the disease or disorder (e.g., for remitting/relapsing diseases, such as multiple sclerosis). Alternatively, non-physical measurements, such as a reported reduction in pain or discomfort or other indicator of disease status can be relied upon to gauge the effectiveness of treatment. Where non-physical measurements are made, various clinically acceptable scales or indices can be used, for example, the Crohn's Disease Activity Index, or CDAI (Best et al., 1976, Gastroenterology 70: 439), which combines both physical indicators, such as hematocrit and the number of liquid or

very soft stools, among others, with patient-reported factors such as the severity of abdominal pain or cramping and general well-being, to assign a disease score.

As the term is used herein, "prophylaxis" performed using a composition as described herein is "effective" if the onset or severity of one or more symptoms is delayed or reduced by at least 10%, or abolished, relative to such symptoms in a similar individual (human or animal model) not treated with the composition.

Whereas the monovalent anti-CD40L antibody polypeptides described herein must bind human CD40L, where one is to evaluate its effect in an animal model system, the polypeptide must cross-react with one or more antigens in the animal model system, preferably at high affinity. One of skill in the art can readily determine if this condition is satisfied for a given animal model system and a given monovalent anti-CD40L antibody polypeptide. If this condition is satisfied, the efficacy of the monovalent anti-CD40L antibody polypeptide can be examined by administering it to an animal model under conditions which mimic a disease state and monitoring one or more indicators of that disease state for at least a 10% improvement.

Animal Models:

Monovalent anti-CD40L antibody polypeptides as described herein are useful for the treatment of autoimmune disorders in which CD40/CD40L signaling is inappropriately active. There are several animal models in which the therapeutic efficacy of a given monovalent anti-CD40L antibody polypeptide can be assessed, as discussed below.

Systemic Lupus Erythematosis (SLE):

Anti-CD40L antibody treatment prevents the development of lupus-like nephritis in NZB/NZW and SNF1 SLE mice. Treatment of SNF1 mice with anti-CD40L antibody reverses established nephritis and preserves kidney function. See, e.g., Mohan et al., 1995, J. Immunol. 154: 1470-1480; Early et al., 1996, J. Immunol. 157: 3159-3164; Kalled et al., 1998, J. Immunol. 160: 2158-2165 ,and Chess, 2001,

"Blockade of the CD40L/CD40 Pathway," in Therapeutic Immunology 2nd Edition, Austen, Burakof, Rosen and Strom, Eds., Blackwell Sciences (Pubs.), pp 441-456.

Multiple Sclerosis:

Specific blockade of CD40L at the time of immunization markedly suppresses the incidence, mortality, day of onset, and clinical scores of experimental autoimmune encephalomyelitis (EAE) in B10P1L and (PLJ x SJL)F1 mice induced by either myelin basic protein or PLP myelin antigens. See, for example, Gerritse, 1996, Proc. Natl. Acad. Sci. U.S.A. 93: 2494; Grewal et al., 1996, Science 273: 186; Laman et al., 1998, Mult. Scler. 4: 14; and Chess, 2001, supra.

Rheumatoid Arthritis:

Anti-CD40L blocks the development of joint inflammation, serum antibody titers to collagen, the infiltration of inflammatory cells into the synovial tissue, ant the erosion of cartilage and bone in collagen-induced arthritis. See, e.g., Durie et al., 1993, Science 261: 132; and Chess, 2001, supra.

Insulin-dependent Type I Diabetes Models:

The non-obese diabetic (NOD) mouse spontaneously develops T cell dependent autoimmune diabetes. Anti-CD40L monoclonal antibody treatment of 3 to 4 week old NOD females (the age at which insulitis typically begins) completely prevented the insulitis and diabetes. Cytokine analysis revealed a dramatic decrease in IFN-g and IL-2 release without a concomitatnt increase in IL-4 production by T cells from anti-CD40L-treated mice. See, e.g., Balasa et al., 1997, J. Immunol. 159: 1420; and Chess, 2001, supra.

Inhibition of Allograft and Xenograft Transplant Rejection:

Anti-CD40L prevents the development of renal rejection of fully allogeneic grafts in mice. Moreover, the survival of renal allografts transplanted into nephrectomized rehsus monkeys is typically prolonged by anti-CD40L therapy alone. Similarly, anti CD40L therapy has prevented graft rejection of skin, islet cells and

cardiac transplants as well as GVHD in rodents. See, e.g., Kirk et al., 1997, Proc. Natl. Acad. Sci. U.S.A. 94: 8789-8794; Parker et al., 1995, Proc. Natl. Acad. Sci. U.S.A. 92: 9560; Larsen et al., 1996, Transplantation 61: 4; and Chess, 2001, supra.

Uses of Monovalent Anti-CD40L Antibody Polypeptides

Anti-CD40L antibody polypeptides as described herein are useful for the treatment or prevention of diseases or disorders in which inappropriate activation of a CD40L/CD40-mediated pathway is involved. In particular, autoimmune diseases frequently involve inappropriate regulation or activity of CD40L/CD40 pathways. Administration of an anti-CD40L antibody polypeptide as described herein to an individual suffering from such a disease, can reduce one or more symptoms of the disease. Non-limiting examples of diseases for which the antibody polypeptides described herein can be therapeutically useful include Systemic Lupus Erythematosus (SLE), Idiotypic Thrombocytopenic Purpura (ITP), transplant rejection, Crohn's Disease, Inflammatory Bowel Disease (IBD), colitis, asthma/allergy, atherosclerosis, Myasthenia Gravis, immune response to recombinant drug products, e.g., factor VII in hemophilia, Multiple Sclerosis, Psoriasis, Rheumatoid Arthritis, Ankylosing Spondylitis, Coronary Heart Disease, and Diabetes, including Type 1 and/or Type 2 Diabetes.

The anti-CD40L antibody polypeptides described herein are additionally useful in the way that generally any antibody preparation is useful, e.g., for in vivo imaging or diagnostic uses, in vitro diagnostic uses, etc. For these and other uses it may be desirable to label the anti-CD40L antibody polypeptides, e.g., with a fluorescent, colorimetric, enzymatic or radioactive label. Methods of labeling antibody polypeptides are well known in the art.

EXAMPLES

Example 1. Biotinylation of Recombinant CD40L

Recombinant human soluble CD40L (PeproTech) was biotinylated and used during phage selections. Reagents, equipment and sources from which they are available are provided in Table 1.

Biotinylation of CD40L was performed by incubating CD40L (0.5mg/ml) with EZ-Link™ Sulfo-NHS-LC-Biotin [Sulfosuccinimidyl-6-(biotinamido)hexanoate] (Pierce) at a molar ratio of 5:1 on ice for 2 hours according to the product instructions. The biotinylation reaction mixture was then dialysed against 3 exchanges of PBS (1000x sample volume) in a Slide-A-Lyzer® Dialysis Cassette at 4°C to remove the unincorporated biotinylation reagent.

The biotinylated-CD40L was tested by receptor binding assay for binding to CD40/Fc to confirm its biological activity. Quality of biotin-CD40L was also monitored by analysing on a NuPaGE 4-12% Bis-Tris gel and detected by Simply Blue Safe-Stain (Invitrogen) (Figure 1a), and western-blotting by probing with Streptavidin-HRP (Figure 1b). The biotinylated-CD40L was further analysed by mass spectrometry with the majority of CD40L subunits containing 1 or 2 biotin moieties (data not shown).

Table 1.

Equipment/Reagent	**Suggested or required supplier**
Recombinant human soluble CD40 ligand/TRAP	PeproTech, Cat No: 310-02 Reconstituted in 5 mM Sodium phosphate, pH5.0 to concentration of 0.5 mg/ml
EZ-Link™ Sulfo-NHS-LC-Biotin	Pierce, Cat No: 21335
Slide-A-Lyzer® Dialysis Cassette	Pierce, Cat No: 66110

Recombinant human CD40/Fc chimera	R&D Systems, Cat No: 1493-CD
NuPAGE® 4-12% Bis-Tris gel	Invitrogen life technologies Ltd Cat. No NP0322
Streptavidin-HRP	Amersham Biosciences Cat No: 1231V
Invitrogen™ Simply Blue Safe-stain	Invitrogen Cat No:LC6065

Example 2. Phage Selections using Biotinylated Antigen

The Domain Antibody (dAb) libraries are based on a single human framework for the V_H (DP47 and JH4b) and for the VK (DPK9 and JK1) with side chain diversity incorporated at positions in the antigen binding site that make contact with antigen in known molecular structures and mirror residues diversified in the human antibody repertoire. The antibodies are displayed as fusion proteins covalently linked to the N – terminus of the Fd-phage protein pIII, using the phage vector pDOM4 (Fd-Tet) with encodes the Fd phage genome with dAb expression under the control of the gene-III promoter. The dAb cassette consists of (5′ to 3'): eukaryotic leader sequence, dAb, myc tag, gIII. The vector contains both the *M13* and *colE1* origins of replication and is selectable using tetracycline. The V_H and V_K libraries each have a calculated size of over $1x10^{10}$ molecules. Reagents, equipment and sources from which they are available are provided in Table 2.

Approximately $1x10^{11}$ phage from the each of the Domantis dAb libraries were incubated in a final volume of 1 ml PBS containing 2% Marvel™ at room temperature for 1 h. Biotinylated antigen was added to the blocked phage such that the phage antigen mixture had a final concentration of 2% Marvel™ in PBS. The antigen concentration used for the first round of selection was 60 nM; the antigen concentration was decreased to 6 nM for round 2, and to 0.6 nM for round 3. The antigen/phage mix was incubated for 1 h at room temperature with rotation at ~40 rpm.

For each selection, 100 µl of streptavidin-coated paramagnetic beads (Dynal Biotech) were prepared by washing once in 1 ml of PBS containing 0.1% Tween-20 followed by a second wash in 1 ml of PBS. The beads were then blocked in 1 ml of PBS containing 2% Marvel™ in a 2 ml eppendorf tube at room temperature on a rotating wheel for 1 h.

The tube containing the blocked streptavidin-coated magnetic beads was placed into a magnetic holder, enabling capture of the magnetic beads. The

supernatant was removed and the beads resuspended in the antigen/phage mix. This mixture was rotated for 10 min to allow for bead capture of phage/antigen complexes.

The beads were captured using a magnetic holder and repeatedly washed 19 times using 1 ml of PBS containing 0.1% Tween-20, followed by a final wash of 1 ml PBS. The eppendorf tubes were changed following washing steps 3, 9, 15 and 19 to minimise background phage carryover.

The washed beads were then recaptured and all washing solution removed. The phage were eluted through resuspension in 500 µl of trypsin solution (50 µl of 10 mg/ml trypsin stock solution added to 450 µl PBS, freshly diluted) and rotated for 10 min at room temperature. The eluted phage were recovered by capturing the beads using the magnetic holder and the liquid containing the eluted phage recovered. The eluted phage were used to infect *E. coli* TG1 to prepare phage for a further round of selection.

The eluted phage (250 µl) were mixed with 1.75 ml of log phase *E. coli* TG1 (OD_{600} between 0.3 and 0.6) and infection allowed to occur for 30 min at 37°C without shaking. The infected *E. coli* TG1 culture was centrifuged at 11,600 g in a micro centrifuge for 1 min at room temperature. The pelleted bacteria were resuspended in 100 µl of 2xTY and plated on regular 9 cm diameter plates containing TYE supplemented with 15 µg/ml tetracycline. Plates were grown at 37°C overnight.

After overnight growth, 2 ml of 2xTY containing 15% glycerol was added to the culture plates and cells loosened with a spreader, ensuring the cells were thoroughly mixed. Two millilitres of the culture were recovered by pipetting into a cryo-vial, from which 50 µl was used to inoculate 50 ml of 2xTY supplemented with 15 µg/ml tetracycline. The remaining cells in the cryo-vial were stored at -80°C.

The 50 ml culture was grown at 37°C for 16 to 24 hours with shaking at 250 rpm.

Following overnight growth, the culture was centrifuged at 3,300 g for 15 min to pellet the bacteria. The phage were then precipitated from the supernatant through

the addition of 10 ml of PEG/NaCl to 40 ml of clarified supernatant. The phage/PEG solution was mixed and incubated on ice for at least 1 h. To pellet the phage, the solution was centrifuged at 3,300 g for 30 min at 4°C. The supernatant was decanted and any remaining supernatant removed by aspiration.

The resulting phage pellet was resuspended in 2 ml PBS and centrifuged at 11,600 g for 10 min in a micro centrifuge to remove any remaining bacterial debris. The supernatant was filtered through a 0.45 μm filter (Sartorius, Minisart). The resuspended phage solution was used for the next round of selection.

Table 2

Equipment/Reagent	**Suggested or required supplier**	**Instrument setting, reagent preparation**
Dynabeads® M-280 Streptavidin (Prod. No.: 112.05)	Dynal Biotech UK 11 Bassendale Road, Croft Business Park, Bromborough, Wirral CH62 3QL UK	Resuspend thoroughly through repeated pipetting.
Tween 20	Sigma Chemical Company Ltd.	0.1% in PBS.
99.5% dried skim milk powder	Marvel™ (premier brands) from supermarkets.	2% in PBS (prepare fresh and do not store).
Trypsin (T-8642) Type XIII from Bovine Pancreas.	Sigma Chemical Company Ltd. Fancy Road Dorset BH17 7NH U.K Tel +44 1202 733114 Fax +44 1202 715460	made up in 50 mM Tris-HCl pH7.4; 1 mM $CaCl_2$ and stored at -20°C. *The trypsin stock solution should be stored aliquotted at -20°C to avoid autoproteolysis.*
PEG/NaCl	Sigma Chemical Company Ltd.	20% Polyethylene glycol 8000 [formally known as 6000], 2.5 M NaCl pre-chilled to 4°C.
Dynal MPC-S magnetic particle concentrator (Prod.	Dynal Biotech UK 11 Bassendale Road, Croft Business Park, Bromborough, Wirral	

No.: 120.20)	CH62 3QL UK	
2xTY		16 g Tryptone, 10 g Yeast Extract and 5 g NaCl in 1 litre. Autoclave (121°C, 15 min) and store at RT

Example 3: Cloning Enriched Phage Selection Outputs into the Soluble dAb Expression Vector pDOM5

Following the second and third rounds of selection, *E. coli* cells infected with the enriched dAb displaying fd-phage populations were obtained. An aliquot of these cells was used to prepare phage DNA and the enriched V-genes excised by digestion using the restriction endonucleases, *Sal*I and *Not*I. The purified V-genes were ligated into the corresponding sites of pDOM5 (expression vector derived from pUC119 with LacZ promoter, eukaryotic leader, dAb cloning site, myc tag), and the ligated DNA used to electro-transform *E. coli* HB2151 cells which were grown overnight on agar plates containing the antibiotic carbenicillin. The resulting colonies were induced to express dAb protein either as 200 µl microcultures or 50 ml cultures. The resulting dAb was analysed for inhibitory activity using the CD40L receptor binding assay.

Following selection of phage, pDOM4 DNA was purified from the cell pellet obtained from a 50 ml overnight *E. coli* culture using the QIAfilter Plasmid Midi DNA purification kit from Qiagen, following the manufacturer's instructions. The dAb genes were excised from the pDOM4 vector by mixing: 10 µl of 10x *Sal*I buffer; 1 µl of 100x BSA; 20 µg of purified DNA fragment; 2.5 µl of *Sal*I enzyme (10 U/µl); 2.5 µl of *Not*I enzyme (10 U/µl); the digestion mix was made up to a final volume of 100 µl using sterile water. The digestion mix was incubated for 5 hours at 37°C.

The digested DNA samples were electrophoresed on a 1.5% agarose gel and the band corresponding to the dAb V-genes (~324 bp to 372 bp) was excised from the gel. The dAb gene DNA was purified from the gel slice using the QIAquick Gel Extraction kit from Qiagen, following the manufacturer's instructions.

The expression vector pDOM5 was digested with *Sal*I and *Not*I as follows: 10 µl of 10x *Sal*I buffer; 1 µl of 100x BSA; 20 µg of plasmid pDOM5; 1.5 µl of *Sal*I enzyme (10 U/µl); 1.5 µl of *Not*I enzyme (10 U/µl); the digestion mix was made up to a final volume of 100 µl using sterile water. The digestion mix was incubated for 2 hours at 37°C. The digested vector fragment was purified using the QIAquick PCR Purification Kit.

The digested pDOM5 and digested dAb genes were ligated by mixing: 2 µl of 10x T4 DNA ligase buffer; 400 ng of digested pDOM5 vector; 100 ng of digested dAb genes;

1 µl of T4 DNA ligase (400 U/µl); the ligation mix was made up to 20 µl with sterile water. The ligation mixes were incubated for 2 hours at 25°C.

Two microlitres of the ligation mix was transferred to the bottom of a pre-chilled (on ice) 0.2 cm electroporation cuvette to which 100 µl of electrocompetent *E. coli* HB2151 cells were added. The DNA / cell mixture was incubated on ice for 1-2 min, then electroporated at 2.5 kV (25 µF, 200 Ω). One millilitre of 2xTY was immediately added to the cuvette and the cells gently resuspended. The resuspended cells were transferred to a 14 ml disposable culture tube and incubated for 1 hour at 37°C with shaking at 250 rpm. Dilutions of the cells from 10^{-0} to 10^{-3} were plated on regular 9 cm diameter plates containing TYE supplemented with 5% glucose and 50 µg/ml carbenicillin. The cells are incubated overnight at 37°C in an inverted position. Reagents, equipment and sources from which they are available are provided in Table 3.

Table 3

Equipment/Reagent	**Suggested or required supplier**	**Instrument setting, reagent preparation**
QIAfilter Plasmid Midi DNA purification kit	Qiagen Ltd Cat. No.: 12143	Supplied as kit
*Sal*I restriction endonuclease + 10x *Sal*I buffer	New England Biolabs Cat. No.: R0138S	
*Not*I restriction endonuclease + 10x *Not*I buffer + 100x BSA	New England Biolabs Cat. No.: R0189S	
QIAquick Gel Extraction kit	Qiagen Ltd Cat. No.: 28706	Supplied as kit
Expression plasmid pDOM5		
T4 DNA ligase + 10x T4 DNA ligase buffer	New England Biolabs Cat. No.: M0202L	The T4 DNA ligase buffer should be stored aliquotted at -20°C. Repeated freeze-thawing should be avoided to minimise the hydrolysis of ATP in the buffer.

Example 4. Microwell Expression of Soluble dAbs

Following cloning of the selected phage dAb outputs into pDOM5, individual bacterial colonies were inoculated as microwell cultures and induced using IPTG to express dAb protein which was analysed for inhibitory activity using the CD40L receptor binding assay. Reagents, equipment and sources from which they are available are provided in Table 4.

Individual bacterial colonies were carefully picked to ensure that contamination from neighbouring colonies was avoided. The picked colonies were used to inoculate 96 well cell culture plates containing 100 µl per well of 2xTY supplemented with 5% glucose and 50 µg/ml carbenicillin. The lids were placed on the cell culture plates which were incubated overnight in a HiGro orbital shaker (GeneMachines, 935 Washington St, San Carlos, CA 94070, USA) under a humidified atmosphere at 37°C with shaking at 450 rpm (4 mm shaking orbital diameter), with gas (30% O_2 + 70% N_2) pulsed for 10 seconds every 5 minutes at a flow rate of 5 SLPM (standard litres per minute). [These plates are referred to as Master Plates].

Following overnight growth, a 96 well transfer device was used to transfer between 1–5 µl of the bacterial culture into a fresh 96 well culture plate containing 100 µl per well of 2xTY supplemented with 0.1% glucose and 50 µg/ml carbenicillin.

The freshly inoculated plates were incubated at 37°C for 3 to 4 h (shaking at 450 rpm, gas (30% O_2 + 70% N_2) pulsed for 10 seconds every 5 minutes at a flow rate of 5 SLPM) until the culture OD_{600} reached approximately 1.0. The cultures were then induced by the addition of 100 µl per well of 2xTY containing 50 µg/ml carbenicillin and 2 mM IPTG (final IPTG concentration of 1 mM) and incubated overnight at 30°C with shaking at 450 rpm, with gas (30% O_2 + 70% N_2) pulsed for 10 seconds every 5 minutes at a flow rate of 5 SLPM. [These plates are referred to a Induction Plates].

Glycerol stocks of the original Master Plates were made by the addition of 100 µl per well of 2xTY containing 50% sterile glycerol. These plates were stored at -80°C.

Following overnight incubation of the Induction Plates, the bacterial cells were pelleted by centrifugation at 1,800 g for 10 min at 4°C. The supernatant (containing expressed dAb) was then analysed to determine if dAbs were capable of inhibiting binding of CD40L to CD40-Fc fusion in a receptor binding assay.

Table 4

Equipment/Reagent	**Suggested or required supplier**	**Instrument setting, reagent preparation**
96 Well Cell Culture Cluster with round bottom and lid, Non-pyrogenic, Polystyrene	Corning Incorporated, Costar. Number:3799	
2xTY		16 g Tryptone, 10 g Yeast Extract and 5 g NaCl in 1 litre. Autoclave (121°C, 15min) and store at RT

Example 5. Expression of dAb in *E. coli* at 50 ml

To generate greater quantities of dAb protein for analysis, 50 ml cultures were used for induction. A single colony of the desired dAb (for example DOM-24) grown on TYE plates was inoculated into 10 ml 2xTY supplemented with 5% glucose and 50 µg/ml carbenicillin in a 30 ml universal tube and grown overnight at 37°C with shaking at 250 rpm. Five hundred microlitres of the overnight culture was added into 50 ml of 2xTY supplemented with 0.1% glucose and 50 µg/ml carbenicillin and grown at 37°C with shaking at 250 rpm. The OD_{600} of the culture was monitored regularly in comparison with sterile 2xTY and at an OD_{600} of 0.9 the culture was induced by the addition of 1 M IPTG to a final concentration of 1 mM. The inoculated culture was incubated at 30°C with shaking at 250 rpm overnight. The next day, the culture was centrifuged at 6000 g for 15 min at 4°C and the clarified supernatant mixed with 100 µl of protein-A streamline or protein-L agarose (pre-washed with 5 mM $MgSO_4$) overnight at 4°C. The supernatant/bead mixture was then centrifuged at 180 g at 4°C for 2 minutes. The supernatant was decanted and the retained beads washed with 10 ml of PBS containing 0.5M NaCl. The bead solution was transferred into a 96 well Whatman filter plate and the beads washed once with 400 µl of PBS containing 0.5M NaCl, then once with 400 µl of PBS, followed by centrifugation for 2 minutes at 180 g after each washing step. dAb protein was eluted using 70 µl of 0.1 M glycine (pH 2.0) and the solution neutralised by the addition of 40 µl of 1 M Tris-HCl (pH 8.0). The purified dAb concentration was determinate by OD_{280}.

Reagents, equipment and sources from which they are available are provided in Table 5.

Table 5

Equipment/Reagent	Suggested or required supplier	Instrument setting, reagent preparation

TYE		15 g Bacto-Agar, 8 g NaCl, 10 g Tryptone, 5 g Yeast Extract in 1 litre water. Autoclave (121°C, 15 min) and store at RT
2xTY		16 g Tryptone, 10 g Yeast Extract and 5 g NaCl in 1 litre. Autoclave (121°C, 15 min) and store at RT
1 M IPTG		stock made up in MQ water is sterile filtered through 0.22 µM filter and stored in aliquots at -20°C
Carbenicillin		50 mg/ml stock made in water, 0.2 µm filter sterilised and stored in aliquots at -20°C
40% glucose solution		0.2 µm filter sterilise, store at RT
5 mM $MgSO_4$		prepare fresh from 1 M stock solution, 0.2 µm filter sterilise and store at RT
0.5M NaCl/PBS		Autoclave or 0.2 µm filter sterilise and store at RT
Protein A agarose	Sigma P3476	store 4°C
Protein L agarose	Sigma P3351	store 4°C

Streamline rProtein A	Amersham Biosciences, cat no. 17-1281-02 (300 ml)	store 4°C
1 M Tris-HCl, pH 8.0		0.2 μm filter sterilise or autoclave and store at RT
0.2 M Glycine, pH2.0		0.2 μm filter sterilise and store at 4°C

Example 6: CD40L Receptor Binding Assay

The CD40L assay was used to measure the binding of CD40L to CD40 and the ability of binding entities (eg, monvalent antibody fragments such a dAbs) to block this interaction, as described below and shown schematically in Figure 7. (The soluble proteins from R&D Systems are CD40/Fc = homodimer and CD40L = homotrimer).

A 96 well Nunc Maxisorp assay plate was coated overnight at 4°C with 100 µl per well of recombinant human CD40/Fc (R&D Systems) at 0.5 µg/ml in carbonate buffer. The plate was washed 3 times with 300 µl of 0.05% Tween/PBS and 3 times with 300 µl of PBS using a Tecan plate washer. The wells were blocked using 200 µl of PBS containing 2% (w/v) BSA and incubated for a minimum of 1 h at room temperature. The wells were washed as above, then 50 µl of purified dAb protein (or unpurified supernatant containing dAb from a micro-culture expression) was added to each well. To each well 50 µl of CD40L, at 6 ng/ml in diluent (for a final concentration of 3 ng/ml), was also added and the plate incubated for 1 hr at room temperature.

The plate was washed as described previously and 100 µl biotinylated anti-CD40L antibody, 0.5 µg/ml in diluent, was added and incubated for 1 hr at room temperature. The plate was washed as described above, then 100 µl HRP conjugated anti-biotin antibody (1:5000 dilution in diluent) added to each well and the plate incubated for 1 hr at room temperature. The plate was washed again as described above using a Tecan plate washer and the assay developed using 100 µl of SureBlue 1-Component TMB MicroWell Peroxidase solution (the plate was left at room temperature for up to 20 min). The reaction was stopped by the addition of 100 µl 1 M hydrochloric acid. The OD_{450nm} of the plate was assayed within 30 minutes of acid addition. The OD_{450nm} is proportional to the amount of bound streptavidin-HRP conjugate, therefore the greater the degree of dAb inhibition the lower the OD_{450nm} of the resulting signal. Reagents, equipment and sources from which they are available are provided in Table 6.

Controls

The following controls were included:

- 0 ng/ml CD40L (diluent only)
- 3 ng/ml CD40L
- 3 ng/ml CD40L with 1 µg/ml anti-CD40L antibody

Table 6

Equipment/Reagent	Suggest or required supplier (specify)	Reagent preparation
F96 Maxisorp 96 well immunoplate	Nunc, Cat No: 439454	
0.2M sodium carbonate bicarbonate buffer pH9.4	Pierce, Cat No: 28382	Dissolve 1 sachet in 500 ml deionised water and keep solution at 4 °C
Recombinant human CD40/Fc chimera	R&D Systems, Cat No: 1493-CD	Stock 50 µg/ml at-80 °C
Phosphate buffered saline (PBS)	Sigma, Cat No: P4417	10x solution100 tablets/L water.
Wash buffer		0.05% Tween-20/PBS
Diluent		0.1% BSA, 0.05% Tween-20 in PBS
Block		2% BSA in PBS
Recombinant human CD40L	R&D Systems, Cat No: 617-CL	Stock 50 µg/ml at-80 °C
Neutralising anti-CD40L antibody	Calbiochem, Cat No: 217595	Stock 1 mg/ml at 4 °C
Biotinylated anti-CD40L antibody	R&D Systems, Cat No: BAF617	Stock 50 µg/ml at -80 °C

Anti-biotin-HRP conjugate	Stratech, Cat No: 200-032-096	Stock 800 µg/ml at -80C, diluted 1:5000 in antibody diluent. Keep for 1 week only.
SureBlue TMB 1-component microwell peroxidase substrate	KPL, Cat No: 52-00-00	at 4 °C .

Example 7: Results

Receptor binding data for the most potent inhibitors is summarised in Figures 2, 3, and 4, and in Table 7, below. Table 8, below, provides DNA and translated amino acid sequence of unique dAbs identified in the receptor binding assay as inhibiting CD40L binding to CD40.

Figure 2 shows a dose response receptor binding assay(RBA) readout, analysing the inhibition of CD40L binding to CD40-Fc by dAbs DOM-10, -20, -27, -30, -31, -62, -77, titrated from 1 μM down to 10 pM. dAbs DOM-20, -30, and -31 are the most potent, with IC_{50} values of approximately 8 nM.

Figure 3 shows a dose response receptor binding assay readout, analysing the inhibition of CD40L binding to CD40-Fc by dAbs DOM-4 and DOM-5, titrated from 1 μM down to 500 pM. The IC_{50} values for dAbs DOM-5 and DOM-4 are approximately 3 nM and 100 nM respectively.

Figure 4 shows a dose response receptor binding assay readout, analysing the inhibition of CD40L binding to CD40-Fc by dAb DOM-24, titrated from 100 nM down to 0.5 pM. The data were curve-fitted using GraphPad Prism software.

Table 7

Clone Name	dAb Type	IC_{50} (nM)
DOM-2	VH	800
DOM-4	VH	100
DOM-5	VH	3
DOM-7	VH	1500
DOM-8	VK	900
DOM-10	VH	50
DOM-20	VH	8

DOM-24	VH	0.417
DOM-27	VH	100 approx.
DOM-30	VH	8
DOM-31	VH	8
DOM-77	VH	40

Table 8: Summary of dAbs exhibiting a range of CD40L inhibitory IC_{50} values as determined using the CD40L / CD40-Fc receptor inhibition assay.

The DNA and translated amino acid sequence of unique dAbs identified in the receptor binding assay as inhibiting CD40L binding to CD40 are detailed below:

DOM-2 SEQ ID NO: 7

```
            E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S D
            Y E M·

      1     GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT
            GATTATGAGA

            CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGA
            CTAATACTCT

            · M W V R Q A P G K G L E W V S T I T S D G I S T Y Y A D S V
            K G R·

    101     TGATGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTACTTCGG ATGGTATTTC TACATACTAC GCAGACTCCG
            TGAAGGGCCG

            ACTACACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAATGAAGCC TACCATAAAG ATGTATGATG CGTCTGAGGC
            ACTTCCCGGC

            · F T I F R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
            K S G

    201     GTTCACCATC TTCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC
            GAAAAGTGGG

            CAAGTGGTAG AAGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG
            CTTTTCACCC

            R F F D Y W G Q G T L V T V S S

    301     AGGTTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 94)
```

TCCAAAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 95)

DOM-4 SEQ ID NO: 8

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D N Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT AATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTA TTAATACTCT

· T W V R Q A P G K G L E W V S S I T S D G T S T Y Y A D S V K G R·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACGAGTG ATGGTACTTC GACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAATGCTCAC TACCATGAAG CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P N

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCTAAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTGGATTA

P P F D Y W G Q G T L V T V S S

301 CCGCCGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 96)

GGCGGCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 97)

DOM-5 SEQ ID NO: 9

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D G Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT GGGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTA CCCATACTCT

· A W V R Q A P G K G L E W V S S I T S D G T S T Y Y A D S V K·G R·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACGAGTG ATGGTACGAG TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAATGCTCAC TACCATGCTC ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCGGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCCCC

L R F D Y W G Q G T L V T V S S

301 CTGCGTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 98)

GACGCAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 99)

DOM-7 SEQ ID NO: 10

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F N L
Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAT
TTGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATTA
AACATACTCT

· T W V R Q A P G K G L E W V S S I T S D G V S T Y Y A D S V
K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACTAGTG ATGGTGTTTC TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACTGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAATGATCAC TACCACAAAG ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K A G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAAGCTGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTCGACCC

V I F D Y W G Q G T L V T V S S

301 GTGATTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 100)

CACTAAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 101)

DOM-8 SEQ ID NO: 11

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q F I D T S L E·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTTTATTGAT ACGTCGTTAG

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CAAATAACTA TGCAGCAATC

· W Y Q Q K P G K A P K L L I Y D G S H L Q S G V P S R F S G S G S·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GGGTCCCATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

TCACCATAGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATACTA CCCAGGGTAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

·G T D F T L T I S S L Q P E D L A T Y Y C Q Q Y W V L P L T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTAG CTACGTACTA CTGTCAACAG TATTGGGTTC TTCCTCTGAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAATC GATGCATGAT GACAGTTGTC ATAACCCAAG AAGGAGACTG CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 102)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 103)

DOM-10 SEQ ID NO: 12

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F I A Y D M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTAGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTATT GCTTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AATCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATAA CGAATACTAT

· S W V R Q A P G K G L E W V S W I D E W G L Q T Y Y A D S V K G R·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCATGG ATTGATGAGT GGGGTCTGCA GACATACTAC GCAGACTCCG TGAAGGGCCG

ACTCAACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTACC TAACTACTCA CCCCAGACGT CTGTATGATG CGTCTGAGGC ACTTCCCGGC

·· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K K T

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAAAGACG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTTTCTGC

P E E F D Y W G Q G T L V T V S S

301 CCTGAGGAGT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C (SEQ ID NO: 104)

GGACTCCTCA AACTGATGAC CCCAGTCCCT TGGGACCAGT GGCAGAGCTC G (SEQ ID NO: 105)

DOM-11 SEQ ID NO: 13

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G D Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGT GATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCA CTAATACTCT

· S W V R Q A P G K G L E W V S G I D G E G S D T Y Y A D S V K G R·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGG ATTGATGGTG AGGGTTCTGA TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTCAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTCCC TAACTACCAC TCCCAAGACT ATGTATGATG CGTCTGAGGC ACTTCCCGGC

·F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCGGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTGGCCCC

R S F D Y W G Q G T L V T V S S

301 AGGAGTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 106)

TCCTCAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 107)

DOM-12 SEQ ID NO: 14

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F R L Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGG TTGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATCC AACATACTCT

· A W V R Q A P G K G L E W V S G I D I L G S R T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGG ATTGATATTT TGGGTTCGAG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTCCC TAACTATAAA ACCCAAGCTC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K D L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAGATCTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTCTAGAC

S W Q G F D Y W G Q G T L V T V S S

301 TCGTGGCAGG GTTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC (SEQ ID NO: 108)

AGCACCGTCC CAAAACTGAT GACCCCAGTC CCTTGGGACC AGTGGCAGAG CTCG (SEQ ID NO: 109)

DOM-13 SEQ ID NO: 15

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S Y Y S M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT TATTATTCGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGA ATAATAAGCT

· Y W V R Q A P G K G L E W V S S I S P F G W G T Y Y A D S V K G R ·

101 TGTATTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTTCGCCTT TTGGTTGGGG TACATACTAC GCAGACTCCG TGAAGGGCCG

ACATAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGC TAAAGCGGAA AACCAACCCC ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K D T L Y L Q M N S L R A E D T A V Y Y C A K Y G

201 GTTCACCATC TCCCGCGACA ATTCCAAGGA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATATGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCCT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTATACCC

E T S G P I S E N F D Y W G Q G T L V T V S S

301 GAGACGAGTG GTCCGATTTC TGAGAATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 110)

CTCTGCTCAC CAGGCTAAAG ACTCTTAAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 111)

DOM-14 SEQ ID NO: 16

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F W S Y D M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTGG TCTTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAACC AGAATACTAT

· T W V R Q A P G K G L E W V S S I M A S G D D T Y Y A D S V K G R·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTATGGCTT CGGGTGATGA TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAATACCGAA GCCCACTACT ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· P T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K W D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAATGGGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTACCCTA

R D F D Y W G Q G T L V T V S S

301 CGGGATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 112)

GCCCTAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 113)

DOM-15 SEQ ID NO: 17

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E E Y V M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GAGTATGTTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC CTCATACAAT

· S W V R Q A P G K G L E W V S T I S P I G L T T Y Y A D S V K G R·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACT ATTTCTCCTA TTGGTCTGAC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACAGCACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGA TAAAGAGGAT AACCAGACTG ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
E F P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GGAATTTCCT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CCTTAAAGGA

L I I L P D F D Y W G Q G T L V T V S S

301 TTGATTATTC TTCCTGATTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC (SEQ ID NO: 114)

AACTAATAAG AAGGACTAAA ACTGATGACC CCAGTCCCTT GGGACCAGTG GCAGAGCTCG (SEQ ID NO: 115)

DOM-16 SEQ ID NO: 18

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F M E
Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC CTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTATG
GAGTATGCGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG GACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATAC
CTCATACGCT

· I W V R Q A P G K G L E W V S I I S P L G L S T Y Y A D S V
K G R ·

101 TGATTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAATT ATTTCTCCGC TTGGTTTGTC TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACTAAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTAA TAAAGAGGCG AACCAAACAG ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K Y Q

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAATATCAG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTATAGTC

D S S D S Q Y T N F D Y W G Q G T L V T V S S

301 GATTCGTCTG ATAGTCAGTA TACGAATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 116)

CTAAGCAGAC TATCAGTCAT ATGCTTAAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 117)

DOM-17 SEQ ID NO: 19

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E D Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GATTATGGGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC CTAATACCCT

· G W A R Q A P G K G L E W V S S I G P L G L W T Y Y A D S A K G R ·

101 TGGGGTGGGC CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTGGTCCTC TGGGTCTTTG GACATACTAC GCAGACTCCG CGAAGGGCCG

ACCCCACCCG GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTCA TAACCAGGAG ACCCAGAAAC CTGTATGATG CGTCTGAGGC GCTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K S P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATCTCCG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTAGAGGC

L E G L I T N F D Y W G Q G T L V T V S S

301 CTTGAGGGTT TGATTACGAA TTTTGACTAC TGGGGTCAGG GAACCCTGGT CACCGTCTCG AGC (SEQ ID NO: 118)

GAACTCCCAA ACTAATGCTT AAAACTGATG ACCCCAGTCC CTTGGGACCA GTGGCAGAGC TCG (SEQ ID NO: 119)

DOM-18 SEQ ID NO: 20

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F P E Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCCT GAGTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAGGA CTCATACTAT

· T W V R Q A P G K G L E W V S Y I S S D G Y S T Y Y A D S V K G R ·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCATAT ATTAGTTCTG ATGGTTATTC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTATA TAATCAAGAC TACCAATAAG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P H

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCGCAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCGTA

G S P R E F D Y W G Q G T L V T V S S

301 GGGAGTCCGC GGGAGTTTGA CTACTGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC (SEQ ID NO: 120)

CCCTCAGGCG CCCTCAAACT GATGACCCCA GTCCCTTGGG ACCAGTGGCA GAGCTCG (SEQ ID NO: 121)

DOM-19 SEQ ID NO: 21

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F P F P Q
Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CCCCTTTCCG
CAGTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GGGGAAAGGC
GTCATAGTCT

· A W V R Q A P G K G L E W V S M I T S D G L D T Y Y A D S V
K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAATG ATTACTTCTG ATGGTCTTGA TACATATTAC GCAGACTCCG
TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTAC TAATGAAGAC TACCAGAACT ATGTATAATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P E

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC
GAAACCTGAG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG
CTTTGGACTC

P L F D Y W G Q G T L V T V S S

301 CCTCTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 122)

GGAGAAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 123)

DOM-20 SEQ ID NO: 22

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S G
Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCG GGTTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGC CCAATAGTCT

· A W V R Q A P G K G L E W V S G I S S E G L T T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGT ATTAGTTCGG AGGGTCTTAC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTCCA TAATCAAGCC TCCCAGAATG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K L G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATTGGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTAACCCC

R R F D Y W G Q G T L V T V S S

301 CGTAGGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 124)

GCATCCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 125)

DOM-21 SEQ ID NO: 23

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A N Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG AATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACGC TTAATACTCT

· G W A R Q A P G K G L E W V S V I S E W G Y S T Y Y A D S A K G R ·

101 TGGGGTGGGC CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGTT ATTTCTGAGT GGGGTTATTC TACATACTAC GCAGACTCCG CGAAGGGCCG

ACCCCACCCG GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTCAA TAAAGACTCA CCCCAATAAG ATGTATGATG CGTCTGAGGC GCTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K L V

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACTTGTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGAACAC

G G T Q Y E F D Y W G Q G T L V T V S S

301 GGTGGGACTC AGTATGAGTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC (SEQ ID NO: 126)

CCACCCTGAG TCATACTCAA ACTGATGACC CCAGTCCCTT GGGACCAGTG GCAGAGCTCG (SEQ ID NO: 127)

DOM-22 SEQ ID NO: 24

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F H N Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCAT AATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAGTA TTAATACTCT

·S W V R Q A P G K G L E W V S S I S S G G S S T Y Y A D S V K G R·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTTCTTCGG GTGGTTCTTC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACAGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTCA TAAAGAAGCC CACCAAGAAG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

·F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGCCCC

V K F D Y W G Q G T L V T V S S

301 GTTAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 128)

CAATTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 129)

DOM-23 SEQ ID NO: 25

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          E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G L
          Y E M·

1         GAGGTGCAGC TGTTGGAGTC CGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGG
          CTGTATGAGA

          CTCCACGTCG ACAACCTCAG GCCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCC
          GACATACTCT


          · T W V R Q A P G K G L E W V S S I T G D G I S T Y Y A D S V
          K G R·

101       TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTACGGGTG ATGGTATTTC GACATACTAC GCAGACTCCG
          TGAAGGGCCG

          ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTCA TAATGCCCAC TACCATAAAG CTGTATGATG CGTCTGAGGC
          ACTTCCCGGC


          · F T I S R D N S R N T L Y L Q M N S L R A E D T A V Y Y C A
          K A G

201       GTTCACCATC TCCCGCGACA ATTCCAGGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
          GAAAGCTGGG

          CAAGTGGTAG AGGGCGCTGT TAAGGTCCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
          CTTTCGACCC


          R K F D Y W G Q G T L V T V S S

301       AGGAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 130)

          TCCTTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 131)
```

DOM-24 SEQ ID NO: 26

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          E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S N
          Y Q M·

1         GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT
          AATTATCAGA

          CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATCA
          TTAATAGTCT


          · A W V R Q A P G K G L E W V S S I T S E G G S T Y Y A D S V
          K G R·

101       TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTACTAGTG AGGGTGGTTC GACATACTAC GCAGACTCCG
          TGAAGGGCCG

          ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTCA TAATGATCAC TCCCACCAAG CTGTATGATG CGTCTGAGGC
          ACTTCCCGGC
```

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACACTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCGGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGTGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCCCA

K N F D Y W G Q G T L V T V S S

301 AAGAATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 132)

TTCTTAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 133)

DOM-25 SEQ ID NO: 27

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D N
Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT
AATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTA
TTAATACTCT

· T W V R Q A P G K G L E W V S T I T S Q G T S T Y Y A D S V
K G R ·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTACGTCGC AGGGTACTAG TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAATGCAGCG TCCCATGATC ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGACTA

R S F D Y W G Q G T L V T V S S

301 CGTTCTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 134)

GCAAGAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 135)

DOM-26 SEQ ID NO: 28

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F R S
Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCGT AGTTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAGCA TCAATACTCT

· T W V R Q A P G K G L E W V S S I T S D G G T T Y Y A D S V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCATCT ATTACGTCGG ATGGTGGTAC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGAACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTAGA TAATGCAGCC TACCACCATG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGACTA

K T F D Y W G Q G T L V T V S S

301 AAGACGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 136)

TTCTGCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 137)

DOM-27 SEQ ID NO: 29

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F N L Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAT TTGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATTA AACATACTCT

· T W V R Q A P G K G L E W V S S I T S D G V S T Y Y A D S V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACTAGTG ATGGTGTTTC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAATGATCAC TACCACAAAG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGCCTA

S P F D Y W G Q G T L V T V S S

301 TCTCCGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 138)

AGAGGCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 139)

DOM-28 SEQ ID NO: 30

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G H Y D M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGG CATTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCC GTAATACTAT

·A W V R Q A P G K G L E W V S T I S D N G N G T Y Y A D S V K G R·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTAGTGATA ATGGTAATGG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAATCACTAT TACCATTACC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

·F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGCCCC

R D F D Y W G Q G T L V T V S S

301 CGTGATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 140)

GCACTAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 141)

DOM-29 SEQ ID NO: 31

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G R Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGT CGTTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCA GCAATAGTCT

· A W V R Q A P G K G L E W V S S I S S D G G G T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTTCTTCTG ATGGTGGGGG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAAAGAAGAC TACCACCCCC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCTGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTGGACCC

R A F D Y W G Q G T L V T V S S

301 CGGGCGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 142)

GCCCGCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 143)

DOM-30 SEQ ID NO: 32

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A R Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG AGGTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACGC TCCATAGTCT

· A W V R Q A P G K G L E W V S T I S D D G D S T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACT ATTTCTGATG ATGGTGATTC GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGA TAAAGACTAC TACCACTAAG CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K L D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACTGGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGACCTA

K L F D Y W G Q G T L V T V S S

301 AAGTTGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 144)

TTCAACAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 145)

DOM-31 SEQ ID NO: 33

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E E
Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG
GAGTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC
CTCATAGTCT

· A W V R Q A P G K G L E W V S T I S D D G S S T Y Y A D S V
K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTTCGGATG ATGGTTCTTC GACATACTAC GCAGACTCCG
TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGC TAAAGCCTAC TACCAAGAAG CTGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGACTA

L Y F D Y W G Q G T L V T V S S

301 CTTTATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 146)

GAAATAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 147)

DOM-32 SEQ ID NO: 34

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E V
Y Q M ·

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1       GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG
        GTGTATCAGA

        CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC
        CACATAGTCT

        · G W V R Q A P G K G L E W V S F I V P G G D L T Y Y A D S V
        K G R ·

101     TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATTT ATTGTGCCTG GGGGTGATTT GACATACTAC GCAGACTCCG
        TGAAGGGCCG

        ACCCAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAAA TAACACGGAC CCCCACTAAA CTGTATGATG CGTCTGAGGC
        ACTTCCCGGC

        · F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
        E T W

201     GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
        GGAAACGTGG

        CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
        CCTTTGCACC

        P E F D Y W G Q G T L V T V S S

301     CCGGAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 148)

        GGCCTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 149)
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DOM-33 SEQ ID NO: 35

```
        D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q T I G E
        S L H ·

1       GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GACGATTGGG
        GAGAGTTTAC

        CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CTGCTAACCC
        CTCTCAAATG

        · W Y Q Q K P G K A P R L L I Y F A S L L Q S G V P S R F S G
        S G S ·

101     ATTGGTACCA GCAGAAACCA GGGAAAGCCC CTAGGCTCCT GATCTATTTT GCTTCCCTGT TGCAAAGTGG GGTCCCATCG CGTTTCAGTG
        GCAGTGGATC

        TAACCATGGT CGTCTTTGGT CCCTTTCGGG GATCCGAGGA CTAGATAAAA CGAAGGGACA ACGTTTCACC CCAGGGTAGC GCAAAGTCAC
        CGTCACCTAG

        · G T D F T L T I S S L Q P E D F A T Y Y C Q Q H H M L P S T
        F G Q

201     TGGGACAGAT TTTACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CATCATATGC TTCCTTCTAC
        GTTCGGCCAA

        ACCCTGTCTA AAATGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC GTAGTATACG AAGGAAGATG
        CAAGCCGGTT
```

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 150)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 151)

DOM-34 SEQ ID NO: 36

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D S L S ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTGGT GATAGTTTAT

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CACCTAACCA CTATCAAATA

· W Y Q Q K P G K A P K L L I Y F A S Y L Q S G V P T R F S G S G S ·

101 CTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT GCTTCCTATT TGCAAAGTGG GGTCCCAACA CGTTTCAGTG GCAGTGGATC

GAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAA CGAAGGATAA ACGTTTCACC CCAGGGTTGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y F E N P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTTTGAGA ATCCTGTTAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATAAAACTCT TAGGACAATG CAAGCCGGTT

G T K V G I K R

301 GGGACCAAGG TGGGAATCAA ACGG (SEQ ID NO: 152)

CCCTGGTTCC ACCCTTAGTT TGCC (SEQ ID NO: 153)

DOM-35 SEQ ID NO: 37

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q F I G D S L S ·

1 GACATCCAAA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTTTATTGGT GATTCTTTAT

CTGTAGGTTT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CAAATAACCA CTAAGAAATA

· W Y Q Q K P G K A P K L L I Y F S S I L Q S G V P S R F S G S G S ·

101 CTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT TCTTCCATTT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

GAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAA AGAAGGTAAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y M D I P I T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATATGGATA TTCCTATTAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATATACCTAT AAGGATAATG CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 154)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 155)

DOM-36 SEQ ID NO: 38

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I D H N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGAT CATAATTTAG

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CCTATAACTA GTATTAAATC

· W Y Q Q K P G K A P K L L I Y D S S M L Q S G V P S R F S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT AGTTCCATGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

TCACCATAGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATACTA TCAAGGTACA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H S I P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATTCTA TTCCTGTTAC
GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATAGTAAGAT AAGGACAATG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 156)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 157)

DOM-37 SEQ ID NO: 39

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q Q I E T
N L E·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GCAGATTGAG
ACGAATTTAG

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CGTCTAACTC
TGCTTAAATC

· W Y Q Q K P G K A P K L L I Y D G S W L Q S G V P S R F S G
S G S·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GGTTCCTGGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG
GCAGTGGATC

TCACCATAGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATACTA CCAAGGACCA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC
CGTCACCTAG

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H S L P A T
F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATAGTT TGCCTGCTAC
GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATAGTATCAA ACGGACGATG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 158)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 159)

DOM-38 SEQ ID NO: 40

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I G N
N L E·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGGT
AATAATTTAG

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CCTATAACCA TTATTAAATC

· W Y Q Q K P G K A P R L L I Y H G S W L Q S G V P S R F S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAGGCTCCT GATCTATCAT GGGTCCTGGT TGCAAAGTGG GGTCCCATCG CGTTTCAGTG GCAGTGGATC

TCACCATGGT CGTCTTTGGT CCCTTTCGGG GATCCGAGGA CTAGATAGTA CCCAGGACCA ACGTTTCACC CCAGGGTAGC GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y D F N P T T F G Q

201 TGGGACAGAT TTCACTCTTA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATGATTTTA ATCCTACTAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAAT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATACTAAAAT TAGGATGATG CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 160)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 161)

DOM-39 SEQ ID NO: 41

D I Q M T Q S P S S L S A S V G D C V T I T C R A S Q N I D G L L W ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CTGTGTCACC ATCACTTGCC GGGCAAGTCA GAATATTGAT GGTCTGTTAT

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GACACAGTGG TAGTGAACGG CCCGTTCAGT CTTATAACTA CCAGACAATA

· W Y Q Q K P G K A P K L L I Y A G S G L Q S G V P S R F S G S G S ·

101 GGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCG GGGTCCGGGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

CCACCATAGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATACGC CCCAGGCCCA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q K A F E P F T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG AAGGCTTTTG AGCCTTTTAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC TTCCGAAAAC TCGGAAAATG CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 162)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 163)

DOM-40 SEQ ID NO: 42

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F K A Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAG GCGTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATTC CGCATACTAT

· G W V R Q A P G K G L E W V S Q I G R D G S F T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCACAG ATTGGGAGGG ATGGTTCTTT TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCCAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTGTC TAACCCTCCC TACCAAGAAA ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P R

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTCGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGAGCA

R Y A I F T F D R G Q G T L V T V S S

301 CGGTATGCTA TTTTTACTTT TGATCGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC (SEQ ID NO: 164)

GCCATACGAT AAAAATGAAA ACTAGCCCCA GTCCCTTGGG ACCAGTGGCA GAGCTCG (SEQ ID NO: 165)

DOM-41 SEQ ID NO: 43

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F F E Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTTT GAGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAAA CTCATACTCT

· T W V R Q A P G K G L E W V S S I A N D G S T T Y Y A D S V K G R ·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTGCGAATG ATGGTTCGAC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAACGCTTAC TACCAAGCTG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGACTA

R Q F D Y W G Q G T L V T V S S

301 CGGCAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 166)

GCCGTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 167)

DOM-42 SEQ ID NO: 44

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G P Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGT CCGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCA GGCATACTCT

· T W V R Q A P G K G L E W V S S I V G D G L D T Y Y A D S V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGTTGGTG ATGGTCTGGA TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGC TAACAACCAC TACCAGACCT ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCGGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCCTA

R V F D Y W G Q G T L V T V S S

301 CGGGTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 168)

GCCCAAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 169)

DOM-43 SEQ ID NO: 45

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A S
Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT
TCTTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACGA
AGAATACTCT

· A W V R Q A P G K G L E W V S S I G S D G G P T Y Y A D S V
K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGGTAGTG ATGGTGGGCC GACATACTAC GCAGACTCCG
TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGC TAACCATCAC TACCACCCGG CTGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D S A V Y Y C A
K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC TCCGCGGTAT ATTACTGTGC
GAAACCTGAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG AGGCGCCATA TAATGACACG
CTTTGGACTA

R A F D Y W G Q G T L V T V S S

301 AGGGCTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 170)

TCCCGAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 171)

DOM-44 SEQ ID NO: 46

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T S Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACG TCTTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATGC AGAATACTCT

· G W V R Q A P G K G L E W V S S I E P T G I T T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTGAGCCTA CTGGTATTAC GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCCCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAACTCGGAT GACCATAATG CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P H

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTCAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGAGTA

F T E L G F D Y W G Q G T L V T V S S

301 TTTACTGAGC TTGGTTTTGA CTACTGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC (SEQ ID NO: 172)

AAATGACTCG AACCAAAACT GATGACCCCA GTCCCTTGGG ACCAGTGGCA GAGCTCG (SEQ ID NO: 173)

DOM-45 SEQ ID NO: 47

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G N Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGT AATTATGCGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCA TTAATACGCT

· A W V R Q A P G K G L E W V S K I G A Q G L H T Y Y A G S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAAG ATTGGGGCGC AGGGTCTTCA TACATACTAC GCAGGCTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTTC TAACCCCGCG TCCCAGAAGT ATGTATGATG CGTCCGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K Q T

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACAGACG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGTCTGC

T M D Y E R F D Y W G Q G T L V T V S S

301 ACGATGGATT ATGAGAGGTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC (SEQ ID NO: 174)

TGCTACCTAA TACTCTCCAA ACTGATGACC CCAGTCCCTT GGGACCAGTG GCAGAGCTCG (SEQ ID NO: 175)

DOM-46 SEQ ID NO: 48

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E L Y A M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG TTGTATGCTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC AACATACGAT

·A W V R Q A P G K G L E W V S G I G A V G E T T Y Y A D S V K G R·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGT ATTGGTGCTG TGGGTGAGAC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTCCA TAACCACGAC ACCCACTCTG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

·F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K E A

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGAGGCT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTCTCCGA

N N L S D N L V F D Y W G Q G T L V T V S S

301 AATAATCTTT CTGATAATCT TGTGTTTGAC TACTGGGGTC AGGGAACCCT GGTCACCGTC TCGAGC (SEQ ID NO: 176)

TTATTAGAAA GACTATTAGA ACACAAACTG ATGACCCCAG TCCCTTGGGA CCAGTGGCAG AGCTCG (SEQ ID NO: 177)

DOM-47 SEQ ID NO: 49

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D S L S·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTGGG GATTCGTTAA

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CACCTAACCC CTAAGCAATT

·W Y Q Q K P G K A P K L L I Y F G S Y L Q S G V P S R F S G S G S·

101 GTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT GGTTCCTATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

CAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAA CCAAGGATAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y L H T P S T
F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTTGCATA CTCCTTCGAC
GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATAAACGTAT GAGGAAGCTG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 178)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 179)

DOM-48 SEQ ID NO: 50

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D
S L S·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTGGG
GATTCGTTAA

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CACCTAACCC
CTAAGCAATT

·W Y Q Q K P G K A P K L L I Y F G S Y L Q N G V P S R F S G
S G S·

101 GTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT GGTTCCTATT TGCAAAATGG GGTCCCATCA CGTTTCAGTG
GCAGTGGATC

CAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAA CCAAGGATAA ACGTTTTACC CCAGGGTAGT GCAAAGTCAC
CGTCACCTAG

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y M I T P T T
F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATATGATTA CTCCTACTAC
GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATATACTAAT GAGGATGATG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 180)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 181)

DOM-49 SEQ ID NO: 51

D V Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D S L S ·

1 GACGTCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTGGG GATTCGTTAA

CTGCAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CACCTAACCC CTAAGCAATT

· W Y Q Q K P G K A P K L L I Y F G S Y L Q S G V P S R F S G S G S ·

101 GTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT GGTTCCTATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

CAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAA CCAAGGATAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y M S A P S T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATATGAGTG CTCCTTCTAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATATACTCAC GAGGAAGATG CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 182)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 183)

DOM-50 SEQ ID NO: 52

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D S L S ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTGGG GATTCGTTAA

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CACCTAACCC CTAAGCAATT

· W Y Q Q K P G K A P K L L I Y F G S Y L Q S G V P S R F S G S G S ·

101 GTTGGTACCA GCAGAAACCA *GGGAAAGCCC CTAAGCTCCT* GATCTATTTT GGTTCCTATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

CAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAA CCAAGGATAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D S A T Y Y C Q Q Y Q Y V P S T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTCTG CTACGTACTA CTGTCAACAG TATCAGTATG TTCCTTCTAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAGAC GATGCATGAT GACAGTTGTC ATAGTCATAC AAGGAAGATG
CAAGCCGGTT

G T K V E I K Q

301 GGGACCAAGG TGGAAATCAA ACAG (SEQ ID NO: 184)

CCCTGGTTCC ACCTTTAGTT TGTC (SEQ ID NO: 185)

DOM-51 SEQ ID NO: 53

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                D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q P I V D
E L D ·

1       GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GCCTATTGTT
GATGAGTTAG

        CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CGGATAACAA
CTACTCAATC

        · W Y Q Q K P G K A P K L L I Y A A S I L Q S G V P S R F S G
S G S ·

101     ATTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCT GCGTCCATTT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG
GCAGTGGATC

        TAACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATACGA CGCAGGTAAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC
CGTCACCTAG

        · G T D F T L T I S S L Q P E D F A T Y Y C H Q W S T Y P T T
F G Q

201     TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCATCAG TGGTCTACTT ATCCTACGAC
GTTCGGCCAA

        ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTAGTC ACCAGATGAA TAGGATGCTG
CAAGCCGGTT

        G T K V E I K R

301     GGGACCAAGG TGGAAATTAA ACGG (SEQ ID NO: 186)

        CCCTGGTTCC ACCTTTAATT TGCC (SEQ ID NO: 187)
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DOM-52 SEQ ID NO: 54

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                D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I G S
A L R ·

1       GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGTGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGGG
TCTGCGTTAA

        CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCACT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CCTATAACCC
AGACGCAATT

        · W Y Q Q K P G K A P K L L I Y L G S D L Q S G V P S R F S G
S G S ·

101     GGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTG GGTTCCGATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG
GCAGTGGATC

        CCACCATAGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAAAC CCAAGGCTAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC
CGTCACCTAG
```

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q T Q Y F P T T
F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ACGCAGTATT TTCCTACGAC
GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC TGCGTCATAA AAGGATGCTG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 188)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 189)

DOM-53 SEQ ID NO: 55

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A I Y G
G L R·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGCGATTTAT
GGGGGGTTAC

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CCGCTAAATA
CCCCCCAATG

·W Y Q Q K P G K A P K L L I Y G E S M L Q S G V P S R F S G
S G S·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGGG GAGTCCATGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG
GCAGTGGATC

CCACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATACCC CTCAGGTACA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC
CGTCACCTAG

·G T D F T L T I S S L H P E D F A T Y Y C Q Q V Y H K P F T
F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCATCCT GAAGATTTTG CTACGTACTA CTGTCAACAG GTTTATCATA AGCCTTTTAC
GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTAGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC CAAATAGTAT TCGGAAAATG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 190)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 191)

DOM-54 SEQ ID NO: 56

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T A Y R M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACG GCGTATAGGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATGC CGCATATCCT

· A W V R Q A P G K G L E W V S W I S P S G S G T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATGG ATTTCGCCTT CTGGTTCGGG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTACC TAAAGCGGAA GACCAAGCCC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K T L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAACTTTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTTGAAAC

T D S P S G H Y E F D Y W G Q G T L V T V S S

301 ACGGATTCGC CGTCGGGGCA TTATGAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 192)

TGCCTAAGCG GCAGCCCCGT AATACTCAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 193)

DOM-55 SEQ ID NO: 57

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A R Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG CGGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACGC GCCATACTCT

· G W V R Q A P G K G L E W V S R I T A Q G L G T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCACGG ATTACTGCTC AGGGTCTTGG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCCCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTGCC TAATGACGAG TCCCAGAACC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K Y L

201 GTTCACCATC TCCCGCGACA ACTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAATATCTT

CAAGTGGTAG AGGGCGCTGT TGAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG
CTTTATAGAA

T D F S S G H Q E F D Y W G Q G T L V T V S S

301 ACTGATTTTA GTAGTGGGCA TCAGGAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 194)

TGACTAAAAT CATCACCCGT AGTCCTCAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 195)

DOM-56 SEQ ID NO: 58

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F N D Y T M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAT GATTATACTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATTA CTAATATGAT

· G W V R Q A P G K G L E W V S W I H G T G G Q T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATGG ATTCATGGGA CTGGTGGTCA GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCCAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTACC TAAGTACCCT GACCACCAGT CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K A L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGCTTTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTCGAAAC

A D R S G G V V E F D Y W G Q G T L V T V S S

301 GCTGATAGGA GTGGGGGGGT TGTTGAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 196)

CGACTATCCT CACCCCCCCA ACAACTCAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 197)

DOM-57 SEQ ID NO: 59

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S E Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT GAGTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGA CTCATACTAT

· Y W V R Q A P G K G L E W V S W I D T D G G D T Y Y A D S V K G R ·

101 TGTATTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATGG ATTGATACTG ATGGTGGGGA TACATACTAC GCAGACTCCG TGAAGGGCCG

ACATAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTACC TAACTATGAC TACCACCCCT ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGACCA

L K F D Y W G Q G T L V T V S S

301 CTGAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 198)

GACTTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 199)

DOM-58 SEQ ID NO: 60

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E V
Y T M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG
GTTTATACTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC
CAAATATGAT

· A W V R Q A P G K G L E W V S T I D E S G R D T Y Y A D S V
K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACG ATTGATGAGT CTGGTCGTGA TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGC TAACTACTCA GACCAGCACT ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGACCA

V W F D Y W G Q G T L V T V S S

301 GTTTGGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 200)

CAAACCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 201)

DOM-59 SEQ ID NO: 61

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F L D Y A M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCTG GATTATGCGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAGAC CTAATACGCT

· G W V R Q A P G K G L E W V S T I S P M G M G T Y Y A D S V K G R·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACT ATTTCTCCGA TGGGTATGGG TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCCAACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGA TAAAGAGGCT ACCCATACCC ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K S S

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATCGAGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTAGCTCA

A I S F T S D I S N F D Y W G Q G T L V T V S S

301 GCTATTTCGT TTACTTCTGA TATTTCTAAT TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC (SEQ ID NO: 202)

CGATAAAGCA AATGAAGACT ATAAAGATTA AAACTGATGA CCCCAGTCCC TTGGGACCAG TGGCAGAGCT CG (SEQ ID NO: 203)

DOM-61 SEQ ID NO: 62

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A A Y A M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT GCTTATGCTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACGA CGAATACGAT

· T W V R Q A P G K G L E W V S Y I S P N G T A T Y Y A D S V K G R·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATAT ATTAGTCCGA ATGGTACGGC GACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTATA TAATCAGGCT TACCATGCCG CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A E Y V

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GGAATATGTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CCTTATACAC

G M R W N S F D Y W G Q G T L V T V S S

301 GGGATGCGTT GGAATTCTTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC (SEQ ID NO: 204)

CCCTACGCAA CCTTAAGAAA ACTGATGACC CCAGTCCCTT GGGACCAGTG GCAGAGCTCG (SEQ ID NO: 205)

DOM-62 SEQ ID NO: 63

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S S Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCG AGTTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGC TCAATACTCT

·A W V R Q A P G K G L E W V S S I T S L G T S T Y Y A D S V K G R·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACGAGTC TTGGTACTTC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAATGCTCAG AACCATGAAG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

·F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGCCCA

R K F D Y W G Q G T L V T V S S

301 AGGAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 206)

TCCTTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 207)

DOM-65 SEQ ID NO: 64

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F N E Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAT GAGTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATTA CTCATACTCT

·T W V R Q A P G K G L E W V S T I T S E G S G T Y Y A D S V K G R·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTACTAGTG AGGGTAGTGG GACATACTAC GCAGACTCCG TAAAGGGCCG

ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGC TAATGATCAC TCCCATCACC CTGTATGATG CGTCTGAGGC ATTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P N

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTAAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGATTA

G K F D Y W G Q G T L V T V S S

301 GGTAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 208)

CCATTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 209)

DOM-66 SEQ ID NO: 65

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S D
Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT
GATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGA
CTAATACTCT

· L W V R Q A P G K G L E W V S T I T S E G H S T Y Y A D S V
K G R ·

101 TGTTGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACT ATTACTAGTG AGGGTCATTC TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACAACACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGA TAATGATCAC TCCCAGTAAG ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGACCC

T S F D Y W G Q G T L V T V S S

301 ACTTCGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 210)

TGAAGCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 211)

DOM-67 SEQ ID NO: 66

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S D Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT GATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATCA CTAATACTCT

· S W V R Q A P G K G L E W V S T I D S D G S F T Y Y A D S V K G R·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTGATTCTG ATGGTAGTTT TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTCAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGC TAACTAAGAC TACCATCAAA ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGCCCA

V K F D Y W G Q G T L V T V S S

301 GTGAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 212)

CACTTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 213)

DOM-68 SEQ ID NO: 67

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F K D Y E M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAG GATTATGAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATTC CTAATACTCT

· T W V R Q A P G K G L E W V S S I S S T G Q S T Y Y A D S V K G R·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTTCTTCTA CTGGTCAGTC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACTGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAAAGAAGAT GACCAGTCAG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCCCA

N K F D Y W G Q G T L V T V S S

301 AATAAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 214)

TTATTCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 215)

DOM-69 SEQ ID NO: 68

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F L D Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCTT GATTATGGTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAGAA CTAATACCAT

· A W V R Q A P G K G L E W V S A I S P L G L S T Y Y A D S V K S R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGCT ATTTCGCCTC TTGGTCTTAG TACATACTAC GCAGACTCCG TGAAGAGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTCGA TAAAGCGGAG AACCAGAATC ATGTATGATG CGTCTGAGGC ACTTCTCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K E V

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAGAGGTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTCTCCAC

R V G R G V H P P K F D Y W G Q G T L V T V S S

301 AGGGTGGGTA GGGGTGTTCA TCCTCCGAAG TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC (SEQ ID NO: 216)

TCCCACCCAT CCCCACAAGT AGGAGGCTTC AAACTGATGA CCCCAGTCCC TTGGGACCAG TGGCAGAGCT CG (SEQ ID NO: 217)

DOM-70 SEQ ID NO: 69

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E N Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG AATTATGCTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC TTAATACGAT

· S W V R Q A P G K G L E W V S T I A P L G V P T Y Y A D S V K G R ·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTGCTCCGC TGGGTGTTCC GACATACTAC GCAGACTCCG TGAAGGGCCG

ACAGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGC TAACGAGGCG ACCCACAAGG CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K K K

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC
GAAAAAGAAG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG
CTTTTTCTTC

V G A W L Q S R S F D Y W G Q G T L V T V S S

301 GTTGGGGCGT GGCTGCAGTC GCGGAGTTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 218)

CAACCCCGCA CCGACGTCAG CGCCTCAAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 219)

DOM-71 SEQ ID NO: 70

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E G
Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG
GGTTATCCTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC
CCAATAGGAT

· S W V R Q A P G K G L E W V S T I S P L G P D T Y Y A D S V
K G R ·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTAGTCCTT TGGGTCCTGA TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACAGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAATCAGGAA ACCCAGGACT ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K L L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC
GAAACTGTTG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG
CTTTGACAAC

M G E Y L N S R T F D Y W G Q G T L V T V S S

301 ATGGGGGAGT ATTTGAATTC TAGGACGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 220)

TACCCCCTCA TAAACTTAAG ATCCTGCAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 221)

DOM-72 SEQ ID NO: 71

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E A Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GCGTATCCTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC CGCATAGGAT

· S W V R Q A P G K G L E W V S S I S P L G L W T Y Y A D S V K G R ·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTTCCCCTC TTGGTTTGTG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACAGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTCA TAAAGGGGAG AACCAAACAC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K L S

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACTTAGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGAATCA

A G A E T H V Y R L F D Y W G Q G T L V T V S S

301 GCTGGGGCGG AGACTCATGT TTATCGGCTT TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC (SEQ ID NO: 222)

CGACCCCGCC TCTGAGTACA AATAGCCGAA AAACTGATGA CCCCAGTCCC TTGGGACCAG TGGCAGAGCT CG (SEQ ID NO: 223)

DOM-73 SEQ ID NO: 72

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S K Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT AAGTATGATA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGA TTCATACTAT

· S W V R Q A P G K G L E W V S T I L E D G L T T Y Y A D S V K G R ·

101 TGTCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTCTGGAGG ATGGTCTGAC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACAGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAAGACCTCC TACCAGACTG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCCCC

R L F D Y W G Q G T L V T V S S

301 CGTTTGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 224)

GCAAACAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 225)

DOM-74 SEQ ID NO: 73

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          E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S D
          Y P M·

1         GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCG
          GATTATCCTA

          CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAAAGC
          CTAATAGGAT


          · T W V R Q A P G K G L E W V S T I L S P G T E T Y Y A D S V
          K G R·

101       TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACT ATTCTGTCTC CGGGTACGGA GACATACTAC GCAGACTCCG
          TGAAGGGCCG

          ACTGCACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGA TAAGACAGAG GCCCATGCCT CTGTATGATG CGTCTGAGGC
          ACTTCCCGGC


          · F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
          K A E

201       GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
          GAAAGCTGAG

          CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
          CTTTCGACTC


          K D F D Y W G Q G T L V T V S S

301       AAGGATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 226)

          TTCCTAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 227)
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DOM-75 SEQ ID NO: 74

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                                                                  AvaI

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          E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F L Q
          Y P M·

1         GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCGGGATT CACCTTTTTG
          CAGTATCCGA

          CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGCCCTAA GTGGAAAAAC
          GTCATAGGCT


          · G W V R Q A P G K G L E W V S T I S P V G L T T Y Y A D S V
          K G R·

101       TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTTCTCCTG TTGGTTTGAC TACATACTAC GCAGACTCCG
          TGAAGGGCCG
```

ACCCAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAAAGAGGAC AACCAAACTG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K L F

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAATTGTTT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTAACAAA

E G S R I Q R D V G F D Y W G Q G T L V T V S S

301 GAGGGGTCGA GGATTCAGCG TGATGTGGGT TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC (SEQ ID NO: 228)

CTCCCCAGCT CCTAAGTCGC ACTACACCCA AAACTGATGA CCCCAGTCCC TTGGGACCAG TGGCAGAGCT CG (SEQ ID NO: 229)

DOM-77 SEQ ID NO: 75

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E E Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GAGTATGGTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC CTCATACCAT

· A W V R Q A P G K G L E W V S T I S P L G I S T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTTCTCCGC TGGGTATTTC GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGA TAAAGAGGCG ACCCATAAAG CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K H A

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACATGCT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGTACGA

T S Q E S L R S F D Y W G Q G T L V T V S S

301 ACGTCTCAGG AGTCTTTGCG GTCTTTTGAC TACTGGGGTC AGGGAACCCT GGTCACCGTC TCGAGC (SEQ ID NO: 230)

TGCAGAGTCC TCAGAAACGC CAGAAAACTG ATGACCCCAG TCCCTTGGGA CCAGTGGCAG AGCTCG (SEQ ID NO: 231)

DOM-78 SEQ ID NO: 76

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E R Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG AGGTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC TCCATAGTCT

· A W V R Q A P G K G L E W V S T I S S D G G G T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCGGGGAAGG GTCTAGAGTG GGTCTCAACG ATTAGTTCTG ATGGTGGGGG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGCCCCTTCC CAGATCTCAC CCAGAGTTGC TAATCAAGAC TACCACCCCC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTGGACCA

H R F D Y W G Q G T L V T V S S

301 CATCGGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 232)

GTAGCCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 233)

DOM-80 SEQ ID NO: 77

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G R Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGT CGTTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACCA GCAATAGTCT

· A W V R Q A P G K G L E W V S S I S S D G G G T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTTCTTCTG ATGGTGGGGG GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGA TAAAGAAGAC TACCACCCCC CTGTATGATG CGTCTGAGGC ACTTCCCGGC

. F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P S

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCGTCT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGCAGA

R R F D Y W G Q G T L V T V S S

301 CGTCGGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 234)

GCAGCCAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 235)

DOM-81 SEQ ID NO: 78

E V Q L L E S G G G L V Q P G G F L R L S C A A S G F T F E L Y P M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTT CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG TTGTATCCGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAA GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACTC AACATAGGCT

·A W V R Q A P G K G L E W V S S I S P V G F L T Y Y A D S V K G R·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCATCG ATTTCTCCGG TTGGTTTTCT GACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTAGC TAAAGAGGCC AACCAAAAGA CTGTATGATG CGTCTGAGGC ACTTCCCGGC

·F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G H

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAGGGCAT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTA TGGCGCCATA TAATGACACG CTTTCCCGTA

E G S Y T P R S A F D Y W G Q G T L V T V S S

301 GAGGGGTCGT ATACTCCGCG GTCGGCTTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 236)

CTCCCCAGCA TATGAGGCGC CAGCCGAAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 237)

DOM-82 SEQ ID NO: 79

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F V A Y P M·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGTG GCGTATCCTA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACAC CGCATAGGAT

·A W V R Q A P G K G L E W V S T I A P L G G N T Y Y A D S V K G R·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAACT ATTGCGCCTC TGGGTGGTAA TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGCACCCA GGCGGTCCGA GGTCCCTTCC CAGACCTCAC CCAGAGTTGA TAACGCGGAG ACCCACCATT ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K R P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACGGCCG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGCCGGC

E G L Q I D S Q N F D Y W G Q G T L V T V S S

301 GAGGGGCTGC AGATTGATTC TCAGAATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC (SEQ ID NO: 238)

CTCCCCGACG TCTAACTAAG AGTCTTAAAA CTGATGACCC CAGTCCCTTG GGACCAGTGG CAGAGCTCG (SEQ ID NO: 239

DOM-83 SEQ ID NO: 80

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A L
Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG
TTGTATCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAACGC
AACATAGTCT

· A W V R Q A P G K G L E W V S S I D S S G S D T Y Y A D S V
K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGATTCTT CTGGTAGTGA TACATACTAC GCAGACTCCG
TGAAGGGCCG

ACCGAACCCA GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTAGC TAACTAAGAA GACCATCACT ATGTATGATG CGTCTGAGGC
ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A
K P E

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC
GAAACCTGAG

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG
CTTTGGACTC

R D F D Y W G Q G T L V T V S S

301 CGTGATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 240)

GCACTAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 241)

DOM-84 SEQ ID NO: 81

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F R Q Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGG CAGTACCAGA

CTCCACGTCG ACAACCTCAG ACCCCCTCCG AACCATGTCG GACCCCCCAG GGACGCAGAG AGGACACGTC GGAGGCCTAA GTGGAAATCC GTCATGGTCT

· A W A R Q A P G K G L E W V S T I A S D G V S T Y Y A D S V K G R ·

101 TGGCTTGGGC CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTGCGTCGG ATGGTGTTTC TACATACTAC GCAGACTCCG TGAAGGGCCG

ACCGAACCCG GGCGGTCCGA GGTCCCTTCC CAGATCTCAC CCAGAGTTGC TAACGCAGCC TACCACAAAG ATGTATGATG CGTCTGAGGC ACTTCCCGGC

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K V G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGTTGGT

CAAGTGGTAG AGGGCGCTGT TAAGGTTCTT GTGCGACATA GACGTTTACT TGTCGGACGC ACGGCTCCTG TGGCGCCATA TAATGACACG CTTTCAACCA

R D F D Y W G Q G T L V T V S S

301 CGTGATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC (SEQ ID NO: 242)

GCACTAAAAC TGATGACCCC AGTCCCTTGG GACCAGTGGC AGAGCTCG (SEQ ID NO: 243)

DOM-116 SEQ ID NO: 82

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q P I G P D L L ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GCCTATTGGT CCTGATTTAC

CTGTAGGTCT ACTGGGTCAG AGGTAGGAGG GACAGACGTA GACATCCTCT GGCACAGTGG TAGTGAACGG CCCGTTCAGT CGGATAACCA GGACTAAATG

· W Y Q Q K P G K A P K L L I Y Q T S I L Q S G V P S R F S G S G S ·

101 TGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAG ACGTCCATTT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

ACACCATGGT CGTCTTTGGT CCCTTTCGGG GATTCGAGGA CTAGATAGTC TGCAGGTAAA ACGTTTCACC CCAGGGTAGT GCAAAGTCAC CGTCACCTAG

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y W A F P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTGGGCTT TTCCTGTGAC GTTCGGCCAA

ACCCTGTCTA AAGTGAGAGT GGTAGTCGTC AGACGTTGGA CTTCTAAAAC GATGCATGAT GACAGTTGTC ATAACCCGAA AAGGACACTG
CAAGCCGGTT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG (SEQ ID NO: 244)

CCCTGGTTCC ACCTTTAGTT TGCC (SEQ ID NO: 245)--

DOM-85 - SEQ ID NO.: 246

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E Q Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG CAGTATGATA

· R W V R Q A P G K G L E W V S W I D E A G H E T Y Y A D S V K G R ·

101 TGAGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATGG ATTGATGAGG CGGGTCATGA GACATACTAT GCAGACTCCG TGAAGGGCCG

· F T I S R D N S R N T L Y L Q M N S L R A E D T A V Y Y C A K G M

201 GTTCACCATC TCCCGCGACA ATTCCAGGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGGATG

D G F D Y W G Q G T L V T V S S

301 GATGGGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC - **SEQ ID NO.: 361**

DOM-86 - SEQ ID NO.: 247

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I G D A L F ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGGG GATGCTTTAT

· W Y Q Q K P G K A P K L L I Y Y S S M L Q S G V P S R F S G G G S ·

101 TTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTAT TCTTCCATGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCGGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q R H S T P A T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CGGCATAGTA CTCCTGCTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG - **SEQ ID NO.: 362**

DOM-87 - SEQ ID NO.: 248

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D
I D E S L M·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTGAT GAGTCTTTAA

·W Y Q Q K P G K A P R L L I Y G V S Y L Q S G V P S R F
S G S G S·

101 TGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAGGCTCCT GATCTATGGG GTGTCCTATT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q R W K A P
F T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTCG CTACGTACTA CTGTCAACAG CGGTGGAAGG
CTCCTTTTAC GTTCGGCCAA

G T K V E I K R **– SEQ ID NO.: 363**

DOM-88 – SEQ ID NO.: 249

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q E
I V E D L Y·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTTACC ATCACTTGCC GGGCAAGTCA
GGAGATTGTG GAGGATTTAT

·W Y Q Q K P G K A A K L L I Y G A S W L Q S G V P S R F
S G S G S·

101 ATTGGTATCA GCAGAAACCA GGGAAAGCCG CTAAGCTCCT GATCTATGGT GCGTCCTGGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q T R R R P
Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ACGCGTAGGC
GTCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **– SEQ ID NO.: 364**

DOM-89 – SEQ ID NO.: 250

D I Q M T Q S P A S L S A S V G D R V T I T C R A S Q D
I D P M L R·

1 GACATCCAGA TGACCCAGTC TCCAGCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGAT CCTATGTTAA

· W Y Q Q K P G K A P K L L I Y A G S I L Q S G V P S R F S G S G S ·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCG GGTTCCATTT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q T L V T P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ACGCTGGTGA CTCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **- SEQ ID NO.: 365**

DOM-90 - SEQ ID NO.: 251

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S I S D A L F ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTCGATTTCG GATGCGTTAT

· W Y Q Q K P G K A P R L L I Y Y G S V L Q S G V P S R F S G S G S ·

101 TTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAGGCTCCT GATCTATTAT GGTTCCGTTT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q R F Q E P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CGTTTTCAGG AGCCTGTGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **- SEQ ID NO.: 366**

DOM-91 - SEQ ID NO.: 252

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q Q I S D E L N ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCCGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GCAGATTAGT GATGAGTTAA

· W Y Q Q K P G K A P K L L I Y A V S I L Q S G V P S R F
S G S G S ·

101 ATTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCT GTGTCCATTT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q W L S F P
S T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TGGTTGAGTT
TTCCTTCGAC GTTTGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 367**

DOM-92 – SEQ ID NO.: 253

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T
F V D Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT
CACCTTTGTT GATTATCCGA

· G W V R Q A P G K G L E W V S T I S T G G F S T Y Y A D
S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTTCTACGG GGGGTTTTTC GACATACTAC
GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y
C A K A R

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT
ATTACTGTGC GAAAGCGCGG

Y Y Y L S Q I K N F D Y W G Q G T L V T V S S

301 TATTATTATC TTAGTCAGAT TAAGAATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 368**

DOM-93 – SEQ ID NO.: 254

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T
F D I Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT ATTTATGGGA

· T W V R Q A P G K G L E W V S S I S P L G L V T Y Y A D P V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAAGT ATTTCGCCTC TTGGTCTTGT TACATACTAC GCAGACCCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K L K

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACTGAAG

E H G D V P F D Y W G Q G T L V T V S S

301 GAGCATGGGG ATGTTCCTTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC **– SEQ ID NO.: 369**

DOM-94 – SEQ ID NO.: 255

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E L Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG CTTTATCCGA

· S W V R Q A P G K G L E W V S T I S P T G L L T Y Y A D S V K G R ·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTTCTCCTA CGGGTTTGTT GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K F K

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATTTAAG

R S G K T D D T N F D Y W G Q G T L V T V S S

301 AGGAGTGGGA AGACTGATGA TACTAATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC **– SEQ ID NO.: 370**

DOM-95 – SEQ ID NO.: 256

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F R E Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCGG GAGTATGATA

· L W V R Q A P G K G L E W V S T I V G D G N G T Y Y A D S V K G R ·

101 TGCTGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTGTGGGGG ATGGTAATGG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K Q D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACAGGAT

R Q F D Y W G Q G T L V T V S S

301 CGTCAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC **- SEQ ID NO.: 371**

DOM-96 - SEQ ID NO.: 257

E V Q P L E S G G G L V Q P G G S L R L S C A A S G F T F T D Y K M ·

1 GAGGTGCAGC CGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACT GATTATAAGA

· L W V R Q A P G K G L E W V S S I S P S G R W T Y Y A D S V K G R ·

101 TGCTTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTTCTCCTA GTGGTCGTTG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K S L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAAGTCTT

F E G S F D Y W G Q G T L V T V S S

301 TTTGAGGGTA GTTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC **- SEQ ID NO.: 372**

DOM-97 - SEQ ID NO.: 258

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E E Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GAGTATGGTA

· S W V R Q A P G K G L E W V S T I S P I G V T T Y Y A D S V K G R ·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTTCGCCTA TTGGTGTTAC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K N A

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAAATGCT

Y D R K S N F D Y W G Q G T L V T V S S

301 TATGATCGGA AGTCTAATTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC **– SEQ ID NO.: 373**

DOM-98 – SEQ ID NO.: 259

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D R Y V M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT CGGTATGTGA

· V W V R Q A P G K D L E W V S G I T P S G R R T Y Y A D S V K G R ·

101 TGGTGTGGGT CCGCCAGGCT CCAGGGAAGG ATCTAGAGTG GGTCTCAGGT ATTACTCCGA GTGGTAGGAG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K D T L Y L Q M N S L R A E D T A V Y Y C A K V L

201 GTTCACCATC TCCCGCGACA ATTCCAAGGA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGTGTTG

G R H F D P L L P S F D Y W G Q G T L V T V S S

301 GGGCGTCATT TTGATCCTCT TCTGCCTTCG TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC **– SEQ ID NO.: 374**

DOM-99 – SEQ ID NO.: 260

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E D Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GATTATGCTA

· S W V R Q A P G K G L E W V S T I T P G G F W T Y Y A D S V K G R ·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTACTCCGG GTGGTTTTTG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K T S

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAACGTCT

S G E L Q L V E D F D Y W G Q G T L V T V S S

301 AGTGGGGAGT TGCAGTTGGT TGAGGATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC **– SEQ ID NO.: 375**

DOM-100 – SEQ ID NO.: 261

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q N I K H S L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GAGCAAGTCA GAATATTAAG CATTCGTTAC

· W Y Q Q K P G K A P R L L I Y H R S Q L Q S G V P S R F S G S G S ·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAGGCTCCT GATCTATCAT CGTTCCCAGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q V R H R P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG GTTAGGCATC GTCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **– SEQ ID NO.: 376**

DOM-101 – SEQ ID NO.: 262

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A I G H R L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCT CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q V A L F P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG GTTGCTTTGT TTCCCTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 377**

DOM-102 – SEQ ID NO.: 263

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q H I G H H L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GCATATTGGT CATCATTTAA

· W Y Q Q K P G K A P K L L I Y H R S H L Q S G V P S R F S G S G S ·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CCAAGCTCCT GATCTATCAT AGGTCCCATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D S A T Y Y C Q Q W D R P P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTCTG CTACGTACTA CTGTCAACAG TGGGATAGGC CGCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 378**

DOM-103 – SEQ ID NO.: 264

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A I G H R L R ·

1 GACATCCAGA TGACCCAGTC CCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F
S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q V R A V P
Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG GTGCGGGCTG
TGCCTTATAC GTTTGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATTAA ACGG **- SEQ ID NO.: 379**

DOM-104 - SEQ ID NO.: 265

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A
I G H R L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F
S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q V R F S P
Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG GTTCGTTTTT
CTCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **- SEQ ID NO.: 380**

DOM-105 - SEQ ID NO.: 266

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A
I G H R L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F
S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q S Y A R P
V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TCTTATGCTA
GGCCTGTGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **- SEQ ID NO.: 381**

DOM-106 - SEQ ID NO.: 267

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S
I N H R L Y ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
AAGTATTAAT CATAGGTTAT

· W Y Q Q K P G K A P K L L I Y H R S R L Q S G V P S R F
S G S G S ·

101 ATTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAGGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y K.V R P
N T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAGGATTTTG CTACGTACTA CTGTCAACAG TATAAGGTTA
GGCCTAATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG **- SEQ ID NO.: 382**

DOM-107 - SEQ ID NO.: 268

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A
I G H R L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
– –GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F
S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATTTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q T Y S S P
H T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ACTTATTCGT
CTCCTCATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 383**

DOM-108 – SEQ ID NO.: 269

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A
I G H R L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F
S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

·G T D F T L T I S S L Q P E D F A T Y Y C Q Q R A V R P
F T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTACAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG AGGGCGGTGA
GGCCTTTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAAG TGGAAATCAA ACGG – **SEQ ID NO.: 384**

DOM-109 – SEQ ID NO.: 270

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A
I G H R L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGCTATTGGG CATCGGTTAC

· W Y Q Q K P G K A P K L L I Y H R S K L Q S G V P S R F S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT CGGTCCAAGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q T Y Y R P L T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ACTTATTATC GTCCTCTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 385**

DOM-110 – SEQ ID NO.: 271

D I Q M T Q S P A S L S A S V G D R V T I T C R A S Q D I D P M L R ·

1 GACATCCAGA TGACCCAGTC TCCAGCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGAT CCTATGTTAA

· W Y Q Q K P G K A P K L L I Y A G S I L Q S G V P S R F S G S G S ·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCG GGTTCCATTT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q T S I R P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ACTAGTATTA GGCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 386**

DOM8-111 – SEQ ID NO.: 272

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E R Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG CGTTATCCTA

· T W V R Q A P G K G L E W V S T I H G S G S A T Y Y A D S V K G R ·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTCATGGTT CTGGTAGTGC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGGCCG

Y T S R H N S L G H F D Y W G Q G T L V T V S S

301 TATACTAGTC GGCATAATAG TCTTGGGCAT TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC – **SEQ ID NO.: 387**

DOM-112 – SEQ ID NO.: 273

· E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F M D Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTATG GATTATCCTA

· G W V R Q A P G K G L E W V S S I G P V G M S T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTGGGCCTG TTGGTATGAG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K Y G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAATATGGG

G T S G R H N T K F D Y W G Q G T L V T V S S

301 GGGACTAGTG GTAGGCATAA TACTAAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 388**

DOM-113 – SEQ ID NO.: 274

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T E Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACT GAGTATCCTA

· S W V R Q A P G K G L E W V S V I S P L G F T T Y Y A D S V K G R ·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGTT ATTTCTCCTC TTGGTTTTAC GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K W T

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAATGGACT

G G S G I L N S S F D Y W G Q G T L V T V S S

301 GGTGGGAGTG GTATTTTGAA TTCTTCTTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 389**

DOM-114 – SEQ ID NO.: 275

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F R V S N Y D L ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT TAGGGTTAGC AATTACGATT

· T W V R Q A P G K G L E W V S T I S A T N G S T Y Y A D S V K G R ·

101 TGACCTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTATCAACC ATTAGTGCCA CAAACGGTAG CACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A A V T

201 GTTCACCATC TCCCGTGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTATTGCGC GGCAGTGACG

W W L L R H N D N L G F W G Q G T L V T V S S

301 TGGTGGTTGT TGCGTCATAA CGACAACTTG GGGTTTTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 390**

DOM-115 – SEQ ID NO.: 276

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F S I S Y K N M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT TAGCATTAGC TATAAGAATA

· A W V R Q A P G K G L E W V S A I K A A N G S T Y Y A D S V K G R ·

101 TGGCCTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTATCAGCC ATTAAGGCGG CAAACGGTAG CACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A T G S

201 GTTCACCATC TCCCGTGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTATTGCGC GACAGGGAGT

Q K K R T Y T F D F W G Q G T L V T V S S

301 CAGAAGAAGC GGACCTACAC GTTCGACTTT TGGGGTCAGG GAACCCTGGT CACCGTCTCG AGC **– SEQ ID NO.: 391**

DOM-120 – SEQ ID NO.: 277

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F R S Y T M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGG TCTTATACGA

· G W V R Q A P G K G L E W V S S I N P M G Y Q T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTAATCCTA TGGGTTATCA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K H G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACATGGG

V G K G T K P H N F D Y W G Q G T L V T V S S

301 GTGGGGAAGG GTACTAAGCC GCATAATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC **– SEQ ID NO.: 392**

DOM-121 – SEQ ID NO.: 278

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E L Y R M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG CTGTATAGGA

· S W V R Q A P G K G L E W V S E I S G S G F P T Y Y A D S V K G R ·

101 TGTCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGAG ATTAGTGGTA GTGGTTTTCC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K S L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAAGTCTG

H D K T Q H H Q E F D Y W G Q G T L V T V S S

301 CATGATAAGA CTCAGCATCA TCAGGAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 393**

DOM-123 – SEQ ID NO.: 279

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F I E Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTATT GAGTATCCTA

· R W V R Q A P G K G L E W V S L I S P S G V F T Y Y A D S V K G R ·

101 TGCGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCACTT ATTTCTCCGT CTGGTGTGTT TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGGGAT

E S S T F D Y W G Q G T L V T V S S

301 GAGTCTAGTA CTTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 394**

DOM-124 – SEQ ID NO.: 280

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F K R Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAG CGGTATGATA

· D W V R Q A P G K G L E W V S T I G S S G Y P T Y Y A D S V K G R ·

101 TGGATTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTGGGAGTT CGGGTTATCC GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A E R M

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GGAAAGGATG

P G Y F P G F A R Q F D Y W G Q G T L V T V S S

301 CCTGGTTATT TTCCTGGGTT TGCTCGGCAG TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC – **SEQ ID NO.: 395**

DOM-125 – SEQ ID NO.: 281

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F W R Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTGG CGGTATGCTA

· G W V R Q A P G K G L E W V S T I N D E G R E T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTAATGATG AGGGTCGGGA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K K R

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAAAGCGG

V S S S V N A P Y E F D Y W G Q G T L V T V S S

301 GTGTCTAGTT CTGTGAATGC TCCGTATGAG TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC – **SEQ ID NO.: 396**

DOM-126 – SEQ ID NO.: 282

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A N Y S M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG AATTATAGTA

· S W V R Q A P G K G L E W V S S I D R L G T H T Y Y A D S V K G R ·

101 TGAGTTGGGT CCGCCAGGCC CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGATCGTC TTGGTACGCA TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K V L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA TACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAGTGCTG

A D L I A G H A E F D Y W G Q G T L V T V S S

301 GCTGATCTTA TTGCTGGGCA TGCGGAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 397**

DOM-127 – SEQ ID NO.: 283

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F P S Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCCG TCGTATGATA

· A W V R Q A P G K G L E W V S G I S R S G S M T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGG ATTTCGAGGT CTGGTTCTAT GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G V

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT TTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGTGTT

D A H V Y Y M E P F F D Y W G Q G T L V T V S S

301 GATGCGCATG TTTATTATAT GGAGCCTTTT TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC – **SEQ ID NO.: 398**

DOM-128 – SEQ ID NO.: 284

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E R Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG AGGTATCAGA

· A W V R Q A P G K G L E W V S T I S S D G G G T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTAGTTCTG ATGGTGGGGG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGT

T V F D Y W G Q G T L V T V S S

301 ACTGTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 399**

DOM-129 – SEQ ID NO.: 285

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F P K Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACATTTCCG AAGTATGAGA

· A W V R Q A P G K G L E W V S S I D G D G K S T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTGATGGTG ATGGTAAGTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCGGAT

Q F F D Y W G Q G T L V T V S S

301 CAGTTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 400**

DOM-130 – SEQ ID NO.: 286

E V Q L L E S G G G L V Q P G G S L R L S C T A S G F T F A G Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTACAG CCTCCGGATT CACCTTTGCG GGTTATCAGA

· S W V R Q A P G K G L E W V S S I T N E G V S T Y Y A D S V K G R ·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTACTAATG AGGGTGTTTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGG

K Y F D Y W G Q G T L V T V S S

301 AAGTATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 401**

DOM-131 – SEQ ID NO.: 287

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G E Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGG GAGTATGAGA

· V W V R Q A P G K G L E W V S S I T S D G L S T Y Y A D S V K G R ·

101 TGGTGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCATCT ATTACGTCGG ATGGTCTGAG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCTGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGT

I R F D Y W G Q G T L V T V S S

301 ATTCGTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 402**

DOM-132 – SEQ ID NO.: 288

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A D Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT GATTATGATA

· A W V R Q A P G K G L E W V S G I V D D G L M T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGT ATTGTTGATG ATGGTCTTAT GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGAT

V A F D Y W G Q G T L V T V S N

301 GTTGCTTTTG ACTACTGGGG TCAGGGGACC CTGGTCACCG TCTCGAAC – **SEQ ID NO.: 403**

DOM-133 – SEQ ID NO.: 289

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F I G Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTATT GGTTATGCTA

· A W V R Q A P G K G L E W V S S I G P L G A T T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTGGTCCTT TGGGTGCGAC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K L P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATTGCCT

A G T S S H S V D F D Y W G Q G T L V T V S S

301 GCTGGTACGA GTAGTCATAG TGTGGATTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 404**

DOM-134 – SEQ ID NO.: 290

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A D Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG GATTATGAGA

· T W V R Q A P G K G L E W V S S I T S D G V S T Y Y A D S V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACTAGTG ATGGTGTTTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P S

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGTCG

V Q F D Y W G Q G T L V T V S S

301 GTTCAGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 405**

DOM-135 – SEQ ID NO.: 291

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F R R Y V M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCGT AGGTATGTTA

· G W V R Q A P G K G L E W V S W I E A D G R T T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATGG ATTGAGGCTG ATGGTCGTAC GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGGCTT

T D Q H V I E F D Y W G Q G T L V T V S S

301 ACGGATCAGC ATGTTATTGA GTTTGACTAC TGGGGTCAGG GAACCCTGGT CACCGTCTCG AGC – **SEQ ID NO.: 406**

DOM-136 – SEQ ID NO.: 292

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D G Y R M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT GGTTATCGTA

· G W V R Q A P G K G L E W V S S I A P D G N Y T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGCTCCGG ATGGTAATTA TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K F W

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATTTTGG

G M Q F D Y W G Q G T L V T V S S

301 GGGATGCAGT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C – **SEQ ID NO.: 407**

DOM-137 – SEQ ID NO.: 293

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A S Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT TCGTATCCGA

· G W V R Q A P G K G L E W V S S I G P I G F T T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTGGTCCTA TTGGTTTTAC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A E M K

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GGAAATGAAG

S P Y K P Q F D Y W G Q G T L V T V S S

301 TCGCCTTATA AGCCGCAGTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC – **SEQ ID NO.: 408**

DOM-138 – SEQ ID NO.: 294

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F L A Y W M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTTG GCTTATTGGA

· V W V R Q A P G K G L E W V S S I S P S G T H T Y Y A D S V K G R ·

101 TGGTTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTTCTCCGT CGGGTACGCA TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R V E D T A V Y Y C A K Y T

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGTCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATATACT

E P G L G S F D Y W G Q G T L V T V S S

301 GAGCCGGGGT TGGGTTCTTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC – **SEQ ID NO.: 409**

DOM-139 – SEQ ID NO.: 295

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S N Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCG AATTATGAGA

· G W V R Q A P G K G L E W V S V I S E V G S L T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGTG ATTTCTGAGG TGGGTTCTCT GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P H

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTCAT

D S S I G F D Y W G Q G T L V T V S S

301 GATAGTTCGA TTGGGTTTGA CTACTGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC – **SEQ ID NO.: 410**

DOM-141 – SEQ ID NO.: 296

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D T L T ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GTGGATTGGG GATACGTTAA

· W Y Q Q K L G K A P K L L I Y G G S E L Q S G V P P R F
S G S G S ·

101 CGTGGTACCA GCAGAAACTA GGGAAAGCCC CTAAGCTCCT GATCTATGGT GGTTCCGAGT TGCAAAGTGG GGTCCCACCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q C I S S P
C T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TGTATTAGTA
GTCCTTGTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 411**

DOM-142 – SEQ ID NO.: 297

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q F
I G D S L S ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GTTTATTGGT GATTCTTTAT

· W Y Q Q K P G K A P K L L I Y F S S I L Q S G V P S R F
S G S G S ·

101 CTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT TCTTCCATTT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H T S P
T T F G R

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATACTT
CGCCTACTAC GTTCGGCCGA

G T K V K I K R

301 GGGACCAAGG TGAAAATCAA ACGG – **SEQ ID NO.: 412**

DOM-143 – SEQ ID NO.: 298

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q T
I E T N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCCGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GACTATTGAG ACTAATTTAG

· W Y Q Q K P G K A P K L L I Y D S S Q L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT TCTTCCCAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D L A T Y Y C Q Q Y H G Y · P
T T F G Q

201 TGGGACAGAT TTTACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTAG CTACGTACTA CTGTCAACAG TATCATGGGT
ATCCTACGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 413**

DOM-144 – SEQ ID NO.: 299

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q M
I D Q D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GATGATTGAT CAGGATTTAG

· W Y Q Q K P G K A P K L L I Y N A S W L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATAAT GCGTCCTGGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGCG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H G Y P
I T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATGGTT
ATCCTATTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 414**

DOM-145 – SEQ ID NO.: 300

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q T
I Y T S L S ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GACGATTTAT ACTTCGTTAA

· W Y Q Q K P G K A P K L L I H Y G S V L Q S G V P S R F S G S G S ·

101 GTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCCATTAT GGTTCCGTGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D S A T Y Y C Q Q V H Q A P T T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTCTG CTACGTACTA CTGTCAACAG GTTCATCAGG CTCCTACGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 415**

DOM-146 – SEQ ID NO.: 301

D I R M T Q S P S S L S A S V G D R V T I T C R A S Q W I G D S L A ·

1 GACATCCGGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTGGG GATTCTTTAG

· W Y Q Q K P G K A P K L L I Y G I S E L Q S G V P S R F S G S G S ·

101 CGTGGTACCA GCAGAAGCCA GGGAAAGCCC CTAAGCTCCT GATCTATGGT ATTTCCGAGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D S A T Y Y C Q L S S S M P H T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTCTG CTACGTACTA CTGTCAACTG TCTAGTAGTA TGCCTCATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 416**

DOM-147 – SEQ ID NO.: 302

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q E I E T N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGAGATTGAG ACGAATTTAG

· W Y Q Q K P G K A P K L L I Y D S S H L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT TCGTCCCATT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H Q N P
P T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATCAGA
ATCCTCCGAC GTTCGGCCAA

G T K V E I K R

301 GGAACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 417**

DOM-149 – SEQ ID NO.: 303

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W
I G R Q L V ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GTGGATTGGG AGGCAGTTAG

· W Y Q Q K P G K A P K L L I Y G A T E L Q S G V P S R F
S G S G S ·

101 TTTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGGG GCGACCGAGT TGCAAAGTGG GGTCCCATCA
CGTTTTAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Q S K G P
L T F G H

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CAGTCGAAGG
GTCCTCTTAC GTTCGGCCAT

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 418**

DOM-150 – SEQ ID NO.: 304

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G
I G T D L N ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGGGATTGGT ACTGATTTAA

· W Y Q Q K P G K A P K L L I Y M G S Y L Q S G V P S R F S G S G S ·

101 ATTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATATG GGTTCCTATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q I Y S F P I T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG ATTTATTCTT TTCCTATTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 419**

DOM-154 – SEQ ID NO.: 305

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I E E M L H ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGAG GAGATGTTAC

· W Y Q Q K P G K A P K L L I Y F G S L L Q S G V P S R F S G S R S ·

101 ATTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTTT GGTTCCCTGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTAGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q H H T R P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CATCATACTC GTCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 420**

DOM-155 – SEQ ID NO.: 306

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I G M D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTGGG ATGGATTTAG

· W Y Q Q I P G K V P K L L I Y D A S Y L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGATACCA GGGAAAGTCC CTAAGCTCCT GATCTATGAT GCGTCCTATT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H K L P
A T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATAAGC
TTCCTGCGAC GTTTGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 421**

DOM-156 – SEQ ID NO.: 307

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D
I M D N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTATG GATAATTTAG

· W Y Q Q K P G K A P K L L I Y A A S W L Q S G V P S R F
S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGCG GCGTCCTGGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H K L P
V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATAAGT
TGCCTGTGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 422**

DOM-157 – SEQ ID NO.: 308

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q N
I G E D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GAGCAAGTCA GAATATTGGG GAGGATTTAG

· W Y Q Q K P G N A P K L L I Y S A S H L Q S G V P S R F S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAATGCCC CTAAGCTCCT GATCTATAGT GCGTCCCATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S S Y P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTCTAGTT ATCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 423**

DOM-158 – SEQ ID NO.: 309

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q P I D E D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CCGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GCCGATTGAT GAGGATTTAG

· W Y Q Q K P G N A P K L L I Y S A S Y L Q S G V P S R F S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAATGCCC CTAAGCTCCT GATCTATAGT GCGTCCTATT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S R L Q P E D F A T Y Y C Q Q Y H L L P A T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG ACTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATCTTC TGCCTGCTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 424**

DOM-159 – SEQ ID NO.: 310

D I Q M I Q S P S S L S A S V G D R V T I T C R A S Q D I N E D L E ·

1 GACATCCAGA TGATCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTAAT GAGGATTTAG

· W Y Q Q K P G K A P K L L I Y N A S M L Q S G V P S R F S G S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATAAT GCTTCCATGT TGCAAAGCGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P K D F A T Y Y C Q Q Y H T N P T T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT AAAGATTTTG CTACGTACTA CTGTCAACAG TATCATACTA ATCCTACTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 425**

DOM-160 – SEQ ID NO.: 311

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I E A D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GGATATTGAG GCGGATTTAG

· W Y Q Q K P G K A P K L L I Y H S S E L Q S G V P S R F S G S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCAT TCTTCCGAGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GAAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H M S P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATATGT CGCCTGTGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 426**

DOM-161 – SEQ ID NO.: 312

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D I D S D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTGAT AGTGATTTAG

· W Y Q Q K P G K A P M L L I Y S S S D L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTATGCTCCT GATCTATTCT TCGTCCGATT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H S L P
V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATAGTC
TGCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 427**

DOM-162 – SEQ ID NO.: 313

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D
I S D D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTTCG GATGATTTAG

· W Y Q Q K P G K A P K L L I Y N S S F L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATAAT TCGTCCTTTT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G A D F T L T I S S L Q P E D F A T Y Y C Q Q Y H S L P
V T F G Q

201 TGGGGCAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATAGTT
TGCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 428**

DOM-163 – SEQ ID NO.: 314

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D
I E G N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTGAG GGTAATTTAG

· W Y Q Q K P G K A P K L L I Y D S S Q L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT TCGTCCCAGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGGTC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H H L P
T T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATCATC
TTCCTACGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 429**

DOM-164 – SEQ ID NO.: 315

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S
I D T D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GAGTATTGAT ACGGATTTAG

· W Y Q Q K P G K A P K L L I Y D G S W L Q S G V P S R F
S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GGGTCCTGGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y R W I P
V T F G Q

201 TGGGACAGAT TTTACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCGGTGGA
TTCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 430**

DOM-165 – SEQ ID NO.: 316

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S
I S T D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GAGTATTAGT ACTGATTTAG

· W Y Q Q K L G K A P K L L I Y D A S L L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACTA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GCTTCCCTTT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S S L P
V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTCGAGTC
TGCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 431**

DOM-166 – SEQ ID NO.: 317

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q P
I T T S L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GCCTATTACG ACGTCTTTAG

· W Y Q Q K P G K A P K L L I Y D A S M L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GCGTCCATGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y W V T P
V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTGGGTTA
CGCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 432**

DOM-167 – SEQ ID NO.: 318

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q N
I H T N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACCTGCC GGGCAAGTCA GAATATTCAT ACGAATTTAG

· W Y Q Q K P G K A P K L L I Y D G S M L Q S G V P S R F S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GGTTCCATGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S A N P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATTCGGCTA ATCCTGTTAC GTTCGGCCAA

G T K V G I K R

301 GGGACCAAGG TGGGAATCAA ACGG – **SEQ ID NO.: 433**

DOM-168 – SEQ ID NO.: 319

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q W I H T D L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTGGATTCAT ACGGATTTAG

· W Y Q Q K P G K A P K L L I Y D G S M L Q S G V P S R F S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAT GGTTCCATGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S V S P V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATAGTGTGT CGCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 434**

DOM-169 – SEQ ID NO.: 320

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S I D N N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GAGTATTGAT AATAATTTAG

· W Y Q Q K P G E A P K L L I Y D G S L L Q S G V P S R F
S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGGAAGCCC CTAAGCTCCT GATCTATGAT GGGTCCCTTT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y H L H P
V T F G Q

201 TGGGACAGAT TTCACTCTTA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATCATCTTC
ATCCTGTTAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 435**

DOM-170 – SEQ ID NO.: 321

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D
I D T N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GGATATTGAT ACGAATTTAG

· W Y Q Q K P G E A P K L L I Y D R S T L Q S G V P S R F
S G S G S ·

101 AGTGGTATCA GCAGAAACCA GGGGAAGCCC CTAAGCTCCT GATCTATGAT CGTTCCACGT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y D S Y P
V T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATGATTCTT
ATCCTGTGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 436**

DOM-171 – SEQ ID NO.: 322

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S
I E S N L E ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA GTCTATTGAG TCTAATTTAG

· W Y Q Q K P G K A P K L L I Y N A S E L Q S G V P S R F S G S G S ·

101 AGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATAAT GCGTCCGAGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L R P E D F A T Y Y C Q Q Y D Q W P T T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCGACCT GAAGATTTTG CTACGTACTA CTGTCAACAG TATGATCAGT GGCCTACGAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 437**

DOM-172 – SEQ ID NO.: 323

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q A I G N T L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACT ATCACTTGCC GGGCAAGTCA GGCTATTGGT AATACTTTAC

· W Y Q Q K P G K A P K L L I Y L S S R L Q S G V P S R F S G S G S ·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATCTT AGTTCCAGGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q L K K P P Y T F G Q

201 TGGGACAGAT TTTACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CTGAAGAAGC CTCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 438**

DOM-173 – SEQ ID NO.: 324

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q K I K N R L A ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GAAGATTAAG AATCGGTTAG

· W Y Q Q K P G K A P K L L I Y E V S H L Q S G V P S R F
S G S G S ·

101 CGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATGAG GTTTCCCATT TGCAAAGTGG GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I G S L Q P E D F A T Y Y C Q Q R R Q S P
Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCGGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG AGGAGGCAGT
CGCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 439**

DOM-174 – SEQ ID NO.: 325

D I Q M T Q S P S S L S A S V G D R V T I T C R A S E D
I G E E L F ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTGA
GGATATTGGG GAGGAGTTAT

· W Y Q Q K P G K A P K L L I Y S A S T L Q S E V P S R F
S G S G S ·

101 TTTGGTATCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTCG GCGTCCACGT TGCAAAGTGA GGTCCCATCA
CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q H E D F A T Y Y C Q Q V Y E W P
Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACAT GAAGATTTTG CTACGTACTA CTGTCAACAG GTTTATGAGT
GGCCTTATAC GTTCGGCCAA

G T K V E I K R

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 440**

DOM-175 – SEQ ID NO.: 326

D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q P
I S G G L R ·

1 GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGAGA CCGTGTCACC ATCACTTGCC GGGCAAGTCA
GCCTATTTCT GGGGGTTTAA

· W Y Q Q K P G K A P K L L I Y S T S M L Q S G V P S R F S G S G S ·

101 GGTGGTACCA GCAGAAACCA GGGAAAGCCC CTAAGCTCCT GATCTATTCT ACTTCCATGT TGCAAAGTGG GGTCCCATCA CGTTTCAGTG GCAGTGGATC

· G T D F T L T I S S L Q P E D F A T Y Y C Q Q L Y S A P Y T F G Q

201 TGGGACAGAT TTCACTCTCA CCATCAGCAG TCTGCAACCT GAAGATTTTG CTACGTACTA CTGTCAACAG CTTTATTCTG CTCCTTATAC GTTCGGCCAA

G T K V E I K R ·

301 GGGACCAAGG TGGAAATCAA ACGG – **SEQ ID NO.: 441**

DOM-176 – SEQ ID NO.: 327

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D A Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT GCGTATGAGA

· G W V R Q A P G K G L E W V S I I D W D G N S T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAATT ATTGATTGGG ATGGTAATTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCTGGG

D N V G I F D Y W G Q G T L V T V S S

301 GATAATGTTG GTATTTTTGA CTACTGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC – **SEQ ID NO.: 442**

DOM-177 – SEQ ID NO.: 328

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S N Y Y M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT AATTATTATA

· V W V R Q A P G K G L E W V S A I D E W G F A T Y Y A D S V K G R ·

101 TGGTGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGCG ATTGATGAGT GGGGTTTTGC GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K H W

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACATTGG

E F T S D T S R F D Y W G Q G T L V T V S S

301 GAGTTTACGT CTGATACGTC GCGTTTTGAC TACTGGGGTC AGGGAACCCT GGTCACCGTC TCGAGC – **SEQ ID NO.: 443**

DOM-178 – SEQ ID NO.: 329

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E D F D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG GATTTTGATA

· A W V R Q A P G K G L E W V S S I N D Q G S L T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTAATGATC AGGGTTCTCT GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGAT

Q F F D Y W G Q G T L V T V S S

301 CAGTTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 444**

DOM-179 – SEQ ID NO.: 330

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S A Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT GCTTATGATA

· M W V R Q A P G K G L E W V S R I S P Q G Q R T Y Y A D S V K G R ·

101 TGATGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCACGG ATTAGTCCTC AGGGTCAGCG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K I R

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAATTCGT

G Q S R I P M R F D Y W G Q G T L V

301 GGGCAGTCGC GGATTCCTAT GAGGTTTGAC TACTGGGGTC AGGGAACCCT GGTC – **SEQ ID NO.: 445**

DOM-180 – SEQ ID NO.: 331

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T D Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACG GATTATGAGA

· G W V R Q A P G K G L E W V S T I T S L G E S T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTACTAGTT TGGGTGAGAG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTGGT

R I F D Y W G Q G T L V T V S S

301 CGTATTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 446**

DOM-181 – SEQ ID NO.: 332

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A F Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT TTTTATCCTA

· M W V R Q A P G K G L E W V S W I D A T G T R T Y Y A D S V K G R ·

101 TGATGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATGG ATTGATGCTA CGGGTACGAG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A E G N

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GGAAGGTAAT

Y G S S Y T M G V F D Y W G Q G T L V T V S S

301 TATGGGAGTT CGTATACTAT GGGGGTTTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 447**

DOM-182 – SEQ ID NO.: 333

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D E Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAT GAGTATCCGA

· Y W V R Q A P G K G L E W V S S I G P S G P N T Y Y A D S V K G R ·

101 TGTATTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGGTCCTT CTGGTCCGAA TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K S P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATCTCCG

Y F D V I P S Y F D Y W G Q G T L V T V S S

301 TATTTGATG TTATTCCTAG TTATTTTGAC TACTGGGGTC AGGGAACCCT GGTCACCGTC TCGAGC– **SEQ ID NO.: 448**

DOM-183 – SEQ ID NO.: 334

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A D Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG GATTACGGTA

· G W V R Q A P G K G L E W V S S I Q S S G L R T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTCAGTCGT CGGGTTTGCG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K R A

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACGGGCT

N S R R G F D Y W G Q G T L V T V S S

301 AATTCTCGTA GGGGTTTTGA CTACTGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC – **SEQ ID NO.: 449**

DOM-184 – SEQ ID NO.: 335

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S D Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT GATTATGAGA

· M W V R Q A P G K G L E W V S S I T S H G G S T Y Y A D S V K G R ·

101 TGATGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACTAGTC ATGGTGGGTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCTGAT

K D F D Y W G Q G T L V T V S S

301 AAGGATTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 450**

DOM-185 – SEQ ID NO.: 336

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A H Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG CATTATCCGA

· S W V R Q A P G K G L E W V S S I G R L G N R T Y Y A D S V K G R ·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGGTAGGC TGGGTAATCG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K R A

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACGTGCT

T P V P I K G L F D Y W G Q G T L V T V S S

301 ACGCCTGTGC CGATTAAGGG TTTGTTTGAC TACTGGGGTC AGGGAACCCT GGTCACCGTC TCGAGC – **SEQ ID NO.: 451**

DOM-186 – SEQ ID NO.: 337

E V Q L L E S G G G L V Q P G G S L R L S C A A S G L T F G R Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGACT CACCTTTGGG AGGTATGAGA

· A W V R Q A P G K G L E W V S S I D S D G W V T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTGATTCGG ATGGTTGGGT GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A Q P D

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GCAACCGGAT

S L F D Y W G Q G T L V T V S S

301 TCGTTGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 452**

DOM-187 – SEQ ID NO.: 338

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S S Y S M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCT AGTTATTCTA

· V W V R Q A P G K G L E W V S G I N R G G T R T Y Y A D S V K G R ·

101 TGGTGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGT ATTAATCGGG GTGGTACTCG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G W

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGTTGG

R R G F D Y W G Q G T L V T V S S

301 AGGAGGGGGT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C – **SEQ ID NO.: 453**

DOM-188 – SEQ ID NO.: 339

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T R Y R M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACG CGTTATAGGA

· S W V R Q A P G K G L E W V S G I S R D G Y R T Y Y A D S V K G R ·

101 TGTCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAGGG ATTTCGAGGG ATGGTTATCG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G M

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGTATG

T A S F D Y W G Q G T L V T V S S

301 ACTGCGTCGT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C – **SEQ ID NO.: 454**

DOM-189 – SEQ ID NO.: 340

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F Q M Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTCAG ATGTATCCGA

· G W V R Q A P G K G L E W V S M I E P A G D L T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAATG ATTGAGCCGG CTGGTGATCT TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K Y Q

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATATCAG

E Q P W F D Y W G Q G T L V T V S S

301 GAGCAGCCTT GGTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 455**

DOM-190 – SEQ ID NO.: 341

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S M Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT ATGTATGATA

· H W V R Q A P G K G L E W V S T I L S D G T D T Y Y A D S V K G R ·

101 TGCATTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTTTGTCTG ATGGTACGGA TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K Y G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATATGGG

A M F D Y W G Q G T L V T V S S

301 GCTATGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 456**

DOM-191 – SEQ ID NO.: 342

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F K L Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAG TTGTATCCGA

· T W V R Q A P G K G L E W V S S I D A G G H E T Y Y A D S V K G R ·

101 TGACGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTGATGCGG GGGGTCATGA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K D W

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAGATTGG

W D Y L F D Y W G Q G T L V T V S S

301 TGGGATTATC TGTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 457**

DOM-192 – SEQ ID NO.: 343

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S R Y P M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTTCG CGGTATCCGA

· S W V R Q A P G K G L E W V S S I N R S G M R T Y Y A D S V K G R ·

101 TGAGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTAATCGTT CGGGTATGCG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A E G H

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GGAAGGGCAT

Q A P F D Y W G Q G T L V T V S S

301 CAGGCGCCGT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C – **SEQ ID NO.: 458**

DOM-193 – SEQ ID NO.: 344

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T G Y A M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACG GGGTATGCTA

· S W V R Q A P G K G L E W V S T I N A N G I R T Y Y A D S V K G R ·

101 TGTCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTAATGCGA ATGGTATTCG GACATACTAC GCCGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N G L R A E D T A V Y Y C A K G G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACGGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGGGGG

V W R W G T G H K F D Y W G Q G T L V T V S S

301 GTTTGGAGGT GGGGGACTGG GCATAAGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 459**

DOM-194 – SEQ ID NO.: 345

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F K Q Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAG CAGTATGATA

· R W V R Q A P G K G L E W V S T I S Q N G T K T Y Y A D S V K G R ·

101 TGCGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACG ATTTCGCAGA ATGGTACTAA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K S R

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATCGAGG

T G R Y F D Y W G Q G T L V T V S S

301 ACTGGTAGGT ATTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 460**

DOM-195 – SEQ ID NO.: 346

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G T Y D M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGG ACTTATGATA

· G W V R Q A P G K G L E W V S R I N W Q G D R T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGG ATTAATTGGC AGGGTGATCG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K A G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGCGGGG

F G H Y V D G L G F D Y W G Q G T L V T V S S

301 TTTGGTCATT ATGTTGATGG TCTTGGGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 461**

DOM-196 – SEQ ID NO.: 347

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S G Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT GGGTATGAGA

· A W V R Q A P G K G L E W V S S I T D M G D S T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTACTGATA TGGGTGATTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCTGGG

T A F D Y W G P G T L V T V S S

301 ACTGCGTTTG ACTACTGGGG TCCGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 462**

DOM-197 – SEQ ID NO.: 348

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A K Y K M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCG AAGTATAAGA

· W W V R Q A P G K G L E W V S S I T P K G H S T Y Y A D S V K G R ·

101 TGTGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTACTCCGA AGGGTCATTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K R P

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAAGGCCG

M T P F D Y W G Q G T L V T V S S

301 ATGACTCCGT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C – **SEQ ID NO.: 463**

DOM-198 – SEQ ID NO.: 349

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E R Y N M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG CGGTATAATA

· S W V R Q A P G K G L E W V S S I R P R G G K T Y Y A D S V K G R ·

101 TGTCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTCGGCCGC GGGGTGGGAA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K W R

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAATGGCGG

R E G Y T G S K F D Y W G Q G T L V T V S S

301 CGGGAGGGGT ATACTGGTTC TAAGTTTGAC TACTGGGGTC AGGGAACCCT GGTCACCGTC TCGAGC – **SEQ ID NO.: 464**

DOM-199 – SEQ ID NO.: 350

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F E R Y G M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGAG AGGTATGGTA

· T W V R Q A P G K G L E W V S S I W P R G Q K T Y Y A D S V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAAGT ATTTGGCCGA GGGGTCAGAA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K G N

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGGGAAT

S R Y V F D Y W G Q G T L V T V S S

301 AGTCGGTATG TTTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 465**

DOM-200 – SEQ ID NO.: 351

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T N Y S M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTACT AATTATAGTA

· G W V R Q A P G K G L E W V S T I R P N G T K T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAACT ATTCGTCCTA ATGGTACTAA GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K R S

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACGGTCG

S A H L Q R F D Y W G Q G T L V T V S S

301 TCTGCGCATC TTCAGAGGTT TGACTACTGG GGTCAGGGAA CCCTGGTCAC CGTCTCGAGC – **SEQ ID NO.: 466**

DOM-201 – SEQ ID NO.: 352

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F G N Y S M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGGT AATTATTCGA

· G W V R Q A P G K G L E W V S S I G R H G G R T Y Y A D S V K G R ·

101 TGGGTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCG ATTGGTCGTC ATGGTGGGCG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K K G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAAAGGGG

S T Y P R F D Y W G Q G T L V T V S S

301 AGTACTTATC CTAGGTTTGA CTACTGGGGT CAGGGAACCC TGGTCACCGT CTCGAGC – **SEQ ID NO.: 467**

DOM-202 – SEQ ID NO.: 353

E V Q L L E S G G G L V Q P G G S L R L S C T A S G F T F S H Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTACAG CCTCCGGATT CACCTTTTCG CATTATGAGA

· G W V R Q A P G K G L E W V S S I E P F G G G T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTGAGCCTT TTGGTGGTGG GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K V Y

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAAGTGTAT

P Q G S F D Y W G Q G T L V T V S S

301 CCTCAGGGTT CTTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 468**

DOM-203 – SEQ ID NO.: 354

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S N Y T M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAGT AATTATACTA

· G W V R Q A P G K G L E W V S S I R P D G K I T Y Y A D S V K G R ·

101 TGGGGTGGGT CCGTCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCATCT ATTCGGCCTG ATGGTAAGAT TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A E V Y

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GGAAGTTTAT

S S C A M C T P L L F D Y W G Q G T L V T V S S

301 TCTTCGTGTG CGATGTGTAC TCCGCTTTTG TTTGACTACT GGGGTCAGGG AACCCTGGTC ACCGTCTCGA GC – **SEQ ID NO.: 469**

DOM-204 – SEQ ID NO.: 355

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F K R Y S M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGGGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTAAG CGGTATTCGA

· A W V R Q A P G K G L E W V S D I G P R G F S T Y Y A D S V K G R ·

101 TGGCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGAT ATTGGGCCGA GGGGTTTTTC GACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K V G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGTGGGT

R G Q R D T S Q P F D Y W G Q G T L V T V S S

301 CGTGGTCAGC GTGATACTAG TCAGCCGTTT GACTACTGGG GTCAGGGAAC CCTGGTCACC GTCTCGAGC – **SEQ ID NO.: 470**

DOM-205 – SEQ ID NO.: 356

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A S Y Q M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT TCTTATCAGA

· A W V R Q A P G K G L E W V S G I T S G G L S T Y Y A D S V K G R ·

101 TGGCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGG ATTACTTCGG GTGGTCTTAG TACGTACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGG

R G F D Y W G Q G T L V T V S S

301 AGGGGTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 471**

DOM-206 – SEQ ID NO.: 357

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A S Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGTGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT TCTTATGAGA

· T W V R Q A P G K G L E W V S G I S S D G L S T Y Y A D S V K G R ·

101 TGACTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGGG ATTTCTTCTG ATGGTCTGTC TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAACCGGGG

V L F D Y W G Q G T L V T V S S

301 GTGTTGTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 472**

DOM-207 – SEQ ID NO.: 358

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F D K Y L M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCTGGATT CACCTTTGAT AAGTATTTGA

· S W V R Q A P G K G L E W V S G I E P L G D V T Y Y A D S V K G R ·

101 TGTCGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTGGAGTG GGTCTCAGGT ATTGAGCCTC TGGGTGATGT TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K E A

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGAGGCT

S G D F D Y W G Q G T L V T V S S

301 TCGGGGGATT TTGACTACTG GGGTCAGGGA ACCCTGGTCA CCGTCTCGAG C – **SEQ ID NO.: 473**

DOM-208 – SEQ ID NO.: 359

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F T E Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGGTT CACCTTTACT GAGTATGAGA

· S W V R Q A P G K G L E W V S S I D N V G S S T Y Y A D S V K G R ·

101 TGTCTTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAAGT ATTGATAATG TGGGTAGTAG TACATACTAC GCAGACTCCG TGAAGGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P G

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAT ACCGCGGTAT ATTACTGTGC GAAACCTGGG

K L F D Y W G Q G T L V T V S S

301 AAGCTTTTTG ACTACTGGGG TCAGGGAACC CTGGTCACCG TCTCGAGC – **SEQ ID NO.: 474**

DOM-209 – SEQ ID NO.: 360

E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F A D Y E M ·

1 GAGGTGCAGC TGTTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGCGTCTC TCCTGTGCAG CCTCCGGATT CACCTTTGCT GATTATGAGA

· W W V R Q A P G K G L E W V S A I S R Q G F A T Y Y A D S V K G R ·

101 TGTGGTGGGT CCGCCAGGCT CCAGGGAAGG GTCTAGAGTG GGTCTCAGCG ATTTCTAGGC AGGGTTTTGC TACATACTAC GCAGACTCCG TGAAAGGCCG

· F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K D L

201 GTTCACCATC TCCCGCGACA ATTCCAAGAA CACGCTGTAT CTGCAAATGA ACAGCCTGCG TGCCGAGGAC ACCGCGGTAT ATTACTGTGC GAAAGATCTG

E R D D F D Y W G Q G T L V T V S S

301 GAGCGGGATG ATTTTGACTA CTGGGGTCAG GGAACCCTGG TCACCGTCTC GAGC – **SEQ ID NO.: 475**

Among the DOM clones described, a number of observations have been made regarding homology among clones, and properties of amino acids predicted at particular positions in the anti-CD40L dAbs. These are shown in Tables 9 and 10 below:

Table 9. Predictions for anti-VH CD40L clones: (using standard single letter amino acid code)

Predicted amino acid number relative to dummy sequence	Predicted Kabat numbering	Predicted dummy amino acid at this position	Properties of amino acids frequently fount at the designated position in anti-CD40L dAbs	Amino acid frequently fount at the designated position in anti-CD40L dAbs
30	30	S	acidic; small	D, E, A, G
33	33	A	mix	Q, E, P, G
35	35	S	mix	A, M, G, T
50	50	A	nucleophilic; or G, W	S, T, G, W
52	52	S	hydrophilic; or G, D, N	T, S, G, D, N
53	53	G	mix	P, R, S, D
54	54	S	acidic; or L, I, Q	D, E, L, I, Q
99*	95	S	hydrophobic; or P, G	P, G, V, L
100	96	Y	mix	G, D, R, L

* If amino acid at position 99 (dummy) is P then G or D is frequently found at position 100.

Table 10. Predictions for anti-VK CD40L clones:

Predicted amino acid number relative to dummy sequence	Predicted Kabat numbering	Predicted dummy amino acid at this position	Properties of amino acids frequently fount at the designated position in anti-CD40L dAbs	Amino acid frequently fount at the designated position in anti-CD40L dAbs
28*	28	S	mix	A, D, W, P
30	30	S	acidic; or G	G, D, E
32	32	Y	mix	R, S, D, N

34**	34	N	acidic or basic	R, E
50	50	A	mix	H, D, G, S
51	51	A	mix	S, G, R
53	53	S	hydrophobic; or K, E	I, L, M, K, E, W
91	91	S	mix	Y, R, V
92	92	Y	basic; or S, W	H, S, W, R
96	96	N	mix	Y, V, A, T

*If amino acid at position 28 (dummy) is D then E is frequently found at position 34.
**If amino acid at position 34 (dummy) is E then Y is frequently found at position 91 and H or D at position 92.

Example 8: PEGylation of DOM8-24

Site specific maleimide-PEGylation of DOM8-24 requires a solvent accessible cysteine to be provided on the surface of the protein, in this example, at the C-terminus. The cysteine residue, once reduced to give the free thiol, can then be used to specifically couple the protein to PEG via a wide range of chemistries such as maleimide or another thiol to give a disulphide. A wide range of chemical modified PEGs of different sizes and branched formats are available from Nektar (formally known as Shearwater Corp). This allows the basic dAb-cys monomer to be formatted in a variety of ways for example as a PEGylated monomer, dimer, trimer, tetramer etc. The size of the PEGs is given in kDa but can also be referred to as K (i.e. "40K PEG" = 40 kDa PEG).

PCR construction of DOM8-24cys

The site of attachment for the PEG may be placed elsewhere on the surface of the dAb as long as the targeted amino acid is solvent accessible and the resultant PEGylated protein still maintains antigen binding. Thus it is also possible to engineer the cys into any one of frameworks 1-4 of the dAb for PEGylation and still maintain some antigen binding. The following oligonucleotides were used to specifically PCR DOM8-24 with a *Sal*I and *Bam*HI sites for cloning and also to introduce a C-terminal cysteine residue.

```
      SalI
    ~~~~~~~~
Ala Ser Thr Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu
Val
```

```
      1  GCG TCG ACG GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG
         GTA
         CGC AGC TGC CTC CAC GTC GAC AAC CTC AGA CCC CCT CCG AAC
         CAT

         Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
         Phe
     46  CAG CCT GGG GGG TCC CTG CGT CTC TCC TGT GCA GCC TCC GGA
         TTC
         GTC GGA CCC CCC AGG GAC GCA GAG AGG ACA CGT CGG AGG CCT
         AAG

         Thr Phe Ser Asn Tyr Gln Met Ala Trp Val Arg Gln Ala Pro
         Gly
     91  ACC TTT AGT AAT TAT CAG ATG GCG TGG GTC CGC CAG GCT CCA
         GGG
         TGG AAA TCA TTA ATA GTC TAC CGC ACC CAG GCG GTC CGA GGT
         CCC

         Lys Gly Leu Glu Trp Val Ser Ser Ile Thr Ser Glu Gly Gly
         Ser
    136  AAG GGT CTA GAG TGG GTC TCA AGT ATT ACT AGT GAG GGT GGT
         TCG
         TTC CCA GAT CTC ACC CAG AGT TCA TAA TGA TCA CTC CCA CCA
         AGC

         Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
         Arg
    181  ACA TAC TAC GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC
         CGC
         TGT ATG ATG CGT CTG AGG CAC TTC CCG GCC AAG TGG TAG AGG
         GCG

         Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
         Arg
    226  GAC AAT TCC AAG AAC ACA CTG TAT CTG CAA ATG AAC AGC CTG
         CGT
         CTG TTA AGG TTC TTG TGT GAC ATA GAC GTT TAC TTG TCG GAC
         GCA

         Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Pro Gly Lys
         Asn
    271  GCC GAG GAC ACC GCG GTA TAT TAC TGT GCG AAA CCG GGT AAG
         AAT
         CGG CTC CTG TGG CGC CAT ATA ATG ACA CGC TTT GGC CCA TTC
         TTA
```

```
        Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Cys
        ***
    316 TTT GAC TAC TGG GGT CAG GGA ACC CTG GTC ACC GTC TCG TGC
        TAA
        AAA CTG ATG ACC CCA GTC CCT TGG GAC CAG TGG CAG AGC ACG
        ATT

             BamHI
            ~~~~~~~
        *** Gly Ser   (SEQ ID NI: 85)
    361 TAA GGA TCC   (SEQ ID NO: 83)
        ATT CCT AGG   (SEQ ID NO: 84)
```

The DNA sequence of the PCR primers used to amplify the engineered dAb are shown below.

Forward primer

5'- AGTGCGTCGACGGAGGTGCAGCTGTTGGAGTCT-3' (SEQ ID NO: 86)

Reverse primer

5'- AAAGGATCCTTATTAGCACGAGACGGTGACCAGGGTTCCCTG-3' (SEQ ID NO: 87)

The PCR reaction (50µL volume) was set up as follows: 200µM dNTP's, 0.4µM of each primer, 5 µL of 10x *Pfu*Turbo buffer (Stratagene), 100 ng of template plasmid (DOM8-24), 1µL of *Pfu*Turbo enzyme (Stratagene) and the volume adjusted to 50µL using sterile water. The following PCR conditions were used: initial denaturing step 94 °C for 2 mins, then 25 cycles of 94 °C for 30 secs, 64 °C for 30 sec and 72 °C for 30 sec. A final extension step was also included of 72 °C for 5 mins. The PCR product was purified and digested with *Sal*I and *Bam*HI and ligated into the vector (pDOM5) which had also been cut with the same restriction enzymes. Correct clones were verified by DNA sequencing.

Expression and purification of DOM8-24

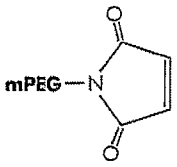
DOM8-24 vector was transformed into HB2151 electro-competent cells. Cells carrying the dAb plasmid were selected for using 100µg/mL carbenicillin. Cultures were set up in 2L baffled flasks containing 500 mL of terrific broth (Sigma-Aldrich) and 100µg/mL carbenicillin.

The cultures were grown at 30 °C at 200rpm to an O.D.600 of 1-1.5 and then induced with 1mM IPTG (isopropyl-beta-D-thiogalactopyranoside, from Melford Laboratories). The expression of the dAb was allowed to continue for 12-16 hrs at 30 °C. It was found that most of the dAb was present in the culture media. Therefore, the cells were separated from the media by centrifugation (8,000x*g* for 30 mins), and the supernatant used to purify the dAb. Per litre of supernatant, 10 mL of Streamline Protein A (Amersham Biosciences) was added and the dAb allowed to batch bind with stirring for 3 hours at room temperature, or overnight at 4 °C. The resin was then allowed to settle under gravity for an hour before the supernatant removed. The agarose was then packed into a XK 16 column (Amersham Pharmacia) and was washed with 10 column volumes of 2xPBS. The bound dAb was eluted with 100 mM glycine pH 3.0 and protein containing fractions were then neutralized by the addition of 1/5 volume of 1 M Tris pH 8.0.

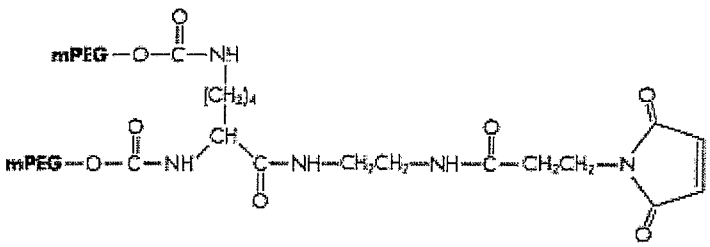
PEGylation of DOM8-24cys using MAL activated PEG

Monomer PEGylation

The cysteine residue which had been engineered onto the surface of the VH dAb was specifically modified with a single linear or branched PEG-MAL to give monomeric modified protein. mPEG-MAL formats which may be used to PEGylate a monomeric VH or Vk dAb. The PEGs may be of MW from 500 to 60,000 (eg, from 2,000 to 40,000) in size.



mPEG-MAL



mPEG2-MAL

2.5 ml of 500 µM DOM8-24cys was reduced with 5 mM dithiothreitol and left at room temperature for 20 minutes. The sample was then buffer exchanged using a PD-10 column (Amersham Pharmacia). The column had been pre-equilibrated with 5 mM EDTA, 20 mM sodium phosphate pH 6.5, 10% glycerol, and the sample applied and eluted following the

manufactures guidelines. The eluted sample (3.5 ml of ~360 μM dAb) was placed on ice until required. A four fold molar excess of 30K PEG-MAL or 40K PEG2-MAL (Nektar) was weighed out and added directly to the reduced dAb solution and gently mixed until the polymer had dissolved. The reaction was left to proceed at room temperature for 3 hours.

Purification of 30K and 40K PEGylated DOM8-24cys monomer

The PEGylated dAb was purified using cation exchange chromatography as the isoelectric point (pI) of the protein is ~8. 40 μL of 40% glacial acetic acid was added per mL of the 30K or 40K PEG DOM8-24cys reaction to reduce the pH to ~4. The sample was then applied to a 1mL Resource S cation exchange column (Amersham Pharmacia), which had been pre-equilibrated with 50 mM sodium acetate pH 4.0. The PEGylated material was separated from the unmodified dAb by running a linear sodium chloride gradient from 0 to 500 mM over 20 column volumes. Fractions containing PEGylated dAb only were identified using SDS-PAGE and then pooled and the pH increased to 8 by the addition of 1/5 volume of 1M Tris pH 8.0

In vitro functional binding assay: CD40 ligand receptor assay (CD40L RBA)

The CD40L assay was used to measure the binding of CD40L to CD40 and the ability of binding entities (including monovalent antibody fragments such a dAbs) to block this interaction, as shown Figure 7.

A 96 well Nunc Maxisorp assay plate was coated overnight at 4°C with 100 μl per well of recombinant human CD40/Fc (R&D Systems) at 0.5 μg/ml in carbonate buffer. The plate was washed 3 times with 300 μl of 0.05% Tween/PBS and 3 times with 300 μl of PBS using a Tecan plate washer. The wells were blocked using 200 μl of PBS containing 2% (w/v) BSA and incubated for a minimum of 1 h at room temperature. The wells were washed as above, then 50 μl of purified dAb protein was added to each well. To each well 50 μl of CD40L, at 6 ng/ml in diluent (for a final concentration of 3 ng/ml), was also added and the plate incubated for 1 hr at room temperature. The plate was washed as described previously and 100 μl biotinylated anti-CD40L antibody, 0.5 μg/ml in diluent, was added and incubated for 1 hr at room temperature. The plate was washed as described above, then 100 μl HRP conjugated anti-biotin antibody (1:5000 dilution in diluent) added to each well and the plate incubated for 1 hr at room

temperature. The plate was washed again as described above using a Tecan plate washer and the assay developed using 100 µl of SureBlue 1-Component TMB MicroWell Peroxidase solution (the plate was left at room temperature for up to 20 min). The reaction was stopped by the addition of 100 µl 1 M hydrochloric acid. The OD_{450nm} of the plate was assayed within 30 minutes of acid addition. The OD_{450nm} is proportional to the amount of bound streptavidin-HRP conjugate, therefore the greater the degree of dAb inhibition the lower the OD_{450nm} of the resulting signal. The following controls were included; 0 ng/ml CD40L (diluent only), 3 ng/ml CD40L and 3 ng/ml CD40L with 1 µg/ml anti-CD40L antibody.

The results of the CD40L RBA are shown in Fig. 9. It can be seen that PEGylated DOM8-24cys has a similar affinity for CD40L as the unmodified protein. The size of the polymer attached (either 30 or 40K) does not seem to significantly affect the IC 50 of~1 nM.

Example 9: Generation and Expression of a Dual Specific dAb Dimer (DOM7h-26—DOM-24)

A dual specific dAb dimer was constructed essentially as described in WO2004/003019 and as described below.

A PCR fragment introducing 5' NotI and 3' EcoRI restriction sites into the DOM-24 DNA sequence was generated using the primers VH-5'-NotI (5'-GTATGTCTGGCGGCCGCAGAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTG-3'; SEQ ID NO: 90) and VH-NO-XhoI (5'-TAGAATTCTTATTAGCTGGAGACGGTGACCAGGGT-3'; SEQ ID NO: 91). The PCR product was electrophoresed on a 1% agarose gel and the appropriate band was excised and gel cleaned using the Qiagen Gel Extraction Kit. The purified DNA was digested with NotI and EcoRI for 1 hour at 37°C and then the digested DNA fragment purified using the Qiagen PCR Cleanup Kit. The digested PCR product was ligated using T4 DNA ligase into the vector pCH3, derived from pPICz-alpha (Invitrogen Ltd, Paisley, UK), which had been previously been digested with NotI and EcoRI, thereby enabling the ligation of the DOM-24 DNA sequence 3' relative to the anti-human serum albumin dAb DNA sequence (DOM7h-26). One micro-litre of the ligation mix was used to electroporate 50 µl of E. coli TOP10F' (0.2 mm diameter cuvettes; 25 µFD; 200Ω; 2.500 kV) using a Biorad Genepulser II with pulse controller module. The electroporated cells were immediately resuspended in 1 ml low salt LB medium (10 g/l tryptone; 5 g/l yeast extract; 5 g/l NaCl; pH to 7.5 with 4 M NaOH;

then autoclaved at 121°C for 15 min) and 100 µl of the cell suspension plated onto low salt LB agar plates (10 g/l tryptone; 5 g/l yeast extract; 5 g/l NaCl; pH to 7.5 with 4 M NaOH; 15 g/l agar; then autoclaved at 121°C for 15 min) supplemented with 25 µg/ml zeocin (Invitrogen Ltd, Paisley, UK) and grown at 37°C overnight.

Several individual clones from the overnight growth plate were analysed by DNA sequencing using the alpha-factor sequencing primer (5'-TACTATTGCCAGCATTGCTGC-3'; SEQ ID NO: 92) and the reverse 3' AOX1 primer (5'-GCAAATGGCATTCTGACATCC-3'; SEQ ID NO: 93). A plasmid prep derived from a single clone containing the desired DNA sequence was made by inoculating 100 ml of low salt LB medium supplemented with 25 µg/ml zeocin and grown overnight at 37°C with shaking at 250 rpm, followed by purification of the plasmid DNA using a Qiagen Plasmid Midi Prep Kit (Qiagen).

The concentration and purity of the DNA was determined by measuring the absorbance at 280 nm and 260 nm. Between 10 and 50 µg of DNA was digested with PmeI, SacI or BstXI restriction enzymes and the DNA digestion confirmed by analysis on a 0.8% agarose gel. The digested DNA was purified using the Qiagen Qiaex II Kit (Qiagen) and the DNA eluted into 10 µl purified water per 20 µl of Qiaex II resin.

Competent yeast cells were made by streaking the wild type Pichia pastoris strain X33 onto a YPD agar plate (for 1 litre of media: 20 g peptone from meat type 1; 10 g yeast extract; 20 g agar dissolved in 900 ml water and sterilise by autoclaving at 121°C for 15 minutes; followed by the aseptic addition of 100ml of 20% destrose and the desired amount of zeocin once the media had cooled below 50°C) and grown at 30°C for 36-48 h until colonies appeared. A single colony was inoculated into 5 ml of YPD medium (as for YPD agar, without the addition of 20 g agar) and grown overnight in a 50 ml baffled flask at 30°C with shaking at 250 rpm. Five hundred millilitres of YPD medium in a 2l baffled flask was inoculated with 0.1-0.5 ml of the overnight culture and grown at 30°C with shaking at 250 rpm overnight to an OD600 of approximately 1.3-1.5. The culture was cooled on ice then the Pichia harvested by centrifugation at 1500 g for 5 min at 4°C. The supernatant was discarded and the pellet resuspended in 500 ml ice cold ultrapure water. The Pichia were recovered again by centrifugation at 1500 g for 5 min at 4°C. The pellet was resuspended and centrifuged for a further three times, the first time in

250 ml ice cold ultrapure water, then twice in 20 ml ice cold 1M sorbitol. The competent Pichia were kept on ice for up to 6 hours prior to use.

To transform the Pichia by electroporation, between 10 to 20 μg of linearised vector in 10 μl of water was mixed with 80 μl of competent Pichia in a precooled microfuge tube and incubate on ice for 5 min. The Pichia mixture was then transferred to a precooled 0.2 cm gap electroporation cuvette, and electroporated: 25 μF, resistance set to infinity, 0.54 kV in a Biorad Genepulser II with pulse controller module. Immediately 1 ml ice cold 1 M sorbitol was added to the electroporation cuvette and the electroporated Pichia transferred into a 15 ml polypropylene tube. This culture was incubated at 30°C without shaking for 2 hours to allow the cells to recover. The cells were then plated onto YPDS agar plates (YPD agar supplemented with 1 M sorbitol) and zeocin added to final concentrations of 100 μg/ml, 500 μg/ml, 1000 μg/ml, and 2000 μg/ml. The plates were incubated at 30°C in the dark for 2-10 days until colonies appeared. Several clones were picked from each plate and restreaked onto YPD plates supplemented with the same amount of zeocin as they were originally selected against. For small to medium scale shaking flask expression the Mut status of each X33 clone was not determined.

Individual restreaked colonies were used to inoculate 50 ml of BMGY medium (for 1 litre of medium: 20 g peptone from meat type 1; 10 g yeast extract were dissolved in 700 ml with water and sterilise by autoclaving at 121°C for 15 minutes; followed by the aseptic addition of 100 ml 1 M KPO4 pH 6.0; 100 ml 10% glycerol; and 100 ml 10xYNB (Yeast Nitrogen Base); 2 ml of 500x biotin) in a 250 ml flask and grown at 30°C with shaking at 250 rpm until an OD600 of 2 to 6 was attained. The Pichia was recovered by centrifugation at 1500 g for 5 min at room temperature. The resulting pellet was resuspended in 20 ml BMMH medium (as for BMGY medium except the 100 ml 10% glycerol was replaced with 100 ml of 5% methanol), and returned to a fresh 250 ml baffled flask and incubated at 30°C with shaking at 250 rpm for up to 5 days post induction. At 24 h intervals, 0.5 ml samples were recovered and assessed by analysis of the supernatant by SDS-PAGE.

Dimer dAb was purified from the culture supernatant using Streamline protein-A matrix (Amersham Biosciences). Following binding of the dimer dAb, the matrix was transferred to an

empty 20 ml chromatography column containing a frit and the supernatant allowed to flow through the column, retaining the matrix. The matrix was washed with 10 times the matrix volume of high salt buffer (10 mM Phosphate buffer, 2.7 mM KCl, 487 mM NaCl, pH 7.4). The bound protein was eluted with 0.1 M glycine pH 3 supplemented with 0.15 M NaCl and 0.8 ml fractions were collected which were immediately neutralised with the addition of 0.2 ml of 1 M Tris-HCl pH 8.0.

Simultaneous Antigen Binding of the Dual Specific dAb Dimer

To demonstrate that the dual specific dimeric dAb was functional and could bind both antigens simultaneously, an antigen binding ELISA was performed. Fifty microlitres per well of human serium albumin at 100 µg/ml in PBS was coated on to a Maxisorb ELISA plate (Nunc) overnight at 4°C. The plate was washed 3 times with 250 µl/well of PBS supplemented with 0.1% (v/v) Tween 20 (Sigma). The plate was then blocked for 1 h at room temperature with 200 µl per well of PBSM (PBS supplemented with 2% (w/v) low fat milk powder). The plate was washed as before, then 50 µl per well of doubling dilutions of the dimer dAb in PBSM were added and incubated for 1 hour at room temperature. The plate was washed as before then 50 µl per well of a 5 nM solution of biotinylated CD40L in PBSM added and incubated for 1 hour at room temperature. The plate was washed as before then 50 µl per well of streptavidin-HRP (Amersham Biosciences) diluted 1 in 4000 in PBSM was added and incubated for 1 hour at room temperature. The plate was washed 4 times with 250 µl/well of PBS supplemented with 0.1% (v/v) Tween 20, followed by two washes with 250 µl/well of PBS. The ELISA was developed by adding 50 µl per well of SureBlue 1-Component TMB MicroWell Peroxidase solution (KPL, Gaithersberg, MD) and incubated at room temperature for several minutes until the desired colour intensity was obtained. The reaction was stopped by the addition of 100 µl of 1 M hydrochloric acid and read at 450 nm (Figure 10).

Generation and Expression of a Dual Specific Fab (DOM7h-2:CK DOM-24:CH)

The produce a dual specific Fab the method used was essentially the same method as described for the dual specific dAb dimer (DOM7h-26—DOM-24) except that the DNA for the DOM7h-2 and DOM-24 dAbs were cloned into separate pPICz-alpha based vectors containing the human CK or human CH1 domain respectively. Each of the two vectors were designed to

enable the expression of a single polypeptide consisting of the CH1 or CK domain fused in frame to the 3' end of the appropriate dAb. To obtain expression of the complete Fab fragment, competent Pichia pastoris strain X33 were cotransformed with two linearised vectors. Purification was as described for the dimer Fab.

Simultaneous Antigen Binding of the Dual Specific Fab

The simultaneous antigen binding of the dual specific Fab was carried out using the same assay as described for the dual specific dAb dimmer above. The results are shown in Figure 11.

Inhibition of CD40 mediated CD54 upregulation disruption of CD40 / CD40L interaction in L3055 cells.

Monomeric dAb molecules were assayed for their ability to disrupt the binding of cellularly associated CD40L to CD40. This was determined by measuring the level inhibition of CD40 mediated CD54 upregulation on group 1 Burkitt lymphoma cell line L3055 by FACS. CD40 displayed on the L3055 cells was stimulated by CD40L expressed on the surface of fibroblasts (typically mouse L-cells) transfected with the CD40L gene as previously described by Garrone et al., 1995 (Garrone P., Neidhardt E.V., Garcia E., Galibert L., van Kooten C., Banchereau J. (1995) J Exp Med 182, 1265-1273.)

Samples containing the dAbs were preincubated with the L-cells cells for 1 hour prior to the addition of the L3055 cells. Both cell lines were co-cultured for 22 hours following which the L-cells were stained for FACS analysis, as described by Baker et al., 1998 (Baker, M.P., Eliopoulos, A.G., Young, L.S., Armitage, R.J., Gregory, C.D., Gordon, J. (1998) Blood, 92 (8), 2830-2843) and by Challa et al., 2002 (Challa, A., Eliopoulos, A.G., Holder, M.J., Burguete, A.S., Pound, J.D., Chamba, A., Grafton, G., Armitage, R.J., Gregory, C.D., Martinez-Valdez, H., Young, L. and Gordon, J. (2002) Blood, 99 (9), 3411-3418.

The FACS analysis of DOM-24 (monomeric form) at a concentration of 12μm showed inhibition.

FACS analysis of the Vk DOM-116 at a concentration of 9μM showed inhibition compared with a control blank dAb and the stimulated cell trace (Figure 13).

Effect of anti-CD40L therapy on primary immune response to KLH immunisation.

To show the effect of anti-CD40L therapy on the primary immune response to KLH immunization a similar study to that described by Gobburu, J.V.S. et al., 1998 can be carried out (Gobburu, J.V.S., Tenhoor, C., Rogge, M.C., Frazier, D.E., Thomas, D., Benjamin, C., Hess, D.M., & Jusko, W.J. (1998) JPET, 286, 925-930.

A study example could be as follows: Cynomolgus monkeys are injected with an anti-CD40L antibody or dAb format at a dosing of 10mg/kg at time 0h, 168h and 336h. At 24h the animals receive a single intradermal injection of 100 µg highly purified keyhole limpet hemocyanin (KLH). The serum is tested for a KLH response prior to the start and following completion of the study. The animals are allowed to recover for a suitable time to ensure that the dAb molecule has cleared the circulation, then injected with KLH (without dAb) to determine if the animal can raise an immune response against KLH. Alternative antigens can be used for the study if an earlier response to KLH is detected prior to the start of the study.

The immune response to KLH will be measured by a suitable ELISA, RIA or antibody titer assay. It is anticipated that the results would show that that the PEglyated monomeric DOM-24 would suppress the animals immune response to KLH. This would be shown in the reduction of the antibody titer compared to a positive control. The results could be normalized such that the positive control was deemed to be 100 and the reduction in immune response after the administration of the dAbs would be in the range of 10 to 90% ie 90 to 10 units.

Sequence of the DOM7h-26—DOM-24

LEKREVQLLESGGGLVQPGGSLRLSCTASGFTFDEYNMSWVRQAPGKGLEWVSTILPH
GDRTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKQDPLYRFDYWGQG
TLVTVSSAAAEVQLLESGGGLVQPGGSLRLSCAASGFTFSNYQMAWVRQAPGKGLEW
VSSITSEGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPGKNFDY
WGQGTLVTVSS (SEQ ID NO: 88)

Sequence of the DOM7h-26

DIQMTQSPSSLSASVGDRVTITCRASQKIATYLNWYQQKPGKAPKLLIYRSSSLQSAVPS
RFSGSGSGTVFTLTISSLQPEDFATYYCQQTYAVPPTFGQGTKVEIKR (SEQ ID NO: 89)

All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

Annex 1; polypeptides which enhance half-life *in vivo*.

Alpha-1 Glycoprotein (Orosomucoid) (AAG)

Alpha-1 Antichyromotrypsin (ACT)

Alpha-1 Antitrypsin (AAT)

Alpha-1 Microglobulin (Protein HC) (AIM)

Alpha-2 Macroglobulin (A2M)

Antithrombin III (AT III)

Apolipoprotein A-1 (Apo A-1)

Apoliprotein B (Apo B)

Beta-2-microglobulin (B2M)

Ceruloplasmin (Cp)

Complement Component (C3)

Complement Component (C4)

C1 Esterase Inhibitor (C1 INH)

C-Reactive Protein (CRP)

Cystatin C (Cys C)

Ferritin (FER)

Fibrinogen (FIB)

Fibronectin (FN)

Haptoglobin (Hp)

Hemopexin (HPX)

Immunoglobulin A (IgA)

Immunoglobulin D (IgD)

Immunoglobulin E (IgE)

Immunoglobulin G (IgG)

Immunoglobulin M (IgM)

Immunoglobulin Light Chains (kapa/lambda)

Lipoprotein(a) [Lp(a)]

Mannose-bindign protein (MBP)

Myoglobin (Myo)

Neonatal Fc (FcRn)

Plasminogen (PSM)

Prealbumin (Transthyretin) (PAL)

Retinol-binding protein (RBP)

Rheomatoid Factor (RF)

Serum Amyloid A (SAA)

Soluble Tranferrin Receptor (sTfR)

Transferrin (Tf)

Annex 2

Pairing	Therapeutic relevant references.
TNF ALPHA/TGF-β	• TGF-b and TNF when injected into the ankle joint of collagen induced arthritis model significantly enhanced joint inflammation. In non-collagen challenged mice there was no effect.
TNF ALPHA/IL-1	• TNF and IL-1 synergize in the pathology of uveitis. • TNF and IL-1 synergize in the pathology of malaria (hypoglycaemia, NO). • TNF and IL-1 synergize in the induction of polymorphonuclear (PMN) cells migration in inflammation. • IL-1 and TNF synergize to induce PMN infiltration into the peritoneum. • IL-1 and TNF synergize to induce the secretion of IL-1 by endothelial cells. Important in inflammation. • IL-1 or TNF alone induced some cellular infiltration into knee synovium. IL-1 induced PMNs, TNF – monocytes. Together they induced a more severe infiltration due to increased PMNs. • Circulating myocardial depressant substance (present in sepsis) is low levels of IL-1 and TNFacting synergistically.
TNF ALPHA/IL-2	• Most relating to synergisitic activation of killer T-cells.
TNF ALPHA/IL-3	• Synergy of interleukin 3 and tumor necrosis factor alpha in stimulating clonal growth of acute myelogenous leukemia blasts is the result of induction of secondary hematopoietic cytokines by tumor necrosis factor alpha. • Cancer Res. 1992 Apr 15;52(8):2197-201.
TNF ALPHA/IL-4	• IL-4 and TNF synergize to induce VCAM expression on endothelial cells. Implied to have a role in asthma. Same for synovium – implicated in RA. • TNF and IL-4 synergize to induce IL-6 expression in

	keratinocytes. • Sustained elevated levels of VCAM-1 in cultured fibroblast-like synoviocytes can be achieved by TNF-alpha in combination with either IL-4 or IL-13 through increased mRNA stability. *Am J Pathol.* 1999 Apr;154(4):1149-58
TNF ALPHA/IL-5	• Relationship between the tumor necrosis factor system and the serum interleukin-4, interleukin-5, interleukin-8, eosinophil cationic protein, and immunoglobulin E levels in the bronchial hyperreactivity of adults and their children. *Allergy Asthma Proc.* 2003 Mar-Apr;24(2):111-8.
TNF ALPHA/IL-6	• TNF and IL-6 are potent growth factors for OH-2, a novel human myeloma cell line. *Eur J Haematol.* 1994 Jul;53(1):31-7.
TNF ALPHA/IL-8	• TNF and IL-8 synergized with PMNs to activate platelets. Implicated in Acute Respiratory Distress Syndrome. • See IL-5/TNF (asthma). Synergism between interleukin-8 and tumor necrosis factor-alpha for neutrophil-mediated platelet activation. *Eur Cytokine Netw.* 1994 Sep-Oct;**5**(5):455-60. (adult respiratory distress syndrome (ARDS))
TNF ALPHA/IL-9	
TNF ALPHA/IL-10	• IL-10 induces and synergizes with TNF in the induction of HIV expression in chronically infected T-cells.
TNF ALPHA/IL-11	• Cytokines synergistically induce osteoclast differentiation: support by immortalized or normal calvarial cells. *Am J Physiol Cell Physiol.* 2002 Sep;**283**(3):C679-87. (Bone loss)
TNF ALPHA/IL-12	
TNF ALPHA/IL-13	• Sustained elevated levels of VCAM-1 in cultured fibroblast-like synoviocytes can be achieved by TNF-alpha in combination with either IL-4 or IL-13 through increased mRNA stability. Am J Pathol. 1999 Apr;154(4):1149-58. • Interleukin-13 and tumour necrosis factor-alpha synergistically induce eotaxin production in human nasal fibroblasts. Clin Exp Allergy. 2000 Mar;30(3):348-55.

	• Interleukin-13 and tumour necrosis factor-alpha synergistically induce eotaxin production in human nasal fibroblasts. *Clin Exp Allergy.* 2000 Mar;**30**(3):348-55 (allergic inflammation) • Implications of serum TNF-beta and IL-13 in the treatment response of childhood nephrotic syndrome. *Cytokine.* 2003 Feb 7;**21**(3):155-9.
TNF ALPHA/IL-14	• Effects of inhaled tumour necrosis factor alpha in subjects with mild asthma. *Thorax.* 2002 Sep;**57**(9):774-8.
TNF ALPHA/IL-15	• Effects of inhaled tumour necrosis factor alpha in subjects with mild asthma. *Thorax.* 2002 Sep;**57**(9):774-8.
TNF ALPHA/IL-16	• Tumor necrosis factor-alpha-induced synthesis of interleukin-16 in airway epithelial cells: priming for serotonin stimulation. *Am J Respir Cell Mol Biol.* 2003 Mar;**28**(3):354-62. (airway inflammation) • Correlation of circulating interleukin 16 with proinflammatory cytokines in patients with rheumatoid arthritis. *Rheumatology* (Oxford). 2001 Apr;**40**(4):474-5. No abstract available. • Interleukin 16 is up-regulated in Crohn's disease and participates in TNBS colitis in mice. *Gastroenterology.* 2000 Oct;**119**(4):972-82.
TNF ALPHA/IL-17	• Inhibition of interleukin-17 prevents the development of arthritis in vaccinated mice challenged with Borrelia burgdorferi. *Infect Immun.* 2003 Jun;**71**(6):3437-42. • Interleukin 17 synergises with tumour necrosis factor alpha to induce cartilage destruction *in vitro*. *Ann Rheum Dis.* 2002 Oct;**61**(10):870-6. • A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-alpha. *Eur Respir J.* 2003 Mar;**21**(3):387-93. (Airway inflammation) • Abstract Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. *Arthritis Rheum.* 2001 Sep;**44**(9):2078-83.
TNF	• Association of interleukin-18 expression with enhanced levels of both interleukin-1beta and tumor necrosis factor alpha in

ALPHA/IL-18	knee synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum.* 2003 Feb;**48**(2):339-47. • Abstract Elevated levels of interleukin-18 and tumor necrosis factor-alpha in serum of patients with type 2 diabetes mellitus: relationship with diabetic nephropathy. *Metabolism.* 2003 May;**52**(5):605-8.
TNF ALPHA/IL-19	• Abstract IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. *J Immunol.* 2002 Oct 15;**169**(8):4288-97.
TNF ALPHA/IL-20	• Abstract Cytokines: IL-20 - a new effector in skin inflammation. *Curr Biol.* 2001 Jul 10;**11**(13):R531-4
TNF ALPHA/Complement	• Inflammation and coagulation: implications for the septic patient. *Clin Infect Dis.* 2003 May 15;**36**(10):1259-65. Epub 2003 May 08. Review.
TNF ALPHA/IFN-γ	• MHC induction in the brain. • Synergize in anti-viral response/IFN–β induction. • Neutrophil activation/ respiratory burst. • Endothelial cell activation • Toxicities noted when patients treated with TNF/IFN-γ as anti-viral therapy • Fractalkine expression by human astrocytes. • Many papers on inflammatory responses – i.e. LPS, also macrophage activation. • Anti-TNF and anti-IFN-γ synergize to protect mice from lethal endotoxemia.

TGF-β/IL-1	• Prostaglndin synthesis by osteoblasts • IL-6 production by intestinal epithelial cells (inflammation model) • Stimulates IL-11 and IL-6 in lung fibroblasts (inflammation model)

	• IL-6 and IL-8 production in the retina
TGF-β/IL-6	• Chondrocarcoma proliferation
IL-1/IL-2	• B-cell activation • LAK cell activation • T-cell activation • IL-1 synergy with IL-2 in the generation of lymphokine activated killer cells is mediated by TNF-alpha and beta (lymphotoxin). *Cytokine.* 1992 Nov;4(6):479-87.
IL-1/IL-3	
IL-1/IL-4	• B-cell activation • IL-4 induces IL-1 expression in endothelial cell activation.
IL-1/IL-5	
IL-1/IL-6	• B cell activation • T cell activation (can replace accessory cells) • IL-1 induces IL-6 expression • C3 and serum amyloid expression (acute phase response) • HIV expression • Cartilage collagen breakdown.
IL-1/IL-7	• IL-7 is requisite for IL-1-induced thymocyte proliferation. Involvement of IL-7 in the synergistic effects of granulocyte-macrophage colony-stimulating factor or tumor necrosis factor with IL-1. *J Immunol.* 1992 Jan 1;148(1):99-105.
IL-1/IL-8	
IL-1/IL-10	
IL-1/IL-11	• Cytokines synergistically induce osteoclast differentiation: support by immortalized or normal calvarial cells. *Am J Physiol Cell Physiol.* 2002 Sep;**283**(3):C679-87. (Bone loss)

IL-1/IL-16	• Correlation of circulating interleukin 16 with proinflammatory cytokines in patients with rheumatoid arthritis. *Rheumatology* (Oxford). 2001 Apr;**40**(4):474-5. No abstract available.
IL-1/IL-17	• Inhibition of interleukin-17 prevents the development of arthritis in vaccinated mice challenged with Borrelia burgdorferi. *Infect Immun.* 2003 Jun;**71**(6):3437-42. • Contribution of interleukin 17 to human cartilage degradation and synovial inflammation in osteoarthritis. *Osteoarthritis Cartilage.* 2002 Oct;**10**(10):799-807. • Abstract Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. *Arthritis Rheum.* 2001 Sep;**44**(9):2078-83.
IL-1/IL-18	• Association of interleukin-18 expression with enhanced levels of both interleukin-1beta and tumor necrosis factor alpha in knee synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum.* 2003 Feb;**48**(2):339-47.
IL-1/IFN-g	
IL-2/IL-3	• T-cell proliferation • B cell proliferation
IL-2/IL-4	• B-cell proliferation • T-cell proliferation • (selectively inducing activation of CD8 and NK lymphocytes)IL-2R beta agonist P1-30 acts in synergy with IL-2, IL-4, IL-9, and IL-15: biological and molecular effects. *J Immunol.* 2000 Oct 15;**165**(8): 4312-8.
IL-2/IL-5	• B-cell proliferation/ Ig secretion • IL-5 induces IL-2 receptors on B-cells
IL-2/IL-6	• Development of cytotoxic T-cells
IL-2/IL-7	

IL-2/IL-9	• See IL-2/IL-4 (NK-cells)
IL-2/IL-10	• B-cell activation
IL-2/IL-12	• IL-12 synergizes with IL-2 to induce lymphokine-activated cytotoxicity and perforin and granzyme gene expression in fresh human NK cells. *Cell Immunol.* 1995 Oct 1;**165**(1):33-43. (T-cell activation)
IL-2/IL-15	• See IL-2/IL-4 (NK cells) • (T cell activation and proliferation) IL-15 and IL-2: a matter of life and death for T cells *in vivo*. *Nat Med.* 2001 Jan;7(1):114-8.
IL-2/IL-16	• Synergistic activation of CD4+ T cells by IL-16 and IL-2. *J Immunol.* 1998 Mar 1;160(5):2115-20.
IL-2/IL-17	• Evidence for the early involvement of interleukin 17 in human and experimental renal allograft rejection. *J Pathol.* 2002 Jul;197(3):322-32.
IL-2/IL-18	• Interleukin 18 (IL-18) in synergy with IL-2 induces lethal lung injury in mice: a potential role for cytokines, chemokines, and natural killer cells in the pathogenesis of interstitial pneumonia. *Blood.* 2002 Feb 15;**99**(4):1289-98.
IL-2/TGF-β	• Control of CD4 effector fate: transforming growth factor beta 1 and interleukin 2 synergize to prevent apoptosis and promote effector expansion. *J Exp Med.* 1995 Sep 1;**182**(3):699-709.
IL-2/IFN-γ	• Ig secretion by B-cells • IL-2 induces IFN-γ expression by T-cells
IL-2/IFN-α/β	• None
IL-3/IL-4	• Synergize in mast cell growth • Synergistic effects of IL-4 and either GM-CSF or IL-3 on the induction of CD23 expression by human monocytes: regulatory effects of IFN-alpha and IFN-gamma. *Cytokine.* 1994 Jul;**6**(4):407-13.
IL-3/IL-5	

IL-3/IL-6	
IL-3/IFN-γ	• IL-4 and IFN-gamma synergistically increase total polymeric IgA receptor levels in human intestinal epithelial cells. Role of protein tyrosine kinases. *J Immunol.* 1996 Jun 15;**156**(12):4807-14.
IL-3/GM-CSF	• Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor alpha-chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor alpha expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor alpha expression. *J Immunol.* 2003 Jun 1;**170**(11):5359-66. (allergic inflammation)
IL-4/IL-2	• IL-4 synergistically enhances both IL-2- and IL-12-induced IFN-{gamma} expression in murine NK cells. *Blood.* 2003 Mar 13 [Epub ahead of print]
IL-4/IL-5	• Enhanced mast cell histamine etc. secretion in response to IgE • A Th2-like cytokine response is involved in bullous pemphigoid. the role of IL-4 and IL-5 in the pathogenesis of the disease. *Int J Immunopathol Pharmacol.* 1999 May-Aug;**12**(2):55-61.
IL-4/IL-6	
IL-4/IL-10	
IL-4/IL-11	• Synergistic interactions between interleukin-11 and interleukin-4 in support of proliferation of primitive hematopoietic progenitors of mice. *Blood.* 1991 Sep 15;**78**(6):1448-51.
IL-4/IL-12	• Synergistic effects of IL-4 and IL-18 on IL-12-dependent IFN-gamma production by dendritic cells. *J Immunol.* 2000 Jan 1;164(1):64-71. (increase Th1/Th2 differentiation) • IL-4 synergistically enhances both IL-2- and IL-12-induced IFN-{gamma} expression in murine NK cells. *Blood.* 2003 Mar 13 [Epub ahead of print]
IL-4/IL-13	• Abstract Interleukin-4 and interleukin-13 signaling connections maps. *Science.* 2003 Jun 6;**300**(5625):1527-8. (allergy, asthma) • Inhibition of the IL-4/IL-13 receptor system prevents allergic

	sensitization without affecting established allergy in a mouse model for allergic asthma. *J Allergy Clin Immunol.* 2003 Jun;**111**(6):1361-1369.
IL-4/IL-16	• (asthma) Interleukin (IL)-4/IL-9 and exogenous IL-16 induce IL-16 production by BEAS-2B cells, a bronchial epithelial cell line. *Cell Immunol.* 2001 Feb 1;**207**(2):75-80
IL-4/IL-17	• Interleukin (IL)-4 and IL-17 synergistically stimulate IL-6 secretion in human colonic myofibroblasts. *Int J Mol Med.* 2002 Nov;**10**(5):631-4. (Gut inflammation)
IL-4/IL-24	• IL-24 is expressed by rat and human macrophages. *Immunobiology.* 2002 Jul;**205**(3):321-34.
IL-4/IL-25	• Abstract New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol.* 2002 Jul 1;**169**(1):443-53. (allergic inflammation) • Abstract Mast cells produce interleukin-25 upon Fcepsilon RI-mediated activation. *Blood.* 2003 May 1;**101**(9):3594-6. Epub 2003 Jan 02. (allergic inflammation)
IL-4/IFN-γ	• Abstract Interleukin 4 induces interleukin 6 production by endothelial cells: synergy with interferon-gamma. *Eur J Immunol.* 1991 Jan;**21**(1):97-101.
IL-4/SCF	• Regulation of human intestinal mast cells by stem cell factor and IL-4. *Immunol Rev.* 2001 Feb;**179**:57-60. Review.
IL-5/IL-3	• Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor alpha-chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor alpha expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor alpha expression. *J Immunol.* 2003 Jun 1;**170**(11):5359-66. (Allergic inflammation see abstract)
IL-5/IL-6	
IL-5/IL-13	• Inhibition of allergic airways inflammation and airway hyperresponsiveness in mice by dexamethasone: role of eosinophils, IL-5, eotaxin, and IL-13. *J Allergy Clin Immunol.* 2003 May;**111**(5):1049-61.
IL-5/IL-17	• Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma.

	Am J Respir Cell Mol Biol. 2003 Jan;**28**(1):42-50.
IL-5/IL-25	• Abstract New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol.* 2002 Jul 1;**169**(1):443-53. (allergic inflammation) • Abstract Mast cells produce interleukin-25 upon Fcepsilon RI-mediated activation. *Blood.* 2003 May 1;**101**(9):3594-6. Epub 2003 Jan 02. (allergic inflammation)
IL-5/IFN-γ	
IL-5/GM-CSF	• Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor alpha-chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor alpha expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor alpha expression. *J Immunol.* 2003 Jun 1;**170**(11):5359-66. (Allergic inflammation)
IL-6/IL-10	
IL-6/IL-11	
IL-6/IL-16	• Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. *Immunology.* 2000 May;**100**(1):63-9.
IL-6/IL-17	• Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem.* 2003 May 9;**278**(19):17036-43. Epub 2003 Mar 06. (airway inflammation, asthma)
IL-6/IL-19	• Abstract IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. *J Immunol.* 2002 Oct 15;**169**(8):4288-97.
IL-6/IFN-g	
IL-7/IL-2	• Interleukin 7 worsens graft-versus-host disease. *Blood.* 2002 Oct 1;**100**(7):2642-9.
IL-7/IL-12	• Synergistic effects of IL-7 and IL-12 on human T cell activation. *J Immunol.* 1995 May 15;**154**(10):5093-102.
IL-7/IL-15	• Interleukin-7 and interleukin-15 regulate the expression of the bcl-2 and c-myb genes in cutaneous T-cell lymphoma cells.

	Blood. 2001 Nov 1;**98**(9):2778-83. (growth factor)
IL-8/IL-11	• Abnormal production of interleukin (IL)-11 and IL-8 in polycythaemia vera. *Cytokine.* 2002 Nov 21;**20**(4):178-83.
IL-8/IL-17	• The Role of IL-17 in Joint Destruction. *Drug News Perspect.* 2002 Jan;**15**(1):17-23. (arthritis) • Abstract Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. *Am J Respir Cell Mol Biol.* 2002 Jun;**26**(6):748-53. (airway inflammation)
IL-8/GSF	• Interleukin-8: an autocrine/paracrine growth factor for human hematopoietic progenitors acting in synergy with colony stimulating factor-1 to promote monocyte-macrophage growth and differentiation. *Exp Hematol.* 1999 Jan;**27**(1):28-36.
IL-8/VGEF	• Intracavitary VEGF, bFGF, IL-8, IL-12 levels in primary and recurrent malignant glioma. *J Neurooncol.* 2003 May;**62**(3):297-303.
IL-9/IL-4	• Anti-interleukin-9 antibody treatment inhibits airway inflammation and hyperreactivity in mouse asthma model. *Am J Respir Crit Care Med.* 2002 Aug 1;**166**(3):409-16.
IL-9/IL-5	• Pulmonary overexpression of IL-9 induces Th2 cytokine expression, leading to immune pathology. *J Clin Invest.* 2002 Jan;**109**(1):29-39. • Th2 cytokines and asthma. Interleukin-9 as a therapeutic target for asthma. *Respir Res.* 2001;**2**(2):80-4. Epub 2001 Feb 15. Review. • Abstract Interleukin-9 enhances interleukin-5 receptor expression, differentiation, and survival of human eosinophils. *Blood.* 2000 Sep 15;**96**(6):2163-71 (asthma)
IL-9/IL-13	• Anti-interleukin-9 antibody treatment inhibits airway inflammation and hyperreactivity in mouse asthma model. *Am J Respir Crit Care Med.* 2002 Aug 1;**166**(3):409-16. • Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med.* 2002 Aug;**8**(8):885-9.

IL-9/IL-16	• See IL-4/IL-16
IL-10/IL-2	• The interplay of interleukin-10 (IL-10) and interleukin-2 (IL-2) in humoral immune responses: IL-10 synergizes with IL-2 to enhance responses of human B lymphocytes in a mechanism which is different from upregulation of CD25 expression. *Cell Immunol.* 1994 Sep;**157**(2):478-88.
IL-10/IL-12	
IL-10/TGF-β	• IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol.* 2003 May;**33**(5):1205-14.
IL-10/IFN-γ	
IL-11/IL-6	• Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. *Bone.* 2003 Jan;**32**(1):1-7. (bone resorption in inflammation)
IL-11/IL-17	• Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol.* 2003 Apr;**111**(4):875-81. (allergic dermatitis) • IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. *J Immunol.* 2003 Mar 1;**170**(5):2655-62.
IL-11/TGF-β	• Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol.* 2003 Apr;**111**(4):875-81. (allergic dermatitis)
IL-12/IL-13	• Relationship of Interleukin-12 and Interleukin-13 imbalance with class-specific rheumatoid factors and anticardiolipin antibodies in systemic lupus erythematosus. *Clin Rheumatol.* 2003 May;**22**(2):107-11.
IL-12/IL-17	• Upregulation of interleukin-12 and -17 in active inflammatory bowel disease. *Scand J Gastroenterol.* 2003 Feb;**38**(2):180-5.
IL-12/IL-18	• Synergistic proliferation and activation of natural killer cells by interleukin 12 and interleukin 18. *Cytokine.* 1999 Nov;**11**(11):822-30. • Inflammatory Liver Steatosis Caused by IL-12 and IL-18. *J*

	Interferon Cytokine Res. 2003 Mar;**23**(3):155-62.
IL-12/IL-23	• nterleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature.* 2003 Feb 13;**421**(6924):744-8. • Abstract A unique role for IL-23 in promoting cellular immunity. *J,Leukoc Biol.* 2003 Jan;**73**(1):49-56. Review.
IL-12/IL-27	• Abstract IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity.* 2002 Jun;**16**(6):779-90.
IL-12/IFN-γ	• IL-12 induces IFN-γ expression by B and T-cells as part of immune stimulation.
IL-13/IL-5	• See IL-5/IL-13
IL-13/IL-25	• Abstract New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol.* 2002 Jul 1;**169**(1):443-53. (allergic inflammation) • Abstract Mast cells produce interleukin-25 upon Fcepsilon RI-mediated activation. *Blood.* 2003 May 1;**101**(9):3594-6. Epub 2003 Jan 02. (allergic inflammation)
IL-15/IL-13	• Differential expression of interleukins (IL)-13 and IL-15 in ectopic and eutopic endometrium of women with endometriosis and normal fertile women. *Am J Reprod Immunol.* 2003 Feb;**49**(2):75-83.
IL-15/IL-16	• IL-15 and IL-16 overexpression in cutaneous T-cell lymphomas: stage-dependent increase in mycosis fungoides progression. *Exp Dermatol.* 2000 Aug;**9**(4):248-51.
IL-15/IL-17	• Abstract IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J Immunol.* 2003 Feb 15;**170**(4):2106-12. (airway inflammation)
IL-15/IL-21	• IL-21 in Synergy with IL-15 or IL-18 Enhances IFN-gamma Production in Human NK and T Cells. *J Immunol.* 2003 Jun 1;**170**(11):5464-9.
IL-17/IL-23	• Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J Biol*

	Chem. 2003 Jan 17;**278**(3):1910-4. Epub 2002 Nov 03
IL-17/TGF-β	• Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol.* 2003 Apr;**111**(4):875-81. (allergic dermatitis)
IL-18/IL-12	• Synergistic proliferation and activation of natural killer cells by interleukin 12 and interleukin 18. *Cytokine.* 1999 Nov;**11**(11):822-30. • Abstract Inhibition of in vitro immunoglobulin production by IL-12 in murine chronic graft-vs.-host disease: synergism with IL-18. *Eur J Immunol.* 1998 Jun;**28**(6):2017-24.
IL-18/IL-21	• IL-21 in Synergy with IL-15 or IL-18 Enhances IFN-gamma Production in Human NK and T Cells. *J Immunol.* 2003 Jun 1;**170**(11):5464-9.
IL-18/TGF-β	• Interleukin 18 and transforming growth factor beta1 in the serum of patients with Graves' ophthalmopathy treated with corticosteroids. *Int Immunopharmacol.* 2003 Apr;**3**(4):549-52.
IL-18/IFN-γ	
Anti-TNF ALPHA/anti-CD4	• Synergistic therapeutic effect in DBA/1 arthritic mice.

Annex 3: Oncology combinations

Target	Disease	Pair with
CD89*	Use as cytotoxic cell recruiter	all
CD19	B cell lymphomas	HLA-DR CD5
HLA-DR	B cell lymphomas	CD89 CD19 CD5
CD38	Multiple myeloma	CD138 CD56 HLA-DR
CD138	Multiple myeloma	CD38 CD56 HLA-DR
CD138	Lung cancer	CD56 CEA
CD33	Acute myelod lymphoma	CD34 HLA-DR
CD56	Lung cancer	CD138 CEA
CEA	Pan carcinoma	MET receptor
VEGF	Pan carcinoma	MET receptor

VEGF receptor	Pan carcinoma	MET receptor
IL-13	Asthma/pulmonary inflammation	IL-4 IL-5 Eotaxin(s) MDC TARC TNFα IL-9 EGFR CD40L IL-25 MCP-1 TGFβ
IL-4	Asthma	IL-13 IL-5 Eotaxin(s) MDC TARC TNFα IL-9 EGFR CD40L IL-25 MCP-1

		TGFβ
Eotaxin	Asthma	IL-5 Eotaxin-2 Eotaxin-3
EGFR	cancer	HER2/neu HER3 HER4
HER2	cancer	HER3 HER4
TNFR1	RA/Crohn's disease	IL-1R IL-6R IL-18R
TNFα	RA/Crohn's disease	IL-1α/β IL-6 IL-18 ICAM-1 IL-15 IL-17
IL-1R	RA/Crohn's disease	IL-6R IL-18R
IL-18R	RA/Crohn's disease	IL-6R

CLAIMS

1. A method of antagonizing an activity of CD40 or CD40L in an individual, the method comprising administering an antibody polypeptide that is monovalent for binding to CD40L (gp39) to said individual wherein said polypeptide antagonizes an activity of CD40 or CD40L or both.

2. A method of antagonizing the binding of CD40 to CD40L in an individual, the method comprising administering an antibody polypeptide that is monovalent for binding to CD40L (gp39) to said individual, wherein said polypeptide antagonizes the binding of CD40 to CD40L in said individual.

3. A method of treating or preventing a symptom of autoimmune disease in an individual, the method comprising administering a monovalent anti-CD40L antibody polypeptide to said individual in an amount effective to treat or prevent a symptom of autoimmune disease.

4. A method of treating or preventing an autoimmune disease in an individual, the method comprising administering a monovalent anti-CD40L antibody polypeptide to said individual in an amount effective to treat or prevent a symptom of autoimmune disease.

5. The method of claim 3, wherein said autoimmune disease is a disease selected from the group consisting of systemic lupus erythematosis, multiple sclerosis, rheumatoid arthritis, Diabetes, allograft rejection, xenograft transplant rejection, and graft vs. host disease.

6. The method of claim 3, wherein said antibody polypeptide inhibits the binding of CD40L to CD40.

7. The method of claim 3, wherein said antibody polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 7-82, SEQ ID NOs 246-360, and DOM8-24.

8. The method of claim 3, wherein said antibody polypeptide has an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs 7-82 and 246-360, which antibody polypeptide specifically and monovalently binds CD40L.

9. The method of claim 6, wherein said antibody polypeptide inhibits binding of CD40L to CD40 with an IC_{50} in the range of 20 pM to 1.5 μM, inclusive.

10. The method of claim 3, wherein said antibody polypeptide is PEG-linked.

11. The method of claim 10, wherein said PEG-linked antibody polypeptide has a hydrodynamic size of at least 24 kD.

12. The method of claim 10 wherein said PEG is linked to said antibody at a cysteine or lysine residue.

13. The method of claim 10 wherein the total PEG size is from 20 to 60 kD, inclusive.

14. The method of claim 10 wherein said PEG-linked antibody polypeptide has a hydrodynamic size of at least 200 kD.

15. The method of claim 10 wherein said PEG-linked antibody polypeptide has an increased in vivo half-life relative to the same polypeptide composition lacking linked polyethylene glycol.

16. The method of claim 15 wherein the tα-half life of the polypeptide composition is increased by 10% or more.

17. The method of claim 15 wherein the tα-half life of the polypeptide composition is in the range of 0.25 minutes to 12 hours.

18. The method of claim 15 wherein the tβ-half life of the polypeptide composition is increased by 10% or more.

19. The method of claim 15 wherein the tβ-half life is in the range of 12 to 48 hours.

20. The method of claim 1 or 2 wherein said antibody polypeptide is a camelid or nurse shark V_{HH} domain.

21. An antibody polypeptide that is monovalent for binding to CD40L (gp39), wherein binding of said antibody polypeptide to CD40L does not substantially agonize CD40 and/or CD40L activity.

22. The antibody of claim 21 wherein said antibody polypeptide inhibits the binding of CD40 to CD40L, and does not substantially agonize signaling by CD40.

23. The antibody polypeptide of claim 21 wherein binding of said antibody polypeptide to CD40L does not substantially induce JNK phosphorylation in Jurkat T-cells.

24. The antibody polypeptide of claim 21 wherein binding of said antibody polypeptide to CD40L does not substantially induce IFN-γ secretion by Jurkat T-cells co-stimulated with anti-CD3 antibody.

25. The antibody polypeptide of claim 21 wherein the presence of said antibody polypeptide in a standard platelet aggregation assay does not result in aggregation of more than 25% over the aggregation observed in a negative control assay.

26. The antibody polypeptide of claim 21 in which said antibody polypeptide is selected from the group consisting of an antibody single variable domain polypeptide, dAb, FAb, an scFv, an Fv, or a disulfide-bonded Fv.

27. An antibody polypeptide that is monovalent for binding to CD40L (gp39), wherein the presence of said antibody polypeptide in a standard platelet aggregation assay does not result in aggregation of more than 25% over the aggregation observed in a negative control assay.

28. A PEG-linked antibody polypeptide of claim 21.

29. The PEG-linked antibody polypeptide of claim 28 which has a hydrodynamic size of at least 24 kD.

30. The PEG-linked antibody polypeptide of claim 28 wherein said PEG is linked to said antibody at a cysteine or lysine residue.

31. The PEG-linked antibody polypeptide of claim 28 wherein the total PEG size is from 20 to 60 kD, inclusive.

32. The PEG-linked antibody polypeptide of claim 28 which has a hydrodynamic size of at least 200 kD.

33. The PEG-linked antibody polypeptide of claim 28 which has an increased in vivo half-life relative to the same polypeptide composition lacking linked polyethylene glycol.

34. The PEG-linked antibody polypeptide of claim 33 wherein the tα-half life of the polypeptide composition is increased by 10% or more.

35. The PEG-linked antibody polypeptide of claim 33 wherein the tα-half life of the polypeptide composition is in the range of 0.25 minutes to 12 hours.

36. The PEG-linked antibody polypeptide of claim 33 wherein the tβ-half life of the polypeptide composition is increased by 10% or more.

37. The PEG-linked antibody polypeptide of claim 33 wherein the tβ-half life is in the range of 12 to 48 hours.

38. The antibody of claim 21 which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 7-82 and 246-360.

39. The antibody polypeptide of claim 21 which is free of an Fc domain.

40. The antibody polypeptide of claim 39 wherein the presence of said antibody polypeptide in a standard platelet aggregation assay does not result in aggregation of more than 25% over the aggregation observed in a negative control assay.

41. The antibody of claim 21 which inhibits binding of CD40L to CD40 with an IC_{50} in the range of 20 pM to 1.5 μM, inclusive.

42. A composition comprising the antibody polypeptide of claim 21 and a second antibody polypeptide which binds a ligand other than CD40L, wherein said antibody polypeptide which binds a ligand other than CD40L binds a ligand selected from the group consisting of HSA, TNFα, IL-1, IL-2, IL-4, IL-6, IL-8, IL-12, IL-18, IFN-γ, CD2, CD4, CD8, LFA1, LFA3, VLA4, CD80, B7-1, CD28, CD86, B7-2, CTLA-4 CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, CD3, CD70, Inducible costimulatory molecule ligand (ICOSL), OX40L, HVEM (Herpes Virus Entry Mediator), LIGHT.

43. An antibody polypeptide which has an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs 7-82, SEQ ID NOs 246-360, and DOM8-24, which antibody polypeptide specifically and monovalently binds CD40L.

44. An antigen-binding polypeptide, said polypeptide comprising a immunoglobulin single variable domain which specifically and monovalently binds CD40L, wherein said polypeptide substantially inhibits the binding of CD40L to CD40.

45. The antigen-binding polypeptide of claim 44 which inhibits the binding of CD40 to CD40L with an IC_{50} in the range of 20 pM to 1.5 μM, inclusive.

46. The antigen-binding polypeptide of claim 44 wherein binding of said polypeptide to CD40L does not substantially agonize CD40 and/or CD40L activity.

47. The antigen-binding polypeptide of claim 44 wherein binding of said polypeptide to CD40L does not substantially induce JNK phosphorylation in Jurkat T-cells.

45. The antigen-binding polypeptide of claim 44 wherein binding of said polypeptide to CD40L does not substantially induce IFN-γ secretion by Jurkat T-cells co-stimulated with anti-CD3 antibody.

46. The antigen-binding polypeptide of claim 44 wherein the presence of said antibody polypeptide in a standard platelet aggregation assay does not result in aggregation of more than 25% over the aggregation observed in a negative control assay.

47. The antigen-binding polypeptide of claim 44 in which said antigen-binding polypeptide is selected from the group consisting of an antibody single variable domain polypeptide, dAb, FAb, an scFv, an Fv, or a disulfide-bonded Fv.

48. A polypeptide according to claim 21 or 44, wherein the anti-CD40L variable domain or dAb comprises one or more framework regions comprising an amino acid sequence that is the same as the amino acid sequence of a corresponding framework region encoded by a human germline antibody gene segment, or the amino acid sequence of one or more of said framework regions

collectively comprises up to 5 amino acid differences relative to the amino acid sequence of said corresponding framework region encoded by a human germline antibody gene segment.

49. A polypeptide according to claim 21 or 44, wherein the amino acid sequences of FW1, FW2, FW3 and FW4 of the anti-CD40L variable domain or dAb are the same as the amino acid sequences of corresponding framework regions encoded by a human germline antibody gene segment, or the amino acid sequences of FW1, FW2, FW3 and FW4 collectively contain up to 10 amino acid differences relative to the amino acid sequences of corresponding framework regions encoded by said human germline antibody gene segment.

50. The polypeptide according to claim 49, wherein the amino acid sequences of said FW1, FW2 and FW3 of the anti-CD40L variable domain or dAb are the same as the amino acid sequences of corresponding framework regions encoded by human germline antibody gene segments.

51. The polypeptide according to claim 49, wherein said human germline antibody gene segment is selected from the group consisting of DP47, DP45, DP48 and DPK9.

52. A PEG-linked antigen-binding polypeptide comprising a single immunoglobulin variable domain which specifically and monovalently binds CD40L.

53. The PEG-linked polypeptide of claim 52 which has a hydrodynamic size of at least 24 kD.

54. The PEG-linked polypeptide of claim 52 wherein said PEG is linked to said antibody at a cysteine or lysine residue.

55. The PEG-linked polypeptide of claim 52 wherein the total PEG size is from 20 to 60 kD, inclusive.

56. The PEG-linked polypeptide of claim 52 which has a hydrodynamic size of at least 200 kD.

57. The PEG-linked polypeptide of claim 52 which has an increased in vivo half-life relative to the same polypeptide composition lacking linked polyethylene glycol.

58. The PEG-linked polypeptide of claim 52 wherein the tα-half life of the polypeptide composition is increased by 10% or more.

59. The PEG-linked polypeptide of claim 52 wherein the tα-half life of the polypeptide composition is in the range of 0.25 minutes to 12 hours.

60. The PEG-linked polypeptide of claim 52 wherein the tβ-half life of the polypeptide composition is increased by 10% or more.

61. The PEG-linked polypeptide of claim 60 wherein the tβ-half life is in the range of 12 to 48 hours.

62. An immunoglobulin variable domain polypeptide, said polypeptide comprising a single immunoglobulin variable domain which specifically and monovalently binds CD40L, and which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 7-82, SEQ ID NOs 246-360, and DOM8-24.

63. An immunoglobulin variable domain polypeptide which has an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs 7-82, SEQ ID NOs 246-360, and DOM8-24, which polypeptide specifically and monovalently binds CD40L.

64. The immunoglobulin variable domain polypeptide of claim 63 which comprises a single immunoglobulin variable domain which specifically and monovalently binds CD40L.

65. The immunoglobulin variable domain polypeptide of claim 63 which antagonizes the binding of CD40L.

66. The immunoglobulin variable domain polypeptide of claim 63 which inhibits the binding of CD40 to CD40L and which has an IC50 in the range of 20 pM to 1.5 μM, inclusive.

67. The immunoglobulin variable domain polypeptide of claim 63 wherein said antigen-binding polypeptide inhibits the interaction of CD40 with CD40L, but does not substantially agonize signaling by CD40.

68. A composition comprising the immunoglobulin variable domain polypeptide of claim 63 and a second immunoglobulin variable domain polypeptide which binds a ligand other than CD40L.

69. The composition of claim 68 wherein said antibody polypeptide which binds a ligand other than CD40L binds a ligand selected from the group consisting of HSA, TNFα, IL-1, IL-2, IL-4, IL-6, IL-8, IL-12, IL-18, IFN-γ, CD2, CD4, CD8, LFA1, LFA3, VLA4, CD80, B7-1, CD28, CD86, B7-2, CTLA-4 CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, CD3, CD70, Inducible costimulatory molecule ligand (ICOSL), OX40L, HVEM (Herpes Virus Entry Mediator), LIGHT.

70. An extended release pharmaceutical formulation comprising a monovalent anti-CD40L antibody polypeptide.

71. The extended release pharmaceutical formulation of claim 70, wherein said antibody polypeptide is selected from the group consisting of a dAb, Fab, scFv, Fv, or a disulfide bonded Fv.

72. The formulation of claim 70 wherein said monovalent anti-CD40L antibody polypeptide comprises a single immunoglobulin variable domain that binds CD40L.

73. An antibody single variable domain polypeptide that binds to CD40L, said polypeptide comprising the sequence of DOM8-24.

74. An antibody single variable domain polypeptide that binds to CD40L, wherein the polypeptide has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a sequence that is at least 80% homologous to the sequence of DOM8-24.

75. An antibody single variable domain polypeptide that binds to CD40L, wherein the polypeptide has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24.

76. An antibody single variable domain polypeptide that binds to CD40L, wherein the polypeptide has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24.

77. An antibody single variable domain polypeptide that binds CD40L, wherein the polypeptide has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

78. An antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 .

79. An antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24 .

80. An antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24 .

81. An antibody single variable domain polypeptide that binds CD40L, wherein the dAb has an amino acid sequence that is identical to the amino acid sequence of DOM8-24, or differs from the amino acid sequence of DOM8-24 at no more than 25 amino acid positions and has a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and has a CDR2

sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and has a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

82. A CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24.

83. A CD40L antagonist having a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24.

84. A CD40L antagonist having a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

85. A CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24.

86. A CD40L antagonist having a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

87. A CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

88. A CD40L antagonist having a CDR1 sequence that is at least 50% homologous to the CDR1 sequence of DOM8-24 and a CDR2 sequence that is at least 50% homologous to the CDR2 sequence of DOM8-24 and a CDR3 sequence that is at least 50% homologous to the CDR3 sequence of DOM8-24.

89. The CD40L antagonist of claim 88, wherein said antagonist is an antibody single variable domain polypeptide selected from the group consisting of an antibody single variable domain polypeptide, dAb, FAb, an scFv, an Fv, or a disulfide-bonded Fv.

90. An antibody polypeptide that inhibits the binding of DOM8-24 to CD40L.

91. An antibody polypeptide that binds to the same epitope of CD40L bound by DOM8-24.

92. A method of treating or preventing a symptom of autoimmune disease in an individual, the method comprising administering the antibody polypeptide of claim 90 or 91 to said individual in an amount effective to treat or prevent a symptom of autoimmune disease.

93. An antibody polypeptide that binds to the same epitope of CD40L bound by an antibody single variable domain polypeptide comprising a sequence selected from the group consisting of SEQ ID Nos: 7-82 and 246-360.

94. An antibody single variable domain polypeptide comprising the sequence of CDR1, CDR2, and/or CDR3 of DOM8-24.

95. An antibody single variable domain polypeptide comprising the sequence of CDR1, CDR2, and/or CDR3 of an antibody single variable domain polypeptide comprising a sequence selected from the group consisting of SEQ ID Nos. 7-82 and 246-360.

96. The antibody single variable domain polypeptide of claim 94, wherein said polypeptide comprises the sequence of two or more of CDR1, CDR2, or CDR3 of DOM8-24.

97. The antibody single variable domain polypeptide of claim 95, wherein said polypeptide comprises the sequence of two or more of CDR1, CDR2, or CDR3 of an antibody single variable domain polypeptide comprising a sequence selected from the group consisting of SEQ ID Nos. 7-82 and 246-360.

98. A dual specific ligand comprising a first immunoglobulin single variable domain having a binding specificity to a first antigen and a second single variable domain having a binding activity to a second antigen, wherein the first antigen is CD40L and the second single variable domain is an Antigen Presenting Cell surface antigen or a T cell surface antigen.

99. A ligand according to claim 98 wherein the Antigen Presenting Cell surface antigen is selected from one of the group consisting of, activated macrophage surface antigens, activated B cell surface antigens, co-stimulatory signal pathway surface antigens, and MHC.

100. A ligand according to claim 99 wherein the MHC is class II.

101. A ligand according to claim 100 wherein the MHC class II is alpha.

102. A ligand according to claim 100 wherein the MHC class II is beta.

103. A ligand according to claim 99 wherein the Antigen Presenting Cell surface antigen is selected from the group consisting of CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, CD3, CD70, Inducible costimulatory molecule ligand (ICOSL), OX40L, CD80, CD86, HVEM (Herpes Virus Entry Mediator), and LIGHT.

104. A ligand according to claim 99 wherein the Antigen Presenting Cell surface antigen is selected from the group consisting of CD28, Inducible costimulatory molecule (ICOS), CD27, CD30, OX40, CD45, CD69, and CD3.

105. A ligand according to claim 99 wherein the surface antigen is a B7 gene surface antigen.

106. A ligand according to claim 105 wherein the B7 gene surface antigen is B7- 2, or B7-1.