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(54) Title: TNF SUPER FAMILY MEMBERS WITH ALTERED IMMUNOGENICITY

(57) Abstract: The present invention relates to non-naturally occurring variant Tumor Necrosis Factor Super Family member proteins with reduced immunogenicity. More specifically, the present invention relates to variant BAFF, RANKL, TRAIL, CD40L and APRIL proteins with reduced immunogenicity.



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TNF Super Family Members with Altered Immunogenicity

[01] This application claims benefit under 35 U.S.C. §119(e) to USSNs 60/573,206, filed May 21, 2004; 60/573,301, filed May 21, 2004; 60/573,395, filed May 21, 2004; 60/588,314, filed July 14, 2004; 60/607,396, filed September 2, 2004; and, 60/607,397, filed September 2, 2004.

Field of the Invention

[02] The present invention relates to variant Tumor Necrosis Factor Super Family member proteins with reduced immunogenicity. More specifically, the present invention relates to variant BAFF, RANKL, TRAIL, CD40L and APRIL proteins with reduced immunogenicity. In particular, variants of BAFF, RANKL, TRAIL, CD40L and APRIL proteins with reduced ability to bind one or more human class II MHC molecules are described.

Background of the Invention

[03] Immunogenicity is a major barrier to the development and utilization of protein therapeutics. Although immune responses are typically most severe for non-human proteins, even therapeutics based on human proteins may be immunogenic. Immunogenicity is a complex series of responses to a substance that is perceived as foreign and may include production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, and anaphylaxis. Several factors can contribute to protein immunogenicity, including but not limited to the protein sequence, the route and frequency of administration, and the patient population.

[04] Immunogenicity may limit the efficacy and safety of a protein therapeutic in multiple ways. Efficacy can be reduced directly by the formation of neutralizing antibodies. Efficacy may also be reduced indirectly, as binding to either neutralizing or non-neutralizing antibodies typically leads to rapid clearance from serum. Severe side effects and even death may occur when an immune reaction is raised. One special class of side effects results when neutralizing antibodies cross-react with an endogenous protein and block its function.

[05] Several methods have been developed to modulate the immunogenicity of proteins. In some cases, PEGylation has been observed to reduce the fraction of patients who raise neutralizing antibodies by sterically blocking access to antibody agretopes (see for example, Hershfield et. al. PNAS 1991 88:7185-7189 (1991); Bailon. et al. Bioconjug. Chem. 12: 195-202(2001); He et al. Life Sci. 65: 355-368 (1999), entirely incorporated by reference). Methods that improve the solution properties of a protein therapeutic may also reduce immunogenicity, as aggregates have been observed to be more immunogenic than soluble proteins.

[06] A more general approach to immunogenicity reduction involves mutagenesis targeted at the agretopes in the protein sequence and structure that are most responsible for stimulating the immune system. Some success has been achieved by randomly replacing solvent-exposed residues to lower binding affinity to panels of known neutralizing antibodies (see for example Laroche et. al. Blood 96: 1425-1432 (2000), entirely incorporated by reference). Due to the incredible diversity of the antibody repertoire, mutations that lower affinity to known antibodies will typically lead to production of another set of antibodies rather than abrogation of immunogenicity. However, in some cases it may be possible to decrease surface antigenicity by replacing hydrophobic and charged residues on the

protein surface with polar neutral residues (see Meyer et. al. Protein Sci. 10: 491-503 (2001), entirely incorporated by reference).

- [07] An alternate approach is to disrupt T-cell activation. Removal of MHC-binding agretopes offers a much more tractable approach to immunogenicity reduction, as the diversity of MHC molecules comprises only ~103 alleles, while the antibody repertoire is estimated to be approximately 10⁸ and the T-cell receptor repertoire is larger still. By identifying and removing or modifying class II MHC-binding peptides within a protein sequence, the molecular basis of immunogenicity can be evaded. The elimination of such agretopes for the purpose of generating less immunogenic proteins has been disclosed previously; see for example WO 98/52976, WO 02/079232, and WO 00/3317, entirely incorporated by reference.
- [08] While mutations in MHC-binding agretopes can be identified that are predicted to confer reduced immunogenicity, most amino acid substitutions are energetically unfavorable. As a result, the vast majority of the reduced immunogenicity sequences identified using the methods described above will be incompatible with the structure and/or function of the protein. In order for MHC agretope removal to be a viable approach for reducing immunogenicity, it is crucial that simultaneous efforts are made to maintain a protein's structure, stability, and biological activity.
- [09] B-cell Activation Factor, BAFF (also known as BLyS, TALL-1, THANK, zTNF4 and TNFSF13B) is a member of the TNF super family (TNFSF) of proteins. BAFF is important for survival of B-cells and humoral immune response; to a lesser extent it also induces T-cell activation and proliferation. Normally, only a small number of B-cells mature due to a vigorous negative selection. Overexpression of BAFF in transgenic (Tg) animals promotes increased B-cell survival, resulting in inappropriate survival of autoreactive lymphocytes and enlarged lymphoid organs and spleen, accompanied by the appearance of anti-DNA antibodies, an increase in antibody secretion, and Ig-deposition in the kidneys. This results in glomerulonephritis and syndromes similar to systemic lupus erythematosus (SLE), Sjogren syndrome (SS), and the like. Correlations between high BAFF concentration and elevated levels of anti-dsDNA Ab, a biochemical marker of several autoimmune diseases, have been observed in SLE, RA, and SS patients.
- [10] BAFF binds three receptors: BAFF-R, TACI, and BCMA. BAFF-R is specific to BAFF while TACI and BCMA are shared with APRIL, another member of TNFSF and the closest homologue of BAFF. Phenotypes of BAFF knockout mice (KO) and a BAFF-R mutant strain of mice (A/WySnJ) suggest that BAFF-R is the main receptor for BAFF and is responsible for control of B-cell maturation. TACI controls B-cell homeostasis and T-cell Independent immune response and appears to act as an inhibitory BAFF receptor. The role of BCMA is unclear thus far.
- [11] BAFF is an attractive drug target because it has been implicated in the pathogenesis of several diseases and because BAFF inhibitors would potentially have few side effects. A previous invention provided variant BAFF proteins that function as dominant negative or competitive inhibitors of endogenous BAFF. Furthermore, superagonist variants of BAFF were generated, which may serve to stimulate the immune system. BAFF and variant BAFF proteins, like all proteins, has the potential to induce unwanted immune responses when used as a therapeutic. Accordingly, the development of

therapeutics based on BAFF may be facilitated by preemptively reducing the potential immunogenicity of BAFF or its variants.

- [12] RANKL is a trimeric TNF family member that binds to the trimeric RANK receptor. RANKL is a key modulator of bone remodeling orchestrated by osteoblasts and osteoclasts. (See US 2003/0013651 and WO 02/080955, entirely incorporated by reference). RANKL activates the receptor RANK upon binding, which leads to the differentiation, survival, and fusion of pre-osteoclasts to form active bone resorbing osteoclasts (see Lacey DL, Timms E, Tan H-L, Kelley MJ, Dunstan CR, Burgess T et al. 1998 *Cell* 93: p. 165-176, entirely incorporated by reference). RANKL also binds to the decoy receptor OPG, which functions as a natural antagonist of RANKL activity.
- [13] The RANKL biochemical axis has been successfully targeted to treat osteoporosis, rheumatoid arthritis, prosthesis-induced osteolysis, cancer-induced bone destruction, metastasis, hypercalcemia, and pain (Hofbauer et. al. 2001 *Cancer* 92:460-470; Takahashi et.al. 1999 *Biochem. Biophys. Res. Comm.* 256:449-455; Honore et al. 2000 *Nat. Med.* 6:521-528; Oyajobi et.al. 2001 *Cancer Res* 61:2572-2578; Childs et. el. *J. Bone Mineral Res.* 17:192-199, entirely incorporated by reference). In addition to being important in bone biology, RANKL plays a role in the immune system by regulating antigen-specific T cell responses (Anderson et al., *Nature* 1997, 390:175-179, entirely incorporated by reference).
- [14] Much work has been done to develop therapeutic entities and reagents for biological research based on RANKL. For example, RANKL fragments, analogs, derivatives, or conformers having the ability to bind OPG, which could be used as treatments for a variety of bone diseases, have been described (See U.S. Patent No. 5,843,678, entirely incorporated by reference). RANKL variants, which induce production of an immune response that down-regulates RANKL activity, have been disclosed (See WO00/15807). In other studies, utilization of RANKL protein and its derivatives as immune modulators has been proposed (See WO99/29865, entirely incorporated by reference). Novel RANKL variants, including variants that express solubly in *E. coli*, dominant negative variants, competitive inhibitor variants, receptor-specific variants, and superagonist variants have been disclosed (USSN 10/338,785, filed January 6, 2003, entirely incorporated by reference.)
- [15] A Proliferation-Inducing Ligand (APRIL), also known as TRDL-1 alpha, TALL-2, and TNFSF-13A, is a member of the TNF Super Family (TNFSF) of proteins. The prototype of the family, Tumor Necrosis Factor Alpha (TNF α), originally discovered for promoting tumor regression in vivo, is a key mediator of inflammation. APRIL also participates in a variety of cellular and intracellular signaling processes involved in autoimmune disease, inflammation, and cancer.
- [16] APRIL is expressed by macrophages, monocytes, dendritic cells, T cells, and a number of human tumors and transformed cell lines. It is synthesized as a type II transmembrane protein, cleaved intracellularly in the Golgi apparatus by a furin convertase, and secreted predominantly as a soluble molecule. A splice variant of the APRIL/TWEAK locus also exists, which results in a functional hybrid molecule (TWE-PRIL) that is primarily retained on the cell surface (Lopez-Fraga et al. *EMBO Rep* 2: 945-951 (2001), entirely incorporated by reference). Structurally, APRIL is a sandwich of two anti-parallel beta-sheets with the "jelly roll" or Greek key topology and forms homotrimers typical of the

TNFSF. In addition, APRIL can also form heterotrimers with BAFF, another member of the TNFSF that is closely related to APRIL.

- [17] APRIL and BAFF share two common receptors, B-cell maturation antigen (BCMA) and transmembrane activator and CAML interactor (TACI). BCMA preferentially binds APRIL versus BAFF. BCMA and TACI are type III transmembrane proteins, lacking N-terminal signal sequences. The receptors are expressed on B cells and TACI has also been detected on the surface of some T cells. TACI controls B-cell homeostasis and T-cell independent immune response and may act as an inhibitory BAFF receptor. Injection of TACI-Ig strongly inhibited or prevented collagen induced arthritis in mice. The role of BCMA is unclear thus far. BCMA and TACI contain intracellular TRAF binding motifs. The signaling mechanisms of these receptors are not fully characterized; however, they appear to mediate activation of the NF- κ B, p38, mitogen-activated protein kinase, JNK, AP-1 and NF-AT pathways. APRIL signaling through BCMA and TACI is triggered by binding in its oligomeric (for the most part, trimeric) form.
- [18] Existence of a third APRIL receptor is suggested from work with mouse NIH 3T3 fibroblasts: these cancer cells express no TACI or BCMA, yet APRIL overexpression stimulates their proliferation in vitro and tumorigenicity in vivo. In a similar assay, BAFF has no effect on tumor cells. Also, soluble BCMA, which can bind and block APRIL, inhibits cancer cell growth (Rennert et al. J Exp Med 192: 1677-1684 (2000), entirely incorporated by reference.) Taken together, these facts suggest the existence of a specific APRIL receptor that has not yet been identified.
- [19] APRIL costimulates B cell proliferation and IgM production and appears to play a role in T-independent type II antigen responses and T cell survival. Accordingly, APRIL may be involved in the pathogenesis of autoimmune and inflammatory conditions. APRIL serum levels inversely correlated with disease in patients with systemic lupus erythematosus (SLE), indicating that APRIL may serve as a down modulator of serological and/or clinical autoimmunity. A polymorphism in the APRIL gene has been associated with SLE (67G allele). See for example Tan et al. Arthritis Rheum 48: 982-992 (2003), Roschke et al. J Immunol 169: 4314-4321 (2002), Stohl et al. Ann Rheum Dis 63: 1096-1103 (2004), Koyama et al. Rheumatology (Oxford) 42: 980-985 (2003), all entirely incorporated by reference.
- [20] APRIL can also induce proliferation/survival of nonlymphoid cells. Elevated expression of APRIL has been found in some tumor cell lines and tumor tissue libraries. Moreover, APRIL-transfected NIH-3T3 cells show an increased rate of tumor growth in nude mice compared with the parental cell line. APRIL can also protect glioma cells against FasL- and TRAIL-induced apoptosis. These findings suggest that APRIL may be involved in the regulation of tumor cell growth. See Mackay and Ambrose Cytokine Growth Factor Rev 14: 311-324 (2003), Medema et al. Cell Death Differ 10: 1121-1125 (2003), entirely incorporated by reference.
- [21] APRIL agonists or antagonists may thus be useful in the treatment of oncological, autoimmune, and inflammatory conditions. For example, engineered variants that act as dominant-negative inhibitors, competitive inhibitors, receptor-specific agonists, or superagonists may be used USSN 10/820,465, entirely incorporated by reference.

- [22] CD40L, also known as CD154, TNFSF5, TRAP, and gp39, is a member of the TNF superfamily and may trimerize to bind and activate CD40, as well as α IIb- β 3 integrin. CD40L is a type II membrane glycoprotein of about 33-kDa; the full-length version has 261 amino acids and the extracellular domain (ECD) comprises amino acids 45-261. In some physiological contexts, CD40L is proteolytically processed to yield a soluble form comprising amino acids 113-261. Elevated levels of this soluble form have been established for a variety of disease conditions, including but not limited to: chronic renal failure, diabetes, inflammatory bowel disease, autoimmune thrombocytopenic purpura, Hodgkin's disease, rheumatoid vasculitis, systemic lupus erythematosus, chronic lymphocytic leukaemia, preeclampsia, sickle cell anemia, atherosclerosis, and numerous cardiovascular conditions. Elevated levels of soluble CD40L have also become well established as a reliable predictor of cardiovascular events.
- [23] CD40L is transiently expressed after MHC/peptide-induced TCR activation on CD4⁺T cells. These cells mediate a signal to B cells through the CD40-CD40L interaction, which results in B cell activation. Effects of B cell activation include antibody isotype switching, rescue from apoptosis, germinal center formation, B-cell differentiation and proliferation, and IgE secretion. Mutations in CD40L cause X-linked hyper IgM syndrome, which causes severe immunodeficiency, low levels of IgA, IgG, and IgE, and inability to mount a thymus-dependent humoral response. See Seyama et al. *J Biol Chem* 274: 11310-11320 (1999), Sacco et al. *Cancer Gene Ther* 7: 1299-1306 (2000), entirely incorporated by reference. The pleiotropic immunologic effects of CD40-CD40L interactions include autoimmunity, transplantation and allograft rejection, as well as control of infection.
- [24] CD40 and CD40L are also expressed in other cell types including dendritic cells, monocytes, macrophages, endothelial cells, and fibroblasts, and are involved in many inflammatory processes including leukocyte adhesion and migration, induction of chemokines and cytokines, and activation of fibroblasts and platelets. CD40L has been implicated in the pathogenesis of atherosclerosis; it promotes microglial activation and may play a role in the development of Alzheimer's disease. Activation of the CD40-CD40L system also has remarkable antitumor and antimetastatic effects on certain carcinomas. See Laman et al. *Crit Rev Immunol* 16: 59-108 (1996), Lutgens and Daemen *Trends Cardiovasc Med* 12: 27-32 (2002), Tan et al. *Curr Opin Pharmacol* 2: 445-451 (2002), Prasad et al. *Curr Opin Hematol* 10: 356-361 (2003), Tolba et al. *Cancer Res* 62: 6545-6551 (2002), entirely incorporated by reference.
- [25] CD40L has many potential therapeutic indications: anti-tumor or oncological conditions, including Hodgkins and non-Hodgkins lymphomas (NHL), pre- and post-transplantation immunosuppression, psoriasis, rheumatoid and collagen-induced arthritis, multiple sclerosis, systemic lupus erythematosus (SLE), allergic encephalitis, acute and chronic graft versus host disease, Crohn's disease, diabetes, chronic renal failure, mixed connective tissue disease, sickle cell anemia, inflammatory bowel disease, Hodgkin disease, rheumatoid vasculitis, chronic lymphocytic leukaemia, preeclampsia, Alzheimer's disease, and cardiovascular conditions including atherosclerosis, thrombocytopenia (Purpura), etc.
- [26] Anti-CD40L monoclonal antibodies have shown promise in animal models for the treatment of several chronic inflammatory diseases, autoimmune diseases, and in allograft and transplant

rejection. However, clinical experience with CD40L (including monoclonal antibodies) has not yet produced an effective therapeutic. See Dumont *Curr Opin Investig Drugs* 3: 725-734 (2002), entirely incorporated by reference, discussing monoclonal antibody IDEC-131; also the Biogen/Columbia University monoclonal antibody ruplizumab (anti-CD40L) Phase II Antova trial was discontinued due to thrombo-embolic adverse effects. Recently, evidence has accumulated indicating that CD40L can activate platelets by signaling through alpha IIb-beta3 integrin (see for example Prasad et al. *Proc Natl Acad Sci U S A* 100: 12367-12371 (2003), entirely incorporated by reference.)

- [27] TNF-related apoptosis inducing ligand (TRAIL), also known as Apo2L and TNFSF10, is a type II (intracellular N terminus and extracellular C terminus) transmembrane protein whose extracellular domain can be proteolytically cleaved at the cell surface to form a soluble ligand (residues 114-281). A member of the TNF superfamily, its extracellular domain shares sequence homology with other family members including Fas ligand, TNF- α , lymphotoxin- α , and lymphotoxin- β . Like most other TNF family members, TRAIL forms a homotrimer that binds three receptor molecules, each at the interface between two of its subunits.
- [28] Although TRAIL mRNA has been found in a variety of tissues and cells (Wiley et al. *Immunity* 3: 673-682 (1995)), studies with anti-mTRAIL mAb suggest that only some liver natural killer cells express TRAIL constitutively. However, TRAIL is highly expressed on most natural killer cells after stimulation with IL-2, interferons (IFNs), or IL-15; type I IFN-activated peripheral blood T cells, CD11c+ DC, and monocytes also express TRAIL (see for example Smyth et al. *Immunity* 18: 1-6 (2003), entirely incorporated by reference).
- [29] Soluble recombinant TRAIL selectively induces apoptosis of a variety of tumor cells and transformed cells, but not most normal cells, and has therefore gained interest as a promising cancer therapeutic, alone or in combination with other cancer treatments. Also, administration to experimental animals including mice and primates produces significant tumor regression without systemic toxicity. TRAIL can induce apoptosis regardless of p53 status, and may be particularly useful in cells where the p53 pathway has been inactivated, thus helping to circumvent resistance to chemo- and radiotherapy. See for example Griffith and Lynch *Curr Opin Immunol* 10: 559-563 (1998), Ashkenazi et al. *J Clin Invest* 104: 155-162 (1999), Almasan and Ashkenazi *Cytokine Growth Factor Rev* 14: 337-348 (2003), Smyth et al. *Immunity* 18: 1-6 (2003), Wang and El-Deiry *Oncogene* 22: 8628-8633 (2003), entirely incorporated by reference.
- [30] TRAIL induces apoptosis through engagement of its death receptors. At least five receptors for TRAIL have been identified in humans. Four are membrane receptors that belong to the TNF receptor family, and two of these, DR4 (TRAIL-R1) and DR5 (apo2, TRAIL-R2) are capable of transducing an apoptotic signal. The other receptors, DcR1 (TRAIL-R3), DcR2 (TRAIL-R4), and a soluble receptor called osteoprotegerin (OPG) lack death domains, but may serve as decoy receptors that inhibit TRAIL-mediated cell death when overexpressed. Most studies suggest DR5 signals through a FADD- and caspase-8-dependent pathway (Bodmer et al. *Nat Cell Biol* 2: 241-243 (2000)). The Bcl-2 family member Bax is required for TRAIL-induced apoptosis of certain cancer cell lines, and Bax mutation in mismatch repair-deficient tumors can cause resistance to TRAIL therapy, but preexposure to chemotherapy can rescue tumor sensitivity. While mRNA expression of TRAIL death receptors is

widely distributed in both normal and malignant tissues (Chaudhary et al. *Immunity* 7: 821-830 (1997), entirely incorporated by reference), cell surface expression of DR5 has been reported to be elevated in malignant tumor cells. Antibodies that immunospecifically bind to TRAIL receptors, particularly DR4 or DR5, have been shown to induce apoptosis in human tumor cells and are being investigated as potential therapeutics either alone or in combination with other anticancer drugs (see Alderson et al. *Proc Amer Assoc Cancer Res* 44: Abs 963 (2003), Kaliberov et al. *Gene Ther* 11: 658-667 (2004), Patents WO-2004016753, WO-03054216, WO-03042367, WO-03038043, WO-02097033, WO-02094880, WO-02085946, WO-02079377, WO-00183560, WO-00067793, WO-00066156, WO-00048619, WO-00051638, WO-09912963, WO-09909165, WO-09907408, WO-09903992, and WO-03037913), entirely incorporated by reference.

[31] Despite pursuit of TRAIL as a selective anticancer therapeutic, little is known about the natural physiological function of TRAIL. TRAIL appears to play an important role in both T-cell and natural killer cell-mediated tumor surveillance and suppression of tumor metastasis, and in anti-viral immune surveillance, often augmented by IFN-regulated induction (Smyth et al. *J Exp Med* 193: 661-670 (2001), Takeda et al. *J Exp Med* 195: 161-169 (2002), Almasan et al. *Cytokine Growth Factor Rev* 14: 337-348 (2003), entirely incorporated by reference). TRAIL also has immunosuppressive and immunoregulatory functions that may be protective against autoimmune disorders including diabetes, rheumatoid arthritis, and multiple sclerosis (Song et al. *J Exp Med* 191: 1095-1104 (2000), Hilliard et al. *J Immunol* 166: 1314-1319 (2001), Lamhamedi-Cherradi et al. *Diabetes* 52: 2274-2278 (2003), Lamhamedi-Cherradi et al. *Nat Immunol* 4: 255-260 (2003), Patent WO-2004001009 (2003), Patent WO-2004039395 (2004), entirely incorporated by reference). It has been suggested that TRAIL inhibits autoimmune inflammation by blocking cell cycle progression of activated T-cells or by inhibiting cytokine production (Song et al. *J Exp Med* 191: 1095-1104 (2000), entirely incorporated by reference).

[32] TRAIL has also been reported to play a critical role in inducing hepatic cell death and hepatic inflammation (Zheng et al. *J Clin Invest* 113: 58-64 (2004), entirely incorporated by reference); thus, TRAIL blockers may be useful in the treatment of hepatitis and other liver diseases. TRA-8, an agonistic monoclonal antibody that binds DR5 but not other TRAIL receptors, is tumoricidal in vitro and in vivo, but unlike TRAIL, does not induce apoptosis of normal hepatocytes; this suggests that specific targeting of DR5 may be a safe and effective strategy for cancer therapy (see Ichikawa et al. *Nat Med* 7: 954-960 (2001), entirely incorporated by reference). The specific targeting of DR5 on the highly proliferative synovial cells has also been suggested as a potential therapy for rheumatoid arthritis (see Ichikawa et al. *J Immunol* 171: 1061-1069 (2003), entirely incorporated by reference).

[33] Daily iv injections of 0.1 to 10 mg/kg soluble human TRAIL in cynomolgus monkeys for 7 days elicited no detectable anti-TRAIL antibodies, suggesting that TRAIL is not highly immunogenic (see Ashkenazi et al. *J Clin Invest* 104: 155-162 (1999), entirely incorporated by reference); similarly no anti-TRAIL antibodies were detected in chimpanzees 14 days post injection of 1-5 mg/kg TRAIL iv (Kelley et al. *J Pharmacol Exp Ther* 299: 31-38 (2001), entirely incorporated by reference). However TRAIL, like all proteins, has the potential to induce unwanted immune responses when used as a

therapeutic. Accordingly, the development of therapeutics based on TRAIL may be facilitated by preemptively reducing the potential immunogenicity of TRAIL.

- [34] TNF Super Family members, like all proteins, has the potential to induce unwanted immune responses when used as a therapeutic. Accordingly, the development of therapeutics based on TNF Super Family members may be facilitated by preemptively reducing the potential immunogenicity of TNF Super Family members. There remains a need for novel TNF super family member proteins, including but not limited to superagonist, dominant negative, and competitive inhibitor variant TNF super family member proteins, having reduced immunogenicity.

Summary of the Invention

- [35] In accordance with the objects outlined above, the present invention provides novel TNF Super Family member proteins having reduced immunogenicity as compared to naturally occurring TNF Super Family member proteins. In an additional aspect, the present invention is directed to methods for engineering or designing less immunogenic proteins with TNF Super Family member activity for therapeutic use.
- [36] An aspect of the present invention are TNF Super Family member variants that show decreased binding affinity for one or more class II MHC alleles relative to a parent TNF Super Family member and which significantly maintain the activity of native naturally occurring TNF Super Family member. In a further aspect, the invention provides recombinant nucleic acids encoding the variant TNF Super Family member proteins, expression vectors, and host cells. In an additional aspect, the invention provides methods of producing a variant TNF Super Family member protein comprising culturing the host cells of the invention under conditions suitable for expression of the variant TNF Super Family member protein.
- [37] In a further aspect, the invention provides pharmaceutical compositions comprising a variant TNF Super Family member protein or nucleic acid of the invention and a pharmaceutical carrier. In a further aspect, the invention provides methods for preventing or treating TNF Super Family member responsive disorders comprising administering a variant TNF Super Family member protein or nucleic acid of the invention to a patient.
- [38] In an additional aspect, the invention provides methods for screening the class II MHC haplotypes of potential patients in order to identify individuals who are particularly likely to raise an immune response to a wild type or variant TNF Super Family member therapeutic.
- [39] In accordance with the objects outlined above, the present invention provides TNF Super Family member variant proteins comprising amino acid sequences with at least one amino acid insertion, deletion, or substitution compared to the wild type TNF Super Family member proteins.

Brief Description of the Drawings

- [40] **Figure 1** shows a method for engineering less immunogenic BAFF derivatives.
- [41] **Figure 2** shows a schematic representation of a method for in vitro testing of the immunogenicity of BAFF peptides or proteins with IVV technology.

Detailed Description of the Invention

- [42] By “9-mer peptide frame” and grammatical equivalents herein is meant a linear sequence of nine amino acids that is located in a protein of interest. 9-mer frames may be analyzed for their propensity

to bind one or more class II MHC alleles. By “**allele**” and grammatical equivalents herein is meant an alternative form of a gene. Specifically, in the context of class II MHC molecules, alleles comprise all naturally occurring sequence variants of DRA, DRB1, DRB3/4/5, DQA1, DQB1, DPA1, and DPB1 molecules. By “**TNF Super Family member responsive disorders or conditions**” and grammatical equivalents herein is meant diseases, disorders, and conditions that can benefit from treatment with TNF Super Family member proteins. Examples of TNF Super Family member-responsive disorders include, but are not limited to, autoimmune diseases such as systemic lupus erythematosus, diabetes, rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, Crohn’s Disease, and psoriasis; transplant rejection and graft versus host disease; hematological cancers such as Hodgkin’s lymphoma, non-Hodgkin’s lymphomas (Burkitt’s lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, hairy cell leukemia and lymphoplasmacytic leukemia), tumors of lymphocyte precursor cells (B-cell acute lymphoblastic leukemia/lymphoma and T-cell acute lymphoblastic leukemia/lymphoma), tumors of the mature T and NK cells (peripheral T-cell leukemias, adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia), Langerhans cell histiocytosis, myeloid neoplasias (acute myelogenous leukemias), and chronic myelogenous leukemia. By “**hit**” and grammatical equivalents herein is meant, in the context of the matrix method, that a given peptide is predicted to bind to a given class II MHC allele. In a preferred embodiment, a hit is defined to be a peptide with binding affinity among the top 5%, or 3%, or 1% of binding scores of random peptide sequences. In an alternate embodiment, a hit is defined to be a peptide with a binding affinity that exceeds some threshold, for instance a peptide that is predicted to bind an MHC allele with at least 100 μ M or 10 μ M or 1 μ M affinity. By “**immunogenicity**” and grammatical equivalents herein is meant the ability of a protein to elicit an immune response, including but not limited to production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, and anaphylaxis. By “**reduced immunogenicity**” and grammatical equivalents herein is meant a decreased ability to activate the immune system, when compared to the wild type protein. For example, a variant protein can be said to have “reduced immunogenicity” if it elicits neutralizing or non-neutralizing antibodies in lower titer or in fewer patients than the wild type protein. In a preferred embodiment, the probability of raising neutralizing antibodies is decreased by at least 5%, with at least 50% or 90% decreases being especially preferred. So, if a wild type produces an immune response in 10% of patients, a variant with reduced immunogenicity would produce an immune response in not more than 9.5% of patients, with less than 5% or less than 1% being especially preferred. A variant protein also can be said to have “reduced immunogenicity” if it shows decreased binding to one or more MHC alleles or if it induces T-cell activation in a decreased fraction of patients relative to the parent protein. In a preferred embodiment, the probability of T-cell activation is decreased by at least 5%, with at least 50% or 90% decreases being especially preferred. By “**matrix method**” and grammatical equivalents thereof herein is meant a method for calculating peptide – MHC affinity in which a matrix is used that contains a score for each possible residue at each position in the peptide, interacting with a given MHC allele. The binding score for a given peptide – MHC

interaction is obtained by summing the matrix values for the amino acids observed at each position in the peptide. By **"MHC-binding agretopes"** and grammatical equivalents herein is meant peptides that are capable of binding to one or more class II MHC alleles with appropriate affinity to enable the formation of MHC – peptide – T-cell receptor complexes and subsequent T-cell activation. MHC-binding agretopes are linear peptide sequences that comprise at least approximately 9 residues. By **"parent protein"** as used herein is meant a protein that is subsequently modified to generate a variant protein. Said parent protein may be a wild-type or naturally occurring protein, or a variant or engineered version of a naturally occurring protein. "Parent protein" may refer to the protein itself, compositions that comprise the parent protein, or any amino acid sequence that encodes it. Accordingly, by **"parent TNF Super Family member protein"** as used herein is meant a TNF Super Family member protein that is modified to generate a variant TNF Super Family member protein. By **"patient"** herein is meant both humans and other animals, particularly mammals, and organisms. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient is a mammal, and in the most preferred embodiment the patient is human. By **"protein"** herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e., "analogs" such as peptoids [see Simon et al., Proc. Natl. Acad. Sci. U.S.A. 89(20):9367-71 (1992), entirely incorporated by reference], generally depending on the method of synthesis. For example, homo-phenylalanine, citrulline, and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes amino acid residues such as proline and hydroxyproline. Both D- and L- amino acids may be utilized. By **"treatment"** herein is meant to include therapeutic treatment, as well as prophylactic, or suppressive measures for the disease or disorder. Thus, for example, successful administration of a variant TNF Super Family member protein prior to onset of the disease may result in treatment of the disease. As another example, successful administration of a variant TNF Super Family member protein after clinical manifestation of the disease to combat the symptoms of the disease comprises "treatment" of the disease. "Treatment" also encompasses administration of a variant TNF Super Family member protein after the appearance of the disease in order to eradicate the disease. Successful administration of an agent after onset and after clinical symptoms have developed, with possible abatement of clinical symptoms and perhaps amelioration of the disease, further comprises "treatment" of the disease. Those "in need of treatment" include mammals already having the disease or disorder, as well as those prone to having the disease or disorder, including those in which the disease or disorder is to be prevented. By **"variant TNF Super Family member nucleic acids"** and grammatical equivalents herein is meant nucleic acids that encode variant TNF Super Family member proteins. Due to the degeneracy of the genetic code, an extremely large number of nucleic acids may be made, all of which encode the variant TNF Super Family member proteins of the present invention, by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the variant TNF Super Family member. By **"variant TNF Super Family member proteins"** and grammatical equivalents thereof herein is meant non-naturally occurring TNF Super Family member proteins which differ from the wild type or parent TNF Super Family member protein

by at least 1 amino acid insertion, deletion, or substitution. TNF Super Family member variants are characterized by the predetermined nature of the variation, a feature that sets them apart from naturally occurring allelic or interspecies variation of the TNF Super Family member protein sequence. The TNF Super Family member variants typically either exhibit biological activity that is comparable to naturally occurring TNF Super Family member or have been specifically engineered to have alternate biological properties. The variant TNF Super Family member proteins may contain insertions, deletions, and/or substitutions at the N-terminus, C-terminus, or internally. In a preferred embodiment, variant TNF Super Family member proteins have at least 1 residue that differs from the naturally occurring TNF Super Family member sequence, with at least 2, 3, 4, or 5 different residues being more preferred. Variant TNF Super Family member proteins may contain further modifications, for instance mutations that alter stability or solubility or which enable or prevent posttranslational modifications such as PEGylation or glycosylation. Variant TNF Super Family member proteins may be subjected to co- or post-translational modifications, including but not limited to synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, fusion to proteins or protein domains, and addition of peptide tags or labels. By “**wild type or wt**” and grammatical equivalents thereof herein is meant an amino acid sequence or a nucleotide sequence that is found in nature and includes allelic variations; that is, an amino acid sequence or a nucleotide sequence that has not been intentionally modified. In a preferred embodiment, the wild type sequence is SEQ_ID NO:1.

[43] Identification of MHC-binding agretopes in TNF Super Family Members

[44] MHC-binding peptides are obtained from proteins by a process called antigen processing. First, the protein is transported into an antigen presenting cell (APC) by endocytosis or phagocytosis. A variety of proteolytic enzymes then cleave the protein into a number of peptides. These peptides can then be loaded onto class II MHC molecules, and the resulting peptide-MHC complexes are transported to the cell surface. Relatively stable peptide-MHC complexes can be recognized by T-cell receptors that are present on the surface of naïve T cells. This recognition event is required for the initiation of an immune response. Accordingly, blocking the formation of stable peptide-MHC complexes is an effective approach for preventing unwanted immune responses.

[45] The factors that determine the affinity of peptide-MHC interactions have been characterized using biochemical and structural methods. Peptides bind in an extended conformation bind along a groove in the class II MHC molecule. While peptides that bind class II MHC molecules are typically approximately 13-18 residues long, a nine-residue region is responsible for most of the binding affinity and specificity. The peptide binding groove can be subdivided into “pockets”, commonly named P1 through P9, where each pocket is comprises the set of MHC residues that interacts with a specific residue in the peptide. A number of polymorphic residues face into the peptide-binding groove of the MHC molecule. The identity of the residues lining each of the peptide-binding pockets of each MHC molecule determines its peptide binding specificity. Conversely, the sequence of a peptide determines its affinity for each MHC allele.

[46] Several methods of identifying MHC-binding agretopes in protein sequences are known in the art and may be used to identify agretopes in TNF Super Family members. Sequence-based information

can be used to determine a binding score for a given peptide – MHC interaction (see for example Mallios, *Bioinformatics* 15: 432-439 (1999); Mallios, *Bioinformatics* 17: p942-948 (2001); Sturniolo *et. al.* *Nature Biotech.* 17: 555-561(1999), all entirely incorporated by reference). It is possible to use structure-based methods in which a given peptide is computationally placed in the peptide-binding groove of a given MHC molecule and the interaction energy is determined (for example, see WO 98/59244 and WO 02/069232, entirely incorporated by reference). Such methods may be referred to as “threading” methods. Alternatively, purely experimental methods can be used; for example a set of overlapping peptides derived from the protein of interest can be experimentally tested for the ability to induce T-cell activation and/or other aspects of an immune response. (see for example WO 02/77187, entirely incorporated by reference).

[47] In a preferred embodiment, MHC-binding propensity scores are calculated for each 9-residue frame along the TNF Super Family sequence using a matrix method (see Sturniolo *et. al., supra*; Marshall *et. al.*, *J. Immunol.* 154: 5927-5933 (1995), and Hammer *et. al.*, *J. Exp. Med.* 180: 2353-2358 (1994), entirely incorporated by reference). It is also possible to consider scores for only a subset of these residues, or to consider also the identities of the peptide residues before and after the 9-residue frame of interest. The matrix comprises binding scores for specific amino acids interacting with the peptide binding pockets in different human class II MHC molecule. In the most preferred embodiment, the scores in the matrix are obtained from experimental peptide binding studies. In an alternate preferred embodiment, scores for a given amino acid binding to a given pocket are extrapolated from experimentally characterized alleles to additional alleles with identical or similar residues lining that pocket. Matrices that are produced by extrapolation are referred to as “virtual matrices”.

[48] In a preferred embodiment, the matrix method is used to calculate scores for each peptide of interest binding to each allele of interest. Several methods can then be used to determine whether a given peptide will bind with significant affinity to a given MHC allele. In one embodiment, the binding score for the peptide of interest is compared with the binding propensity scores of a large set of reference peptides. Peptides whose binding propensity scores are large compared to the reference peptides are likely to bind MHC and may be classified as “hits”. For example, if the binding propensity score is among the highest 1% of possible binding scores for that allele, it may be scored as a “hit” at the 1% threshold. The total number of hits at one or more threshold values is calculated for each peptide. In some cases, the binding score may directly correspond with a predicted binding affinity. Then, a hit may be defined as a peptide predicted to bind with at least 100 μ M or 10 μ M or 1 μ M affinity.

[49] In a preferred embodiment, the number of hits for each 9-mer frame in the protein is calculated using one or more threshold values ranging from 0.5% to 10%. In an especially preferred embodiment, the number of hits is calculated using 1%, 3%, and 5% thresholds. In a preferred embodiment, MHC-binding agretopes are identified as the 9-mer frames that bind to several class II MHC alleles. In an especially preferred embodiment, MHC-binding agretopes are predicted to bind at least 10 alleles at 5% threshold and/or at least 5 alleles at 1% threshold. Such 9-mer frames may be especially likely to elicit an immune response in many members of the human population. In a preferred embodiment, MHC-binding agretopes are predicted to bind MHC alleles that are present in

at least 0.01 – 10 % of the human population. Alternatively, to treat conditions that are linked to specific class II MHC alleles, MHC-binding agretopes are predicted to bind MHC alleles that are present in at least 0.01 – 10 % of the relevant patient population.

[50] Data about the prevalence of different MHC alleles in different ethnic and racial groups has been acquired by groups such as the National Marrow Donor Program (NMDP); for example see Mignot et al. *Am. J. Hum. Genet.* 68: 686-699 (2001), Southwood et al. *J. Immunol.* 160: 3363-3373 (1998), Hurley et al. *Bone Marrow Transplantation* 25: 136-137 (2000), Sintasath *Hum. Immunol.* 60: 1001 (1999), Collins et al. *Tissue Antigens* 55: 48 (2000), Tang et al. *Hum. Immunol.* 63: 221 (2002), Chen et al. *Hum. Immunol.* 63: 665 (2002), Tang et al. *Hum. Immunol.* 61: 820 (2000), Gans et al. *Tissue Antigens* 59: 364-369, and Baldassarre et al. *Tissue Antigens* 61: 249-252 (2003), all entirely incorporated by reference.

[51] In a preferred embodiment, MHC binding agretopes are predicted for MHC heterodimers comprising highly prevalent MHC alleles. Class II MHC alleles that are present in at least 10 % of the US population include but are not limited to: DPA1*0103, DPA1*0201, DPB1*0201, DPB1*0401, DPB1*0402, DQA1*0101, DQA1*0102, DQA1*0201, DQA1*0501, DQB1*0201, DQB1*0202, DQB1*0301, DQB1*0302, DQB1*0501, DQB1*0602, DRA*0101, DRB1*0701, DRB1*1501, DRB1*0301, DRB1*0101, DRB1*1101, DRB1*1301, DRB3*0101, DRB3*0202, DRB4*0101, DRB4*0103, and DRB5*0101.

[52] In a preferred embodiment, MHC binding agretopes are also predicted for MHC heterodimers comprising moderately prevalent MHC alleles. Class II MHC alleles that are present in 1% to 10% of the US population include but are not limited to: DPA1*0104, DPA1*0302, DPA1*0301, DPB1*0101, DPB1*0202, DPB1*0301, DPB1*0501, DPB1*0601, DPB1*0901, DPB1*1001, DPB1*1101, DPB1*1301, DPB1*1401, DPB1*1501, DPB1*1701, DPB1*1901, DPB1*2001, DQA1*0103, DQA1*0104, DQA1*0301, DQA1*0302, DQA1*0401, DQB1*0303, DQB1*0402, DQB1*0502, DQB1*0503, DQB1*0601, DQB1*0603, DRB1*1302, DRB1*0404, DRB1*0801, DRB1*0102, DRB1*1401, DRB1*1104, DRB1*1201, DRB1*1503, DRB1*0901, DRB1*1601, DRB1*0407, DRB1*1001, DRB1*1303, DRB1*0103, DRB1*1502, DRB1*0302, DRB1*0405, DRB1*0402, DRB1*1102, DRB1*0803, DRB1*0408, DRB1*1602, DRB1*0403, DRB3*0301, DRB5*0102, and DRB5*0202.

[53] MHC binding agretopes may also be predicted for MHC heterodimers comprising less prevalent alleles. Information about MHC alleles in humans and other species can be obtained, for example, from the IMGT/HLA sequence database ([ebi.ac.uk/imgt/hla/](http://www.ebi.ac.uk/imgt/hla/)).

[54] In an especially preferred embodiment, an immunogenicity score is determined for each peptide, wherein said score depends on the fraction of the population with one or more MHC alleles that are hit at multiple thresholds. For example, the equation

$$\text{Iscore} = N(W_1P_1 + W_3P_3 + W_5P_5)$$

may be used, where P_1 is the percent of the population hit at 1%, P_3 is the percent of the population hit at 3%, P_5 is the percent of the population hit at 5%, each W is a weighting factor, and N is a normalization factor. In a preferred embodiment, $W_1 = 10$, $W_3 = 5$, $W_5 = 2$, and N is selected so that possible scores range from 0 to 100. In this embodiment, agretopes with Iscore greater than or equal

to 10 are preferred and agretopes with Iscore greater than or equal to 25 are especially preferred. Preferred MHC-binding agretopes are those agretopes that are predicted to bind at a 3% threshold to MHC alleles and are present in at least 5% of the population.

[55] In an additional preferred embodiment, MHC-binding agretopes are identified as the 9-mer frames that are located among "nested" agretopes, or overlapping 9-residue frames that are each predicted to bind a significant number of alleles. Such sequences may be especially likely to elicit an immune response. Preferred MHC-binding agretopes are those agretopes that are predicted to bind, at a 3% threshold, to MHC alleles that are present in at least 5% of the population. Especially preferred MHC-binding agretopes are those agretopes that are predicted to bind, at a 1% threshold, to MHC alleles that are present in at least 10% of the population.

[56] Preferred MHC-binding agretopes in BAFF include, but are not limited to, agretope 2: residues 168-176; agretope 3: residues 169-177; agretope 6: residues 192-200; agretope 7: residues 193-201; agretope 9: residues 200-208; agretope 10: residues 212-220; agretope 12: residues 226-234; agretope 14: residues 230-238; agretope 16: residues 276-284. Especially preferred MHC-binding agretopes in BAFF include, but are not limited to, agretope 2: residues 168-176; agretope 6: residues 192-200; agretope 9: residues 200-208; agretope 10: residues 212-220; agretope 12: residues 226-234; agretope 16: residues 276-284.

[57] Preferred MHC-binding agretopes in RANKL include, but are not limited to, agretope 2: residues 207-215; agretope 3: residues 213-221; agretope 4: residues 214-222; agretope 5: residues 215-223; agretope 6: residues 222-230; agretope 9: residues 238-246; agretope 10: residues 239-247; agretope 12: residues 241-249; agretope 14: residues 270-278; agretope 15: residues 277-285; agretope 17: residues 289-297; agretope 18: residues 308-316. Especially preferred MHC-binding agretopes in RANKL include, but are not limited to, agretope 3: residues 213-221; agretope 4: residues 214-222; agretope 10: residues 239-247; agretope 12: residues 241-249; agretope 15: residues 277-285; agretope 17: residues 289-297.

[58] Preferred MHC-binding agretopes in APRIL include, but are not limited to, agretope 1: residues 117-125; agretope 2: residues 120-128; agretope 4: residues 138-146; agretope 5: residues 142-150; agretope 6: residues 155-163; agretope 7: residues 162-170; agretope 9: residues 164-172; agretope 10: residues 170-178; agretope 11: residues 194-202; agretope 12: residues 197-205; agretope 15: residues 228-236. Especially preferred MHC-binding agretopes in APRIL include, but are not limited to, agretope 5: residues 142-150; agretope 9: residues 164-172; agretope 10: residues 170-178; agretope 11: residues 194-202.

[59] Preferred MHC-binding agretopes in CD40L include, but are not limited to, agretope 1: residues 145-153; agretope 2: residues 146-154; agretope 3: residues 152-160; agretope 4: residues 168-176; agretope 5: residues 169-177; agretope 6: residues 170-178; agretope 7: residues 171-179; agretope 9: residues 189-197; agretope 10: residues 204-212; agretope 11: residues 205-213; agretope 12: residues 206-214; agretope 13: residues 223-231; agretope 14: residues 229-237; agretope 15: residues 237-245. Especially preferred MHC-binding agretopes in CD40L include, but are not limited to, agretope 12: residues 206-214.

[60] Preferred MHC-binding agretopes in TRAIL include, but are not limited to, agretope 1: residues 151-159; agretope 2: residues 174-182; agretope 3: residues 181-189; agretope 6: residues 206-214; agretope 7: residues 207-215; agretope 9: residues 220-228; agretope 10: residues 221-229; agretope 12: residues 237-245; agretope 14: residues 256-264; agretope 15: residues 257-265. Especially preferred MHC-binding agretopes in TRAIL include, but are not limited to, agretope 2: residues 174-182; agretope 7: residues 207-215; agretope 10: residues 221-229; agretope 14: residues 256-264; agretope 15: residues 257-265.

[61] Confirmation of MHC-binding agretopes

[62] In a preferred embodiment, the immunogenicity of the above-predicted MHC-binding agretopes is experimentally confirmed by measuring the extent to which peptides comprising each predicted agretope can elicit an immune response. However, it is possible to proceed from agretope prediction to agretope removal without the intermediate step of agretope confirmation.

[63] Several methods, discussed in more detail below, can be used for experimental confirmation of agretopes. For example, sets of naïve T cells and antigen presenting cells from matched donors can be stimulated with a peptide containing an agretope of interest, and T-cell activation can be monitored. It is also possible to first stimulate T cells with the whole protein of interest, and then re-stimulate with peptides derived from the whole protein. If sera are available from patients who have raised an immune response to TNF Super Family, it is possible to detect mature T cells that respond to specific epitopes. In a preferred embodiment, interferon gamma or IL-5 production by activated T-cells is monitored using Elispot assays, although it is also possible to use other indicators of T-cell activation or proliferation such as tritiated thymidine incorporation or production of other cytokines.

[64] Patient genotype analysis and screening

[65] HLA genotype is a major determinant of susceptibility to specific autoimmune diseases (see for example Nepom Clin. Immunol. Immunopathol. 67: S50-S55 (1993), entirely incorporated by reference) and infections (see for example Singh et. al. Emerg. Infect. Dis. 3: 41-49 (1997), entirely incorporated by reference). Furthermore, the set of MHC alleles present in an individual can affect the efficacy of some vaccines (see for example Cailat-Zucman et. al. Kidney Int. 53: 1626-1630 (1998) and Poland et. al. Vaccine 20: 430-438 (2001), both entirely incorporated by reference). HLA genotype may also confer susceptibility for an individual to elicit an unwanted immune response to a TNF Super Family therapeutic.

[66] In a preferred embodiment, class II MHC alleles that are associated with increased or decreased susceptibility to elicit an immune response to TNF Super Family proteins are identified. For example, patients treated with TNF Super Family therapeutics may be tested for the presence of anti-TNF Super Family antibodies and genotyped for class II MHC. Alternatively, T-cell activation assays such as those described above may be conducted using cells derived from a number of genotyped donors. Alleles that confer susceptibility to TNF Super Family immunogenicity may be defined as those alleles that are significantly more common in those who elicit an immune response versus those who do not. Similarly, alleles that confer resistance to TNF Super Family immunogenicity may be defined as those that are significantly less common in those who do not elicit an immune response versus those that do. It is also possible to use purely computational techniques to identify which alleles are likely to

recognize TNF Super Family therapeutics. In one embodiment, the genotype association data is used to identify patients who are especially likely or especially unlikely to raise an immune response to a TNF Super Family therapeutic.

[67] Design of active, less-immunogenic variants

[68] In a preferred embodiment, the above-determined MHC-binding agretopes are replaced with alternate amino acid sequences to generate active variant TNF Super Family proteins with reduced or eliminated immunogenicity. Alternatively, the MHC-binding agretopes are modified to introduce one or more sites that are susceptible to cleavage during protein processing. If the agretope is cleaved before it binds to a MHC molecule, it will be unable to promote an immune response. There are several possible strategies for integrating methods for identifying less immunogenic sequences with methods for identifying structured and active sequences, including but not limited to those presented below.

[69] In one embodiment, for one or more 9-mer agretope identified above, one or more possible alternate 9-mer sequences are analyzed for immunogenicity as well as structural and functional compatibility. The preferred alternate 9-mer sequences are then defined as those sequences that have low predicted immunogenicity and a high probability of being structured and active. It is possible to consider only the subset of 9-mer sequences that are most likely to comprise structured, active, less immunogenic variants. For example, it may be unnecessary to consider sequences that comprise highly non-conservative mutations or mutations that increase predicted immunogenicity.

[70] In a preferred embodiment, less immunogenic variants of each agretope are predicted to bind MHC alleles in a smaller fraction of the population than the wild type agretope. In an especially preferred embodiment, the less immunogenic variant of each agretope is predicted to bind to MHC alleles that are present in not more than 5 % of the population, with not more than 1 % or 0.1 % being most preferred.

[71] Substitution matrices

[72] In another especially preferred embodiment, substitution matrices or other knowledge-based scoring methods are used to identify alternate sequences that are likely to retain the structure and function of the wild type protein. Such scoring methods can be used to quantify how conservative a given substitution or set of substitutions is. In most cases, conservative mutations do not significantly disrupt the structure and function of proteins (see for example, Bowie et. al. Science 247: 1306-1310 (1990), Bowie and Sauer Proc. Nat. Acad. Sci. USA 86: 2152-2156 (1989), and Reidhaar-Olson and Sauer Proteins 7: 306-316 (1990), entirely incorporated by reference). However, non-conservative mutations can destabilize protein structure and reduce activity (see for example, Lim et. al. Biochem. 31: 4324-4333 (1992)). Substitution matrices including but not limited to BLOSUM62 provide a quantitative measure of the compatibility between a sequence and a target structure, which can be used to predict non-disruptive substitution mutations (see Topham et al. Prot. Eng. 10: 7-21 (1997), entirely incorporated by reference). The use of substitution matrices to design peptides with improved properties has been disclosed; see Adenot et al. J. Mol. Graph. Model. 17: 292-309 (1999), entirely incorporated by reference.

- [73] Substitution matrices include, but are not limited to, the BLOSUM matrices (Henikoff and Henikoff, Proc. Nat. Acad. Sci. USA 89: 10917 (1992), entirely incorporated by reference, the PAM matrices, the Dayhoff matrix, and the like. For a review of substitution matrices, see for example Henikoff Curr. Opin. Struct. Biol. 6: 353-360 (1996), entirely incorporated by reference. It is also possible to construct a substitution matrix based on an alignment of a given protein of interest and its homologs; see for example Henikoff and Henikoff Comput. Appl. Biosci. 12: 135-143 (1996), entirely incorporated by reference. In a preferred embodiment, each of the substitution mutations that are considered has a BLOSUM 62 score of zero or higher. According to this metric, preferred substitutions include, but are not limited to:

Table 1. Conservative mutations	
Wild type residue	Preferred substitutions
A	C S T A G V
C	C A
D	S N D E Q
E	S N D E Q H R K
F	M I L F Y W
G	S A G N
H	N E Q H R Y
I	M I L V F
K	S N E Q R K
L	M I L V F

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

- [74] In addition, it is preferred that the total BLOSUM 62 score of an alternate sequence for a nine residue MHC-binding epitope is decreased only modestly when compared to the BLOSUM 62 score of the wild type nine residue epitope. In a preferred embodiment, the score of the variant 9-mer is at least 50 % of the wild type score, with at least 67%, 75%, 80% or 90% being more preferred.
- [75] Alternatively, alternate sequences can be selected that minimize the absolute reduction in BLOSUM score; for example it is preferred that the score decrease for each 9-mer is less than 20, with score decreases of less than about 10 or about 5 being especially preferred. The exact value may be chosen to produce a library of alternate sequences that is experimentally tractable and also sufficiently diverse to encompass a number of active, stable, less immunogenic variants.
- [76] In a preferred embodiment, substitution mutations are preferentially introduced at positions that are substantially solvent exposed. As is known in the art, solvent exposed positions are typically more tolerant of mutation than positions that are located in the core of the protein.
- [77] In another preferred embodiment, substitution mutations are preferentially introduced at positions that are not highly conserved. As is known in the art, positions that are highly conserved among members of a protein family are often important for protein function, stability, or structure, while positions that are not highly conserved often may be modified without significantly impacting the structural or functional properties of the protein.
- [78] Alanine substitutions
- [79] In an alternate embodiment, one or more alanine substitutions may be made, regardless of whether an alanine substitution is conservative or non-conservative. As is known in the art, incorporation of sufficient alanine substitutions may be used to disrupt intermolecular interactions.

- [80] In a preferred embodiment, variant 9-mers are selected such that residues that have been or can be identified as especially critical for maintaining the structure or function of TNF Super Family retain their wild type identity. In alternate embodiments, it may be desirable to produce variant TNF Super Family proteins that do not retain wild type activity. In such cases, residues that have been identified as critical for function may be specifically targeted for modification.
- [81] Positions that mediate binding to the receptors BAFF-R, TACI, and BCMA include, but are not limited to, Q159, Y163, D203, T205, Y206, A207, L211, R231, I233, P264, R265, and D275, more preferably D203, T205, Y206, I233, R265, and D275. Residues that may impact the oligomer subunit exchange properties of BAFF include, but are not limited to, T205, Y206, F220, E223, V227, T228, I233, L240, D273 and D275.
- [82] RANKL contacts its receptor, RANK, and its decoy receptor, OPG, through three dimensional epitopes located in the Large Domain, the Small Domain, and the DE loop. Modifications to the receptor contact positions are expected to have direct effects on receptor binding or signaling. Positions that contact receptor include, but are not limited to, the Large Domain positions 172, 187-193, 222-228, 267-270, 297, and 300-302; the Small Domain positions 179-183 and 233-241; and the DE Loop positions 246-253 and 284.
- [83] RANKL is active as a trimer. Accordingly, modifications to the trimer interface positions are expected to have direct effects on RANKL activity. The trimer Interface includes positions 163, 165, 167, 193, 195, 213, 215, 217, 219, 221, 235, 237, 239, 244, 253-264, 268, 271-282, 300, 302, 304-305, 307, 311, and 313-314.
- [84] Homology modeling with APRIL's closest homolog (BAFF) and sequence alignment with homologous TNF ligands can be used to predict positions important for structure, receptor binding and activity (Karpusas et al. J Mol Biol 315: 1145-1154 (2002)). A polymorphism in the APRIL gene resulting in amino acid substitution G67R is associated with SLE (Koyama et al. Rheumatology (Oxford) 42: 980-985 (2003), entirely incorporated by reference).
- [85] Furthermore, a number of residues may be targeted for mutagenesis in order to yield a APRIL variant that functions as an antagonist, receptor specific agonist, or superagonist. Suitable residues include but are not limited to Large Domain receptor contact residues (positions 121, 139-142, 170-174, 205-208, and 237-241), Small Domain receptor contact residues (positions 175-181 and 195-197), and DE loop receptor contact residues (positions 186-190). In addition, trimer interface positions may be modified, for example to promote trimer exchange or to stabilize desired trimeric structures. Trimer interface positions include but are not limited to residues 115, 117, 119, 142, 144, 162, 164, 166, 168, 170, 176, 177, 192, 194, 201, 208-216, 237, 239, 241, 242, 245, 248, 250, and 251. Especially preferred trimer interface positions are APRIL positions 142, 144, 162, 164, 216 and 251.
- [86] Mutagenesis studies indicate that CD40L residues K143, Y145, Y146, R203, R207, and Q220 are important for CD40 receptor binding and/or activity (Bajorath et al. Biochemistry 34: 1833-1844 (1995), Bajorath et al. Biochemistry 34: 9884-9892 (1995), Singh et al. Protein Sci 7: 1124-1135 (1998)), entirely incorporated by reference. CD40L mutations associated with the X-linked form of hyper-IgM syndrome disrupt the normal function of CD40L; these mutations include A123E, H125R, V126D, V126A, W140C, W140G, W140R, W140X, G144E, T147N, L155P, Y170P, A173D, Q174R,

T176I, A183@, S184X, Q186X, L193@, L195P, R200X, E202X, A208D, C218X, Q220X, Q221X, H224Y, G226A, G227V, L231S, Q232X, A235P, S236X, V237E, T254M, G257D, G257S, and L258S, where X denotes a deletion of the amino acid and @ denotes insertions of one or more amino acids at these locations (uta.fi/imt/bioinfo/CD40Lbase).

[87] Furthermore, a number of residues may be targeted for mutagenesis in order to yield a CD40L variant that functions as an antagonist of wild type CD40L protein or as a superagonist of CD40. Suitable residues include but are not limited to Large Domain receptor contact residues (positions 28-34, 63-69, 112-115, and 137-14), Small Domain receptor contact residues (positions 72-79 and 95-98), and DE loop receptor contact residues (positions 84-89). In addition, trimer interface positions may be modified, for example to promote trimer exchange or to stabilize desired trimeric structures. Trimer interface positions include but are not limited to residues 11, 13, 15, 34, 36, 53-55, 57, 59, 61, 63, 72, 73, 75, 77, 119, 87, 91-99, 102-104, 109, 112-125, 147-149, 151, and 155-157. Especially preferred trimer interface positions to be modified are positions 57, 34, and 91.

[88] Alanine scanning mutagenesis of TRAIL reveals two clusters of residues essential for receptor binding and biological activity; these are located along the walls of a surface crevice formed by adjoining monomers that runs from the wider part of the trimer to the variable loops at the tip. Substitutions at Tyr216 at the top and Gln 205 at the tip each decreased apoptotic activity more than 300-fold, while substitutions at Val207, Glu236, or Tyr237 decreased activity more than 5-fold; all but one of these point mutants showed at least a 5-fold decreased affinity for DR4, DR5, and DcR2. Mutants D218A and D269A slightly increased apoptotic activity, but did not affect receptor binding. A zinc atom coordinated by three symmetry-related Cys230 residues in the trimerization interface appears essential for trimer stability and optimal biological activity; mutation of Cys230 to alanine or serine results in 20- and 70-fold reductions in apoptotic activity, respectively, decreases receptor binding by at least 200-fold, and reduces the stability of the trimeric structure. Removal of zinc from wild type TRAIL by dialysis with chelating agents results in a significant decrease in receptor binding affinity and a 90-fold reduction in apoptotic activity; zinc depleted TRAIL forms poorly active, disulfide-linked dimers. See Bodmer et al. *J Biol Chem* 275: 20632-20637 (2000), Hymowitz et al. *Biochemistry* 39: 633-640 (2000), entirely incorporated by reference.

[89] Based on a model of the TRAIL-sDR4 complex, deletion of the AA" insertion loop (TRAIL residues 137-152) and point mutants of residues believed to interact with the receptor (E144N/K on the β turn of the AA" loop, D218N/K on the DE loop and D267N/K on the GH loop) resulted in decreased or no cytotoxic activity using a Jurkat T cell assay. Decreased cytotoxic activity or sDR5 binding was also obtained with other TRAIL variants containing deletions in the AA" loop (residues 132-135, Ser-Leu-Leu sequence instead of residues 135-153). It has therefore been suggested that the frame insertion of 12-16 amino acids in the AA" loop, unique to TRAIL among TNF family ligands, is critical in providing the conformational flexibility required for translocation of the AA" loop to the central binding interface upon complex formation, and may be important in conferring receptor recognition specificity. See Cha et al. *Immunity* 11: 253-261 (1999), Mongkolsapaya et al. *Nat Struct Biol* 6: 1048-1053 (1999), Cha et al. *J Biol Chem* 275: 31171-31177 (2000), entirely incorporated by reference.

[90] Leucine zippers introduced to the N-terminus to facilitate multimerization result in mutants (LZ-TRAIL) that are superior to normal and cross-linked TRAIL in causing cell lysis in human and mouse cell lines; LZ-TRAIL also confers survival to tumor challenge in mice without hepatotoxicity. See Walczak et al. Nat Med 5: 157-163 (1999), entirely incorporated by reference. It has been suggested that substitution of Asn228 with a large hydrophobic residue could induce stronger intersubunit interactions with Tyr240 and improve stability.

[91] Protein Design Methods

[92] Protein design methods and MHC agretope identification methods may be used together to identify stable, active, and minimally immunogenic protein sequences (see WO03/006154, entirely incorporated by reference). The combination of approaches provides significant advantages over the prior art for immunogenicity reduction, as most of the reduced immunogenicity sequences identified using other techniques fail to retain sufficient activity and stability to serve as therapeutics.

[93] Protein design methods may identify non-conservative or unexpected mutations that nonetheless confer desired functional properties and reduced immunogenicity, as well as identifying conservative mutations. Nonconservative mutations are defined herein to be all substitutions not included in Table 1 above; nonconservative mutations also include mutations that are unexpected in a given structural context, such as mutations to hydrophobic residues at the protein surface and mutations to polar residues in the protein core.

[94] Furthermore, protein design methods may identify compensatory mutations. For example, if a given first mutation that is introduced to reduce immunogenicity also decreases stability or activity, protein design methods may be used to find one or more additional mutations that serve to recover stability and activity while retaining reduced immunogenicity. Similarly, protein design methods may identify sets of two or more mutations that together confer reduced immunogenicity and retained activity and stability, even in cases where one or more of the mutations, in isolation, fails to confer desired properties.

[95] A wide variety of methods are known for generating and evaluating sequences. These include, but are not limited to, sequence profiling (Bowie and Eisenberg, Science 253(5016): 164-70, (1991)), residue pair potentials (Jones, Protein Science 3: 567-574, (1994)), and rotamer library selections (Dahiyat and Mayo, Protein Sci 5(5): 895-903 (1996); Dahiyat and Mayo, Science 278(5335): 82-7 (1997); Desjarlais and Handel, Protein Science 4: 2006-2018 (1995); Harbury et al, PNAS USA 92(18): 8408-8412 (1995); Kono et al., Proteins: Structure, Function and Genetics 19: 244-255 (1994); Hellinga and Richards, PNAS USA 91: 5803-5807 (1994), entirely incorporated by reference).

[96] Protein Design Automation® (PDA®) technology

[97] In an especially preferred embodiment, rational design of improved TNF Super Family variants is achieved by using Protein Design Automation® (PDA®) technology. (See U.S. Patent Nos. 6,188,965; 6,269,312; 6,403,312; WO98/47089 and USSNs 09/058,459, 09/127,926, 60/104,612, 60/158,700, 09/419,351, 60/181,630, 60/186,904, 09/419,351, 09/782,004 and 09/927,790, 60/347,772, and 10/218,102; and PCT/US01/218,102 and U.S.S.N. 10/218,102, U.S.S.N. 60/345,805; U.S.S.N. 60/373,453 and U.S.S.N. 60/374,035, all entirely incorporated by reference.)

- [98] PDA® technology couples computational design algorithms that generate quality sequence diversity with experimental high-throughput screening to discover proteins with improved properties. PDA® utilizes three-dimensional structural information. The computational component uses atomic level scoring functions, side chain rotamer sampling, and advanced optimization methods to accurately capture the relationships between protein sequence, structure, and function. Calculations begin with the three-dimensional structure of the protein and a strategy to optimize one or more properties of the protein. PDA® technology then explores the sequence space comprising all pertinent amino acids (including unnatural amino acids, if desired) at the positions targeted for design. This is accomplished by sampling conformational states of allowed amino acids and scoring them using a parameterized and experimentally validated function that describes the physical and chemical forces governing protein structure. Powerful combinatorial search algorithms are then used to search through the initial sequence space, which may constitute 10^{50} sequences or more, and quickly return a tractable number of sequences that are predicted to satisfy the design criteria. Useful modes of the technology span from combinatorial sequence design to prioritized selection of optimal single site substitutions. PDA® technology has been applied to numerous systems including important pharmaceutical and industrial proteins and has a demonstrated record of success in protein optimization.
- [99] In a most preferred embodiment, the structure of a TNF Super Family member is determined using X-ray crystallography or NMR methods, which are well known in the art. Crystal structures of some human TNF Super Family members have been solved to high resolution: human BAFF (PDB code 1KXG; Oren et al. 2002 Nat. Struct. Biol. 9: 288), human TRAIL (PDB code 1D4V; Mongkolsapaya et al. 1999 Nat. Struct. Biol. 6:1043), human CD40L (PDB code 1ALY; Karpusas et al. 1995 Structure 3:1426), all entirely incorporated by reference. Using homology modeling methods known in the art, the structures of human RANKL and APRIL were determined using the sequences of human RANKL and human APRIL and the structures of murine RANKL (PDB code 1IQA; Ito et al. 2002 J. Biol. Chem. 277: 6631) and murine APRIL (PDB code 1XU2; Hymowitz et al. 2005 J. Biol. Chem. 280:7218), both entirely incorporated by reference. Furthermore, crystal structures of the BAFF / BAFF-R complex (PDB codes 1OTZ and 1P0T; Kim et al. 2003 Nat. Struct. Biol. 10:342), the BAFF / BCMA complex (PDB code 1OQD; Liu et al. 2003 Nature 423:49), the TRAIL / Death Receptor 5 complex (PDB code 1D0G; Hymowitz et al. 1999 Mol. Cell 4:563), the APRIL / TACI complex (PDB code 1XU1; Hymowitz et al. 2005 J. Biol. Chem. 280:7218), and the APRIL / BCMA complex (PDB code 1Xu2; Hymowitz et al. 2005 J. Biol. Chem. 280:7218) have been determined, all entirely incorporated by reference.
- [100] In a preferred embodiment, the results of matrix method calculations are used to identify which of the 9 amino acid positions within the agretope(s) contribute most to the overall binding propensities for each particular allele "hit". This analysis considers which positions (P1-P9) are occupied by amino acids which consistently make a significant contribution to MHC binding affinity for the alleles scoring above the threshold values. Matrix method calculations are then used to identify amino acid substitutions at said positions that would decrease or eliminate predicted immunogenicity and PDA®

technology is used to determine which of the alternate sequences with reduced or eliminated immunogenicity are compatible with maintaining the structure and function of the protein.

[101] In an alternate preferred embodiment, the residues in each agretope are first analyzed by one skilled in the art to identify alternate residues that are potentially compatible with maintaining the structure and function of the protein. Then, the set of resulting sequences are computationally screened to identify the least immunogenic variants. Finally, each of the less immunogenic sequences are analyzed more thoroughly in PDA® technology protein design calculations to identify protein sequences that maintain the protein structure and function and decrease immunogenicity.

[102] In an alternate preferred embodiment, each residue that contributes significantly to the MHC binding affinity of an agretope is analyzed to identify a subset of amino acid substitutions that are potentially compatible with maintaining the structure and function of the protein. This step may be performed in several ways, including PDA® calculations or visual inspection by one skilled in the art. Sequences may be generated that contain all possible combinations of amino acids that were selected for consideration at each position. Matrix method calculations can be used to determine the immunogenicity of each sequence. The results can be analyzed to identify sequences that have significantly decreased immunogenicity. Additional PDA® calculations may be performed to determine which of the minimally immunogenic sequences are compatible with maintaining the structure and function of the protein.

[103] In an alternate preferred embodiment, pseudo-energy terms derived from the peptide binding propensity matrices are incorporated directly into the PDA® technology calculations. In this way, it is possible to select sequences that are active and less immunogenic in a single computational step.

[104] Combining immunogenicity reduction strategies

[105] In a preferred embodiment, more than one method is used to generate variant proteins with desired functional and immunological properties. For example, substitution matrices may be used in combination with PDA® technology calculations. Strategies for immunogenicity reduction include, but are not limited to, those described in USSN 11/004,590, filed December 3, 2004, entirely incorporated by reference.

[106] In a preferred embodiment, a variant protein with reduced binding affinity for one or more class II MHC alleles is further engineered to confer improved solubility. As protein aggregation may contribute to unwanted immune responses, increasing protein solubility may reduce immunogenicity (see for example SIFN).

[107] In an additional preferred embodiment, a variant protein with reduced binding affinity for one or more class II MHC alleles is further modified by derivitization with PEG or another molecule. As is known in the art, PEG may sterically interfere with antibody binding or improve protein solubility, thereby reducing immunogenicity. In an especially preferred embodiment, rational PEGylation methods are used USSN 10/956,352, filed September 30, 2004, entirely incorporated by reference. In a preferred embodiment, PDA® technology and matrix method calculations are used to remove more than one MHC-binding agretope from a protein of interest.

[108] Generating the variants

- [109] Variant TNF Super Family proteins of the invention and nucleic acids encoding them may be produced using a number of methods known in the art. In a preferred embodiment, nucleic acids encoding the TNF Super Family variants are prepared by total gene synthesis, or by site-directed mutagenesis of a nucleic acid encoding a parent TNF Super Family protein. Methods including template-directed ligation, recursive PCR, cassette mutagenesis, site-directed mutagenesis or other techniques that are well known in the art may be utilized (see for example Strizhov et al. PNAS 93:15012-15017 (1996), Prodromou and Perl, Prot. Eng. 5: 827-829 (1992), Jayaraman and Puccini, Biotechniques 12: 392-398 (1992), and Chalmers et al. Biotechniques 30: 249-252 (2001)), entirely incorporated by reference.
- [110] In a preferred embodiment, TNF Super Family variants are cloned into an appropriate expression vector and expressed in *E. coli* (see McDonald, J.R., Ko, C., Mismar, D., Smith, D.J. and Collins, F. Biochim. Biophys. Acta 1090: 70-80 (1991), entirely incorporated by reference). In an alternate preferred embodiment, TNF Super Family variants are expressed in mammalian cells, yeast, baculovirus, or *in vitro* expression systems. A number of expression systems and methods for their use are well known in the art (see Current Protocols in Molecular Biology, Wiley & Sons, and Molecular Cloning - A Laboratory Manual - 3rd Ed., Cold Spring Harbor Laboratory Press, New York (2001), entirely incorporated by reference). The choice of codons, suitable expression vectors and suitable host cells will vary depending on a number of factors, and may be easily optimized as needed.
- [111] In a preferred embodiment, the TNF Super Family variants are purified or isolated after expression. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing. For example, a TNF Super Family variant may be purified using a standard anti-recombinant protein antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., Protein Purification, Springer-Verlag, NY, 3rd ed. (1994), entirely incorporated by reference. The degree of purification necessary will vary depending on the desired use, and in some instances no purification will be necessary.
- [112] Assaying the activity of the variants
- [113] The variant TNF Super Family proteins of the invention may be tested for activity using any of a number of methods, including but not limited to those described below. Suitable binding assays may be used. The kinetic association rate (K_{on}) and dissociation rate (K_{off}), and the equilibrium binding constants (K_d) may be determined using surface plasmon resonance on a BIAcore instrument following the standard procedure in the literature [Pearce et al., Biochemistry 38:81-89 (1999), entirely incorporated by reference]. Binding affinity and kinetics may also be characterized using proximity assays such as AlphaScreen™ (Packard BioScience®) or microcalorimetry (Isothermal Titration Calorimetry, Differential Scanning Calorimetry), entirely incorporated by reference. Cell-based activity assays include but are not limited to, NF- κ B nuclear translocation (Wei et al., Endocrinology 142, 1290-1295, (2001)) or c-Jun (Srivastava et al., JBC 276, 8836-8840 (2001), entirely incorporated by reference) transcription factor activation assays, B-cell proliferation assays, and IgE secretion assays.

[114] Determining the immunogenicity of the variants

[115] In a preferred embodiment, the immunogenicity of the TNF Super Family variants is determined experimentally to confirm that the variants do have reduced or eliminated immunogenicity relative to the parent protein. In a preferred embodiment, *ex vivo* T-cell activation assays are used to experimentally quantitate immunogenicity. In this method, antigen presenting cells and naïve T cells from matched donors are challenged with a peptide or whole protein of interest one or more times. Then, T cell activation can be detected using a number of methods, for example by monitoring production of cytokines or measuring uptake of tritiated thymidine. In the most preferred embodiment, interferon gamma production is monitored using Elispot assays (see Schmittl *et. al.* J. Immunol. Meth., 24: 17-24 (2000), entirely incorporated by reference). Other suitable T-cell assays include those disclosed in Meidenbauer, et al. Prostate 43, 88-100 (2000); Schultes, B.C and Whiteside, T.L., J. Immunol. Methods 279, 1-15 (2003); and Stickler, et al., J. Immunotherapy, 23, 654-660 (2000), all entirely incorporated by reference.

[116] In a preferred embodiment, the PBMC donors used for the above-described T-cell activation assays will comprise class II MHC alleles that are common in patients requiring treatment for TNF Super Family responsive disorders. For example, for most diseases and disorders, it is desirable to test donors comprising all of the alleles that are prevalent in the population. However, for diseases or disorders that are linked with specific MHC alleles, it may be more appropriate to focus screening on alleles that confer susceptibility to TNF Super Family responsive disorders. In a preferred embodiment, the MHC haplotype of PBMC donors or patients that raise an immune response to the wild type or variant TNF Super Family are compared with the MHC haplotype of patients who do not raise a response. This data may be used to guide preclinical and clinical studies as well as aiding in identification of patients who will be especially likely to respond favorably or unfavorably to the TNF Super Family therapeutic.

[117] In an alternate preferred embodiment, immunogenicity is measured in transgenic mouse systems. For example, mice expressing fully or partially human class II MHC molecules may be used. In an alternate embodiment, immunogenicity is tested by administering the TNF Super Family variants to one or more animals, including rodents and primates, and monitoring for antibody formation. Non-human primates with defined MHC haplotypes may be especially useful, as the sequences and hence peptide binding specificities of the MHC molecules in non-human primates may be very similar to the sequences and peptide binding specificities of humans. Similarly, genetically engineered mouse models expressing human MHC peptide-binding domains may be used (see for example Sonderstrup *et. al.* Immunol. Rev. 172: 335-343 (1999) and Forsthuber *et. al.* J. Immunol. 167: 119-125 (2001), entirely incorporated by reference).

[118] Formulation and administration to patients

[119] Once made, the variant TNF Super Family proteins and nucleic acids of the invention find use in a number of applications. In a preferred embodiment, the variant TNF Super Family proteins are administered to a patient to treat a TNF Super Family responsive disorder. Administration may be therapeutic or prophylactic.

[120] The pharmaceutical compositions of the present invention comprise a variant TNF Super Family protein in a form suitable for administration to a patient. In a preferred embodiment, the pharmaceutical compositions are in a water soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[121] The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers such as NaOAc; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations. Combinations of pharmaceutical compositions may be administered. Moreover, the compositions may be administered in combination with other therapeutics.

[122] The administration of the variant TNF Super Family proteins of the present invention, preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, parenterally, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, the variant TNF Super Family protein may be directly applied as a solution or spray. Depending upon the manner of introduction, the pharmaceutical composition may be formulated in a variety of ways. In a preferred embodiment, a therapeutically effective dose of a variant TNF Super Family protein is administered to a patient in need of treatment. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. In a preferred embodiment, the concentration of the therapeutically active variant TNF Super Family protein in the formulation may vary from about 0.1 to about 100 weight %. In another preferred embodiment, the concentration of the variant TNF Super Family protein is in the range of 0.003 to 1.0 molar, with dosages from 0.03, 0.05, 0.1, 0.2, and 0.3 millimoles per kilogram of body weight being preferred. As is known in the art, adjustments for variant TNF Super Family protein degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the

condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[123] In an alternate embodiment, variant TNF Super Family nucleic acids may be administered; i.e., “gene therapy” approaches may be used. In this embodiment, variant TNF Super Family nucleic acids are introduced into cells in a patient in order to achieve in vivo synthesis of a therapeutically effective amount of variant TNF Super Family protein. Variant TNF Super Family nucleic acids may be introduced using a number of techniques, including but not limited to transfection with liposomes, viral (typically retroviral) vectors, and viral coat protein-liposome mediated transfection [Dzau et al., Trends in Biotechnology 11:205-210 (1993), entirely incorporated by reference]. In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu et al., J. Biol. Chem. 262:4429-4432 (1987); and Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 87:3410-3414 (1990), entirely incorporated by reference. For review of gene marking and gene therapy protocols see Anderson et al., Science 256:808-813 (1992), entirely incorporated by reference.

Examples

[124] Example 1. Identification of MHC-binding agretopes in TNF SF members

[125] Matrix method calculations (Sturniolo, supra) were conducted using the parent TNF SF members sequences: BAFF (SEQ_ID_NO:1); RANKL (SEQ_ID_NO:2); and APRIL (SEQ_ID_NO:3).

[126] Agretopes were predicted for the following alleles, each of which is present in at least 1% of the US population: DRB1*0101, DRB1*0102, DRB1*0301, DRB1*0401, DRB1*0402, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*0701, DRB1*0801, DRB1*1101, DRB1*1102, DRB1*1104, DRB1*1301, DRB1*1302, DRB1*1501, and DRB1*1502.

[127] **Table 2.** Predicted MHC-binding agretopes in TNF SF members. Iscore, the number of alleles, and the percent of the population hit at 1%, 3%, and 5% thresholds are shown. Especially preferred agretopes are predicted to affect at least 10% of the population, using a 1% threshold.

Table 2.A. Predicted MHC-binding agretopes in BAFF.									
Agretope number	Residues	Sequence	Iscore	1% hits	3% hits	5% hits	1% pop	3% pop	5% pop
Ag. A1	163 - 171	YTFVPWLLS	2.8	0	0	1	0.00	0.00	0.11
Ag. A2	168 - 176	WLLSFKRGS	24.2	2	4	4	0.10	0.29	0.29
Ag. A3	169 - 177	LLSFKRGSA	14.3	0	1	2	0.00	0.23	0.24
Ag. A4	185 - 193	ILVKETGYF	1.3	0	0	1	0.00	0.00	0.05
Ag. A5	186 - 194	LVKETGYFF	1.2	0	0	1	0.00	0.00	0.05
Ag. A6	192 - 200	YFFIYGQVL	42.5	2	2	3	0.34	0.34	0.37
Ag. A7	193 - 201	FFIYGQVLY	3.6	0	1	2	0.00	0.05	0.07
Ag. A8	194 - 202	FIYGQVLYT	0.5	0	0	1	0.00	0.00	0.02
Ag. A9	200 - 208	LYTDKTYAM	26.2	1	1	1	0.21	0.21	0.21

Ag. A10	212 - 220	IQRKKVHVF	26.4	2	4	5	0.19	0.24	0.25
Ag. A11	219 - 227	VFGDELSLV	5.2	0	0	1	0.00	0.00	0.21
Ag. A12	226 - 234	LVTLFRCIQ	27.9	4	4	5	0.21	0.21	0.29
Ag. A13	227 - 235	VTLFRCIQN	5.6	0	0	1	0.00	0.00	0.23
Ag. A14	230 - 238	FRCIQNMPE	16.6	2	3	7	0.03	0.17	0.35
Ag. A15	259 - 267	LQLAIPREN	3.3	0	0	3	0.00	0.00	0.14
Ag. A16	276 - 284	VTFFGALKL	40.9	1	3	4	0.23	0.43	0.46

Table 2.B. Predicted MHC-binding agretopes in RANKL.

Agrelope number	Residues	Sequence	Iscore	1% hits	3% hits	5% hits	1% pop	3% pop	5% pop
Ag. B1	193 - 201	WAKISNMTF	0.7	0	0	2	0%	0%	3%
Ag. B2	207 - 215	IVNQDGFYY	8.1	0	1	3	0%	5%	25%
Ag. B3	213 - 221	FYYLYANIC	27.9	2	5	6	13%	31%	35%
Ag. B4	214 - 222	YYLYANICF	34.7	2	2	5	24%	24%	45%
Ag. B5	215 - 223	YLYANICFR	5.5	0	1	1	0%	9%	9%
Ag. B6	222 - 230	FRHHETSGD	15.5	1	3	4	2%	23%	24%
Ag. B7	235 - 243	YLQLMVYVT	3.0	0	0	1	0%	0%	12%
Ag. B8	236 - 244	LQLMVYVTK	1.5	0	0	1	0%	0%	6%
Ag. B9	238 - 246	LMVYVTKTS	13.9	0	4	6	0%	18%	30%
Ag. B10	239 - 247	MVYVTKTSI	44.6	1	3	6	21%	43%	63%
Ag. B11	240 - 248	VYVTKTSIK	4.7	0	0	2	0%	0%	19%
Ag. B12	241 - 249	YVTKTSIKI	35.4	1	3	5	25%	30%	37%
Ag. B13	247 - 255	IKIPSSHTL	10.3	0	0	2	0%	0%	42%
Ag. B14	270 - 278	FHFYSINVG	9.4	0	3	3	0%	15%	15%
Ag. B15	277 - 285	VGGFFKLRS	45.1	4	6	7	26%	48%	49%
Ag. B16	280 - 288	FFKLRSCEE	0.4	0	0	1	0%	0%	2%
Ag. B17	289 - 297	ISIEVSNPS	18.5	1	1	2	14%	14%	19%
Ag. B18	308 - 316	FGAFKVRDI	18.5	1	1	4	9%	9%	40%

Table 2.C. Predicted MHC-binding agretopes in APRIL.

Agrelope number	Residues	Sequence	Iscore	1% hits	3% hits	5% hits	1% pop	3% pop	5% pop
Ag. C1	117 - 125	VLHLVPINA	22.2	1	4	5	5%	30%	34%
Ag. C2	120 - 128	LVPINATSK	4.3	0	2	2	0%	7%	7%
Ag. C3	121 - 129	VPINATSKD	0.4	0	0	1	0%	0%	2%
Ag. C4	138 - 146	WQPALRRGR	13.3	1	1	2	9%	9%	19%
Ag. C5	142 - 150	LRRGRGLQA	36.5	1	4	4	23%	37%	37%
Ag. C6	155 - 163	VRIQDAGVY	17.8	0	2	3	0%	25%	34%
Ag. C7	162 - 170	VYLLYSQVL	19.5	1	2	5	5%	17%	42%
Ag. C8	163 - 171	YLLYSQVLF	3.8	0	0	3	0%	0%	15%
Ag. C9	164 - 172	LLYSQVLFQ	35.4	2	6	8	18%	34%	49%
Ag. C10	170 - 178	LFQDVTFTM	26.2	1	1	1	21%	21%	21%
Ag. C11	194 - 202	FRCIRSMPS	56.7	7	9	14	35%	44%	78%
Ag. C12	197 - 205	IRSMPSHPD	7.6	1	2	5	2%	8%	15%
Ag. C13	217 - 225	FHLHQGDIL	0.5	0	0	1	0%	0%	2%
Ag. C14	227 - 235	VIIPRARAK	0.4	0	0	1	0%	0%	2%
Ag. C15	228 - 236	IIPRARAKL	3.1	0	1	1	0%	5%	5%
Ag. C16	236 - 244	LNLSPHGTF	5.2	0	0	1	0%	0%	21%

Ag. C17	238 - 246	LSPHGTFLG	3.0	0	0	2	0%	0%	12%
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Table 2.D. Predicted MHC-binding agretopes in CD40L.

Agrelope number	Residues	Sequence	Iscore	1% hits	3% hits	5% hits	1% pop	3% pop	5% pop
Ag. D1	145 - 153	YYTMSNNLV	17.74	2	4	6	0.03	0.22	0.33
Ag. D2	146 - 154	YTMSNNLVT	14.14	0	1	3	0.00	0.14	0.37
Ag. D3	152 - 160	LVTLENGKQ	9.35	0	3	5	0.00	0.09	0.25
Ag. D4	168 - 176	LYYIYAQVT	10.25	0	2	2	0.00	0.17	0.17
Ag. D5	169 - 177	YYIYAQVTF	7.7	0	2	3	0.00	0.11	0.15
Ag. D6	170 - 178	YIYAQVTFC	13.76	0	3	5	0.00	0.17	0.31
Ag. D7	171 - 179	IYAQVTFCS	18.95	0	6	7	0.00	0.27	0.37
Ag. D8	175 - 183	VTFCNSREA	1.04	0	1	1	0.00	0.02	0.02
Ag. D9	189 - 197	FIASLCLKS	14.62	0	2	2	0.00	0.24	0.24
Ag. D10	204 - 212	ILLRAANTH	4.51	0	2	3	0.00	0.07	0.08
Ag. D11	205 - 213	LLRAANTHS	12.4	1	3	5	0.06	0.09	0.23
Ag. D12	206 - 214	LRAANTHSS	48.23	6	10	10	0.31	0.48	0.48
Ag. D13	223 - 231	IHLGGVFEL	17.86	0	1	2	0.00	0.21	0.41
Ag. D14	229 - 237	FELQPGASV	7.56	0	1	1	0.00	0.12	0.12
Ag. D15	237 - 245	VFVNVTDP	8.6	0	1	1	0.00	0.14	0.14
Ag. D16	253 - 261	FTSFGLLKL	12.5	1	1	3	0.02	0.02	0.43

Table 2.E. Predicted MHC-binding agretopes in TRAIL.

Agrelope number	Residues	Sequence	Iscore	1% hits	3% hits	5% hits	1% pop	3% pop	5% pop
Ag. E1	151 - 159	INSWESSRS	5.9	1	2	3	2%	8%	9%
Ag. E2	174 - 182	LVIHEKGFY	46.1	4	4	5	37%	37%	40%
Ag. E3	181 - 189	FYYIYSQTY	21.0	0	3	5	0%	27%	45%
Ag. E4	182 - 190	YYIYSQTYF	1.2	0	1	1	0%	2%	2%
Ag. E5	183 - 191	YIYSQTYFR	3.7	0	0	2	0%	0%	15%
Ag. E6	206 - 214	MVQYIYKYT	14.3	0	1	2	0%	23%	24%
Ag. E7	207 - 215	VQYIYKYTS	47.2	3	5	6	33%	41%	47%
Ag. E8	209 - 217	YIYKYTSYP	1.3	0	0	1	0%	0%	5%
Ag. E9	220 - 228	ILLMKSARN	7.6	1	3	4	2%	9%	13%
Ag. E10	221 - 229	LLMKSARNS	28.5	4	5	5	21%	25%	25%
Ag. E11	223 - 231	MKSARNSCW	1.5	0	0	1	0%	0%	6%
Ag. E12	237 - 245	YGLYSIQQG	6.3	0	2	3	0%	7%	15%
Ag. E13	240 - 248	YSIQGGIF	1.2	0	1	1	0%	2%	2%
Ag. E14	256 - 264	IFVSVTNEH	21.8	1	3	5	14%	21%	23%
Ag. E15	257 - 265	FVSVTNEHL	33.9	1	3	4	25%	27%	36%

[128] **Table 3.** Predicted MHC-binding agretopes in TNF SF members. DRB1 alleles that are predicted to bind to each allele at 1%, 3%, 5% and 10% cutoffs are marked with "1", "3", "5" or "10" respectively.

Table 3.A. Alleles predicted to bind MHC agretopes in BAFF.

Agrelope number	101	102	301	401	402	404	405	408	701	801	1101	1102	1104	1301	1302	1501	1502
Ag. A1	-	-	-	-	-	-	-	10	-	-	5	-	-	-	-	-	-
Ag. A2	-	-	10	-	-	-	-	-	-	10	3	1	10	3	1	-	-

Ag. A3	-	-	-	-	-	-	-	-	-	-	10	-	10	-	-	3	5
Ag. A4	-	-	-	-	-	-	-	-	-	5	-	-	-	10	-	-	-
Ag. A5	10	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. A6	1	5	-	-	-	-	-	-	1	-	-	-	-	-	-	-	10
Ag. A7	-	-	-	-	-	-	-	-	-	3	-	-	-	-	10	-	5
Ag. A8	10	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	5
Ag. A9	-	-	1	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. A10	-	-	-	-	5	-	-	-	-	3	-	3	-	1	1	-	-
Ag. A11	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. A12	-	-	10	-	-	-	-	-	-	1	1	1	1	5	10	-	-
Ag. A13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	10
Ag. A14	-	-	-	3	-	5	1	1	10	5	5	-	-	-	-	-	5
Ag. A15	-	10	-	-	5	-	-	-	-	-	-	5	-	5	10	-	-
Ag. A16	10	5	-	-	-	-	-	-	3	-	-	-	10	-	-	1	3

Table 3.B. Alleles predicted to bind MHC agretopes in RANKL.

Agrelope number	101	102	301	401	402	404	405	408	701	801	1101	1102	1104	1301	1302	1501	1502
Ag. B1	-	-	-	10	-	-	5	5	10	-	-	-	-	-	-	-	10
Ag. B2	5	3	-	-	-	-	-	-	-	-	-	10	-	5	10	-	-
Ag. B3	1	3	-	3	-	5	3	1	-	-	10	-	-	-	10	-	10
Ag. B4	-	-	-	-	-	-	5	5	5	10	-	-	-	-	10	1	1
Ag. B5	-	-	-	-	-	-	-	-	-	-	10	-	-	-	3	-	-
Ag. B6	-	-	-	3	10	-	1	5	-	10	-	-	-	-	3	-	-
Ag. B7	5	-	-	-	-	-	10	10	-	-	10	-	-	-	-	-	-
Ag. B8	-	-	-	-	-	5	-	10	-	-	-	-	-	-	-	-	-
Ag. B9	-	-	-	-	3	5	-	10	-	3	10	3	10	3	5	10	-
Ag. B10	10	5	1	-	-	-	-	-	3	-	5	10	3	10	10	5	10
Ag. B11	-	-	-	5	10	5	-	10	-	-	-	-	-	-	-	-	-
Ag. B12	10	-	-	-	5	-	-	10	1	3	10	-	-	-	5	10	3
Ag. B13	-	10	-	-	10	-	-	-	5	-	-	-	-	-	-	5	10
Ag. B14	-	-	-	-	-	-	10	-	-	3	-	-	-	-	3	10	3
Ag. B15	-	-	-	-	-	-	-	-	-	10	1	1	1	1	3	3	5
Ag. B16	-	-	-	-	-	-	5	-	-	10	-	-	-	-	-	-	-
Ag. B17	-	-	-	1	-	5	-	10	-	-	-	-	-	-	-	-	-
Ag. B18	10	-	-	-	10	-	-	10	5	-	10	5	-	5	1	-	10

Table 3.C. Alleles predicted to bind MHC agretopes in APRIL.

Agrelope number	101	102	301	401	402	404	405	408	701	801	1101	1102	1104	1301	1302	1501	1502
Ag. C1	3	1	-	-	-	5	-	10	-	-	3	10	3	-	-	10	-
Ag. C2	10	10	-	10	10	3	10	3	-	-	-	-	-	-	-	-	-
Ag. C3	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-
Ag. C4	-	-	-	-	-	-	-	-	-	-	-	10	-	5	1	-	-
Ag. C5	3	3	10	-	10	-	-	-	-	-	-	-	10	-	-	1	3
Ag. C6	5	3	3	-	-	10	-	-	-	-	-	10	10	10	10	-	-
Ag. C7	3	1	-	10	5	5	10	10	5	-	-	-	-	-	-	10	-
Ag. C8	-	-	-	-	-	-	-	-	-	5	-	-	-	-	5	-	5

Ag. C9	-	-	5	1	3	3	10	5	-	10	3	3	1	10	-	-	-
Ag. C10	-	-	1	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. C11	3	10	5	1	3	1	1	1	5	5	1	-	1	-	5	5	1
Ag. C12	-	10	-	10	5	3	1	5	-	5	-	10	-	10	-	-	-
Ag. C13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Ag. C14	-	-	-	-	5	-	-	-	-	-	-	10	-	-	-	-	-
Ag. C15	-	-	-	-	-	-	-	-	-	3	-	10	-	10	-	10	-
Ag. C16	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. C17	-	-	-	-	5	-	-	-	-	-	-	10	-	5	10	-	-

Table 3.D. Alleles predicted to bind MHC agretopes in CD40L.

Agrelope number	101	102	301	401	402	404	405	408	701	801	1101	1102	1104	1301	1302	1501	1502
Ag. D1	5	-	-	3	-	3	1	1	-	-	-	-	-	-	-	-	5
Ag. D2	-	-	-	3	-	-	-	-	5	-	-	-	-	-	-	-	5
Ag. D3	-	-	-	5	-	3	3	3	-	-	-	-	5	-	-	-	-
Ag. D4	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. D5	-	-	-	-	-	-	-	-	-	5	-	-	-	-	3	-	3
Ag. D6	5	-	-	3	-	5	3	3	-	-	-	-	-	-	-	-	-
Ag. D7	-	-	-	5	3	3	-	3	-	-	-	3	-	3	3	-	-
Ag. D8	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Ag. D9	-	-	-	3	-	-	-	-	-	-	3	-	-	-	-	-	-
Ag. D10	-	-	-	-	3	-	-	-	-	3	-	5	-	-	-	-	-
Ag. D11	-	-	-	5	3	1	5	3	-	-	-	-	-	-	-	-	-
Ag. D12	-	-	-	1	1	1	3	1	-	-	3	1	3	1	3	-	-
Ag. D13	-	-	3	-	-	-	-	-	5	-	-	-	-	-	-	-	-
Ag. D14	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. D15	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag. D16	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	5	1

Table 3.E. Alleles predicted to bind MHC agretopes in TRAIL.

Agrelope number	101	102	301	401	402	404	405	408	701	801	1101	1102	1104	1301	1302	1501	1502
Ag. E1	-	-	-	-	1	3	10	5	-	-	-	-	-	-	-	-	-
Ag. E2	-	-	1	-	-	-	-	-	-	10	10	1	5	1	1	10	10
Ag. E3	5	-	-	5	10	10	3	3	3	-	-	-	-	-	-	-	-
Ag. E4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10	3
Ag. E5	-	-	10	5	10	-	10	5	10	-	-	-	-	-	10	-	-
Ag. E6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	5
Ag. E7	-	10	1	-	-	-	-	-	-	10	1	3	1	3	5	-	-
Ag. E8	-	-	-	-	-	-	10	10	-	5	-	-	-	-	10	-	-
Ag. E9	10	10	-	10	10	3	1	3	-	5	-	-	-	-	-	-	-
Ag. E10	-	-	-	-	1	-	-	-	-	3	-	1	10	1	1	-	-
Ag. E11	-	-	-	-	-	5	10	10	-	-	-	-	-	-	-	-	-
Ag. E12	-	-	-	-	-	-	-	-	-	3	-	-	-	-	5	10	3
Ag. E13	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	3
Ag. E14	-	-	-	1	5	3	3	5	-	-	-	10	-	-	-	-	-
Ag. E15	5	-	-	10	-	-	3	3	1	-	-	-	-	-	-	-	10

[129] Example 2. Identification of suitable less immunogenic sequences for MHC-binding agretopes in TNF SF members.

[130] MHC-binding agretopes that were predicted to bind alleles present in at least 10% of the US population, using a 1% threshold, were analyzed to identify suitable less immunogenic variants. At each agretope, all possible combinations of amino acid substitutions were considered, with the following requirements: (1) each substitution has a score of 0 or greater in the BLOSUM62 substitution matrix, (2) each substitution is capable of conferring reduced binding to at least one of the MHC alleles considered, and (3) once sufficient substitutions are incorporated to prevent any allele hits at a 1% threshold, no additional substitutions are added to that sequence.

[131] Alternate sequences were scored for immunogenicity and structural compatibility. Preferred alternate sequences were defined to be those sequences that are not predicted to bind to any of the 17 MHC alleles tested above using a 1% threshold, and that have a total BLOSUM62 score that is at least 80% of the wild type score.

[132] **Table 4.** Suitable less immunogenic variants of of TNF SF members. B(wt) is the BLOSUM62 score of the wild type 9-mer, I(alt) is the percent of the US population containing one or more MHC alleles that are predicted to bind the alternate 9-mer at a 1% threshold and is 0 for all variants listed in Table 4, and B(alt) is the BLOSUM62 score of the alternate 9-mer.

[133] **Table 4.A.i:** Suitable less immunogenic variants of BAFF agretope 2 (residues 168 – 176; WLLSFKRGS); B(wt)=49.

Variant	Variant sequence	B(alt)
Var:A1	WLLSFSRGS	44
Var:A2	WLLSFNRGS	44
Var:A3	WLLSFERGS	45
Var:A4	WLLSFQRGS	45
Var:A5	WLLSFKEGS	44
Var:A6	WLLSFKQGS	45
Var:A7	WLLSFKRGT	46
Var:A8	WLLSFKRGN	46
Var:A9	WLLSFKRGD	45
Var:A10	WLLSFKRGE	45
Var:A11	WLLSFKRGQ	45
Var:A12	WLLSFKRGK	45
Var:A13	WFLSFKNGS	40
Var:A14	WFLSFKKGS	42
Var:A15	WFLSFKRGA	42
Var:A16	WLVGFKRGS	42
Var:A17	WLVDFKRGS	42
Var:A18	WLVSFKNGS	41
Var:A19	WLVSFKHGS	41
Var:A20	WLVSFKKGS	43
Var:A21	WLVSFKRGA	43
Var:A22	WLFSFKNGS	40
Var:A23	WLFSFKKGS	42

Var:A24	WLFSFKRGA	42
Var:A25	WLLTFKNGS	41
Var:A26	WLLTFKHGS	41
Var:A27	WLLTFKKGS	43
Var:A28	WLLTFKRGGA	43
Var:A29	WLLGFKNGS	40
Var:A30	WLLGFKHGS	40
Var:A31	WLLGFKKGS	42
Var:A32	WLLGFKRGA	42
Var:A33	WLLGFKRGG	41
Var:A34	WLLDFKRGGA	42
Var:A35	WLLDFKRGG	41
Var:A36	WLLEFKNGS	40
Var:A37	WLLEFKHGS	40
Var:A38	WLLEFKKGS	42
Var:A39	WLLEFKRGA	42
Var:A40	WLLSFRNGS	41
Var:A41	WLLSFRKGS	43
Var:A42	WLLSFRRGA	43
Var:A43	WLLSFKNGA	41
Var:A44	WLLSFKKGG	40
Var:A45	WLLSFKHGA	41
Var:A46	WLLSFKHGG	40
Var:A47	WLLSFKKGA	43
Var:A48	WLLSFKKGG	42

Var:A49	WLVTFRRGS	40
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[134] Table 4.A.ii lists suitable less immunogenic variants of BAFF agretope 6 (residues 192 - 200; YFFIYGQVL); B(wt)=49.

Variant	Variant sequence	B(alt)
Var:A50	YWFIYGQVL	44
Var:A51	YFWIYGQVL	44
Var:A52	YFFVYGQVL	48
Var:A53	YFFIYGQVL	44
Var:A54	YFFIYGEVL	46
Var:A55	YFFIYGQVM	47

Var:A56	YFFIYGQVF	45
Var:A57	YFFLYNQVL	41
Var:A58	YFFFYGRVL	41
Var:A59	YFFFYGQVV	42
Var:A60	YFFIYSQVV	40
Var:A61	YFFIYNQVV	40
Var:A62	YFFIYGSVV	41
Var:A63	YFFIYGKVV	42

[135] Table 4.A.iii: Suitable less immunogenic variants of BAFF agretope 9 (residues 200 - 208; LYTDKTYAM); B(wt)=48.

Variant	Variant sequence	B(alt)
Var:A64	FYTDKTYAM	44
Var:A65	LWTDKTYAM	43
Var:A66	LYTSKTYAM	42
Var:A67	LYTNKTYAM	43
Var:A68	LYTEKTYAM	44
Var:A69	LYTQKTYAM	42
Var:A70	LYTDKSYAM	44

Var:A71	LYTDKAYAM	43
Var:A72	LYTDKNYAM	43
Var:A73	LYTDKTHAM	43
Var:A74	LYTDKTWAM	43
Var:A75	LYTDKTYAQ	44
Var:A76	LYTDKTYAI	44
Var:A77	LYTDKTYAL	45
Var:A78	LYTDKTYAV	44

[136] Table 4.A.iv: Suitable less immunogenic variants of BAFF agretope 10 (residues 212 - 220; IQRKKVHVF); B(wt)=46.

Variant	Variant sequence	B(alt)
Var:A79	ISRKKVHVF	41
Var:A80	IDRKKVHVF	41
Var:A81	IERKKVHVF	43
Var:A82	IQEKKVHVF	41
Var:A83	IQRSKVHVF	41
Var:A84	IQREKVHVF	42
Var:A85	IQRKKAHVF	43
Var:A86	IQRKKMHVF	43
Var:A87	IQRKKLHVF	43
Var:A88	IQRKKVEVF	38
Var:A89	IQRKKVHVL	40
Var:A90	IQRKKVHVW	41
Var:A91	INQKKVHVF	37
Var:A92	INKKKVHVF	38
Var:A93	INRRKVHVF	38
Var:A94	INRKKIHVF	40
Var:A95	INRKKVHVY	38
Var:A96	IHQKKVHVF	37

Var:A97	IHKKKVHVF	38
Var:A98	IHRKVHVF	38
Var:A99	IHRKKIHVF	40
Var:A100	IHRKKVHVY	38
Var:A101	IQQNKVHVF	37
Var:A102	IQQQKVHVF	38
Var:A103	IQQRKVHVF	39
Var:A104	IQQKKTHVF	39
Var:A105	IQQKKIHVF	41
Var:A106	IQQKKVHVY	39
Var:A107	IQHRKVHVF	38
Var:A108	IQHKKTHVF	38
Var:A109	IQHKKIHVF	40
Var:A110	IQHKKVHVY	38
Var:A111	IQKNKVHVF	38
Var:A112	IQKQKVHVF	39
Var:A113	IQKRKVHVF	40
Var:A114	IQKKKTHVF	40
Var:A115	IQKKKIHVF	42

Var:A116	IQKKKVYVF	37
Var:A117	IQKKKVHVI	37
Var:A118	IQKKKVHVY	40
Var:A119	IQRNKTHVF	38
Var:A120	IQRNKIHVF	40
Var:A121	IQRNKVHVY	38
Var:A122	IQRKRIHVF	42
Var:A123	IQRKRVYVF	37

Var:A124	IQRKRVHVY	40
Var:A125	IQRKKTYVF	37
Var:A126	IQRKKTHVY	40
Var:A127	IQRKKIQVF	37
Var:A128	IQRKKIYVF	39
Var:A129	IQRKKIHVY	42
Var:A130	IQRKKVYVY	37

[137] **Table 4.A.v:** Suitable less immunogenic variants of BAFF agretope 12 (residues 226 - 234; LVTLFRCIQ); B(wt)=46.

Variant	Variant sequence	B(alt)
Var:A131	LTTLFRCIQ	43
Var:A132	LATLFRCIQ	43
Var:A133	LMTLFRCIQ	43
Var:A134	LITLFRCIQ	45
Var:A135	LLTLFRCIQ	43
Var:A136	LVTLFECIQ	41
Var:A137	LVTLFQCIQ	42
Var:A138	LVTLFHCIQ	41

Var:A139	LVTLFRCIN	41
Var:A140	LVTLFRCID	41
Var:A141	LVTLFRCIR	42
Var:A142	LVTIFRCIE	41
Var:A143	LVTIFRCIK	40
Var:A144	LVTVFRCIE	40
Var:A145	LVTVFRCIH	38
Var:A146	LVTVFRCIK	39
Var:A147	LVTVFRCIM	38
Var:A148	LVTFRCIE	39

[138] **Table 4.A.vi:** Suitable less immunogenic variants of BAFF agretope 16 (residues 276 - 284; VTFFGALKL); B(wt)=44.

Variant	Variant sequence	B(alt)
Var:A149	VTWFGALKL	39
Var:A150	VTFMGALKL	38
Var:A151	VTFIGALKL	38
Var:A152	VTFWGALKL	39
Var:A153	VTFFGAVKL	41
Var:A154	VTFFGALKF	40
Var:A155	VSFFGVCLKL	36
Var:A156	VSFFGAIKL	38
Var:A157	VSFFGAFKL	36

Var:A158	VSFFGALKM	38
Var:A159	VTFLGAMKL	36
Var:A160	VTFLGALKM	36
Var:A161	VTFFGTFLK	36
Var:A162	VTFFGVIKL	38
Var:A163	VTFFGVFKL	36
Var:A164	VTFFGAMKM	40
Var:A165	VTFFGAIKM	40
Var:A166	VTFFGAIKV	39
Var:A167	VTFFGAFKM	38
Var:A168	VTFFGAFKV	37

[139] **Table 4.B.i:** Suitable less immunogenic variants of RANKL agretope 3 (residues 213 - 221; FYYLYANIC); B(wt)=54.

Variant	Variant sequence	B(alt)
Var:B1	FWYLYANIC	49
Var:B2	FYWLYANIC	49
Var:B3	FYYVYANIC	51
Var:B4	FYYLYAGIC	48
Var:B5	FYYLYADIC	49
Var:B6	FYYLYAEIC	48

Var:B7	MYYIYANIC	46
Var:B8	MYYFYANIC	44
Var:B9	MYYLYGNIC	44
Var:B10	IYYIYANIC	46
Var:B11	IYYFYANIC	44
Var:B12	IYYLYGNIC	44
Var:B13	LYYIYANIC	46
Var:B14	LYYFYANIC	44

Var:B15	LYYLYGNIC	44
Var:B16	FHHLYANIC	44
Var:B17	FFHLYANIC	45
Var:B18	FYHIYANIC	47
Var:B19	FYHIFYANIC	45
Var:B20	FYHLYGNIC	45
Var:B21	FYHLYASIC	44
Var:B22	FYYIYGNIC	48
Var:B23	FYYIYASIC	47

Var:B24	FYYIYAKIC	46
Var:B25	FYYFYASIC	45
Var:B26	FYYFYATIC	44
Var:B27	FYYFYAQIC	44
Var:B28	FYYFYAHIC	45
Var:B29	FYYFYARIC	44
Var:B30	FYYFYAKIC	44
Var:B31	FYYLYSRIC	45
Var:B32	FYYLYSKIC	45

[140] **Table 4.B.ii:** Suitable less immunogenic variants of RANKL agretope 4 (residues 214 - 222; YYLYANICF); B(wt)=54.

Variant	Variant sequence	B(alt)
Var:B33	YYLWANICF	49
Var:B34	YYLYAEICF	48
Var:B35	YFLYAQICF	44
Var:B36	YWVYANICF	46
Var:B37	YWLHANICF	44
Var:B38	YWLYADICF	44
Var:B39	YWLYAHICF	44
Var:B40	YWLYANVCF	48
Var:B41	YWLYANICY	46
Var:B42	YWLYANICW	44
Var:B43	YYVHANICF	46
Var:B44	YYVYADICF	46
Var:B45	YYVYAQICF	45
Var:B46	YYVYAHICF	46
Var:B47	YYVYANVCF	50
Var:B48	YYVYANICW	46
Var:B49	YYFHANICF	45
Var:B50	YYFYAQICF	44
Var:B51	YYLHADICF	44

Var:B52	YYLHAHICF	44
Var:B53	YYLHANVCF	48
Var:B54	YYLHANICY	46
Var:B55	YYLHANICW	44
Var:B56	YYLFAQICF	44
Var:B57	YYLYADVCF	48
Var:B58	YYLYADICY	46
Var:B59	YYLYADICW	44
Var:B60	YYLYAQVCF	47
Var:B61	YYLYAQFCF	44
Var:B62	YYLYAQICY	45
Var:B63	YYLYAHVCF	48
Var:B64	YYLYAHICY	46
Var:B65	YYLYAHICW	44
Var:B66	YYLYANVCY	50
Var:B67	YYLYANVCW	48
Var:B68	YHFYANVCF	44
Var:B69	YFFYANVCF	45
Var:B70	YFFFANVCF	45
Var:B71	YFFYASVCF	44

[141] **Table 4.B.iii:** Suitable less immunogenic variants of RANKL agretope 10 (residues 239 - 247; MVYVTKTSI); B(wt)=43.

Variant	Variant sequence	B(alt)
Var:B72	MTYVTKTSI	40
Var:B73	MAYVTKTSI	40
Var:B74	MMYVTKTSI	40
Var:B75	MIYVTKTSI	42
Var:B76	MLYVTKTSI	40
Var:B77	MVHVTKTSI	38
Var:B78	MVWVTKTSI	38

Var:B79	MVYTTKTSI	40
Var:B80	MVYVTETSI	39
Var:B81	MVYVTQTSI	39
Var:B82	MVYVTRTSI	40
Var:B83	MVYVTKTSL	41
Var:B84	MVYVTKTSV	42
Var:B85	FVYVTKSSI	35
Var:B86	FVYVTKTSM	36
Var:B87	FVYVTKTSF	35

[142] Table 4.B.iv: Suitable less immunogenic variants of RANKL agretope 12 (residues 212 - 220; YVTKTSIKI); B(wt)=43.

Variant	Variant sequence	B(alt)
Var:B88	YTTKTSIKI	40
Var:B89	YATKTSIKI	40
Var:B90	YMTKTSIKI	40
Var:B91	YITKTSIKI	42
Var:B92	YLTKTSIKI	40
Var:B93	YVTKTGIKI	39

Var:B94	YVTKTNIKI	40
Var:B95	YVTKTDIKI	39
Var:B96	YVTKTEIKI	39
Var:B97	YVTKTQIKI	39
Var:B98	YVTKTSVKI	42
Var:B99	YVTKTSIKV	42
Var:B100	YVTKTSIKF	39
Var:B101	YVTETKIKI	35

[143] Table 4.B.v: Suitable less immunogenic variants of RANKL agretope 15 (residues 212 - 220; VGGFFKLRS); B(wt)=46.

Variant	Variant sequence	B(alt)
Var:B102	VSGFFKLRS	40
Var:B103	VGGYFKLRS	43
Var:B104	VGGFFELRS	42
Var:B105	VGGFFQLRS	42
Var:B106	VGGFFKVRS	43
Var:B107	VGGFFKLRT	43
Var:B108	VGGFFKLRG	42
Var:B109	VGGFFKL RN	43
Var:B110	VGGFFKL RD	42
Var:B111	VGGFFKL RE	42
Var:B112	VGGFFKL RK	42
Var:B113	VAGFFKIRS	38
Var:B114	VAGFFKLRA	37
Var:B115	VGAFFKIRS	38

Var:B116	VGAFFKLRA	37
Var:B117	VGGWFRLRS	38
Var:B118	VGGWFKIRS	39
Var:B119	VGGWFKLRA	38
Var:B120	VGGFFSIRS	39
Var:B121	VGGFFSFRS	37
Var:B122	VGGFFSLRQ	37
Var:B123	VGGFFNIRS	39
Var:B124	VGGFFNFRS	37
Var:B125	VGGFFKMRA	41
Var:B126	VGGFFKIRA	41
Var:B127	VGGFFKIRQ	40
Var:B128	VGGFFKFRA	39

[144] Table 4.B.vi: Suitable less immunogenic variants of RANKL agretope 17 (residues 212 - 220; ISIEVSNPS); B(wt)=42.

Variant	Variant sequence	B(alt)
Var:B129	IDIEVSNPS	38
Var:B130	ISLEVSNPS	40
Var:B131	ISVEVSNPS	41
Var:B132	ISFEVSNPS	38
Var:B133	ISISVSNPS	37
Var:B134	ISINVSNP S	37
Var:B135	ISIQVSNPS	39
Var:B136	ISIKVSNPS	38
Var:B137	ISIEVANPS	39
Var:B138	ISIEVGNPS	38
Var:B139	ISIEVDNPS	38
Var:B140	ISIEVENPS	38

Var:B141	ISIEVQNPS	38
Var:B142	ISIEVKNPS	38
Var:B143	ISIEVSSPS	37
Var:B144	ISIEVSTPS	36
Var:B145	ISIEVSGPS	36
Var:B146	ISIEVSDPS	37
Var:B147	ISIEVSQPS	36
Var:B148	ISIEVSHPS	37
Var:B149	ISIEVSRPS	36
Var:B150	ISIEVSKPS	36
Var:B151	ISIEVSNPT	39
Var:B152	ISIEVSNPA	39
Var:B153	ISIEVSNPG	38
Var:B154	ISIEVSNPN	39

Var:B155	ISIEVSNPD	38
Var:B156	ISIEVSNPE	38

Var:B157	ISIEVSNPK	38
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[145] **Table 4.C.i:** Suitable less immunogenic variants of APRIL agretope 5 (residues 142 - 150; LRRGRGLQA); B(wt)=44.

Variant	Variant sequence	B(alt)
Var:C1	LNRGRGLQA	39
Var:C2	LERGRGLQA	39
Var:C3	LQRGRGLQA	40
Var:C4	LHRGRGLQA	39
Var:C5	LKRGRGLQA	41
Var:C6	LREGRGLQA	39
Var:C7	LRQGRGLQA	40
Var:C8	LRHGRGLQA	39
Var:C9	LRKGRGLQA	41
Var:C10	LRRSRGLQA	38

Var:C11	LRRGRGFQA	40
Var:C12	LRRGRGLQS	41
Var:C13	LRRGRGLQG	40
Var:C14	LRNGRGMQA	37
Var:C15	LRNGRGIQA	37
Var:C16	LRNGRGVQA	36
Var:C17	LRRGRSIQA	36
Var:C18	LRRGRAMQA	36
Var:C19	LRRGRGMQT	38
Var:C20	LRRGRGIQT	38
Var:C21	LRRGRGVQT	37

[146] **Table 4.C.ii:** Suitable less immunogenic variants of APRIL agretope 9 (residues 164 - 172; LLYSQVLFQ); B(wt)=43.

Variant	Variant sequence	B(alt)
Var:C22	LLHSQVLFQ	38
Var:C23	LLWSQVLFQ	38
Var:C24	LLYGQVLFQ	39
Var:C25	LLYSQALFQ	40
Var:C26	LLYSQMLFQ	40
Var:C27	LLYSQILFQ	42
Var:C28	LLYSQLLFQ	40
Var:C29	LLYSQVIFQ	41

Var:C30	LLYSQVVFFQ	40
Var:C31	LLYSQVFFQ	39
Var:C32	LLYSQVLFN	38
Var:C33	LLYSQVLFQ	38
Var:C34	LLYSQVLFQ	40
Var:C35	LLYSQVLFH	38
Var:C36	LLYSQVLFQ	39
Var:C37	LLYSQVLFK	39
Var:C38	LLYTQVLFM	35

[147] **Table 4.C.iii:** Suitable less immunogenic variants of APRIL agretope 10 (residues 170 - 178; LFQDVTFTM); B(wt)=46.

Variant	Variant sequence	B(alt)
Var:C39	FFQDVTFTM	42
Var:C40	LWQDVTFTM	41
Var:C41	LFDDVTFTM	41
Var:C42	LFEDVTFTM	43
Var:C43	LFQSVTFTM	40
Var:C44	LFQNVFTFTM	41
Var:C45	LFQEVFTFTM	42

Var:C46	LFQQVTFTM	40
Var:C47	LFQDVSFTM	42
Var:C48	LFQDVAFTM	41
Var:C49	LFQDVNFTM	41
Var:C50	LFQDVTWTM	41
Var:C51	LFQDVTFTQ	42
Var:C52	LFQDVTFTL	43
Var:C53	LFQDVTFTV	42
Var:C54	LFQDVITYTI	39

[148] **Table 4.C.iv:** Suitable less immunogenic variants of APRIL agretope 11 (residues 194 - 202; FRCIRSMPS); B(wt)=49.

Variant	Variant sequence	B(alt)
Var:C55	FNCIRGMPS	40
Var:C56	FNCIRDMP	40
Var:C57	FNCIREMPS	40
Var:C58	FNCIRSMPT	41
Var:C59	FNCIRSMPG	40
Var:C60	FNCIRSMPK	40
Var:C61	FECVRSMPS	43
Var:C62	FECIRAMPS	41
Var:C63	FECIRGMPS	40
Var:C64	FECIRDMP	40
Var:C65	FECIREMPS	40
Var:C66	FECIRQMPS	40
Var:C67	FECIRSQPS	40
Var:C68	FECIRSIPS	40
Var:C69	FECIRSLPS	41
Var:C70	FECIRSVPS	40
Var:C71	FECIRSFPS	40
Var:C72	FECIRSMPT	41
Var:C73	FECIRSMPA	41
Var:C74	FECIRSMPG	40
Var:C75	FECIRSMPN	41
Var:C76	FECIRSMPD	40
Var:C77	FECIRSMPK	40
Var:C78	FQCIRDMP	41
Var:C79	FQCIREMPS	41
Var:C80	FQCIRSMPT	42
Var:C81	FHCIRGMPS	40
Var:C82	FHCIRDMP	40
Var:C83	FHCIREMPS	40
Var:C84	FHCIRSMPT	41
Var:C85	FHCIRSMPG	40
Var:C86	FHCIRSMPK	40
Var:C87	FKCIRGMPS	42
Var:C88	FKCIRDMP	42
Var:C89	FKCIREMPS	42
Var:C90	FKCIRSMPT	43
Var:C91	FRCVRDMP	44
Var:C92	FRCVREMPS	44
Var:C93	FRCIRGMPG	41
Var:C94	FRCIRGMPN	42
Var:C95	FRCIRGMPD	41
Var:C96	FRCIRGMPE	41
Var:C97	FRCIRDQPS	41
Var:C98	FRCIRDIPS	41
Var:C99	FRCIRDLPS	42

Var:C100	FRCIRDVPS	41
Var:C101	FRCIRDFPS	41
Var:C102	FRCIRDMPT	42
Var:C103	FRCIRDMPA	42
Var:C104	FRCIRDMPG	41
Var:C105	FRCIRDMPN	42
Var:C106	FRCIRDMPD	41
Var:C107	FRCIRDMPK	41
Var:C108	FRCIREQPS	41
Var:C109	FRCIREIPS	41
Var:C110	FRCIREVPS	41
Var:C111	FRCIREFPS	41
Var:C112	FRCIREMPT	42
Var:C113	FRCIREMPA	42
Var:C114	FRCIREMPG	41
Var:C115	FRCIREMPN	42
Var:C116	FRCIREMPD	41
Var:C117	FRCIREMPE	41
Var:C118	FRCIREMPK	41
Var:C119	FRCIRQQPS	41
Var:C120	FRCIRQVPS	41
Var:C121	FRCIRQFPS	41
Var:C122	FRCIRQMPT	42
Var:C123	FRCIRQMPP	41
Var:C124	FRCIRQMPPN	42
Var:C125	FRCIRQMPPD	41
Var:C126	FRCIRQMPE	41
Var:C127	FNCVRAMPS	40
Var:C128	FNCVRSLPS	40
Var:C129	FNCVRSMPPA	40
Var:C130	FNCVRSMPPN	40
Var:C131	FQCVRGMP	40
Var:C132	FQCVRQMPS	40
Var:C133	FQCVRSQPS	40
Var:C134	FQCVRSVPS	40
Var:C135	FQCVRSFPS	40
Var:C136	FQCVRSMPG	40
Var:C137	FQCVRSMPN	41
Var:C138	FQCVRSMPK	40
Var:C139	FHCVRAMPS	40
Var:C140	FHCVRSLPS	40
Var:C141	FHCVRSMPPA	40
Var:C142	FHCVRSMPPN	40
Var:C143	FKCVRQMPS	41
Var:C144	FKCVRSQPS	41
Var:C145	FKCVRSIPS	41
Var:C146	FKCVRSVPS	41

Var:C147	FKCVRSFPS	41
Var:C148	FKCVRSMPA	42
Var:C149	FKCVRSMPG	41
Var:C150	FKCVRSMPN	42
Var:C151	FKCVRSMPK	41
Var:C152	FKCIRAMPA	40
Var:C153	FKCIRAMPN	40
Var:C154	FRCVRAQPS	41
Var:C155	FRCVRAMPT	42
Var:C156	FRCVRAMPG	41
Var:C157	FRCVRAMPN	42
Var:C158	FRCVRGQPS	40
Var:C159	FRCVRGFPS	40
Var:C160	FRCVRGMPT	41

Var:C161	FRCVRGMPA	41
Var:C162	FRCVRGMPK	40
Var:C163	FRCVRQMPK	40
Var:C164	FRCVRSQPT	41
Var:C165	FRCVRSQPG	40
Var:C166	FRCVRSQPK	40
Var:C167	FRCVRSIPT	41
Var:C168	FRCVRSIPG	40
Var:C169	FRCVRSVPT	41
Var:C170	FRCVRSVPG	40
Var:C171	FRCVRSVPK	40
Var:C172	FRCVRSFPT	41
Var:C173	FRCVRSFPG	40
Var:C174	FRCVRSFPK	40

[149] Table 4.D.i: Suitable less immunogenic variants of CD40L agretope 12 (residues 84-92; LRAANTHSS); B(wt)=44.

Variant	Variant sequence	B(alt)
Var:D1	LEAANTHSS	39
Var:D2	LRAANAHSS	39
Var:D3	LRAANTHST	41
Var:D4	LRAANTHSG	40
Var:D5	LRAANTHSN	41
Var:D6	LRAANTHSD	40
Var:D7	LRAANTHSE	40
Var:D8	FRAGNTHSS	36
Var:D9	LNASNTHSS	36
Var:D10	LNAANTHSA	36
Var:D11	LQASNTHSS	37
Var:D12	LQATNTHSS	36
Var:D13	LQAGNTHSS	36
Var:D14	LQAANSHSS	36
Var:D15	LQAANTHSA	37

Var:D16	LQAANTHSSK	36
Var:D17	LHASNTHSS	36
Var:D18	LHAANTHSA	36
Var:D19	LKASNTHSS	38
Var:D20	LKATNTHSS	37
Var:D21	LKAGNTHSS	37
Var:D22	LKAANSHSS	37
Var:D23	LKAANNHSS	36
Var:D24	LKAANVHSS	36
Var:D25	LKAANTHSA	38
Var:D26	LKAANTHSSK	37
Var:D27	LRATNTHSSK	36
Var:D28	LRAGNSHSS	36
Var:D29	LRAGNTHSSK	36
Var:D30	LRAANSHSA	37
Var:D31	LRAANSHSSK	36

[150] Table 4.E.i: Suitable less immunogenic variants of TRAIL agretope 2 (residues 174-182; LVIHEKGFY); B(wt)=49.

Variant	Variant sequence	B(alt)
Var:E1	LTIHEKGFY	46
Var:E2	LAIHEKGFY	46
Var:E3	LMIHEKGFY	46
Var:E4	LIIHEKGFY	48
Var:E5	LLIHEKGFY	46
Var:E6	LVVHEKGFY	48
Var:E7	LVIEEKGFY	41

Var:E8	LVIHESGFY	44
Var:E9	LVIHENGIFY	44
Var:E10	LVIHEEGIFY	45
Var:E11	LVIHEQGFY	45
Var:E12	LVLHEKGFY	42
Var:E13	LVFHERGFY	42
Var:E14	LVFHEKGFY	40
Var:E15	LVIHERGFY	41

[151] **Table 4.E.ii:** Suitable less immunogenic variants of TRAIL agretope 7 (residues 207-215; VQYIYKYTS); B(wt)=48.

Variant	Variant sequence	B(alt)
Var:E16	VSIIYKYTS	43
Var:E17	VDIYKYTS	43
Var:E18	VEIYKYTS	45
Var:E19	VQHIYKYTS	43
Var:E20	VQWIIKYTS	43
Var:E21	VQYIYEYTS	44
Var:E22	VQYIQYTS	44
Var:E23	VQYIYKYTT	45
Var:E24	VQYIYKYTA	45

Var:E25	VQYIYKYTG	44
Var:E26	VQYIYKYTN	45
Var:E27	VQYIYKYTD	44
Var:E28	VQYIYKYTE	44
Var:E29	VQYIYKYTK	44
Var:E30	VNYVYKYTS	42
Var:E31	VNYIYRYTS	40
Var:E32	VHYVYKYTS	42
Var:E33	VHYIYRYTS	40
Var:E34	VQYVYRYTS	44
Var:E35	VQYVYKYTQ	43

[152] **Table 4.E.iii:** Suitable less immunogenic variants of TRAIL agretope 10 (residues 221-229; LLMKSARNS); B(wt)=41.

Variant	Variant sequence	B(alt)
Var:E36	LLMESARNS	37
Var:E37	LLMKSAENS	36
Var:E38	LLMK SARNT	38
Var:E39	LLMK SARNN	38
Var:E40	LFQKSARNS	33
Var:E41	LFMK SARND	33
Var:E42	LLQQSARNS	33
Var:E43	LLQKSGRNS	33
Var:E44	LLQKSAQNS	33
Var:E45	LLQKSAKNS	34
Var:E46	LLQKSARNA	34
Var:E47	LLQKSARNG	33
Var:E48	LLQKSARND	33
Var:E49	LLQKSARNE	33
Var:E50	LLQKSARNQ	33
Var:E51	LLQKSARNK	33
Var:E52	LLLSSARNS	33
Var:E53	LLLKSANNS	33
Var:E54	LLLKSAKNS	35
Var:E55	LLLKSARND	34
Var:E56	LLVKSGRNS	33
Var:E57	LLVKSAQNS	33
Var:E58	LLVKSAKNS	34
Var:E59	LLVKSARNA	34
Var:E60	LLVKSARNG	33
Var:E61	LLVKSARND	33
Var:E62	LLVKSARNE	33
Var:E63	LLVKSARNK	33

Var:E64	LLFKSAQNS	33
Var:E65	LLFKSAKNS	34
Var:E66	LLFKSARNG	33
Var:E67	LLFKSARND	33
Var:E68	LLFKSARNE	33
Var:E69	LLFKSARNK	33
Var:E70	LLMSSAKNS	33
Var:E71	LLMSSARNA	33
Var:E72	LLMNSAKNS	33
Var:E73	LLMQSAQNS	33
Var:E74	LLMQSARND	33
Var:E75	LLMQSARNE	33
Var:E76	LLMKSSRND	34
Var:E77	LLMKSGQNS	33
Var:E78	LLMKSGKNS	34
Var:E79	LLMKSGRNG	33
Var:E80	LLMKSGRND	33
Var:E81	LLMKSGRNE	33
Var:E82	LLMKSGRNK	33
Var:E83	LLMKSANNA	33
Var:E84	LLMKSAQNA	34
Var:E85	LLMKSAQNG	33
Var:E86	LLMKSAQND	33
Var:E87	LLMKSAQNE	33
Var:E88	LLMKSAQNK	33
Var:E89	LLMKSAHNA	33
Var:E90	LLMKSAKNA	35
Var:E91	LLMKSAKNG	34
Var:E92	LLMKSAKND	34
Var:E93	LLMKSAKNK	34

[153] **Table 4.E.iv:** Suitable less immunogenic variants of TRAIL agretope 14 (residues 256-264; IFVSVTNEH); B(wt)=46.

Variant	Variant sequence	B(alt)
Var:E94	IWVSVTNEH	41
Var:E95	IFTSVTNEH	43
Var:E96	IFASVTNEH	43
Var:E97	IFVTVTNEH	43
Var:E98	IFVGVTVNEH	42
Var:E99	IFVSVSNEH	42
Var:E100	IFVSVANEH	41
Var:E101	IFVSVNNEH	41
Var:E102	IFVSVVNEH	41
Var:E103	IFVSVTSEH	41

Var:E104	IFVSVTTEH	40
Var:E105	IFVSVTGEH	40
Var:E106	IFVSVTDEH	41
Var:E107	IFVSVTEEH	40
Var:E108	IFVSVTQEH	40
Var:E109	IFVSVTHEH	41
Var:E110	IFVSVTKEH	40
Var:E111	IFVSVTNEN	39
Var:E112	IFVSVTNEE	38
Var:E113	IFVSVTNER	38
Var:E114	IFVSVTNEY	40

[154] **Table 4.E.v:** Suitable less immunogenic variants of TRAIL agretope 15 (residues 257-265; FVSVTNEHL); B(wt)=46.

Variant	Variant sequence	B(alt)
Var:E115	MVSVTNEHL	40
Var:E116	IVSVTNEHL	40
Var:E117	LVSVTNEHL	40
Var:E118	FTSVTNEHL	43
Var:E119	FASVTNEHL	43
Var:E120	FMSVTNEHL	43
Var:E121	FISVTNEHL	45
Var:E122	FLSVTNEHL	43
Var:E123	FVDVTNEHL	42
Var:E124	FVEVTNEHL	42
Var:E125	FVSATNEHL	43
Var:E126	FVSVTDEHL	41
Var:E127	FVSVTEEHL	40
Var:E128	FVSVTQEHL	40
Var:E129	FVSVTREHL	40
Var:E130	FVSVTKEHL	40
Var:E131	FVSVTNDHL	43
Var:E132	FVSVTNKHL	42
Var:E133	FVSVTNEHV	43
Var:E134	FVSVTNEHF	42

Var:E135	FVTLTNEHL	40
Var:E136	FVTVTHEHL	38
Var:E137	FVTVTNRHL	38
Var:E138	FVTVTNEHM	41
Var:E139	FVALTNEHL	40
Var:E140	FVAVTNEHL	38
Var:E141	FVAVTNRHL	38
Var:E142	FVAVTNEHM	41
Var:E143	FVQLTNEHL	39
Var:E144	FVQVTNEHL	37
Var:E145	FVQVTNRHL	37
Var:E146	FVQVTNEHM	40
Var:E147	FVKLTNEHL	39
Var:E148	FVKVTNEHL	37
Var:E149	FVKVTNRHL	37
Var:E150	FVKVTNEHM	40
Var:E151	FVSMTHEHL	38
Var:E152	FVSLTHEHL	38
Var:E153	FVSVTGEHM	38
Var:E154	FVSVTHEHM	39
Var:E155	FVSVTNRHM	39

[155] Example 3: Identification of suitable less immunogenic sequences for MHC-binding agretopes as determined by PDA® technology.

[156] **Table 5.** Each position in the agretopes of interest was analyzed to identify a subset of amino acid substitutions that are potentially compatible with maintaining the structure and function of the protein. PDA® technology calculations were run for each position of each nine-mer agretope and compatible

amino acids for each position were saved. In these calculations, side-chains within 5 Angstroms of the position of interest were permitted to change conformation but not amino acid identity. The variant agretopes were then analyzed for immunogenicity. The PDA® energies and Iscore values for the wild-type nine-mer agretope were compared to the variants and the subset of variant sequences with lower predicted immunogenicity and PDA® energies within 5.0 kcal/mol of the wild-type were noted. In the tables below, E(PDA) is the energy determined using PDA® technology calculations compared against the wild-type, Iscore: Anchor is the Iscore for the agretope, and Iscore: Overlap is the sum of the Iscores for all of the overlapping agretopes.

[157] Table 5.A.i. Less immunogenic variants of BAFF agretope 2.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	24.2	17.1
L169A	-0.70	11.3	0.0
L169D	1.11	2.2	0.0
L169E	0.99	15.9	0.0
L169F	0.76	23.3	4.0
L169G	0.65	19.9	0.0
L169H	-0.44	23.3	0.0
L169N	-0.12	23.3	0.0
L169S	-0.06	5.8	0.0
L169T	0.17	11.3	0.0
L169W	3.28	11.3	4.0
L169Y	0.81	23.3	4.0
L170A	3.78	11.3	3.5
L170D	4.20	2.2	2.8
L170E	3.18	2.2	8.4
L170G	4.54	16.4	8.4
L170P	2.77	16.4	3.5
L170S	4.17	16.4	7.1
L170T	4.16	11.3	15.7
S171G	3.53	13.6	14.3
K173N	3.80	14.7	17.1
R174D	2.51	5.2	2.8

R174E	1.77	16.8	2.8
R174G	4.31	18.6	8.4
R174H	2.55	19.7	2.8
R174K	0.07	17.7	3.9
R174Q	1.89	19.4	2.8
R174S	2.61	16.8	10.2
R174T	2.32	16.8	6.7
R174Y	3.32	22.4	8.4
S176A	0.57	14.4	17.1
S176D	0.94	6.6	17.1
S176E	0.84	6.6	17.1
S176F	1.25	21.8	17.1
S176G	0.74	13.3	17.1
S176H	1.18	9.9	17.1
S176K	1.21	5.8	17.1
S176M	2.12	22.0	17.1
S176N	0.48	5.5	17.1
S176P	2.25	5.5	17.1
S176Q	1.22	13.7	17.1
S176R	1.15	13.3	17.1
S176T	0.34	5.5	17.1
S176V	1.15	18.2	17.1
S176W	3.15	2.2	17.1
S176Y	1.20	13.3	17.1

[158] Table 5.A.ii. Less immunogenic variants of BAFF agretope 6.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	42.6	32.7
Y192D	2.99	0.0	31.5
Y192E	1.50	0.0	31.5
Y192K	4.04	0.0	31.5
Y192Q	3.25	0.0	31.5
F194H	4.45	20.9	31.0

I195D	4.69	0.0	28.6
I195L	2.08	17.8	29.9
I195N	3.77	7.6	28.6
I195T	3.67	32.6	28.6
L200E	3.31	0.0	6.1
L200K	3.96	0.0	6.1
L200N	4.29	3.0	6.1
L200Q	3.46	0.0	6.1

[159] Table 5.A.iii. Less immunogenic variants of BAFF agretope 9.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	26.2	46.7
L200E	3.31	0.0	3.6
L200K	3.96	0.0	3.6
L200N	4.29	0.0	6.6
L200Q	3.46	0.0	3.6
D203A	4.22	2.5	46.7
T205D	-3.46	0.0	46.7
T205E	-4.94	0.0	46.7
T205G	-2.83	5.2	46.7
T205N	-5.81	5.2	46.7
T205Q	-6.91	13.1	46.7

T205S	-3.93	13.1	46.7
Y206D	-0.04	15.8	46.7
Y206H	0.05	19.8	46.7
Y206K	-1.35	15.8	46.7
Y206R	-0.71	13.1	46.7
Y206W	-0.75	13.1	46.7
M208A	-0.15	13.1	46.7
M208E	4.79	5.2	46.7
M208G	2.51	13.1	46.7
M208K	4.37	5.2	46.7
M208R	4.25	13.1	46.7
M208T	1.85	5.2	46.7

[160] Table 5.A.iv. Less immunogenic variants of BAFF agretope 10.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	26.4	5.2
I212D	4.99	0.0	5.2
I212E	3.42	0.0	5.2
I212N	4.17	0.0	5.2
I212Q	2.86	0.0	5.2
I212T	3.49	0.0	5.2
Q213A	4.44	8.4	5.2
Q213D	1.08	0.0	5.2
Q213E	0.70	8.4	5.2
R214E	3.57	2.6	5.2
R214N	3.20	26.0	5.2
K215A	-1.05	18.2	5.2
K215D	-0.04	26.2	5.2
K215E	-2.16	5.7	5.2
K215G	0.27	5.2	5.2
K215H	0.36	25.2	5.2
K215I	-2.09	17.8	5.2
K215L	-2.87	24.8	5.2
K215N	-0.94	22.3	5.2
K215Q	-1.33	23.6	5.2
K215R	-1.47	21.4	5.2
K215T	-0.71	8.0	5.2
K215V	-2.02	8.0	5.2
K215W	0.43	25.2	5.2
V217D	2.63	0.0	5.2

V217E	2.85	0.0	5.2
V217I	1.34	19.1	5.2
V217K	2.80	26.0	5.2
V217N	1.11	2.9	5.2
V217Q	0.40	8.4	5.2
V217S	-0.49	8.8	5.2
V217T	-0.27	20.5	5.2
V217Y	2.10	0.0	5.2
H218D	-0.78	2.6	5.2
H218E	0.48	8.4	5.2
H218G	-1.07	5.6	5.2
H218K	0.42	26.0	5.2
H218N	-0.65	24.9	5.2
H218Q	0.28	20.0	5.2
H218S	-0.85	9.4	5.2
H218T	-0.06	8.4	5.2
F220A	-1.88	16.5	0.0
F220D	-0.60	12.8	0.0
F220E	-0.61	14.6	0.0
F220G	-1.58	21.4	5.2
F220H	-0.44	16.9	5.2
F220N	-0.98	13.3	5.2
F220P	1.46	9.3	0.0
F220T	-1.38	12.0	0.0
F220W	2.11	6.9	0.0

[161] Table 5.A.v. Less immunogenic variants of BAFF agretope 12.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	28.0	27.4
L226A	2.43	0.0	27.4

L226D	2.01	0.0	27.4
L226E	0.92	0.0	27.4
L226F	3.02	27.0	27.4
L226G	3.17	0.0	27.4

L226H	2.88	0.0	27.4
L226K	2.74	0.0	27.4
L226N	1.94	0.0	27.4
L226P	4.55	0.0	27.4
L226Q	2.25	0.0	27.4
L226R	2.93	0.0	27.4
L226S	2.55	0.0	27.4
L226T	1.73	0.0	27.4
L226Y	2.59	27.0	27.4
V227A	2.29	1.1	21.9
V227D	0.44	0.0	21.9
V227E	0.23	1.1	16.6
V227H	2.71	6.8	17.7
V227K	-0.25	13.0	17.1
V227L	-0.14	11.4	27.4
V227N	1.76	6.8	16.6
V227Q	0.84	13.0	16.6
V227T	0.88	1.1	16.6
V227W	1.72	1.1	17.9
L229A	4.89	14.0	21.9

L229D	4.85	26.2	21.9
L229E	3.47	0.4	21.9
L229K	4.71	20.3	21.9
L229N	3.60	14.0	23.3
L229Q	3.28	13.2	21.9
L229T	4.16	5.8	26.2
R231A	0.46	20.4	13.0
R231D	2.68	1.4	11.2
R231E	0.90	0.0	13.0
R231G	2.30	7.0	13.3
R231L	-0.52	16.9	17.1
R231M	-0.57	2.5	17.1
R231Q	0.22	8.0	17.1
Q234A	1.84	19.4	27.4
Q234D	3.47	4.2	27.4
Q234E	1.73	9.7	27.4
Q234G	3.86	17.5	27.4
Q234K	-3.82	15.2	27.4
Q234R	1.11	19.6	27.4

[162] Table 5.A.vi. Less immunogenic variants of BAFF agretope 16.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	40.9	0.0
V276A	2.87	0.0	0.0
V276N	4.97	0.0	0.0
V276S	2.50	0.0	0.0
V276T	1.63	0.0	0.0
T277S	4.03	34.0	0.0
F278H	4.43	19.4	0.0
F278K	4.93	14.8	0.0
F279H	3.94	25.9	0.0
F279Y	1.94	28.7	0.0
A281G	3.12	34.9	0.0
L282A	4.64	6.5	0.0

L282D	4.80	0.0	0.0
L282E	3.72	0.0	0.0
L282K	3.66	0.0	0.0
L282M	2.38	34.0	0.0
L282N	3.87	15.2	0.0
L282Q	2.31	1.2	0.0
L282T	3.74	6.5	0.0
L284E	3.17	0.0	0.0
L284M	4.65	30.7	0.0
L284N	4.29	5.6	0.0
L284Q	4.69	7.3	0.0
L284T	4.74	14.8	0.0
L284V	3.80	29.6	0.0

[163] Table 5.B.i. Less immunogenic variants of RANKL agretope 3.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	28.0	48.2
F213A	-0.17	0.0	41.3
F213D	-0.35	0.0	40.1
F213E	-0.91	0.0	40.1
F213G	1.97	0.0	40.1
F213H	-3.87	0.0	41.3
F213L	2.83	16.9	48.2
F213M	-3.20	16.9	41.3

F213N	0.20	0.0	41.3
F213Q	-0.13	0.0	43.2
F213S	1.31	0.0	41.3
F213T	-0.83	0.0	46.6
Y214F	2.40	25.9	48.2
Y215H	4.99	19.1	31.9
L216D	4.81	17.2	9.3
L216E	3.19	23.7	9.3
L216N	3.78	15.1	26.3

L216Q	3.97	15.7	21.4
L216T	4.00	11.2	16.0
L216V	1.70	8.6	44.6
A218G	2.76	16.8	42.7
N219A	4.55	25.5	45.6

N219D	4.46	3.4	21.4
N219H	0.97	26.4	23.9
N219K	-1.38	24.7	39.3
N219Q	4.21	21.3	17.2
N219T	2.06	21.0	41.0

[164] Table 5.B.ii. Less immunogenic variants of RANKL agretope 4.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	34.6	57.0
I220S	-5.55	8.5	51.6
I220A	-4.58	5.5	54.6
I220G	-2.89	3.7	51.6
I220N	-2.07	19.1	51.9
F222Y	-1.52	17.5	57.0
N219K	-1.38	25.3	54.2
I220L	-0.60	32.6	51.6
I220E	-0.23	1.2	51.6
I220D	0.11	1.2	51.6
I220M	0.52	32.0	51.6
I220Q	0.61	1.2	51.6
N219H	0.97	8.8	56.9

I220H	1.15	17.7	51.6
L216V	1.70	16.0	52.7
N219T	2.06	27.4	50.1
F222H	2.91	20.9	41.5
L216E	3.19	1.2	47.3
L216N	3.78	16.0	40.9
L216Q	3.97	7.9	44.7
L216T	4.00	7.9	34.8
N219Q	4.21	3.7	50.3
N219D	4.46	7.9	32.5
N219A	4.55	32.0	54.6
F222K	4.56	8.6	45.4
F222M	4.59	33.4	50.2
L216D	4.81	1.2	40.8
Y215H	4.99	29.6	36.8

[165] Table 5.B.iii. Less immunogenic variants of RANKL agretope 10.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	44.6	68.7
I247A	-3.98	17.6	34.5
I247S	-3.84	41.5	30.3
I247N	-3.78	5.2	43.9
I247E	-3.67	5.2	38.2
I247T	-3.62	5.2	38.2
I247G	-3.47	13.1	26.2
I247D	-3.24	5.2	23.0
T245H	-3.02	42.1	64.0
I247Q	-2.96	21.2	43.9
I247H	-2.72	10.1	45.1
I247R	-2.58	13.1	55.0
I247K	-2.50	17.3	36.6
I247V	-1.54	13.9	54.7
I247Y	-1.34	13.1	61.2
T245K	-0.15	39.0	64.0
T245E	0.12	28.3	64.0
M239T	0.59	0.0	54.2
I247W	0.63	0.0	65.8
T245D	0.64	13.1	64.0
V240N	1.10	5.2	64.6

V240T	1.19	0.0	57.5
V242T	1.19	19.1	36.1
M239E	1.24	0.0	54.2
V240E	1.50	0.0	47.1
V242K	1.80	24.6	44.1
V240A	1.85	0.0	67.9
T245G	1.86	40.4	64.0
V240D	1.89	0.0	47.1
Y241H	1.96	17.9	56.3
V240S	2.44	0.0	68.5
V242E	2.59	5.2	33.2
M239K	2.71	0.0	67.2
M239N	2.73	0.0	67.2
M239A	2.98	0.0	54.2
V240K	3.32	10.0	52.7
Y241E	3.38	0.0	27.4
Y241T	3.40	17.9	62.8
M239S	3.46	0.0	53.8
Y241D	3.48	0.0	51.4
V242A	3.59	38.4	36.1
M239D	3.61	0.0	53.4
V242Q	3.73	43.6	44.1
V242N	3.73	38.4	50.9

Y241N	3.85	26.4	63.3
M239H	3.86	0.0	67.2
V240G	4.25	0.0	68.5

Y241A	4.29	17.9	45.1
Y241Q	4.50	17.9	57.0
V242D	4.64	26.2	27.1

[166] Table 5.B.iv. Less immunogenic variants of RANKL agretope 12.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	35.4	77.9
I247A	-3.98	11.4	40.7
I247S	-3.84	7.2	64.6
I247N	-3.78	20.9	28.3
I247E	-3.67	15.2	28.3
I247T	-3.62	15.2	28.3
I247G	-3.47	3.1	36.1
I249E	-3.25	6.7	67.6
I247D	-3.24	0.0	28.3
I249N	-3.03	6.3	67.6
I249D	-3.03	6.3	67.6
I247Q	-2.96	20.9	44.2
I249L	-2.74	33.6	67.6
I247H	-2.72	22.0	33.2
I247R	-2.58	31.9	36.1
I247K	-2.50	13.6	40.4
I249H	-2.39	9.6	67.6
I249S	-2.18	18.9	67.6
I249Y	-2.10	10.5	76.2
I249T	-2.04	3.6	67.6
I249A	-1.95	6.5	67.6
I249K	-1.85	0.4	67.6
I249Q	-1.83	14.4	67.6
I249F	-1.60	21.4	76.2
I249R	-1.56	2.2	67.6
I247V	-1.54	21.4	47.2

I249V	-1.49	20.0	67.6
I247Y	-1.34	22.0	52.2
I249G	-1.29	5.2	67.6
I249M	-1.11	24.9	77.9
S246N	-0.39	17.3	75.0
S246D	0.16	0.5	65.9
S246T	0.17	35.3	64.3
I249W	0.22	3.1	76.2
S246E	0.39	0.0	74.2
I247W	0.63	26.6	39.1
S246R	0.82	35.0	64.0
T243E	0.96	15.2	68.5
V242T	1.19	6.1	49.1
V242K	1.80	15.5	53.1
Y241H	1.96	0.0	74.2
S246G	2.17	19.1	64.9
V242E	2.59	6.1	32.4
S246P	2.69	31.1	59.3
I249P	3.25	1.3	67.6
Y241E	3.38	0.0	27.4
Y241D	3.48	0.0	51.4
V242A	3.59	6.1	68.5
V242Q	3.73	15.5	72.1
V242N	3.73	15.2	74.1
Y241A	4.29	0.0	63.0
Y241Q	4.50	0.0	74.9
V242D	4.64	0.0	53.3

[167] Table 5.B.v. Less immunogenic variants of RANKL agretope 15.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	45.2	9.8
F280Y	-1.69	29.3	9.8
K282E	-0.21	0.0	9.4
S285A	0.16	27.1	9.4
K282Q	0.67	10.4	9.8
S285E	0.67	0.0	9.4
S285D	0.78	0.0	9.4
K282A	1.07	20.4	9.8
K282D	1.23	14.3	9.4
L283E	1.32	0.4	9.4
S285Q	1.34	20.6	9.4

S285G	1.40	13.6	9.4
K282T	1.50	38.8	9.8
S285H	1.58	13.1	9.4
K282S	1.72	37.0	9.8
L283N	1.73	12.2	9.4
F280T	2.09	9.5	9.4
G279A	2.11	39.8	9.8
V277N	2.18	0.0	9.8
K282H	2.36	18.7	9.8
K282G	2.44	29.5	9.8
V277D	2.84	0.0	9.8
V277A	3.11	0.0	9.8

S285W	3.24	1.1	9.4
L283H	3.46	14.2	9.4
L283Q	3.49	7.4	9.4
L283D	3.99	0.0	9.4

V277P	4.27	0.0	9.8
L283T	4.36	0.4	9.4
L283S	4.74	0.4	9.4
L283A	4.83	13.8	9.4

[168] Table 5.B.vi. Less immunogenic variants of RANKL agretope 17.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	18.5	0.0
S297K	-0.99	8.6	0.0
S297R	-0.72	3.4	0.0
N295H	-0.57	8.6	0.0
S297A	0.13	8.6	0.0
S294K	0.14	0.0	0.0
S294Q	0.45	0.0	0.0
S294R	0.55	0.0	0.0
S294E	0.80	0.0	0.0
S297D	0.86	0.0	0.0
N295E	1.10	17.2	0.0
I291V	1.21	8.6	0.0
S297G	1.66	3.4	0.0
S294I	1.70	8.6	0.0
S294A	1.73	8.6	0.0
E292A	1.80	8.4	0.0
E292S	2.17	12.3	0.0
I291P	2.21	8.6	0.0
N295Q	2.30	8.6	0.0

N295L	2.46	17.2	0.0
N295T	2.49	9.9	0.0
I291Q	2.49	3.4	0.0
N295K	2.68	8.6	0.0
I289T	2.68	0.0	0.0
I291T	3.04	3.4	0.0
N295R	3.08	3.4	0.0
N295D	3.19	8.6	0.0
I289K	3.21	0.0	0.0
I289N	3.54	0.0	0.0
I291E	3.56	0.0	0.0
N295A	3.62	8.6	0.0
S290D	3.62	8.6	0.0
I291K	3.82	3.4	0.0
I289A	3.90	0.0	0.0
E292G	3.98	0.4	0.0
S294H	4.19	0.0	0.0
N295S	4.27	9.9	0.0
I291N	4.32	8.6	0.0
I289S	4.51	0.0	0.0
I291A	4.79	3.4	0.0

[169] Table 5.C.i. Less immunogenic variants of APRIL agretope 5.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	36.5	13.3
L142A	3.52	0.0	13.3
L142D	3.81	0.0	13.3
L142E	2.15	0.0	13.3
L142F	1.49	31.1	13.3
L142G	4.69	0.0	13.3
L142H	2.23	0.0	13.3
L142K	3.05	0.0	13.3
L142N	3.23	0.0	13.3
L142Q	3.35	0.0	13.3
L142S	4.28	0.0	13.3
L142T	3.11	0.0	13.3
L142W	4.37	31.1	13.3
L142Y	1.93	31.1	13.3
R143A	-0.18	0.0	5.5
R143D	0.24	0.0	0.0

R143E	-0.15	0.0	0.0
R143F	1.98	5.6	6.7
R143G	0.93	0.0	0.0
R143H	1.61	5.6	5.5
R143M	0.64	5.9	9.2
R143N	-1.91	5.6	2.6
R143P	-1.58	0.0	5.5
R143Q	-1.30	14.3	2.2
R143S	0.13	0.0	5.5
R143T	-0.40	0.0	11.9
R143W	2.95	0.0	6.7
R143Y	2.05	5.6	6.7
R144A	-1.00	15.2	6.7
R144D	-0.92	0.0	0.0
R144E	-1.45	0.0	0.0
R144G	-0.27	17.1	0.0
R144H	-0.68	17.1	5.5
R144K	0.05	15.2	5.5

R144N	-1.31	33.2	-5.5
R144P	4.14	19.3	0.0
R144Q	-1.22	15.2	3.7
R144S	-1.37	17.1	8.8
R144T	-1.33	15.2	1.2
R144V	-1.83	33.2	8.1
R144W	1.63	15.2	5.5
G147A	-1.63	30.1	13.3
G147D	4.67	14.3	13.3
G147E	1.41	5.6	13.3
G147P	-2.40	33.9	13.3
L148A	4.23	5.9	13.3

L148D	2.78	0.0	13.3
L148E	2.54	1.2	13.3
L148K	1.52	3.1	13.3
L148N	2.46	10.6	13.3
L148P	1.37	13.9	13.3
L148Q	3.06	5.9	13.3
L148S	4.36	8.4	13.3
A150G	2.53	15.2	13.3
A150K	3.66	0.0	13.3
A150P	0.50	14.3	13.3
A150S	0.38	29.1	13.3
A150T	1.99	33.2	13.3

[170] Table 5.C.ii. Less immunogenic variants of APRIL agretope 9.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	35.4	49.5
L164A	3.67	0.0	32.1
L164D	4.25	0.0	26.2
L164E	2.77	0.0	26.2
L164F	-0.19	35.3	48.1
L164H	2.10	0.0	34.7
L164K	0.56	0.0	39.2
L164N	4.13	0.0	40.3
L164Q	3.22	0.0	39.8
L164S	4.94	0.0	32.1
L164Y	-2.17	35.3	48.1
L165A	2.37	13.6	46.5
L165D	2.49	0.0	42.0
L165E	2.63	13.6	30.7
L165G	4.83	18.8	33.2
L165N	1.00	25.5	37.4
S167E	3.86	23.7	29.9
S167K	0.45	34.1	33.6
S167T	-0.44	28.7	48.0

S167V	3.73	30.5	47.5
V169D	5.00	0.0	45.7
V169G	3.53	0.0	49.3
V169S	1.90	26.0	46.2
L170A	2.01	15.6	25.0
L170D	0.53	8.6	4.9
L170E	3.01	8.6	4.9
L170F	-1.24	15.3	27.0
L170G	4.88	5.2	7.1
L170H	0.76	11.9	17.8
L170N	2.18	26.0	6.3
L170Q	2.78	15.2	22.7
L170S	3.08	16.4	27.9
L170T	1.97	16.4	15.7
L170Y	0.80	7.6	22.9
Q172A	-0.06	27.9	49.5
Q172E	-1.41	5.2	36.4
Q172G	1.16	18.2	49.5
Q172K	0.14	21.0	49.5
Q172N	0.60	0.0	49.5
Q172T	-0.49	10.7	49.5

[171] Table 5.C.iii. Less immunogenic variants of APRIL agretope 10.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	26.2	58.7
L170A	2.01	0.0	40.5
L170D	0.53	0.0	13.5
L170E	3.01	0.0	13.5
L170F	-1.24	15.8	26.5
L170G	4.88	0.0	12.4
L170H	0.76	0.0	29.7
L170N	2.18	0.0	32.3

L170Q	2.78	0.0	37.9
L170S	3.08	0.0	44.4
L170T	1.97	0.0	32.1
L170Y	0.80	15.8	14.7
Q172D	0.61	13.1	49.5
Q172E	-1.41	13.1	28.5
D173E	3.33	0.0	58.7
D173N	0.58	7.6	58.7
D173T	4.41	6.1	58.7
T175D	0.56	0.0	58.7

T175E	1.00	0.0	58.7
T175G	2.90	13.1	58.7
T175S	0.86	13.1	58.7
T175Y	3.15	0.0	58.7
F176D	-2.14	15.8	58.7
F176H	-0.33	15.8	58.7
F176K	-0.51	15.8	58.7
F176R	-0.15	13.1	58.7

F176W	-0.13	13.1	58.7
M178A	4.85	13.1	58.7
M178E	4.13	13.1	58.7
M178K	2.64	13.1	58.7
M178N	4.80	5.2	58.7
M178Q	3.37	15.8	58.7
M178T	3.94	5.2	58.7

[172] Table 5.C.iv. Less immunogenic variants of APRIL agretope 11.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	56.7	7.5
F194A	2.04	0.0	7.5
F194D	3.83	0.0	7.5
F194G	4.42	0.0	7.5
F194H	4.32	0.0	7.5
F194N	3.40	0.0	7.5
F194S	2.28	0.0	7.5
F194T	2.74	0.0	7.5
R195A	0.39	17.8	7.5
R195D	-0.16	0.3	7.5
R195E	-1.22	17.8	7.5
R195G	2.17	27.9	7.5
R195I	-0.93	36.6	7.5
R195K	-0.73	36.6	7.5
R195L	3.22	36.1	7.5
R195M	-1.47	36.6	7.5
R195N	-0.35	35.8	7.5
R195Q	-1.24	39.7	7.5
R195S	0.63	17.1	7.5
R195T	-0.99	17.8	7.5
R195V	-1.65	56.2	7.5
I197A	0.27	46.1	0.0
I197D	-0.07	44.8	0.0
I197E	-1.00	25.2	0.0
I197G	2.02	16.9	0.0
I197H	0.26	56.2	0.0

I197K	3.09	38.8	0.0
I197N	1.26	47.9	0.0
I197Q	0.98	49.0	0.0
I197R	3.80	33.2	0.0
I197S	1.05	52.8	0.0
I197T	0.11	45.0	0.0
I197V	-0.65	49.6	7.5
S199G	3.35	42.9	7.5
S199H	1.50	34.5	7.5
S199W	-0.80	26.4	7.5
M200A	-3.91	45.5	0.4
M200D	-5.12	22.4	0.0
M200E	-3.68	31.2	3.4
M200G	0.13	19.4	0.0
M200K	-5.10	39.9	3.1
M200N	-8.27	43.1	0.0
M200Q	-6.10	38.6	0.4
M200S	-5.91	34.8	0.0
M200T	-3.41	35.2	0.0
S202D	-0.39	20.8	0.4
S202E	-0.76	24.7	0.0
S202G	-0.11	38.5	0.0
S202H	1.29	53.4	1.3
S202N	0.17	26.1	7.0
S202Q	-0.01	42.3	1.3
S202W	2.60	40.1	5.7
S202Y	1.69	56.6	5.2

[173] Table 5.D.i. Less immunogenic variants of CD40L agretope 12.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	48.3	16.9
L206A	4.78	0.0	12.3
L206D	4.72	0.0	0.0
L206E	0.74	0.0	4.3
L206N	3.82	0.0	15.4
L206T	4.91	0.0	12.5

R207A	3.31	2.5	7.5
R207D	2.19	0.0	13.1
R207E	2.96	2.5	10.0
R207K	1.61	16.7	9.8
R207N	1.51	9.4	15.8
R207Q	1.46	17.4	10.7
R207S	1.93	0.4	8.7

R207T	3.41	2.5	8.7
A209G	0.86	10.2	12.4
T211D	-1.51	0.0	9.2
T211E	-1.01	0.0	16.8
T211F	1.17	0.0	11.2
T211G	2.35	3.5	6.0
T211K	2.72	37.8	10.1
T211R	2.80	30.7	13.7
T211Y	-0.55	0.0	10.8
H212D	4.55	16.7	13.6
H212F	1.50	46.2	15.3
H212N	3.76	46.9	12.4
S214A	1.01	18.1	16.9
S214D	0.28	1.5	16.9
S214E	0.21	1.5	16.9

S214F	4.42	13.7	16.9
S214G	1.84	11.1	16.9
S214H	1.19	16.7	16.9
S214I	2.11	29.0	16.9
S214K	1.70	15.8	16.9
S214L	3.12	11.1	16.9
S214M	2.87	30.5	16.9
S214N	0.83	3.6	16.9
S214P	-0.23	2.5	16.9
S214Q	1.20	32.3	16.9
S214R	1.76	10.8	16.9
S214T	1.22	2.5	16.9
S214V	1.20	14.8	16.9
S214W	3.73	2.9	16.9
S214Y	3.88	9.4	16.9

[174] Table 5.E.i. Less immunogenic variants of TRAIL agretope 2.

Var.	E(PDA)	I score Anchor	I score Overlap
wt	0.00	46.0	22.2
L174E	3.54	0.0	22.2
L174Q	4.46	0.0	22.2
V175A	2.45	0.0	22.2
V175D	2.36	0.0	22.2
V175E	1.99	2.6	22.2
V175G	4.63	11.4	22.2
V175I	1.79	22.7	22.2
V175K	2.19	22.7	22.2
V175N	2.48	20.0	22.2
V175Q	1.86	29.1	22.2
V175S	2.08	0.0	22.2
V175T	1.85	0.0	22.2
I176L	4.00	40.8	22.2
I176N	4.62	22.7	22.2

I176T	3.41	12.9	22.2
I176V	0.65	22.7	22.2
H177D	-0.64	26.2	22.2
H177E	-1.84	13.1	22.2
H177N	-1.84	41.2	22.2
H177T	-0.48	13.9	22.2
H177W	1.19	34.4	22.2
K179A	2.42	23.5	22.2
K179D	1.13	0.0	22.2
K179E	-0.20	0.0	22.2
K179G	0.98	16.2	22.2
K179H	1.99	20.0	22.2
K179N	1.04	25.8	22.2
K179P	-1.03	29.1	22.2
K179Q	-0.01	11.4	22.2
K179S	-0.58	31.4	22.2

[175] Table 5.E.ii. Less immunogenic variants of TRAIL agretope 7.

Var.	E(PDA)	I score Anchor	I score Overlap
wt	0.00	47.2	15.5
V207A	3.18	0.0	1.3
V207D	2.82	0.0	1.3
V207E	3.43	0.0	1.3
V207K	3.60	0.0	1.3
V207N	1.45	0.0	1.3
V207Q	2.31	0.0	1.3
V207S	3.86	0.0	1.3
V207T	1.86	0.0	1.3
Q208A	1.05	9.9	15.5

Q208D	-0.04	0.0	1.3
Q208E	-0.91	9.9	1.3
Q208T	3.88	9.9	15.5
Y209E	4.60	1.1	0.0
Y209H	3.29	20.8	0.0
Y209K	4.53	20.8	0.0
I210E	4.71	2.9	14.3
I210K	4.80	33.5	15.5
I210L	3.04	44.3	15.5
I210N	4.45	36.0	14.3
I210Q	2.45	38.3	15.5

I210T	4.22	7.9	14.3
K212G	4.84	12.3	14.8
Y213H	3.26	38.9	15.5
Y213K	4.85	36.2	15.5

S215D	-0.24	0.0	14.3
S215E	-0.27	6.2	14.3
S215T	0.50	1.1	15.5

[176] Table 5.E.iii. Less immunogenic variants of TRAIL agretope 10.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	28.5	9.1
L221A	4.71	0.0	4.0
L221K	4.03	0.0	9.1
L221N	3.25	0.0	8.6
L221P	2.86	0.0	1.9
L221T	4.75	0.0	4.0
L222E	2.83	22.3	1.4
L222T	4.97	22.3	4.0
M223Q	4.87	14.1	0.0
K224A	0.97	27.7	7.6
K224E	1.61	13.2	7.6
K224G	3.12	6.7	7.6

K224S	1.60	15.0	7.6
K224T	1.21	9.3	7.6
A226G	4.02	26.2	1.9
R227A	0.51	26.4	9.1
R227D	-0.27	0.4	9.1
R227E	-2.18	3.6	9.1
R227F	4.22	27.7	9.1
R227G	1.50	3.5	9.1
R227K	-1.11	22.9	9.1
R227Q	-2.75	13.8	9.1
R227Y	2.44	17.6	9.1
S229A	0.48	22.3	7.6
S229G	1.10	26.8	7.6
S229T	1.84	11.2	7.6

[177] Table 5.E.iv. Less immunogenic variants of TRAIL agretope 14.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	21.8	33.9
I256A	4.28	0.0	33.9
I256K	4.54	0.0	33.9
I256N	4.88	0.0	33.9
I256Q	4.15	0.0	33.9
I256T	2.70	0.0	33.9
V258A	4.82	10.2	0.0
V258P	0.48	13.2	0.0
V258S	4.73	13.2	0.0
V258T	1.49	10.2	0.0
S259A	-1.64	18.7	33.6
S259G	2.50	1.0	33.9
T261E	3.90	0.0	33.9
T261N	4.15	10.2	33.9
T261Q	4.76	0.0	33.9
T261S	3.39	9.9	33.9
N262D	0.02	8.6	6.1
N262E	-0.35	8.6	6.1

N262F	0.02	2.3	30.3
N262H	-1.07	10.2	30.3
N262K	1.84	8.6	15.2
N262Q	2.18	10.7	15.2
N262R	1.59	2.5	15.5
N262W	3.07	1.0	30.3
N262Y	0.54	2.5	15.2
H264A	0.99	20.9	33.9
H264D	0.63	1.1	33.9
H264E	-0.12	1.1	33.9
H264G	1.13	4.7	33.9
H264K	1.71	12.3	33.9
H264L	2.22	18.7	33.9
H264M	2.04	20.4	33.9
H264N	0.56	3.8	33.9
H264P	2.79	3.4	33.9
H264R	1.98	4.7	33.9
H264T	1.36	3.4	33.9
H264V	1.76	14.8	33.9

[178] Table 5.E.v. Less immunogenic variants of TRAIL agretope 15.

Var.	E(PDA)	Iscore Anchor	Iscore Overlap
wt	0.00	33.9	21.8
F257H	3.50	0.0	21.8

V258A	4.82	0.0	10.2
V258N	4.72	15.2	21.8
V258P	0.48	0.0	13.2
V258S	4.73	0.0	13.2

V258T	1.49	0.0	10.2
S259A	-1.64	33.6	18.7
V260A	4.64	24.0	21.8
V260D	2.13	17.2	21.8
V260N	4.81	24.4	21.8
N262D	0.02	6.1	8.6
N262E	-0.35	6.1	8.6
N262F	0.02	30.3	2.3
N262H	-1.07	30.3	10.2
N262K	1.84	15.2	8.6
N262Q	2.18	15.2	10.7
N262R	1.59	15.5	2.5
N262W	3.07	30.3	1.0
N262Y	0.54	15.2	2.5
E263G	2.63	16.6	21.8

E263K	0.22	21.7	21.8
E263P	-1.97	22.1	21.8
L265A	3.27	12.1	21.8
L265D	2.41	2.2	21.8
L265E	0.97	2.2	21.8
L265F	3.23	16.5	21.8
L265G	5.00	1.8	21.8
L265H	2.95	13.2	21.8
L265K	1.90	5.4	21.8
L265M	4.40	20.2	21.8
L265N	2.16	1.4	21.8
L265Q	2.61	15.0	21.8
L265R	4.55	0.7	21.8
L265S	3.18	23.4	21.8
L265Y	2.71	1.8	21.8

[179] Example 4. MHC agretopes in TNF SF member variants designed for soluble expression, superagonism, dominant negative inhibition, and competitive inhibition.

[180] Previously described TNF SF member variants have been designed for a number of improved properties, including but not limited to superagonism, dominant negative inhibition, competitive inhibition, and receptor specificity. All 9-mers for which I score is altered, relative to wild type, in one or more variants is shown below.

Table 10. I scores of MHC agretopes in wild type human BAFF versus designed BAFF variants.																				
Var.	170-178	171-179	203-211	207-215	208-216	213-221	216-224	217-225	228-236	234-242	235-243	236-244	238-246	250-258	267-275	269-277	271-279	274-282	278-286	280-288
WT	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q167E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q167K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q167R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q167D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
S170N	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
S170L	5.6	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
S170D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171A	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171E	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171H	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171K	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171R	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171D	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171T	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171F	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171L	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y171I	0	0	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
D211S	0	2.8	0.4	0	10	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
D211N	0	2.8	0	0	0.4	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
D211E	0	2.8	0	0	0	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
D211K	0	2.8	0	0	3.6	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0

D211G	0	2.8	0	0	0.4	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
K212E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
K212Q	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213A	0	2.8	0	1.1	13	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213N	0	2.8	0	0	5.2	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213S	0	2.8	0	0	13	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213D	0	2.8	0	0	0	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213I	0	2.8	0	5.4	26	11	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T213L	0	2.8	0	13	13	11	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214A	0	2.8	0	0	33	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214E	0	2.8	0	0	33	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214K	0	2.8	0	0	16	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214Q	0	2.8	0	0	33	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214S	0	2.8	0	0	29	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214F	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Y214I	0	2.8	0	0	33	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
A215S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
A215T	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
G217A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
G217V	0	2.8	0	0	26	0	3.6	1.1	0	28	5.6	0	17	0	3.3	0	0	0	0	0
G217S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
G217T	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L219K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L219V	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L219D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L219E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
T236N	0	2.8	0	0	26	0	0	0	0	36	14	0	17	0	3.3	0	0	0	0	0
T236V	0	2.8	0	0	26	0	0	0	0	36	42	6.5	17	0	3.3	0	0	0	0	0
T236K	0	2.8	0	0	26	0	0	0	0	28	16	0	17	0	3.3	0	0	0	0	0
T236Q	0	2.8	0	0	26	0	0	0	8.6	28	16	0	17	0	3.3	0	0	0	0	0
T236E	0	2.8	0	0	26	0	0	0	0	11	5.6	0	17	0	3.3	0	0	0	0	0
T236D	0	2.8	0	0	26	0	0	0	0	6.8	0	0	17	0	3.3	0	0	0	0	0
R239A	0	2.8	0	0	26	0	0	0	0	20	5.6	0	2.2	0	3.3	0	0	0	0	0
R239K	0	2.8	0	0	26	0	0	0	0	31	5.6	0	6.3	0	3.3	0	0	0	0	0
R239E	0	2.8	0	0	26	0	0	0	0	0	5.6	0	2.2	0	3.3	0	0	0	0	0
R239D	0	2.8	0	0	26	0	0	0	0	1.5	5.6	0	0.4	0	3.3	0	0	0	0	0
I241A	0	2.8	0	0	26	0	0	0	0	28	0	0	7.3	0	3.3	0	0	0	0	0
I241E	0	2.8	0	0	26	0	0	0	0	28	0	0	19	0	3.3	0	0	0	0	0
I241L	0	2.8	0	0	26	0	0	0	0	28	5.6	0	18	0	3.3	0	0	0	0	0
I241T	0	2.8	0	0	26	0	0	0	0	28	0	0	11	0	3.3	0	0	0	0	0
I241V	0	2.8	0	0	26	0	0	0	0	28	0	0	4.2	0	3.3	0	0	0	0	0
I241Q	0	2.8	0	0	26	0	0	0	0	28	0	0	11	0	3.3	0	0	0	0	0
I241Y	0	2.8	0	0	26	0	0	0	0	28	0	0	24	0	3.3	0	0	0	0	0
E246Q	0	2.8	0	0	26	0	0	0	0	28	5.6	0	41	0	3.3	0	0	0	0	0
E246K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	39	0	3.3	0	0	0	0	0
L248A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0

L248K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248N	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248Q	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248Y	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
L248F	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
N250A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
N250S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
N250D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
N250Y	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	8.3	3.3	0	0	0	0	0
P272N	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	1	0	8.6	0	0	0
P272D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	0	0	0	0	0	0
P272S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	1	0	0	0	0	0
P272A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	0.4	0	0	0	0	0
R273A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	0	0	0	0	0	0
R273K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	0	0	0	0	0	0
R273L	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	1.1	0	0	0	0	0
R273E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	0	0	0	0	0	0
R273H	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	0	0	0	0	0	0
E274A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
E274L	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
E274Q	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
E274T	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	5.3	0	0	0	0
E274K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
E274R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	1	0	0	0
E274D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	5.2	0	0	0
E274I	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
E274F	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	3.4	0	0
N275D	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	2.6	0	0	0	0	0
N275E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	8.4	0	0	0	0	0
N275R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	19	0	0	0	0	0
N275S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	31	0	0	0	0	0
Q277H	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q277K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q277S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q277R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
Q277E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
S279R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	13	0
S279E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0
D281A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	13
D281E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	13
D281S	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	13
D281K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	26
D281R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	30
D281H	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	26
D281N	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	26
D283A	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	13	0

D283V	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	26	0
D283K	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	26	0
D283R	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	26	0
D283H	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	13	0
D283N	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	5.2	0
D283E	0	2.8	0	0	26	0	0	0	0	28	5.6	0	17	0	3.3	0	0	0	0	0

Table 12. MHC agretopes in RANKL variants designed for soluble expression, superagonism, dominant negative inhibition, and competitive inhibition.

residues	215-223	220-228	222-230	235-243	236-244	240-248	241-249	247-255	264-272	270-278	281-289	291-299
wild type	5.5	0.0	15.5	3.0	1.5	4.7	35.4	10.3	0.0	9.4	0.0	0.0
C221S/I247E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H180E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/K181Q	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/K181E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H189R	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/R191Q	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/G192A	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/S197E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/R223M	2.9	0.0	4.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/R223E	1.1	0.0	0.4	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/R223Q	1.8	0.0	4.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225T	0.0	0.4	10.9	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225N	0.0	0.0	12.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225E	0.0	0.0	19.4	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225R	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E226Q	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E226D	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E226R	0.0	0.8	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/T227K	0.0	0.0	5.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/S228E	0.0	0.0	22.8	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/S228H	0.0	0.0	30.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/Q237T	0.0	0.0	15.5	3.0	0.0	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/Q237K	0.0	0.0	15.5	3.0	0.0	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/Q237E	0.0	0.0	15.5	0.0	0.0	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/K248E	0.0	0.0	15.5	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/I249Q	0.0	0.0	15.5	3.0	1.5	4.7	3.1	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H253S	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H253T	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/S268D	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E269R	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E269T	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E269Q	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E269K	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	2.2	9.4	0.0	0.0
C221S/I247E/F270T	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/F270K	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/F270E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/F270V	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	7.6	0.0	0.0
C221S/I247E/R284E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	7.6	0.0
C221S/I247E/S297Q	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	6.1
C221S/I247E/D300N	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D302H	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0

C221S/I247E/D302E	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E269T/F270T	0.0	0.0	15.5	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/H225E/E269K	0.0	0.0	19.4	3.0	1.5	4.7	15.2	0.0	2.2	9.4	0.0	0.0
C221S/I247E/H225E/F270T	0.0	0.0	19.4	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/H225R/E269K	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	2.2	9.4	0.0	0.0
C221S/I247E/H225R/F270T	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/R191E/I249R	0.0	0.0	15.5	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0
C221S/I247E/G192AH225N	0.0	0.0	12.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225NI249R	0.0	0.0	12.3	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225RI249R	0.0	0.0	7.3	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0
C221S/I247E/E226RI249R	0.0	0.8	15.5	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0
C221S/I247E/T227QK248E	0.0	0.0	0.0	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/S228EI249R	0.0	0.0	22.8	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D190T/K248E	0.0	0.0	15.5	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D190T/H225R	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D190Q/K248E	0.0	0.0	15.5	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D190Q/H225R	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D190Q/K248E	0.0	0.0	15.5	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/G192A/H225R	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/D190Q/H225R	0.0	0.0	7.3	3.0	1.5	4.7	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/G192A/K248E	0.0	0.0	15.5	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/H225E/E226D/F270T	0.0	0.0	19.4	3.0	1.5	4.7	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/R191K/E226R/K248E	0.0	0.8	15.5	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/R191K/E226Q/K248E	0.0	0.0	11.3	3.0	1.5	1.1	15.2	0.0	0.0	9.4	0.0	0.0
C221S/I247E/G192A/K248E/F270T	0.0	0.0	15.5	3.0	1.5	1.1	15.2	0.0	0.0	0.0	0.0	0.0
C221S/I247E/G192A/H225N/I249R	0.0	0.0	12.3	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0
C221S/I247E/G192A/H225R/I249R	0.0	0.0	7.3	3.0	1.5	4.7	0.0	0.0	0.0	9.4	0.0	0.0

Table 5.D Comparison of MHC binding agretopes in wild type human CD40L and designed CD40L variants.

	145-153	146-154	152-160	168-176	169-177	170-178	171-179	175-183	189-197	204-212	205-213	206-214	223-231	229-237	237-245	253-261
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wt	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
A123E	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
H125R	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
V126A	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
V126D	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
W140C	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
W140G	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
W140R	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
W140X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
K143E	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
G144E	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
T147N	22	32	9	10	8	14	19	1	15	5	12	48	18	8	9	13
L155P	18	14	0	10	8	14	19	1	15	5	12	48	18	8	9	13
Y170P	18	14	9	3	0	0	19	1	15	5	12	48	18	8	9	13
A173D	18	14	9	0	8	29	2	1	15	5	12	48	18	8	9	13
Q174R	18	14	9	6	35	14	12	1	15	5	12	48	18	8	9	13
T176I	18	14	9	34	8	30	9	4	15	5	12	48	18	8	9	13
S184X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
Q186X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
L195P	18	14	9	10	8	14	19	1	0	5	12	48	18	8	9	13
R200X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
E202X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
R203E	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
R207E	18	14	9	10	8	14	19	1	15	9	1	2	18	8	9	13
A208D	18	14	9	10	8	14	19	1	15	5	31	11	18	8	9	13
C218X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
Q220X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
Q221X	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
H224Y	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
G226A	18	14	9	10	8	14	19	1	15	5	12	48	24	8	9	13
G227V	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
L231S	18	14	9	10	8	14	19	1	15	5	12	48	30	0	9	13
Q232X	18	14	9	10	8	14	19	1	15	5	12	48	18	0	9	13
A235P	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
S236X	18	14	9	10	8	14	19	1	15	5	12	48	18	0	9	13
V237E	18	14	9	10	8	14	19	1	15	5	12	48	18	0	0	13
T254M	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	44
G257D	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
G257S	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	13
L258S	18	14	9	10	8	14	19	1	15	5	12	48	18	8	9	42

[181] In a preferred embodiment, variants that do not have any new agretopes (when compared to the wild type human TNF SF member) are developed for therapeutic use. In an especially preferred embodiment, variants in which Iscore is reduced for one or more agretopes relative to wild type human TNF SF member are developed for therapeutic use.

[182] While the foregoing invention has been described above, it will be clear to one skilled in the art that various changes and additional embodiments made be made without departing from the scope of the invention. All references cited herein, including patents, patent applications (provisional, utility and PCT), and publications are entirely incorporated by reference.

[183] SEQUENCE ID 1: (wild type human BAFF, extra-cellular domain underlined)

MDDSTEREQSRLTSCLKKREEMKLKECVSILPRKESPSVRSSKDGKLLAATLLALLSCCLTVVSFYQ
VAALQGD LASLRAELQGHHA EKLPAGAGAPKAGLEEAPAVTAGLKIFEPPAPGEGNSSQNSRNKRAV
QGPEETVTQDCLQLIADSETPTIQKGSYTFVPWLLSFKRGSAALEEKENKILVKETGYFFIYGQVLYTDK
TYAMGHLIQRKKVHVFGDELSLVTLFRCIQNMPETLPNNSCYSAGIAKLEEGDELQLAIPRENAQISLD
GDVTFFGALKLL

[184] SEQUENCE ID 2: (wild type human RANKL, extra-cellular domain underlined)

MRRASRDYTKYLRGSEEMGGGPGAPHEGPLHAPPPAPHQPPAASRSMFVALLGLGLGQVVCSSVA
LFFYFRAQMDPNRISEDGTHCIYRILRLHENADFQD TTLESQDTKLIPDSCRRIKQAFQGA VQKELQHI
VGSQH IRAEKAMVDG SWLDLAKRSKLEAQPF AHL TINATDIPSGSHKVSLS SSWYH DRGWAKISNMTF
SNGLIVNQDGFYYLYANICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTLMKGGSTKYWSGNSE
FHFYSINVG GFFKLRS GEEISIEVSNPSLLDPDQDATYFGAFKVRDID

[185] SEQUENCE ID 3: (wild type human APRIL, extra-cellular domain underlined)

MPASSPFLAPKGPPGNMGGPVREPALSVALWLSWGAALGAVACAMALLTQQTELQSLRREVSRLQ
GTGGPSQNGEGYPWQSLPEQSSDALEAWENGERSRKRRAVLTQKQKKQH SVLHLVPINATSKDDS
DVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFR
IRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARA KLNLSPHGTFLGFVKL

[186] SEQUENCE ID 4: (wild type human CD40L, extra-cellular domain underlined)

MIETYNQTS PRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQ
RCNTGERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVL
QWAEKGYTMSNNLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILL
RAANTHSSAKPCGQQSIHLGGVFELQPGASVFNVTDP SQVSHGTGFTSFGLLKL

[187] SEQUENCE ID 5: (wild type human TRAIL, extra-cellular domain underlined)

MAMMEVQGGPSLGQTCVLIVFTVLLQSLCVAVTYVYFTNELKQM QDKYSKSGIACFLKEDDSYWD P
NDEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVA AHITGTRGRS
NTLSSPN SKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYIYSQTYFRFQEEIKENTK
NDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLYSIQGGIFELKENDRIFVSVTNEHLIDMD
HEASFFGAFLVG

Claims

[188] Claims:

1. A non-naturally occurring variant TNF Super Family member protein having reduced immunogenicity as compared with a parent protein, wherein said variant protein comprises at least two amino acid modifications; and, wherein said parent protein is selected from the group consisting of human BAFF (SEQ. ID. 1), human RANKL (SEQ. ID. 2), human APRIL (SEQ. ID. 3), human CD40L (SEQ. ID. 4), and human TRAIL (SEQ. ID. 5).
2. A variant protein of claim 1 wherein said parent protein is human BAFF.
3. A variant protein of claim 2 wherein at least one amino acid modification is made to the group consisting of Agretope A1 (163-171), Agretope A2 (168-176), Agretope A3 (169-177), Agretope A4 (185-193), Agretope A5 (186-194), Agretope A6 (192-200), Agretope A7 (193-201), Agretope A8 (194-202), Agretope A9 (200-208), Agretope A10 (212-220), Agretope A11 (219-227), Agretope A12 (226-234), Agretope A13 (227-235), Agretope A14 (230-238), Agretope A15 (259-267), and Agretope A16 (276-284).
4. A variant protein of claim 2, wherein at least one amino acid modification is made to the group consisting of positions 192, 194, 195, 200, 203, 205, 206, 208, 212, 213, 214, 215, 217, 218, 220, 226, 227, 229, 231, 234, 276, 277, 278, 279, 281, 282, and 284; and, wherein the possible modifications at position 192 are selected from the group consisting of E, D, Q, and K; wherein the possible modification at position 194 is H; wherein the possible modifications at position 195 are selected from the group consisting of L, T, N, and D; wherein the possible modifications at position 200 are selected from the group consisting of E, Q, K, and N; wherein the possible modification at position 203 is A; wherein the possible modifications at position 205 are selected from the group consisting of Q, N, E, S, D, and G; wherein the possible modifications at position 206 are selected from the group consisting of K, W, R, D, and H; wherein the possible modifications at position 208 are selected from the group consisting of A, T, G, R, K, and E; wherein the possible modifications at position 212 are selected from the group consisting of Q, E, T, N, and D; wherein the possible modifications at position 213 are selected from the group consisting of E, D, and A; wherein the possible modifications at position 214 are selected from the group consisting of N and E; wherein the possible modifications at position 215 are selected from the group consisting of L, E, I, V, R, Q, A, N, T, D, G, H, and W; wherein the possible modifications at position 217 are selected from the group consisting of S, T, Q, N, I, Y, D, K, and E; wherein the possible modifications at position 218 are selected from the group consisting of G, S, D, N, T, Q, K, and E; wherein the possible modifications at position 220 are selected from the group consisting of A, G, T, N, E, D, H, P, and W; wherein the possible modifications at position 226 are selected from the group consisting of E, T, N, D, Q, A, S, Y, K, H, R, F, G, and P; wherein the possible modifications at position 227 are selected from the group consisting of K, L, E, D, Q, T, W, N, A, and H; wherein the possible modifications at position 229 are selected from the group consisting of Q, E, N, T, K, D, and A; wherein the possible modifications at position 231 are selected from the group consisting of M, L, Q, A, E, G, and D; wherein the possible modifications at position 234 are selected from the group consisting of K, R, E, A, D, and G; wherein the possible modifications at position 276 are selected from the group consisting of T, S, A, and N;

wherein the possible modification at position 277 is S; wherein the possible modifications at position 278 are selected from the group consisting of H and K; wherein the possible modifications at position 279 are selected from the group consisting of Y and H; wherein the possible modification at position 281 is G; wherein the possible modifications at position 282 are selected from the group consisting of Q, M, K, E, T, N, A, and D; and, wherein the possible modifications at position 284 are selected from the group consisting of E, V, N, M, Q, and T.

5. A variant protein of claim 2 wherein at least one amino acid modification is made to the group consisting of positions 192, 195, 200, 205, 212, 213, 217, 226, 227, 231, 276, 282 and 284; and wherein the possible modifications at position 192 are selected from the group consisting of E, D, Q, and K; wherein the possible modification at position 195 is D; wherein the possible modifications at position 200 are selected from the group consisting of E, Q, K, and N; wherein the possible modifications at position 205 are selected from the group consisting of E and D; wherein the possible modifications at position 212 are selected from the group consisting of Q, E, T, N, and D; wherein the possible modification at position 213 is D; wherein the possible modifications at position 217 are selected from the group consisting of Y, D, and E; wherein the possible modifications at position 226 are selected from the group consisting of E, T, N, D, Q, A, S, K, H, R, G, and P; wherein the possible modification at position 227 is D; wherein the possible modification at position 231 is E; wherein the possible modifications at position 276 are selected from the group consisting of T, S, A, and N; wherein the possible modifications at position 282 are selected from the group consisting of K, E, and D; wherein the possible modification at position 284 is E.
6. A variant protein of claim 1 wherein said parent protein is human RANKL.
7. A variant protein of claim 6 wherein at least one amino acid modification is made to the group consisting of Agretope B1 (193-201), Agretope B2 (207-215), Agretope B3 (213-221), Agretope B4 (214-222), Agretope B5 (215-223), Agretope B6 (222-230), Agretope B7 (235-243), Agretope B8 (236-244), Agretope B9 (238-246), Agretope B10 (239-247), Agretope B11 (240-248), Agretope B12 (241-249), Agretope B13 (247-255), Agretope B14 (270-278), Agretope B15 (277-285), Agretope B16 (280-288), Agretope B17 (289-297), and, Agretope B18 (308-316).
8. A variant protein of claim 6, wherein at least one amino acid modification is made to the group consisting of positions 213, 214, 215, 216, 218, 219, 220, 222, 239, 240, 241, 242, 243, 245, 246, 247, 249, 277, 279, 280, 282, 283, 285, 289, 290, 291, 292, 294, 295, and 297; and, wherein the possible modifications at position 213 are selected from the group consisting of H, M, E, T, D, A, Q, N, S, G, and L; wherein the possible modification at position 214 is F; wherein the possible modification at position 215 is H; wherein the possible modifications at position 216 are selected from the group consisting of V, E, N, Q, T, and D; wherein the possible modification at position 218 is G; wherein the possible modifications at position 219 are selected from the group consisting of K, H, T, Q, D, and A; wherein the possible modifications at position 220 are selected from the group consisting of S, A, G, N, L, E, D, M, Q, and H; wherein the possible modifications at position 222 are selected from the group consisting of Y, H, K, and M; wherein the possible modifications at position 239 are selected from the group consisting of T, E, K, N, A, S, D, and H; wherein the possible modifications at position 240 are selected from the group consisting of N, T, E, A, D, S, K, and G; wherein the possible

modifications at position 241 are selected from the group consisting of H, E, T, D, N, A, and Q; wherein the possible modifications at position 242 are selected from the group consisting of T, K, E, A, Q, N, and D; wherein the possible modification at position 243 is E; wherein the possible modifications at position 245 are selected from the group consisting of H, K, E, D, and G; wherein the possible modifications at position 246 are selected from the group consisting of N, D, T, E, R, G, and P; wherein the possible modifications at position 247 are selected from the group consisting of A, S, N, E, T, G, D, Q, H, R, K, V, Y, and W; wherein the possible modifications at position 249 are selected from the group consisting of E, N, D, L, H, S, Y, T, A, K, Q, F, R, V, G, M, W, and P; wherein the possible modifications at position 277 are selected from the group consisting of N, D, A, and P; wherein the possible modification at position 279 is A; wherein the possible modifications at position 280 are selected from the group consisting of Y and T; wherein the possible modifications at position 282 are selected from the group consisting of E, Q, A, D, T, S, H, and G; wherein the possible modifications at position 283 are selected from the group consisting of E, N, H, Q, D, T, S, and A; wherein the possible modifications at position 285 are selected from the group consisting of A, E, D, Q, G, H, and W; wherein the possible modifications at position 289 are selected from the group consisting of T, K, N, A, and S; wherein the possible modification at position 290 is D; wherein the possible modifications at position 291 are selected from the group consisting of V, P, Q, T, E, K, N, and A; wherein the possible modifications at position 292 are selected from the group consisting of A, S, and G; wherein the possible modifications at position 294 are selected from the group consisting of K, Q, R, E, I, A, and H; wherein the possible modifications at position 295 are selected from the group consisting of H, E, Q, L, T, K, R, D, A, and S; wherein the possible modifications at position 297 are selected from the group consisting of K, R, A, D, and G.

9. A variant protein of claim 6, wherein at least one amino acid modifications is selected from a group consisting of positions 213, 239, 240, 241, 242, 246, 247, 277, 282, 283, 285, 289, 291, 294 and 297; and, wherein the possible modifications at position 213 are selected from the group consisting of H, E, T, D, A, Q, N, S, and G; wherein the possible modifications at position 239 are selected from the group consisting of T, E, K, N, A, S, D, and H; wherein the possible modifications at position 240 are selected from the group consisting of T, E, A, D, S, and G; wherein the possible modifications at position 241 are selected from the group consisting of H, E, D, A, and Q; wherein the possible modification at position 242 is D; wherein the possible modifications at position 246 are selected from the group consisting of E; wherein the possible modifications at position 247 are selected from the group consisting of W and D; wherein the possible modifications at position 277 are selected from the group consisting of N, D, A, and P; wherein the possible modifications at position 282 is E; wherein the possible modification at position 283 is D; wherein the possible modifications at position 285 are selected from the group consisting of E and D; wherein the possible modifications at position 289 are selected from the group consisting of T, K, N, A, and S; wherein the possible modification at position 291 is E; wherein the possible modifications at position 294 are selected from the group consisting of K, Q, R, E, and H; and, wherein the possible modification at position 297 is D.
10. A variant protein of claim 1 wherein said parent protein is human APRIL.

11. A variant protein of claim 10 wherein at least one amino acid modification is made to the group consisting of Agretope C1 (117-125), Agretope C2 (120-128), Agretope C3 (121-129), Agretope C4 (138-146), Agretope C5 (142-150), Agretope C6 (155-163), Agretope C7 (162-170), Agretope C8 (163-171), Agretope C9 (164-172), Agretope C10 (170-178), Agretope C11 (194-202), Agretope C12 (197-205), Agretope C13 (217-225), Agretope C14 (227-235), Agretope C15 (228-236), Agretope C16 (236-244), and, Agretope C17 (238-246).
12. A variant protein of claim 10, wherein at least one amino acid modification is made to the group consisting of positions 142, 143, 144, 147, 148, 150, 164, 165, 167, 169, 170, 172, 173, 175, 176, 178, 194, 195, 197, 199, 200, and 202; and, wherein the possible modifications at position 142 are selected from the group consisting of F, Y, E, H, K, T, N, Q, A, D, S, W, and G; wherein the possible modifications at position 143 are selected from the group consisting of N, P, Q, T, A, E, S, D, M, G, H, F, Y, and W; wherein the possible modifications at position 144 are selected from the group consisting of V, E, S, T, N, Q, A, D, H, G, K, W, and P; wherein the possible modifications at position 147 are selected from the group consisting of P, A, E, and D; wherein the possible modifications at position 148 are selected from the group consisting of P, K, N, E, D, Q, A, and S; wherein the possible modifications at position 150 are selected from the group consisting of S, P, T, G, and K; wherein the possible modifications at position 164 are selected from the group consisting of Y, F, K, H, E, Q, A, N, D, and S; wherein the possible modifications at position 165 are selected from the group consisting of N, A, D, E, and G; wherein the possible modifications at position 167 are selected from the group consisting of T, K, V, and E; wherein the possible modifications at position 169 are selected from the group consisting of S, G, and D; wherein the possible modifications at position 170 are selected from the group consisting of F, D, H, Y, T, A, N, Q, E, S, and G; wherein the possible modifications at position 172 are selected from the group consisting of E, D, T, A, K, N, and G; wherein the possible modifications at position 173 are selected from the group consisting of N, E, and T; wherein the possible modifications at position 175 are selected from the group consisting of D, S, E, G, and Y; wherein the possible modifications at position 176 are selected from the group consisting of D, K, H, R, and W; wherein the possible modifications at position 178 are selected from the group consisting of K, Q, T, E, N, and A; wherein the possible modifications at position 194 are selected from the group consisting of A, S, T, N, D, H, and G; wherein the possible modifications at position 195 are selected from the group consisting of V, M, Q, E, T, I, K, N, D, A, S, G, and L; wherein the possible modifications at position 197 are selected from the group consisting of E, V, D, T, H, A, Q, S, N, G, K, and R; wherein the possible modifications at position 199 are selected from the group consisting of W, H, and G; wherein the possible modifications at position 200 are selected from the group consisting of N, Q, S, D, K, A, E, T, and G; wherein the possible modifications at position 202 are selected from the group consisting of E, D, G, Q, N, H, W, and Y.
13. A variant protein of claim 10, wherein at least one amino acid modification is made to the group consisting of positions 142, 143, 144, 148, 150, 164, 165, 169, 170, 172, 173, 175, and 194; and wherein the possible modifications at position 142 are selected from the group consisting of E, H, K, T, N, Q, A, D, S, and G; wherein the possible modifications at position 143 are selected from the group consisting of P, T, A, E, S, D, G, and W; wherein the possible modifications at position 144 are

selected from the group consisting of E and D; wherein the possible modification at position 148 is D; wherein the possible modification at position 150 is K; wherein the possible modifications at position 164 are selected from the group consisting of K, H, Q, A, N, D, and S; wherein the possible modification at position 165 is D; wherein the possible modifications at position 169 are selected from the group consisting of G and D; wherein the possible modifications at position 170 are selected from the group consisting of D, H, T, A, N, Q, E, S, and G; wherein the possible modification at position 172 is N; wherein the possible modification at position 173 is E; wherein the possible modifications at position 175 are selected from the group consisting of D, E, and Y; and, wherein the possible modifications at position 194 are selected from the group consisting of A, S, T, N, D, H, and G.

14. A variant protein of claim 1 wherein said parent protein is human CD40L, and, wherein at least one amino acid modification is made to the group consisting of Agretope D1 (145-153), Agretope D2 (146-154), Agretope D3 (152-160), Agretope D4 (168-176), Agretope D5 (169-177), Agretope D6 (170-178), Agretope D7 (171-179), Agretope D8 (175-183), Agretope D9 (189-197), Agretope D10 (204-212), Agretope D11 (205-213), Agretope D12 (206-214), Agretope D13 (223-231), Agretope D14 (229-237), Agretope D15 (237-245), and, Agretope D16 (253-261).
15. A variant protein of claim 14, wherein said at least one amino acid modification is made from the group consisting of positions 206, 207, 209, 211, 212 and 214; wherein the possible modifications at position 206 are selected from the group consisting of E, N, D, A, T; wherein the possible modifications at position 207 are selected from the group consisting of Q, N, K, S, D, E, A, T; wherein the possible modification at position 209 is G; wherein the possible modifications at position 211 are selected from the group consisting of D, E, Y, F, G, K, R; wherein the possible modifications at position 212 are selected from the group consisting of F, N, D; and, wherein the possible modifications at position 214 are selected from the group consisting of P, E, D, N, A, H, Q, V, T, K, R, G, I, M, L, W, Y, F.
16. A variant protein of claim 14, wherein said at least one amino acid modification is made from the group consisting of positions 206, 207, and 211; and wherein the possible modifications at position 206 are selected from the group consisting of E, N, D, A, T; wherein the possible modification at position 207 is D; and, wherein the possible modifications at position 211 are selected from the group consisting of D, E, Y, F.
17. A variant protein of claim 1, wherein said parent protein is human TRAIL.
18. A variant protein of claim 17, wherein at least one amino acid modification is made to the group consisting of Agretope E1 (151-159), Agretope E2 (174-182), Agretope E3 (181-189), Agretope E4 (182-190), Agretope E5 (183-191), Agretope E6 (206-214), Agretope E7 (207-215), Agretope E8 (209-217), Agretope E9 (220-228), Agretope E10 (221-229), Agretope E11 (223-231), Agretope E12 (237-245), Agretope E13 (240-248), Agretope E14 (256-264), and, Agretope E15 (257-265).
19. A variant protein of claim 17, wherein at least one amino acid modification is made to the group consisting of 174, 175, 176, 177, 179, 207, 208, 209, 210, 212, 213, 215, 221, 222, 223, 224, 226, 227, 229, 256, 257, 258, 259, 260, 261, 262, 263, 264, and 265; and, wherein the possible modifications at position 174 are selected from the group consisting of E and Q; wherein the possible modifications at position 175 are selected from the group consisting of I, T, Q, E, S, K, D, A, N, and G;

wherein the possible modifications at position 176 are selected from the group consisting of V, T, L, and N; wherein the possible modifications at position 177 are selected from the group consisting of E, N, D, T, and W; wherein the possible modifications at position 179 are selected from the group consisting of P, S, E, Q, G, N, D, H, and A; wherein the possible modifications at position 207 are selected from the group consisting of N, T, Q, D, A, E, K, and S; wherein the possible modifications at position 208 are selected from the group consisting of E, D, A, and T; wherein the possible modifications at position 209 are selected from the group consisting of H, K, and E; wherein the possible modifications at position 210 are selected from the group consisting of Q, L, T, N, E, and K; wherein the possible modification at position 212 is G; wherein the possible modifications at position 213 are selected from the group consisting of H and K; wherein the possible modifications at position 215 are selected from the group consisting of E, D, and T; wherein the possible modifications at position 221 are selected from the group consisting of P, N, K, A, and T; wherein the possible modifications at position 222 are selected from the group consisting of E and T; wherein the possible modification at position 223 is Q; wherein the possible modifications at position 224 are selected from the group consisting of A, T, S, E, and G; wherein the possible modification at position 226 is G; wherein the possible modifications at position 227 are selected from the group consisting of Q, E, K, D, A, G, Y, and F; wherein the possible modifications at position 229 are selected from the group consisting of A, G, and T; wherein the possible modifications at position 256 are selected from the group consisting of T, Q, A, K, and N; wherein the possible modification at position 257 is H; wherein the possible modifications at position 258 are selected from the group consisting of P, T, N, S, and A; wherein the possible modifications at position 259 are selected from the group consisting of A and G; wherein the possible modifications at position 260 are selected from the group consisting of D, A, and N; wherein the possible modifications at position 261 are selected from the group consisting of S, E, N, and Q; wherein the possible modifications at position 262 are selected from the group consisting of H, E, F, D, Y, R, K, Q, and W; wherein the possible modifications at position 263 are selected from the group consisting of P, K, and G; wherein the possible modifications at position 264 are selected from the group consisting of E, N, D, A, G, T, K, V, R, M, L, and P; and, wherein the possible modifications at position 265 are selected from the group consisting of E, K, N, D, Q, Y, H, S, F, A, M, R, and G.

20. A variant protein of claim 17 wherein at least one amino acid modification is made to the group consisting of 174, 175, 179, 207, 208, 215, 221, 256, 257, 258, and 261; and, wherein the possible modifications at position 174 are selected from the group consisting of E and Q; wherein the possible modifications at position 175 are selected from the group consisting of T, S, D, and A; wherein the possible modifications at position 179 are selected from the group consisting of E and D; wherein the possible modifications at position 207 are selected from the group consisting of N, T, Q, D, A, E, K, and S; wherein the possible modification at position 208 is D; wherein the possible modification at position 215 is D; wherein the possible modifications at position 221 are selected from the group consisting of P, N, K, A, and T; wherein the possible modifications at position 256 are selected from the group consisting of T, Q, A, K, and N; wherein the possible modification at position 257 is H; wherein the possible modifications at position 258 are selected from the group consisting of P, T, S,

and A; and, wherein the possible modifications at position 261 are selected from the group consisting of E and Q;.

Figure 1

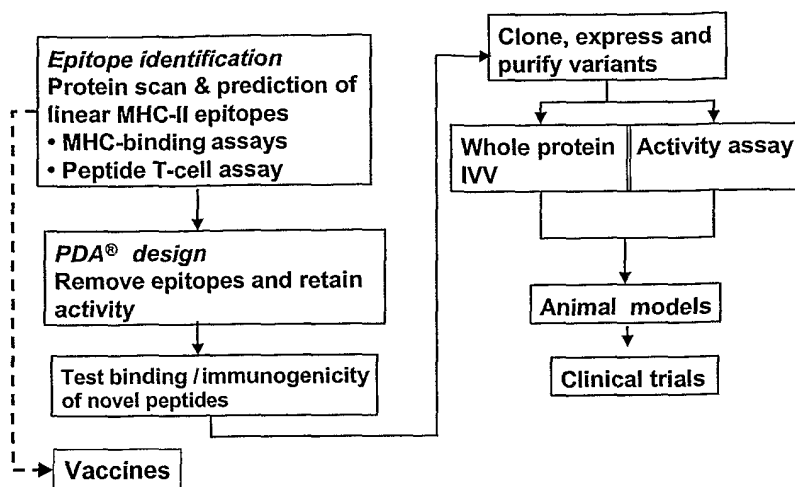


Figure 2

