# PROTEINS BINDING HER2, NKG2D AND CD16

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/461,146, filed February 20, 2017, the entire contents of which are incorporated by reference herein for all purposes.

## SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 16, 2018, is named DFY-008PC_SL.txt and is 92,807 bytes in size.

## FIELD OF THE INVENTION

**[0003]** The invention relates to multi-specific binding proteins that bind to human epidermal growth factor receptor 2 (HER2 or ErbB2), the NKG2D receptor, and CD16.

## BACKGROUND

**[0004]** Cancer continues to be a significant health problem despite the substantial research efforts and scientific advances reported in the literature for treating this disease. Some of the most frequently diagnosed cancers include prostate cancer, breast cancer, and lung cancer. Prostate cancer is the most common form of cancer in men. Breast cancer remains a leading cause of death in women. Current treatment options for these cancers are not effective for all patients and/or can have substantial adverse side effects. Other types of cancer also remain challenging to treat using existing therapeutic options.

**[0005]** Cancer immunotherapies are desirable because they are highly specific and can facilitate destruction of cancer cells using the patient's own immune system. Fusion proteins such as bi-specific T-cell engagers are cancer immunotherapies described in the literature that bind to tumor cells and T-cells to facilitate destruction of tumor cells. Antibodies that bind to certain tumor-associated antigens and to certain immune cells have been described in the literature. *See, e.g.*, WO 2016/134371 and WO 2015/095412.

**[0006]** Natural killer (NK) cells are a component of the innate immune system and make up approximately 15% of circulating lymphocytes. NK cells infiltrate virtually all tissues and were originally characterized by their ability to kill tumor cells effectively without the need for prior sensitization. Activated NK cells kill target cells by means similar to cytotoxic T

cells – *i.e.*, via cytolytic granules that contain perforin and granzymes as well as via death receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN-γ and chemokines that promote the recruitment of other leukocytes to the target tissue.

**[0007]** NK cells respond to signals through a variety of activating and inhibitory receptors on their surface. For example, when NK cells encounter healthy self-cells, their activity is inhibited through activation of the killer-cell immunoglobulin-like receptors (KIRs). Alternatively, when NK cells encounter foreign cells or cancer cells, they are activated via their activating receptors (*e.g.*, NKG2D, NCRs, DNAM1). NK cells are also activated by the constant region of some immunoglobulins through CD16 receptors on their surface. The overall sensitivity of NK cells to activation depends on the sum of stimulatory and inhibitory signals.

**[0008]** HER2 (ErbB2) is a transmembrane glycoprotein, which belongs to the epidermal growth factor receptor family. It is a receptor tyrosine kinase and regulates cell survival, proliferation, and growth. HER2 plays an important role in human malignancies. The *erbB2* gene is amplified or overexpressed in approximately 30% of human breast cancers. Patients with HER2-overexpressing breast cancer have substantially lower overall survival rates and shorter disease-free intervals than patients whose cancer does not overexpress HER2. Moreover, overexpression of HER2 leads to increased breast cancer metastasis. Over-expression of HER2 is also known to occur in many other cancer types, including breast, ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

## SUMMARY

**[0009]** The invention provides multi-specific binding proteins that bind to HER2 on a cancer cell and to the NKG2D receptor and CD16 receptor on natural killer cells. Such proteins can engage more than one kind of NK activating receptor, and may block the binding of natural ligands to NKG2D. In certain embodiments, the proteins can agonize NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various aspects and embodiments of the invention are described in further detail below.

**[0010]** Accordingly, one aspect of the invention provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds to HER2; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. The antigen-binding sites may each incorporate an

antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.*, arranged as in an antibody, or fused together to from an scFv, or one or more of the antigen-binding sites may be a single domain antibody, such as a $V_HH$ antibody like a camelid antibody or a $V_{NAR}$ antibody like those found in cartilaginous fish.

**[0011]** The first antigen-binding site, which binds to NKG2D, in one embodiment, can incorporate a heavy chain variable domain related to SEQ ID NO:1, such as by having an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1, and/or incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:62), CDR2 (SEQ ID NO:63), and CDR3 (SEQ ID NO:64) sequences of SEQ ID NO:1. Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:41 and a light chain variable domain related to SEQ ID NO:42. For example, the heavy chain variable domain of the first antigen binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:41, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:65), CDR2 (SEQ ID NO:66), and CDR3 (SEQ ID NO:67) sequences of SEQ ID NO:41. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:42, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:68), CDR2 (SEQ ID NO:69), and CDR3 (SEQ ID NO:70) sequences of SEQ ID NO:42. In other embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:43 and a light chain variable domain related to SEQ ID NO:44. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:43, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73) sequences of SEQ ID NO:43. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:44, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:74), CDR2 (SEQ ID NO:75), and CDR3 (SEQ ID NO:76) sequences of SEQ ID NO:44.

**[0012]** Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:45 and a light chain variable domain related to SEQ ID NO:46, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:45 and at least 90%

(*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:46 respectively. In another embodiment, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:47 and a light chain variable domain related to SEQ ID NO:48, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:47 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:48 respectively.

**[0013]** The second antigen-binding site can optionally incorporate a heavy chain variable domain related to SEQ ID NO:49 and a light chain variable domain related to SEQ ID NO:53. For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:49, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:50), CDR2 (SEQ ID NO:51), and CDR3 (SEQ ID NO:52) sequences of SEQ ID NO:49. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:53 and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:54), CDR2 (SEQ ID NO:55), and CDR3 (SEQ ID NO:56) sequences of SEQ ID NO:53.

**[0014]** Alternatively, the second antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:57 and a light chain variable domain related to SEQ ID NO:58. For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:57, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:77), CDR2 (SEQ ID NO:78), and CDR3 (SEQ ID NO:79) sequences of SEQ ID NO:57. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:58, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:80), CDR2 (SEQ ID NO:81), and CDR3 (SEQ ID NO:82) sequences of SEQ ID NO:58.

**[0015]** In another embodiment, the second antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:59 and a light chain variable domain related to SEQ ID NO:60. For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:59, and/or incorporate amino acid sequences identical to the

CDR1 (SEQ ID NO:83), CDR2 (SEQ ID NO:84), and CDR3 (SEQ ID NO:85) sequences of SEQ ID NO:59. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:60, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:86), CDR2 (SEQ ID NO:87), and CDR3 (SEQ ID NO:88) sequences of SEQ ID NO:60.

**[0016]** In some embodiments, the second antigen-binding site incorporates a light chain variable domain having an amino acid sequence identical to the amino acid sequence of the light chain variable domain present in the first antigen-binding site.

**[0017]** In some embodiments, the protein incorporates a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains, and/or amino acid sequences at least 90% identical to amino acid sequence 234-332 of a human IgG antibody.

**[0018]** Formulations containing one of these proteins; cells containing one or more nucleic acids expressing these proteins, and methods of enhancing tumor cell death using these proteins are also provided.

**[0019]** Another aspect of the invention involves a method of treating cancer in a patient. The method comprises administering to a patient in need thereof a therapeutically effective amount of the multi-specific binding protein described herein. Exemplary cancers for treatment using the multi-specific binding proteins include, for example, breast, ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0020]** **FIG. 1** is a representation of a multi-specific binding protein that contains an NKG2D-binding domain (right arm), a tumor associated antigen-binding domain (left arm) and an Fc domain or a portion thereof that binds to CD16.

**[0021]** **FIG. 2** is a representation of a multi-specific binding protein that contains an NKG2D-binding domain in a scFv format (right arm), a tumor associated antigen-binding domain (left arm) and an Fc domain or a portion thereof that binds to CD16.

**[0022]** **FIG. 3** are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to human recombinant NKG2D in an ELISA assay.

**[0023]** **FIG. 4** are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to cynomolgus recombinant NKG2D in an ELISA assay.

**[0024]** **FIG. 5** are line graphs demonstrating the binding affinity of NKG2D-binding domains (listed as clones) to mouse recombinant NKG2D in an ELISA assay.

**[0025]** **FIG. 6** are bar graphs demonstrating the binding of NKG2D-binding domains (listed as clones) to EL4 cells expressing human NKG2D by flow cytometry showing mean fluorescence intensity (MFI) fold over background.

**[0026]** **FIG. 7** are bar graphs demonstrating the binding of NKG2D-binding domains (listed as clones) to EL4 cells expressing mouse NKG2D by flow cytometry showing mean fluorescence intensity (MFI) fold over background.

**[0027]** **FIG. 8** are line graphs demonstrating specific binding affinity of NKG2D-binding domains (listed as clones) to recombinant human NKG2D-Fc by competing with natural ligand ULBP-6.

**[0028]** **FIG. 9** are line graphs demonstrating specific binding affinity of NKG2D-binding domains (listed as clones) to recombinant human NKG2D-Fc by competing with natural ligand MICA.

**[0029]** **FIG. 10** are line graphs demonstrating specific binding affinity of NKG2D-binding domains (listed as clones) to recombinant mouse NKG2D-Fc by competing with natural ligand Rae-1 delta.

**[0030]** **FIG. 11** are bar graphs showing activation of human NKG2D by NKG2D-binding domains (listed as clones) by quantifying the percentage of TNFα-positive cells, which express human NKG2D-CD3 zeta fusion proteins.

**[0031]** **FIG. 12** are bar graphs showing activation of mouse NKG2D by NKG2D-binding domains (listed as clones) by quantifying the percentage of TNFα-positive cells, which express mouse NKG2D-CD3 zeta fusion proteins.

**[0032]** **FIG. 13** are bar graphs showing activation of human NK cells by NKG2D-binding domains (listed as clones).

**[0033]** **FIG. 14** are bar graphs showing activation of human NK cells by NKG2D-binding domains (listed as clones).

**[0034]** **FIG. 15** are bar graphs showing activation of mouse NK cells by NKG2D-binding domains (listed as clones).

**[0035]** **FIG. 16** are bar graphs showing activation of mouse NK cells by NKG2D-binding domains (listed as clones).

**[0036]** **FIG. 17** are bar graphs showing the cytotoxic effect of NKG2D-binding domains (listed as clones) on tumor cells.

**[0037]** **FIG. 18** are bar graphs showing the melting temperature of NKG2D-binding domains (listed as clones) measured by differential scanning fluorimetry.

**[0038]** **FIG. 19** is a graph showing enhanced activation of human NK cells by multi-specific binding proteins.

**[0039]** **FIG. 20** is a graph showing multi-specific binding proteins induced higher levels of cytotoxicity towards tumor target cells by human NK cells.

**[0040]** **FIG. 21** is a graph showing multi-specific binding proteins induced higher levels of cytotoxicity towards tumor target cells by human NK cells.

**[0041]** **FIG. 22** is a graph showing multi-specific binding proteins induced higher levels of cytotoxicity towards tumor target cells by human NK cells.

**[0042]** **FIG. 23** is a graph showing multi-specific binding proteins induced higher levels of cytotoxicity towards tumor target cells by human NK cells.

**[0043]** **FIG. 24** is a graph showing multi-specific binding proteins induced higher levels of cytotoxicity towards tumor target cells by mouse NK cells.

**[0044]** **FIG. 25** is a graph showing multi-specific binding proteins induced higher levels of cytotoxicity towards tumor target cells by mouse NK cells.

**[0045]** **FIG. 26** is a binding profile of HER2-targeting TriNKETs to NKG2D expressed on EL4 cells. FIG. 26 represents the same two NKG2D-binding domains now paired with a HER2 second targeting arm.

**[0046]** **FIG. 27A** is a binding profile of HER2-targeting TriNKETs to HER2 expressed on human 786-O renal cell carcinoma cells; **FIG. 27B** shows that NKG2D binding clone C26 containing TriNKET binds to RMA cells transduced with human HER2; **FIG. 27C** shows NKG2D binding clone F04 containing TriNKET binds to RMA cells transduced with human HER2.

**[0047]** **FIGs. 28A – 28C** are bar graphs demonstrating that TriNKETs and trastuzumab were able to activate primary human NK cells in co-culture with HER2-positive human tumor cells, indicated by an increase in CD107a degranulation and IFNγ cytokine production. Compared to the monoclonal antibody trastuzumab, both TriNKETs showed superior activation of human NK cells with a variety of human HER2 cancer cells. FIG. 28A shows that human NK cells are activated by TriNKETs when cultured with SkBr-3 cells. FIG. 28B shows that human NK cells are activated by TriNKETs when cultured with Colo201 cells. FIG. 28C shows that human NK cell are activated by TriNKETs when cultured with HCC1954 cells.

**[0048]** **FIGs. 29A-29B** are graphs demonstrating TriNKETs provide the greater advantage against HER2 medium and low cancers compared to trastuzumab. FIG. 29A shows activated human NK cell killing of HER2 high-SkBr-3 tumor cells. FIG. 29B shows human NK cell killing of HER2 low-786-O tumor cells. TriNKETs provide a greater advantage compared to trastuzumab against cancer cells with low HER2 expression.

**[0049]** **FIGs. 30A-30C** are bar graphs of synergistic activation of NK cells using CD16 and NKG2D. FIG. 30A demonstrates levels of CD107a; FIG. 30B demonstrates levels of IFNγ; FIG. 30C demonstrates levels of CD107a and IFNγ. Graphs indicate the mean (n = 2) ±SD. Data are representative of five independent experiments using five different healthy donors.

**[0050]** **FIG. 31** is a bar graph showing activation of NK cells using TriNKETs targeting NKG2D and CD16. Antibodies tested were of human IgG1 isotypes. Graphs indicate the mean (n = 2) ± SD.

**[0051]** **FIGs. 32A – 32C** are graphs demonstrating TriNKET enhancement of cytotoxic activity using IL-2-activated and rested human NK cells. FIG. 32A shows percent specific lysis of SkBr-3 tumor cells by rested human NK cells. FIG. 32B shows percent specific lysis of SkBr-3 tumor cells by IL-2-activated human NK cells. FIG. 32C shows percent specific lysis of NCI-H661 lung cancer cells by IL-2-activated human NK cells.

**[0052]** **FIGs. 33A & 33B** are bar graphs showing B cells from a health donor are sensitive to TriNKET-mediated lysis. **FIGs. 33C & 33D** are bar graphs showing myeloid cells are resistant to TriNKET-mediated lysis.

**[0053]** **FIG. 34** are line graphs of TriNKETs-mediated hPBMC killing of SkBr-3 tumor cells in long-term co-cultures.

**[0054]** **FIG. 35** is a line graph showing tri-specific binding in one molecule is important for maximal NK cell activity.

**[0055]** **FIG. 36** is a flowchart of study design of RMA/S-HER2 subcutaneous SC2.2 efficacy.

**[0056]** **FIG. 37** are line graphs showing that SC2.2 has no effect on subcutaneous RMA/S-HER2 tumor growth.

**[0057]** **FIG. 38A** shows that HER2-TriNKET-C26 bridges hNKG2D-Fc to RMA-HER2 cells. **FIG. 38B** shows HER2-TriNKET-F04 bridges hNKG2D-Fc to RMA-HER2 cells. Dotted line represents isotype control. Solid line without fill represents unstained control. Solid line with fill represents the TriNKETs.

**[0058]** **FIG. 39** is a representation of a TriNKET in the Triomab form, which is a trifunctional, bispecific antibody that maintains an IgG-like shape. This chimera consists of two half antibodies, each with one light and one heavy chain, that originate from two parental antibodies. Triomab form may be an heterodimeric construct containing ½ of rat antibody and ½ of mouse antibody.

**[0059]** **FIG. 40** is a representation of a TriNKET in the KiH Common Light Chain (LC) form, which involves the knobs-into-holes (KIHs) technology. KiH is a heterodimer containing 2 Fabs binding to target 1 and 2, and an Fc stabilized by heterodimerization mutations. TriNKET in the KiH format may be an heterodimeric construct with 2 fabs binding to target 1 and target 2, containing two different heavy chains and a common light chain that pairs with both heavy chains.

**[0060]** **FIG. 41** is a representation of a TriNKET in the dual-variable domain immunoglobulin (DVD-Ig™) form, which combines the target binding domains of two monoclonal antibodies via flexible naturally occurring linkers, and yields a tetravalent IgG - like molecule. DVD-Ig™ is an homodimeric construct where variable domain targeting antigen 2 is fused to the N terminus of variable domain of Fab targeting antigen 1 Construct contains normal Fc.

**[0061]** **FIG. 42** is a representation of a TriNKET in the Orthogonal Fab interface (Ortho-Fab) form, which is an heterodimeric construct that contains 2 Fabs binding to target1 and target 2 fused to Fc. LC-HC pairing is ensured by orthogonal interface. Heterodimerization is ensured by mutations in the Fc.

**[0062]** **FIG. 43** is a representation of a TrinKET in the 2-in-1 Ig format.

**[0063]** **FIG. 44** is a representation of a TriNKET in the ES form, which is an heterodimeric construct containing two different Fabs binding to target 1 and target 2 fused to the Fc. Heterodimerization is ensured by electrostatic steering mutations in the Fc.

**[0064]** **FIG. 45** is a representation of a TriNKET in the Fab Arm Exchange form: antibodies that exchange Fab arms by swapping a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule, resulting in bispecific antibodies. Fab Arm Exchange form (cFae) is a heterodimer containing 2 Fabs binding to target 1 and 2, and an Fc stabilized by heterodimerization mutations.

**[0065]** **FIG. 46** is a representation of a TriNKET in the SEED Body form, which is an heterodimer containing 2 Fabs binding to target 1 and 2, and an Fc stabilized by heterodimerization mutations.

**[0066]** **FIG. 47** is a representation of a TriNKET in the LuZ-Y form, in which leucine zipper is used to induce heterodimerization of two different HCs. LuZ-Y form is a heterodimer containing two different scFabs binding to target 1 and 2, fused to Fc. Heterodimerization is ensured through leucine zipper motifs fused to C-terminus of Fc.

**[0067]** **FIG. 48** is a representation of a TriNKET in the Cov-X-Body form.

**[0068]** **FIGs. 49A-49B** are representations of TriNKETs in the κλ-Body forms, which are an heterodimeric constructs with two different Fabs fused to Fc stabilized by heterodimerization mutations: Fab1 targeting antigen 1 contains kappa LC, while second Fab targeting antigen 2 contains lambda LC. FIG. 49A is an exemplary representation of one form of a κλ-Body; FIG. 49B is an exemplary representation of another κλ-Body.

**[0069]** **FIG. 50** is an Oasc-Fab heterodimeric construct that includes Fab binding to target 1 and scFab binding to target 2 fused to Fc. Heterodimerization is ensured by mutations in the Fc.

**[0070]** **FIG. 51** is a DuetMab, which is an heterodimeric construct containing two different Fabs binding to antigens 1 and 2, and Fc stabilized by heterodimerization mutations. Fab 1 and 2 contain differential S-S bridges that ensure correct light chain (LC) and heavy chain (HC) pairing.

**[0071]** **FIG. 52** is a CrossmAb, which is an heterodimeric construct with two different Fabs binding to targets 1 and 2 fused to Fc stabilized by heterodimerization. CL and CH1 domains and VH and VL domains are switched, *e.g.*, CH1 is fused in-line with VL, while CL is fused in-line with VH.

**[0072]** **FIG. 53** is a Fit-Ig, which is an homodimeric constructs where Fab binding to antigen 2 is fused to the N terminus of HC of Fab that binds to antigen 1. The construct contains wild-type Fc.

## DETAILED DESCRIPTION

**[0073]** The invention provides multi-specific binding proteins that bind a HER2 on a cancer cell and the NKG2D receptor and CD16 receptor on natural killer cells to activate the natural killer cell, pharmaceutical compositions comprising such multi-specific binding proteins, and therapeutic methods using such multi-specific proteins and pharmaceutical compositions, including for the treatment of cancer. Various aspects of the invention are set forth below in sections; however, aspects of the invention described in one particular section are not to be limited to any particular section.

**[0074]** To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

**[0075]** The terms “a” and “an” as used herein mean “one or more” and include the plural unless the context is inappropriate. As used herein, the term "antigen-binding site" refers to the part of the immunoglobulin molecule that participates in antigen binding. In human antibodies, the antigen-binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions," or "FRs." Thus the term "FR" refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In a human antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." In certain animals, such as camels and cartilaginous fish, the antigen-binding site is formed by a single antibody chain providing a “single domain antibody.” Antigen-binding sites can exist in an intact antibody, in an antigen-binding fragment of an antibody that retains the antigen-binding surface, or in a recombinant polypeptide such as an scFv, using a peptide linker to connect the heavy chain variable domain to the light chain variable domain in a single polypeptide.

**[0076]** The term “tumor associated antigen” as used herein means any antigen including but not limited to a protein, glycoprotein, ganglioside, carbohydrate, lipid that is associated with cancer. Such antigen can be expressed on malignant cells or in the tumor microenvironment such as on tumor-associated blood vessels, extracellular matrix, mesenchymal stroma, or immune infiltrates.

**[0077]** As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably include humans.

**[0078]** As used herein, the term “effective amount” refers to the amount of a compound (*e.g.*, a compound of the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or

dosages and is not intended to be limited to a particular formulation or administration route. As used herein, the term "treating" includes any effect, *e.g.*, lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

**[0079]** As used herein, the term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

**[0080]** As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, s*ee e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975].

**[0081]** As used herein, the term "pharmaceutically acceptable salt" refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound of the present invention which, upon administration to a subject, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, "salts" of the compounds of the present invention may be derived from inorganic or organic acids and bases. Exemplary acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

**[0082]** Exemplary bases include, but are not limited to, alkali metal (*e.g.*, sodium) hydroxides, alkaline earth metal (*e.g.*, magnesium) hydroxides, ammonia, and compounds of formula $NW_4^+$, wherein W is $C_{1-4}$ alkyl, and the like.

**[0083]** Exemplary salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate,

persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as $Na^+$, $NH_4^+$, and $NW_4^+$ (wherein W is a $C_{1-4}$ alkyl group), and the like.

**[0084]** For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

**[0085]** Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

**[0086]** As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

## I. PROTEINS

**[0087]** The invention provides multi-specific binding proteins that bind HER2 on a cancer cell and the NKG2D receptor and CD16 receptor on natural killer cells to activate the natural killer cell. The multi-specific binding proteins are useful in the pharmaceutical compositions and therapeutic methods described herein. Binding of the multi-specific binding protein to the NKG2D receptor and CD16 receptor on natural killer cell enhances the activity of the natural killer cell toward destruction of a cancer cell. Binding of the multi-specific binding protein to HER2 on a cancer cell brings the cancer cell into proximity with the natural killer cell, which facilitates direct and indirect destruction of the cancer cell by the natural killer cell. Further description of exemplary multi-specific binding proteins is provided below.

**[0088]** The first component of the multi-specific binding proteins binds to NKG2D receptor-expressing cells, which can include but are not limited to NK cells, $\gamma\delta$ T cells and $CD8^+$ $\alpha\beta$ T cells. Upon NKG2D binding, the multi-specific binding proteins may block natural ligands, such as ULBP6 and MICA, from binding to NKG2D and activating NKG2D receptors.

**[0089]** The second component of the multi-specific binding proteins binds to HER2-expressing cells, which can include but are limited to breast, ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma, adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

**[0090]** The third component for the multi-specific binding proteins binds to cells expressing CD16, an Fc receptor on the surface of leukocytes including natural killer cells, macrophages, neutrophils, eosinophils, mast cells, and follicular dendritic cells.

**[0091]** The multi-specific binding proteins described herein can take various formats. For example, one format is a heterodimeric, multi-specific antibody including a first immunoglobulin heavy chain, a first immunoglobulin light chain, a second immunoglobulin heavy chain and a second immunoglobulin light chain (FIG. 1). The first immunoglobulin heavy chain includes a first Fc (hinge-CH2-CH3) domain, a first heavy chain variable domain and optionally a first CH1 heavy chain domain. The first immunoglobulin light chain includes a first light chain variable domain and a first light chain constant domain. The first immunoglobulin light chain, together with the first immunoglobulin heavy chain, forms an antigen-binding site that binds NKG2D. The second immunoglobulin heavy chain comprises a second Fc (hinge-CH2-CH3) domain, a second heavy chain variable domain and optionally a second CH1 heavy chain domain. The second immunoglobulin light chain includes a second light chain variable domain and a second light chain constant domain. The second immunoglobulin light chain, together with the second immunoglobulin heavy chain, forms an antigen-binding site that binds HER2. The first Fc domain and second Fc domain together are able to bind to CD16 (FIG. 1). In some embodiments, the first immunoglobulin light chain can be identical to the second immunoglobulin light chain.

**[0092]** Another exemplary format involves a heterodimeric, multi-specific antibody including a first immunoglobulin heavy chain, a second immunoglobulin heavy chain and an immunoglobulin light chain (FIG. 2). The first immunoglobulin heavy chain includes a first Fc (hinge-CH2-CH3) domain fused via either a linker or an antibody hinge to a single-chain variable fragment (scFv) composed of a heavy variable domain and light chain variable domain which pair and bind NKG2D or HER2. The second immunoglobulin heavy chain includes a second Fc (hinge-CH2-CH3) domain, a second heavy chain variable domain and optionally a CH1 heavy chain domain. The immunoglobulin light chain includes a light chain variable domain and a constant light chain domain. The second immunoglobulin heavy chain pairs with the immunoglobulin light chain and binds to NKG2D or HER2. The first Fc domain and the second Fc domain together are able to bind to CD16 (FIG. 2).

**[0093]** One or more additional binding motifs may be fused to the C-terminus of the constant region CH3 domain, optionally via a linker sequence. In certain embodiments, the antigen-binding site could be a single-chain or disulfide-stabilized variable region (scFv) or could form a tetravalent or trivalent molecule.

**[0094]** In some embodiments, the multi-specific binding protein is in the Triomab form, which is a trifunctional, bispecific antibody that maintains an IgG-like shape. This chimera consists of two half antibodies, each with one light and one heavy chain, that originate from two parental antibodies.

**[0095]** In some embodiments, the multi-specific binding protein is the KiH Common Light Chain (LC) form, which involves the knobs-into-holes (KIHs) technology. The KIH involves engineering $C_H3$ domains to create either a "knob" or a "hole" in each heavy chain to promote heterodimerization. The concept behind the "Knobs-into-Holes (KiH)" Fc technology was to introduce a "knob" in one CH3 domain (CH3A) by substitution of a small residue with a bulky one (*e.g.*, $T366W_{CH3A}$ in EU numbering). To accommodate the "knob," a complementary "hole" surface was created on the other CH3 domain (CH3B) by replacing the closest neighboring residues to the knob with smaller ones (*e.g.*, $T366S/L368A/Y407V_{CH3B}$). The "hole" mutation was optimized by structured-guided phage library screening (Atwell S, Ridgway JB, Wells JA, Carter P., Stable heterodimers from remodeling the domain interface of a homodimer using a phage display library, *J. Mol. Biol.* (1997) 270(1):26–35). X-ray crystal structures of KiH Fc variants (Elliott JM, Ultsch M, Lee J, Tong R, Takeda K, Spiess C, *et al.*, Antiparallel conformation of knob and hole aglycosylated half-antibody homodimers is mediated by a CH2-CH3 hydrophobic interaction. *J. Mol. Biol.* (2014) 426(9):1947–57; Mimoto F, Kadono S, Katada H, Igawa T, Kamikawa T, Hattori K. Crystal structure of a novel asymmetrically engineered Fc variant with improved affinity for FcgammaRs. *Mol. Immunol.* (2014) 58(1):132–8) demonstrated that heterodimerization is thermodynamically favored by hydrophobic interactions driven by steric complementarity at the inter-CH3 domain core interface, whereas the knob–knob and the hole–hole interfaces do not favor homodimerization owing to steric hindrance and disruption of the favorable interactions, respectively.

**[0096]** In some embodiments, the multi-specific binding protein is in the dual-variable domain immunoglobulin (DVD-Ig™) form, which combines the target binding domains of two monoclonal antibodies via flexible naturally occurring linkers, and yields a tetravalent IgG-like molecule.

**[0097]** In some embodiments, the multi-specific binding protein is in the Orthogonal Fab interface (Ortho-Fab) form. In the ortho-Fab IgG approach (Lewis SM, Wu X, Pustilnik A, Sereno A, Huang F, Rick HL, *et al.*, Generation of bispecific IgG antibodies by structure-based design of an orthogonal Fab interface. *Nat. Biotechnol.* (2014) 32(2):191–8), structure-based regional design introduces complementary mutations at the LC and $HC_{VH\text{-}CH1}$ interface in only one Fab, without any changes being made to the other Fab.

**[0098]** In some embodiments, the multi-specific binding protein is in the 2-in-1 Ig format. In some embodiments, the multi-specific binding protein is in the ES form, which is a heterodimeric construct containing two different Fabs binding to targets 1 and target 2 fused to the Fc. Heterodimerization is ensured by electrostatic steering mutations in the Fc. In some embodiments, the multi-specific binding protein is in the κλ-Body form, which is an heterodimeric constructs with two different Fabs fused to Fc stabilized by heterodimerization mutations: Fab1 targeting antigen 1 contains kappa LC, while second Fab targeting antigen 2 contains lambda LC. FIG. 49A is an exemplary representation of one form of a κλ-Body; FIG. 49B is an exemplary representation of another κλ-Body.

**[0099]** In some embodiments, the multi-specific binding protein is in Fab Arm Exchange form (antibodies that exchange Fab arms by swapping a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule, which results in bispecific antibodies). In some embodiments, the multi-specific binding protein is in the SEED Body form. The strand-exchange engineered domain (SEED) platform was designed to generate asymmetric and bispecific antibody-like molecules, a capability that expands therapeutic applications of natural antibodies. This protein engineered platform is based on exchanging structurally related sequences of immunoglobulin within the conserved CH3 domains. The SEED design allows efficient generation of AG/GA heterodimers, while disfavoring homodimerization of AG and GA SEED CH3 domains. (Muda M. *et al.*, *Protein Eng. Des. Sel.* (2011, 24(5):447-54)). In some embodiments, the multi-specific binding protein is in the LuZ-Y form, in which a leucine zipper is used to induce heterodimerization of two different HCs. (Wranik, BJ. *et al.*, *J. Biol. Chem.* (2012), 287:43331-9).

**[00100]** In some embodiments, the multi-specific binding protein is in the Cov-X-Body form. In bispecific CovX-Bodies, two different peptides are joined together using a branched azetidinone linker and fused to the scaffold antibody under mild conditions in a site-specific manner. Whereas the pharmacophores are responsible for functional activities, the antibody scaffold imparts long half-life and Ig-like distribution. The pharmacophores can be

chemically optimized or replaced with other pharmacophores to generate optimized or unique bispecific antibodies. (Doppalapudi VR *et al.*, *PNAS* (2010), 107(52);22611-22616).

**[00101]** In some embodiments, the multi-specific binding protein is in an Oasc-Fab heterodimeric form that includes Fab binding to target 1, and scFab binding to target 2 fused to Fc. Heterodimerization is ensured by mutations in the Fc.

**[00102]** In some embodiments, the multi-specific binding protein is in a DuetMab form, which is an heterodimeric construct containing two different Fabs binding to antigens 1 and 2, and Fc stabilized by heterodimerization mutations. Fab 1 and 2 contain differential S-S bridges that ensure correct LC and HC pairing.

**[00103]** In some embodiments, the multi-specific binding protein is in a CrossmAb form, which is an heterodimeric construct with two different Fabs binding to targets 1 and 2, fused to Fc stabilized by heterodimerization. CL and CH1 domains and VH and VL domains are switched, *e.g.*, CH1 is fused in-line with VL, while CL is fused in-line with VH.

**[00104]** In some embodiments, the multi-specific binding protein is in a Fit-Ig form, which is an homodimeric constructs where Fab binding to antigen 2 is fused to the N terminus of HC of Fab that binds to antigen 1. The construct contains wild-type Fc.

**[00105]** Additional formats of the multi-specific binding proteins can be devised by combining various formats of NKG2D- and HER2-binding fragments described herein.

**[00106]** Table 1 lists peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to NKG2D.

| Table 1 | | |
|---|---|---|
| Clones | Heavy chain variable region amino acid sequence | Light chain variable region amino acid sequence |
| ADI-27705 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:1)<br><br>CDR1 (SEQ ID NO:62) – GSFSGYYWS<br>CDR2 (SEQ ID NO:63) – EIDHSGSTNYNPSLKS | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYNSYPI TFGGGTKVEIK (SEQ ID NO:2) |

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| | CDR3 (SEQ ID NO:64) – ARARGPWSFDP | |
| ADI-27724 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:3) | EIVLTQSPGTLSLSPGERATLSCRA SQSVSSSYLAWYQQKPGQAPRLL IYGASSRATGIPDRFSGSGSGTDFT LTISRLEPEDFAVYYCQQYGSSPIT FGGGTKVEIK (SEQ ID NO:4) |
| ADI-27740 (A40) | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:5) | DIQMTQSPSTLSASVGDRVTITCR ASQSIGSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYHSFYT FGGGTKVEIK (SEQ ID NO:6) |
| ADI-27741 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:7) | DIQMTQSPSTLSASVGDRVTITCR ASQSIGSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQSNSYYT FGGGTKVEIK (SEQ ID NO:8) |
| ADI-27743 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:9) | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYNSYPT FGGGTKVEIK (SEQ ID NO:10) |
| ADI-28153 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW GFDPWGQGTLVTVSS (SEQ ID NO:11) | ELQMTQSPSSLSASVGDRVTITCR TSQSISSYLNWYQQKPGQPPKLLI YWASTRESGVPDRFSGSGSGTDF TLTISSLQPEDSATYYCQQSYDIP YTFGQGTKLEIK (SEQ ID NO:12) |

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| ADI-28226 (C26) | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:13) | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYGSFPIT FGGGTKVEIK (SEQ ID NO:14) |
| ADI-28154 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:15) | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTDFT LTISSLQPDDFATYYCQQSKEVP WTFGQGTKVEIK (SEQ ID NO:16) |
| ADI-29399 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:17) | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYNSFPT FGGGTKVEIK (SEQ ID NO:18) |
| ADI-29401 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:19) | DIQMTQSPSTLSASVGDRVTITCR ASQSIGSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYDIYPT FGGGTKVEIK (SEQ ID NO:20) |
| ADI-29403 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ FSLKLSSVTAADTAVYYCARARGPW SFDPWGQGTLVTVSS (SEQ ID NO:21) | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT LTISSLQPDDFATYYCQQYDSYPT FGGGTKVEIK (SEQ ID NO:22) |
| ADI-29405 | QVQLQQWGAGLLKPSETLSLTCAVY GGSFSGYYWSWIRQPPGKGLEWIGEI DHSGSTNYNPSLKSRVTISVDTSKNQ | DIQMTQSPSTLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLL IYKASSLESGVPSRFSGSGSGTEFT |

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| | FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:23) | LTISSLQPDDFATYYCQQYGSFPT<br>FGGGTKVEIK<br>(SEQ ID NO:24) |
| ADI-29407 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:25) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYQSFPT<br>FGGGTKVEIK<br>(SEQ ID NO:26) |
| ADI-29419 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:27) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYSSFST<br>FGGGTKVEIK<br>(SEQ ID NO:28) |
| ADI-29421 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:29) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYESYST<br>FGGGTKVEIK<br>(SEQ ID NO:30) |
| ADI-29424 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:31) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYDSFITF<br>GGGTKVEIK<br>(SEQ ID NO:32) |
| ADI-29425 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:33) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYQSYPT<br>FGGGTKVEIK<br>(SEQ ID NO:34) |

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| ADI-29426 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:35) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSIGSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYHSFPT<br>FGGGTKVEIK<br>(SEQ ID NO:36) |
| ADI-29429 | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:37) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSIGSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYELYSY<br>TFGGGTKVEIK<br>(SEQ ID NO:38) |
| ADI-29447<br>(F47) | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:39) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCQQYDTFIT<br>FGGGTKVEIK<br>(SEQ ID NO:40) |
| ADI-27727 | QVQLVQSGAEVKKPGSSVKVSCKAS<br>GGTFSSYAISWVRQAPGQGLEWMGG<br>IIPIFGTANYAQKFQGRVTITADESTS<br>TAYMELSSLRSEDTAVYYCARGDSSI<br>RHAYYYYGMDVWGQGTTVTVSS<br>(SEQ ID NO:41)<br>CDR1 (SEQ ID NO:65) –<br>GTFSSYAIS<br>CDR2 (SEQ ID NO:66) –<br>GIIPIFGTANYAQKFQG<br>CDR3 (SEQ ID NO:67) –<br>ARGDSSIRHAYYYYGMDV | DIVMTQSPDSLAVSLGERATINCK<br>SSQSVLYSSNNKNYLAWYQQKP<br>GQPPKLLIYWASTRESGVPDRFSG<br>SGSGTDFTLTISSLQAEDVAVYYC<br>QQYYSTPITFGGGTKVEIK<br>(SEQ ID NO:42)<br>CDR1 (SEQ ID NO:68) –<br>KSSQSVLYSSNNKNYLA<br>CDR2 (SEQ ID NO:69) –<br>WASTRES<br>CDR3 (SEQ ID NO:70) –<br>QQYYSTPIT |
| ADI-29443<br>(F43) | QLQLQESGPGLVKPSETLSLTCTVSG<br>GSISSSSYYWGWIRQPPGKGLEWIGSI<br>YYSGSTYYNPSLKSRVTISVDTSKNQ | EIVLTQSPATLSLSPGERATLSCRA<br>SQSVSRYLAWYQQKPGQAPRLLI<br>YDASNRATGIPARFSGSGSGTDFT |

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| | FSLKLSSVTAADTAVYYCARGSDRF<br>HPYFDYWGQGTLVTVSS<br>(SEQ ID NO:43)<br>CDR1 (SEQ ID NO:71) –<br>GSISSSSYYWG<br>CDR2 (SEQ ID NO:72) –<br>SIYYSGSTYYNPSLKS<br>CDR3 (SEQ ID NO:73) –<br>ARGSDRFHPYFDY | LTISSLEPEDFAVYYCQQFDTWPP<br>TFGGGTKVEIK<br>(SEQ ID NO:44)<br>CDR1 (SEQ ID NO:74) –<br>RASQSVSRYLA<br>CDR2 (SEQ ID NO:75) –<br>DASNRAT<br>CDR3 (SEQ ID NO:76) –<br>QQFDTWPPT |
| ADI-29404<br>(F04) | QVQLQQWGAGLLKPSETLSLTCAVY<br>GGSFSGYYWSWIRQPPGKGLEWIGEI<br>DHSGSTNYNPSLKSRVTISVDTSKNQ<br>FSLKLSSVTAADTAVYYCARARGPW<br>SFDPWGQGTLVTVSS<br>(SEQ ID NO:89) | DIQMTQSPSTLSASVGDRVTITCR<br>ASQSISSWLAWYQQKPGKAPKLL<br>IYKASSLESGVPSRFSGSGSGTEFT<br>LTISSLQPDDFATYYCEQYDSYPT<br>FGGGTKVEIK<br>(SEQ ID NO:90) |
| ADI-28200 | QVQLVQSGAEVKKPGSSVKVSCKAS<br>GGTFSSYAISWVRQAPGQGLEWMGG<br>IIPIFGTANYAQKFQGRVTITADESTS<br>TAYMELSSLRSEDTAVYYCARRGRK<br>ASGSFYYYYGMDVWGQGTTVTVSS<br>(SEQ ID NO:91) | DIVMTQSPDSLAVSLGERATINCE<br>SSQSLLNSGNQKNYLTWYQQKP<br>GQPPKPLIYWASTRESGVPDRFSG<br>SGSGTDFTLTISSLQAEDVAVYYC<br>QNDYSYPYTFGQGTKLEIK<br>(SEQ ID NO:92) |
| ADI-29379<br>(E79) | QVQLVQSGAEVKKPGASVKVSCKAS<br>GYTFTSYYMHWVRQAPGQGLEWM<br>GIINPSGGSTSYAQKFQGRVTMTRDT<br>STSTVYMELSSLRSEDTAVYYCARG<br>APNYGDTTHDYYYMDVWGKGTTVT<br>VSS<br>(SEQ ID NO:94)<br><br>CDR1 (SEQ ID NO:96) - YTFTSYYMH<br>CDR2 (SEQ ID NO:97) -<br>IINPSGGSTSYAQKFQG<br>CDR3 (SEQ ID NO:98) -<br>ARGAPNYGDTTHDYYYMDV | EIVMTQSPATLSVSPGERATLSCR<br>ASQSVSSNLAWYQQKPGQAPRLL<br>IYGASTRATGIPARFSGSGSGTEFT<br>LTISSLQSEDFAVYYCQQYDDWP<br>FTFGGGTKVEIK<br>(SEQ ID NO:95)<br><br>CDR1 (SEQ ID NO:99) -<br>RASQSVSSNLA<br>CDR2 (SEQ ID NO:100) -<br>GASTRAT<br>CDR3 (SEQ ID NO:101) -<br>QQYDDWPFT |

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|---|---|---|
| ADI-27749 (A49) | EVQLVESGGGLVKPGGSLRLSCAAS GFTFSSYSMNWVRQAPGKGLEWVSS ISSSSSYIYYADSVKGRFTISRDNAKN SLYLQMNSLRAEDTAVYYCARGAP MGAAAGWFDPWGQGTLVTVSS (SEQ ID NO:102)<br><br>CDR1 (SEQ ID NO:104) - FTFSSYSMN<br>CDR2 (SEQ ID NO:105) - SISSSSSYIYYADSVKG<br>CDR3 (SEQ ID NO:106) - ARGAPMGAAAGWFDP | DIQMTQSPSSVSASVGDRVTITCR ASQGISSWLAWYQQKPGKAPKLL IYAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQGVSFP RTFGGGTKVEIK (SEQ ID NO:103)<br><br>CDR1 (SEQ ID NO:107) - RASQGISSWLA<br>CDR2 (SEQ ID NO:108) - AASSLQS<br>CDR3 (SEQ ID NO:109) - QQGVSFPRT |

**[00107]** Alternatively, a heavy chain variable domain defined by SEQ ID NO:45 can be paired with a light chain variable domain defined by SEQ ID NO:46 to form an antigen-binding site that can bind to NKG2D, as illustrated in US 9,273,136.

QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFIRYDGS NKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDRGLGDGTYFDYW GQGTTVTVSS (SEQ ID NO:45)

QSALTQPASVSGSPGQSITISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSG VSDRFSGSKSGTSAFLAISGLQSEDEADYYCAAWDDSLNGPVFGGGTKLTVL (SEQ ID NO:46)

**[00108]** Alternatively, a heavy chain variable domain defined by SEQ ID NO:47 can be paired with a light chain variable domain defined by SEQ ID NO:48 to form an antigen-binding site that can bind to NKG2D, as illustrated in US 7,879,985.

QVHLQESGPGLVKPSETLSLTCTVSDDSISSYYWSWIRQPPGKGLEWIGHISYSGSAN YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCANWDDAFNIWGQGTMVTVS S (SEQ ID NO:47)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID NO:48)

**[00109]** Table 2 lists peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to HER2.

| Table 2 | | |
|---|---|---|
| Clones | Heavy chain variable domain amino acid sequence | Light chain variable domain amino acid sequence |
| Trastuzumab | EVQLVESGGGLVQPGGSLRLSCAA SGFNIKDTYIHWVRQAPGKGLEWV ARIYPTNGYTRYADSVKGRFTISAD TSKNTAYLQMNSLRAEDTAVYYCS RWGGDGFYAMDYWGQGTLVTVS S (SEQ ID NO:49)<br><br>CDR1(SEQ ID NO:50) - GFNIKDT<br>CDR2 (SEQ ID NO:51) - YPTNGY<br>CDR3 (SEQ ID NO:52) - WGGDGFYAMDY | DIQMTQSPSSLSASVGDRVTITCRA SQDVNTAVAWYQQKPGKAPKLLI YSASFLYSGVPSRFSGSRSGTDFTL TISSLQPEDFATYYCQQHYTTPPTF GQGTKVEIK (SEQ ID NO:53)<br><br>CDR1(SEQ ID NO:54) - QDVNTAVA<br>CDR2 (SEQ ID NO:55) - SASFLYS<br>CDR3 (SEQ ID NO:56) - QQHYTTPPT |
| Pertuzumab | EVQLVESGGGLVQPGGSLRLSCAA SGFTFTDYTMDWVRQAPGKGLEW VADVNPNSGGSIYNQRFKGRFTLS VDRSKNTLYLQMNSLRAEDTAVY YCARNLGPSFYFDYWGQGTLVTVS SA (SEQ ID NO:57)<br><br>CDR1 (SEQ ID NO:77) - GFTFTDY<br>CDR2 (SEQ ID NO:78) - NPNSGG<br>CDR3 (SEQ ID NO:79) - NLGPSFYFDY | DIQMTQSPSSLSASVGDRVTITCKA SQDVSIGVAWYQQKPGKAPKLLIY SASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPYTFG QGTKVEIKR (SEQ ID NO:58)<br><br>CDR1 (SEQ ID NO:80) - QDVSIGVA<br>CDR2 (SEQ ID NO:81) - SASYRYT<br>CDR3 (SEQ ID NO:82) - QQYYIYPYT |
| MGAH22 (US 8,802,093) | QVQLQQSGPELVKPGASLKLSCTA SGFNIKDTYIHWVKQRPEQGLEWI GRIYPTNGYTRYDPKFQDKATITAD TSSNTAYLQVSRLTSEDTAVYYCS | DIVMTQSHKFMSTSVGDRVSITCK ASQDVNTAVAWYQQKPGHSPKLL IYSASFRYTGVPDRFTGSRSGTDFT FTISSVQAEDLAVYYCQQHYTTPP |

| | | |
|---|---|---|
| | RWGGDGFYAMDYWGQGASVTVS SA (SEQ ID NO:59)<br><br>CDR1 (SEQ ID NO:83) - GFNIKDT<br>CDR2 (SEQ ID NO:84) - YPTNGY<br>CDR3 (SEQ ID NO:85) - WGGDGFYAMDY | TFGGGTKVEIKR (SEQ ID NO:60)<br><br>CDR1 (SEQ ID NO:86) - QDVNTAVA<br>CDR2 (SEQ ID NO:87) - SASFRYT<br>CDR3 (SEQ ID NO:88) - QQHYTTPPT |

**[00110]** Alternatively, novel antigen-binding sites that can bind to HER2 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:61.

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQV
VQGNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNY
ALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILW
KDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCAR
CKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFESMP
NPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCA
RVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQL
QVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLR
SLRELGSGLALIHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLACH
QLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQ
PQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQ
PCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIR
KYTMRRLLQETELVEPLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWI
PDGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVT
QLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDVRLVHRDLAARNVL
VKSPNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYG
VTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRP
RFRELVSEFSRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYL
VPQQGFFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEGA
GSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLTCSPQPE
YVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPE
YLTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLD
VPV (SEQ ID NO:61).

**[00111]** Within the Fc domain, CD16 binding is mediated by the hinge region and the CH2 domain. For example, within human IgG1, the interaction with CD16 is primarily focused on amino acid residues Asp 265 – Glu 269, Asn 297 – Thr 299, Ala 327 – Ile 332, Leu 234 – Ser 239, and carbohydrate residue N-acetyl-D-glucosamine in the CH2 domain (*see*, Sondermann *et al*, Nature, 406 (6793):267-273). Based on the known domains, mutations can be selected to enhance or reduce the binding affinity to CD16, such as by using phage-

displayed libraries or yeast surface-displayed cDNA libraries, or can be designed based on the known three-dimensional structure of the interaction.

**[00112]** The assembly of heterodimeric antibody heavy chains can be accomplished by expressing two different antibody heavy chain sequences in the same cell, which may lead to the assembly of homodimers of each antibody heavy chain as well as assembly of heterodimers. Promoting the preferential assembly of heterodimers can be accomplished by incorporating different mutations in the CH3 domain of each antibody heavy chain constant region as shown in US13/494870, US16/028850, US11/533709, US12/875015, US13/289934, US14/773418, US12/811207, US13/866756, US14/647480, and US14/830336. For example, mutations can be made in the CH3 domain based on human IgG1 and incorporating distinct pairs of amino acid substitutions within a first polypeptide and a second polypeptide that allow these two chains to selectively heterodimerize with each other. The positions of amino acid substitutions illustrated below are all numbered according to the EU index as in Kabat.

**[00113]** In one scenario, an amino acid substitution in the first polypeptide replaces the original amino acid with a larger amino acid, selected from arginine (R), phenylalanine (F), tyrosine (Y) or tryptophan (W), and at least one amino acid substitution in the second polypeptide replaces the original amino acid(s) with a smaller amino acid(s), chosen from alanine (A), serine (S), threonine (T), or valine (V), such that the larger amino acid substitution (a protuberance) fits into the surface of the smaller amino acid substitutions (a cavity). For example, one polypeptide can incorporate a T366W substitution, and the other can incorporate three substitutions including T366S, L368A, and Y407V.

**[00114]** An antibody heavy chain variable domain of the invention can optionally be coupled to an amino acid sequence at least 90% identical to an antibody constant region, such as an IgG constant region including hinge, CH2 and CH3 domains with or without CH1 domain. In some embodiments, the amino acid sequence of the constant region is at least 90% identical to a human antibody constant region, such as an human IgG1 constant region, an IgG2 constant region, IgG3 constant region, or IgG4 constant region. In some other embodiments, the amino acid sequence of the constant region is at least 90% identical to an antibody constant region from another mammal, such as rabbit, dog, cat, mouse, or horse. One or more mutations can be incorporated into the constant region as compared to human IgG1 constant region, for example at Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411 and/or K439. Exemplary substitutions include, for example, Q347E, Q347R, Y349S,

Y349K, Y349T, Y349D, Y349E, Y349C, T350V, L351K, L351D, L351Y, S354C, E356K, E357Q, E357L, E357W, K360E, K360W, Q362E, S364K, S364E, S364H, S364D, T366V, T366I, T366L, T366M, T366K, T366W, T366S, L368E, L368A, L368D, K370S, N390D, N390E, K392L, K392M, K392V, K392F, K392D, K392E, T394F, T394W, D399R, D399K, D399V, S400K, S400R, D401K, F405A, F405T, Y407A, Y407I , Y407V, K409F, K409W, K409D, T411D, T411E, K439D, and K439E.

**[00115]** In certain embodiments, mutations that can be incorporated into the CH1 of a human IgG1 constant region may be at amino acid V125, F126, P127, T135, T139, A140, F170, P171, and/or V173. In certain embodiments, mutations that can be incorporated into the Cκ of a human IgG1 constant region may be at amino acid E123, F116, S176, V163, S174, and/or T164.

**[00116]** Amino acid substitutions could be selected from the following sets of substitutions shown in Table 3.

| Table 3 | | |
|---|---|---|
| | First Polypeptide | Second Polypeptide |
| Set 1 | S364E/F405A | Y349K/T394F |
| Set 2 | S364H/D401K | Y349T/T411E |
| Set 3 | S364H/T394F | Y349T/F405A |
| Set 4 | S364E/T394F | Y349K/F405A |
| Set 5 | S364E/T411E | Y349K/D401K |
| Set 6 | S364D/T394F | Y349K/F405A |
| Set 7 | S364H/F405A | Y349T/T394F |
| Set 8 | S364K/E357Q | L368D/K370S |
| Set 9 | L368D/K370S | S364K |
| Set 10 | L368E/K370S | S364K |
| Set 11 | K360E/Q362E | D401K |
| Set 12 | L368D/K370S | S364K/E357L |
| Set 13 | K370S | S364K/E357Q |
| Set 14 | F405L | K409R |
| Set 15 | K409R | F405L |

**[00117]** Alternatively, amino acid substitutions could be selected from the following sets of substitutions shown in Table 4.

| Table 4 | | |
|---|---|---|
| | First Polypeptide | Second Polypeptide |
| Set 1 | K409W | D399V/F405T |
| Set 2 | Y349S | E357W |
| Set 3 | K360E | Q347R |
| Set 4 | K360E/K409W | Q347R/D399V/F405T |
| Set 5 | Q347E/K360E/K409W | Q347R/D399V/F405T |
| Set 6 | Y349S/K409W | E357W/D399V/F405T |

**[00118]** Alternatively, amino acid substitutions could be selected from the following set of substitutions shown in Table 5.

| Table 5 | | |
|---|---|---|
| | First Polypeptide | Second Polypeptide |
| Set 1 | T366K/L351K | L351D/L368E |
| Set 2 | T366K/L351K | L351D/Y349E |
| Set 3 | T366K/L351K | L351D/Y349D |
| Set 4 | T366K/L351K | L351D/Y349E/L368E |
| Set 5 | T366K/L351K | L351D/Y349D/L368E |
| Set 6 | E356K/D399K | K392D/K409D |

**[00119]** Alternatively, at least one amino acid substitution in each polypeptide chain could be selected from Table 6.

| Table 6 | |
|---|---|
| First Polypeptide | Second Polypeptide |
| L351Y, D399R, D399K, S400K, S400R, Y407A, Y407I, Y407V | T366V, T366I, T366L, T366M, N390D, N390E, K392L, K392M, K392V, K392F K392D, K392E, K409F, K409W, T411D and T411E |

**[00120]** Alternatively, at least one amino acid substitutions could be selected from the following set of substitutions in Table 7, where the position(s) indicated in the First

Polypeptide column is replaced by any known negatively-charged amino acid, and the position(s) indicated in the Second Polypeptide Column is replaced by any known positively-charged amino acid.

| Table 7 | |
|---|---|
| First Polypeptide | Second Polypeptide |
| K392, K370, K409, or K439 | D399, E356, or E357 |

**[00121]** Alternatively, at least one amino acid substitutions could be selected from the following set of in Table 8, where the position(s) indicated in the First Polypeptide column is replaced by any known positively-charged amino acid, and the position(s) indicated in the Second Polypeptide Column is replaced by any known negatively-charged amino acid.

| Table 8 | |
|---|---|
| First Polypeptide | Second Polypeptide |
| D399, E356, or E357 | K409, K439, K370, or K392 |

**[00122]** Alternatively, amino acid substitutions could be selected from the following set in Table 9.

| Table 9 | |
|---|---|
| First Polypeptide | Second Polypeptide |
| T350V, L351Y, F405A, and Y407V | T350V, T366L, K392L, and T394W |

**[00123]** Alternatively, or in addition, the structural stability of a heteromultimer protein may be increased by introducing S354C on either of the first or second polypeptide chain, and Y349C on the opposing polypeptide chain, which forms an artificial disulfide bridge within the interface of the two polypeptides.

**[00124]** The multi-specific proteins described above can be made using recombinant DNA technology well known to a skilled person in the art. For example, a first nucleic acid sequence encoding the first immunoglobulin heavy chain can be cloned into a first expression vector; a second nucleic acid sequence encoding the second immunoglobulin heavy chain can be cloned into a second expression vector; a third nucleic acid sequence encoding the immunoglobulin light chain can be cloned into a third expression vector; the first, second,

and third expression vectors can be stably transfected together into host cells to produce the multimeric proteins.

**[00125]** To achieve the highest yield of the multi-specific protein, different ratios of the first, second, and third expression vector can be explored to determine the optimal ratio for transfection into the host cells. After transfection, single clones can be isolated for cell bank generation using methods known in the art, such as limited dilution, ELISA, FACS, microscopy, or Clonepix.

**[00126]** Clones can be cultured under conditions suitable for bio-reactor scale-up and maintained expression of the multi-specific protein. The multi-specific proteins can be isolated and purified using methods known in the art including centrifugation, depth filtration, cell lysis, homogenization, freeze-thawing, affinity purification, gel filtration, ion exchange chromatography, hydrophobic interaction exchange chromatography, and mixed-mode chromatography.

**II. Characteristics of the multi-specific proteins**

**[00127]** In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a HER2-binding domain, bind to cells expressing human NKG2D. In certain embodiments, the multi-specific binding proteins which include an NKG2D-binding domain and a HER2-binding domain, bind to HER2 at a comparable level to that of a monoclonal antibody having the same HER2-binding domain. For example, the multi-specific binding proteins that include an NKG2D-binding domain and a HER2-binding domain from Trastuzumab can bind to HER2 expressed on cells at a level comparable to that of Trastuzumab.

**[00128]** However, the multi-specific binding proteins described herein are more effective in reducing tumor growth and killing cancer cells. For example, a multi-specific binding protein of the present disclosure that targets HER2-expressing tumor/cancer cells is more effective than SC2.2 — a single chain bispecific molecule built from an scFv derived from trastuzumab linked to ULBP-6, a ligand for NKG2D. SC2.2 binds HER2+ cancer cells and NKG2D+ NK cells simultaneously. Therefore, effectiveness of SC2.2 in reducing HER2+ cancer cell number was investigated. *In vitro* activation and cytotoxity assays demonstrated that SC2.2 was effective in activating and killing NK cells. However, SC2.2 failed to demonstrate efficacy in the RMA/S-HER2 subcutaneous tumor model. The efficacy of SC2.2 was also tested *in vivo* using an RMA/S-HER2 overexpressing syngeneic mouse model (FIG. 36). In this mouse model, SC2.2 failed to demonstrate control of tumor growth compared to

vehicle control (FIG. 37). Thus, although SC2.2 was able to activate and kill NK cells, and binds to HER2+ cancer cells, these properties were insufficient to effectively control HER2+ tumor growth.

**[00129]** In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a binding domain for tumor associated antigen, activate primary human NK cells when culturing with tumor cells expressing the antigen. NK cell activation is marked by the increase in CD107a degranulation and IFNγ cytokine production. Furthermore, compared to a monoclonal antibody that includes the tumor associated antigen-binding domain, the multi-specific binding proteins show superior activation of human NK cells in the presence of tumor cells expressing the antigen. For example, compared to the monoclonal antibody trastuzumab, the multi-specific binding proteins of the present disclosure having a HER2-binding domain, have a superior activation of human NK cells in the presence of HER2-expressing cancer cells.

**[00130]** In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a binding domain for a tumor associated antigen, enhance the activity of rested and IL-2-activated human NK cells in the presence of tumor cells expressing the antigen. Rested NK cells showed less background IFNγ production and CD107a degranulation than IL-2-activated NK cells. In certain embodiments, rested NK cells show a greater change in IFNγ production and CD107a degranulation compared to IL-2-activated NK cells. In certain embodiments, IL-2-activated NK cells show a greater percentage of cells becoming IFNγ+; CD107a+ after stimulation with TriNKETs.

**[00131]** In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a binding domain for a tumor associated antigen (non-limiting examples of tumor associated antigens including CD20, BCMA, and HER2), enhance the cytotoxic activity of rested and IL-2-activated human NK cells in the presence of tumor cells expressing the antigen. Furthermore, the multi-specific binding proteins (*e.g.*, A40-multi-specific binding protein, A49-multi-specific binding protein, C26-multi-specific binding protein, F04-multi-specific binding protein, F43-multi-specific binding protein, F47-multi-specific binding protein, and E79-multi-specific binding protein), which include a binding domain for HER2, more potently direct, activated and rested NK cell responses against the tumor cells, compared to a monoclonal antibody that includes HER2-binding site. In certain embodiments, the multi-specific binding proteins offer advantage against tumor cells expressing medium and low HER2, compared to monoclonal antibodies

that HER2-binding site. Therefore, a therapy including multi-specific binding proteins can be superior to a monoclonal antibody therapy.

**[00132]** In certain embodiments, compared to monoclonal antibodies, the multi-specific binding proteins described herein (*e.g.*, A40-multi-specific binding protein, A49-multi-specific binding protein, C26-multi-specific binding protein, F04-multi-specific binding protein, F43-multi-specific binding protein, F47-multi-specific binding protein, and E79-multi-specific binding protein), which include a binding domain for HER2 are advantageous in treating cancers with high expression of Fc receptor (FcR), or cancers residing in a tumor microenvironment with high levels of FcR. Monoclonal antibodies exert their effects on tumor growth through multiple mechanisms including ADCC, CDC, phagocytosis, and signal blockade amongst others. Amongst FcγRs, CD16 has the lowest affinity for IgG Fc; FcγRI (CD64) is the high-affinity FcR, which binds about 1000 times more strongly to IgG Fc than CD16. CD64 is normally expressed on many hematopoietic lineages such as the myeloid lineage, and can be expressed on tumors derived from these cell types, such as acute myeloid leukemia (AML). Immune cells infiltrating into the tumor, such as MDSCs and monocytes, also express CD64 and are known to infiltrate the tumor microenvironment. Expression of CD64 by the tumor or in the tumor microenvironment can have a detrimental effect on monoclonal antibody therapy. Expression of CD64 in the tumor microenvironment makes it difficult for these antibodies to engage CD16 on the surface of NK cells, as the antibodies prefer to bind the high-affinity receptor. The multi-specific binding proteins, through targeting two activating receptors on the surface of NK cells, can overcome the detrimental effect of CD64 expression (either on tumor or tumor microenvironment) on monoclonal antibody therapy. Regardless of CD64 expression on the tumor cells, the multi-specific binding proteins are able to mediate human NK cell responses against all tumor cells, because dual targeting of two activating receptors on NK cells provides stronger specific binding to NK cells.

**[00133]** In some embodiments, the multi-specific binding proteins described herein (*e.g.*, A40-multi-specific binding protein, A49-multi-specific binding protein, C26-multi-specific binding protein, F04-multi-specific binding protein, F43-multi-specific binding protein, F47-multi-specific binding protein, and E79-multi-specific binding protein), which include a binding domain for HER2 provide a better safety profile through reduced on-target off-tumor side effects. Natural killer cells and CD8 T cells are both able to directly lyse tumor cells, although the mechanisms through which NK cells and CD8 T cell recognize normal self from tumor cells differ. The activity of NK cells is regulated by the balance of signals from

activating (NCRs, NKG2D, CD16, *etc.*) and inhibitory (KIRs, NKG2A, *etc.*) receptors. The balance of these activating and inhibitory signals allow NK cells to determine healthy self-cells from stressed, virally infected, or transformed self-cells. This "built-in" mechanism of self-tolerance will help protect normal heathy tissue from NK cell responses. To extend this principle, the self-tolerance of NK cells will allow the multi-specific binding proteins to target antigens expressed both on self and tumor without off tumor side effects, or with an increased therapeutic window. Unlike natural killer cells, T cells require recognition of a specific peptide presented by MHC molecules for activation and effector functions. T cells have been the primary target of immunotherapy, and many strategies have been developed to redirect T cell responses against the tumor. T cell bispecifics, checkpoint inhibitors, and CAR-T cells have all been approved by the FDA, but often suffer from dose-limiting toxicities. T cell bispecifics and CAR-T cells work around the TCR-MHC recognition system by using binding domains to target antigens on the surface of tumor cells, and using engineered signaling domains to transduce the activation signals into the effector cell. Although effective at eliciting an anti-tumor immune response these therapies are often coupled with cytokine release syndrome (CRS), and on-target off-tumor side effects. The multi-specific binding proteins are unique in this context as they will not "override" the natural systems of NK cell activation and inhibition. Instead, the multi-specific binding proteins are designed to sway the balance, and provide additional activation signals to the NK cells, while maintaining NK tolerance to healthy self.

**[00134]** In some embodiments, the multi-specific binding proteins described herein including an NKG2D-binding domain (*e.g.*, A40-multi-specific binding protein, A49-multi-specific binding protein, C26-multi-specific binding protein, F04-multi-specific binding protein, F43-multi-specific binding protein, F47-multi-specific binding protein, and E79-multi-specific binding protein), which include a binding domain for HER2 delay progression of the tumor more effectively than monoclonal antibodies that include the same tumor antigen-binding domain. In some embodiments, the multi-specific binding proteins including an NKG2D-binding domain and a tumor antigen-binding domain are more effective against cancer metastases than monoclonal antibodies that include the same tumor antigen-binding domain.

## III. THERAPEUTIC APPLICATIONS

**[00135]** The invention provides methods for treating cancer using a multi-specific binding protein described herein and/or a pharmaceutical composition described herein. The methods

may be used to treat a variety of cancers which express HER2 by administering to a patient in need thereof a therapeutically effective amount of a multi-specific binding protein described herein.

**[00136]** The therapeutic method can be characterized according to the cancer to be treated. For example, in certain embodiments, the cancer is breast, ovarian, esophageal, bladder or gastric cancer, salivary duct carcinoma, salivary duct carcinomas, adenocarcinoma of the lung or aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

**[00137]** In certain other embodiments, the cancer is brain cancer, breast cancer, cervical cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, leukemia, lung cancer, liver cancer, melanoma, ovarian cancer, pancreatic cancer, rectal cancer, renal cancer, stomach cancer, testicular cancer, or uterine cancer. In yet other embodiments, the cancer is a squamous cell carcinoma, adenocarcinoma, small cell carcinoma, melanoma, neuroblastoma, sarcoma (*e.g.*, an angiosarcoma or chondrosarcoma), larynx cancer, parotid cancer, bilary tract cancer, thyroid cancer, acral lentiginous melanoma, actinic keratoses, acute lymphocytic leukemia, acute myeloid leukemia, adenoid cystic carcinoma, adenomas, adenosarcoma, adenosquamous carcinoma, anal canal cancer, anal cancer, anorectum cancer, astrocytic tumor, bartholin gland carcinoma, basal cell carcinoma, biliary cancer, bone cancer, bone marrow cancer, bronchial cancer, bronchial gland carcinoma, carcinoid, cholangiocarcinoma, chondosarcoma, choriod plexus papilloma/carcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, clear cell carcinoma, connective tissue cancer, cystadenoma, digestive system cancer, duodenum cancer, endocrine system cancer, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, endothelial cell cancer, ependymal cancer, epithelial cell cancer, Ewing's sarcoma, eye and orbit cancer, female genital cancer, focal nodular hyperplasia, gallbladder cancer, gastric antrum cancer, gastric fundus cancer, gastrinoma, glioblastoma, glucagonoma, heart cancer, hemangiblastomas, hemangioendothelioma, hemangiomas, hepatic adenoma, hepatic adenomatosis, hepatobiliary cancer, hepatocellular carcinoma, Hodgkin's disease, ileum cancer, insulinoma, intaepithelial neoplasia, interepithelial squamous cell neoplasia, intrahepatic bile duct cancer, invasive squamous cell carcinoma, jejunum cancer, joint cancer, Kaposi's sarcoma, pelvic cancer, large cell carcinoma, large intestine cancer, leiomyosarcoma, lentigo maligna melanomas, lymphoma, male genital cancer, malignant melanoma, malignant mesothelial tumors, medulloblastoma, medulloepithelioma, meningeal cancer, mesothelial cancer, metastatic carcinoma, mouth cancer, mucoepidermoid carcinoma, multiple myeloma, muscle cancer, nasal tract cancer,

nervous system cancer, neuroepithelial adenocarcinoma nodular melanoma, non-epithelial skin cancer, non-Hodgkin's lymphoma, oat cell carcinoma, oligodendroglial cancer, oral cavity cancer, osteosarcoma, papillary serous adenocarcinoma, penile cancer, pharynx cancer, pituitary tumors, plasmacytoma, pseudosarcoma, pulmonary blastoma, rectal cancer, renal cell carcinoma, respiratory system cancer, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, sinus cancer, skin cancer, small cell carcinoma, small intestine cancer, smooth muscle cancer, soft tissue cancer, somatostatin-secreting tumor, spine cancer, squamous cell carcinoma, striated muscle cancer, submesothelial cancer, superficial spreading melanoma, T cell leukemia, tongue cancer, undifferentiated carcinoma, ureter cancer, urethra cancer, urinary bladder cancer, urinary system cancer, uterine cervix cancer, uterine corpus cancer, uveal melanoma, vaginal cancer, verrucous carcinoma, VIPoma, vulva cancer, well differentiated carcinoma, or Wilms tumor.

**[00138]** In certain other embodiments, the cancer is non-Hodgkin's lymphoma, such as a B-cell lymphoma or a T-cell lymphoma. In certain embodiments, the non-Hodgkin's lymphoma is a B-cell lymphoma, such as a diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, or primary central nervous system (CNS) lymphoma. In certain other embodiments, the non-Hodgkin's lymphoma is a T-cell lymphoma, such as a precursor T-lymphoblastic lymphoma, peripheral T-cell lymphoma, cutaneous T-cell lymphoma, angioimmunoblastic T-cell lymphoma, extranodal natural killer/T-cell lymphoma, enteropathy type T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, anaplastic large cell lymphoma, or peripheral T-cell lymphoma.

**[00139]** The cancer to be treated can be characterized according to the presence of a particular antigen expressed on the surface of the cancer cell. In certain embodiments, the cancer cell can express one or more of the following in addition to HER2: CD2, CD19, CD20, CD30, CD38, CD40, CD52, CD70, EGFR/ERBB1, IGF1R, HER3/ERBB3, HER4/ERBB4, MUC1, cMET, SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, and PD1.

## IV. COMBINATION THERAPY

**[00140]** Another aspect of the invention provides for combination therapy. Multi-specific binding proteins described herein be used in combination with additional therapeutic agents to treat the cancer.

**[00141]** Exemplary therapeutic agents that may be used as part of a combination therapy in treating cancer, include, for example, radiation, mitomycin, tretinoin, ribomustin, gemcitabine, vincristine, etoposide, cladribine, mitobronitol, methotrexate, doxorubicin, carboquone, pentostatin, nitracrine, zinostatin, cetrorelix, letrozole, raltitrexed, daunorubicin, fadrozole, fotemustine, thymalfasin, sobuzoxane, nedaplatin, cytarabine, bicalutamide, vinorelbine, vesnarinone, aminoglutethimide, amsacrine, proglumide, elliptinium acetate, ketanserin, doxifluridine, etretinate, isotretinoin, streptozocin, nimustine, vindesine, flutamide, drogenil, butocin, carmofur, razoxane, sizofilan, carboplatin, mitolactol, tegafur, ifosfamide, prednimustine, picibanil, levamisole, teniposide, improsulfan, enocitabine, lisuride, oxymetholone, tamoxifen, progesterone, mepitiostane, epitiostanol, formestane, interferon-alpha, interferon-2 alpha, interferon-beta, interferon-gamma, colony stimulating factor-1, colony stimulating factor-2, denileukin diftitox, interleukin-2, luteinizing hormone releasing factor and variations of the aforementioned agents that may exhibit differential binding to its cognate receptor, and increased or decreased serum half-life.

**[00142]** An additional class of agents that may be used as part of a combination therapy in treating cancer is immune checkpoint inhibitors. Exemplary immune checkpoint inhibitors include agents that inhibit one or more of (i) cytotoxic T−lymphocyte-associated antigen 4 (CTLA4), (ii) programmed cell death protein 1 (PD1), (iii) PDL1, (iv) LAG3, (v) B7-H3, (vi) B7-H4, and (vii) TIM3. The CTLA4 inhibitor ipilimumab has been approved by the United States Food and Drug Administration for treating melanoma.

**[00143]** Yet other agents that may be used as part of a combination therapy in treating cancer are monoclonal antibody agents that target non-checkpoint targets (*e.g.*, herceptin) and non-cytotoxic agents (*e.g.*, tyrosine-kinase inhibitors).

**[00144]** Yet other categories of anti-cancer agents include, for example: (i) an inhibitor selected from an ALK Inhibitor, an ATR Inhibitor, an A2A Antagonist, a Base Excision Repair Inhibitor, a Bcr-Abl Tyrosine Kinase Inhibitor, a Bruton's Tyrosine Kinase Inhibitor, a CDC7 Inhibitor, a CHK1 Inhibitor, a Cyclin-Dependent Kinase Inhibitor, a DNA-PK Inhibitor, an Inhibitor of both DNA-PK and mTOR, a DNMT1 Inhibitor, a DNMT1 Inhibitor plus 2-chloro-deoxyadenosine, an HDAC Inhibitor, a Hedgehog Signaling Pathway Inhibitor,

an IDO Inhibitor, a JAK Inhibitor, a mTOR Inhibitor, a MEK Inhibitor, a MELK Inhibitor, a MTH1 Inhibitor, a PARP Inhibitor, a Phosphoinositide 3-Kinase Inhibitor, an Inhibitor of both PARP1 and DHODH, a Proteasome Inhibitor, a Topoisomerase-II Inhibitor, a Tyrosine Kinase Inhibitor, a VEGFR Inhibitor, and a WEE1 Inhibitor; (ii) an agonist of OX40, CD137, CD40, GITR, CD27, HVEM, TNFRSF25, or ICOS; and (iii) a cytokine selected from IL-12, IL-15, GM-CSF, and G-CSF.

**[00145]** Proteins of the invention can also be used as an adjunct to surgical removal of the primary lesion.

**[00146]** The amount of multi-specific binding protein and additional therapeutic agent and the relative timing of administration may be selected in order to achieve a desired combined therapeutic effect. For example, when administering a combination therapy to a patient in need of such administration, the therapeutic agents in the combination, or a pharmaceutical composition or compositions comprising the therapeutic agents, may be administered in any order such as, for example, sequentially, concurrently, together, simultaneously and the like. Further, for example, a multi-specific binding protein may be administered during a time when the additional therapeutic agent(s) exerts its prophylactic or therapeutic effect, or *vice versa.*

## V. PHARMACEUTICAL COMPOSITIONS

**[00147]** The present disclosure also features pharmaceutical compositions that contain a therapeutically effective amount of a protein described herein. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present disclosure are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, *see, e.g.*, Langer (Science 249:1527-1533, 1990).

**[00148]** The intravenous drug delivery formulation of the present disclosure may be contained in a bag, a pen, or a syringe. In certain embodiments, the bag may be connected to a channel comprising a tube and/or a needle. In certain embodiments, the formulation may be a lyophilized formulation or a liquid formulation. In certain embodiments, the formulation may freeze-dried (lyophilized) and contained in about 12-60 vials. In certain embodiments, the formulation may be freeze-dried and 45 mg of the freeze-dried formulation may be contained in one vial. In certain embodiments, the about 40 mg – about 100 mg of freeze-dried formulation may be contained in one vial. In certain embodiments, freeze dried

formulation from 12, 27, or 45 vials are combined to obtained a therapeutic dose of the protein in the intravenous drug formulation. In certain embodiments, the formulation may be a liquid formulation and stored as about 250 mg/vial to about 1000 mg/vial. In certain embodiments, the formulation may be a liquid formulation and stored as about 600 mg/vial. In certain embodiments, the formulation may be a liquid formulation and stored as about 250 mg/vial.

**[00149]** This present disclosure could exist in a liquid aqueous pharmaceutical formulation including a therapeutically effective amount of the protein in a buffered solution forming a formulation.

**[00150]** These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as-is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents. The composition in solid form can also be packaged in a container for a flexible quantity.

**[00151]** In certain embodiments, the present disclosure provides a formulation with an extended shelf life including the protein of the present disclosure, in combination with mannitol, citric acid monohydrate, sodium citrate, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, polysorbate 80, water, and sodium hydroxide.

**[00152]** In certain embodiments, an aqueous formulation is prepared including the protein of the present disclosure in a pH-buffered solution. The buffer of this invention may have a pH ranging from about 4 to about 8, *e.g.*, from about 4.5 to about 6.0, or from about 4.8 to about 5.5, or may have a pH of about 5.0 to about 5.2. Ranges intermediate to the above recited pH's are also intended to be part of this disclosure. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. Examples of buffers that will control the pH within this range include acetate (*e.g.* sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers.

**[00153]** In certain embodiments, the formulation includes a buffer system which contains citrate and phosphate to maintain the pH in a range of about 4 to about 8. In certain embodiments the pH range may be from about 4.5 to about 6.0, or from about pH 4.8 to about

5.5, or in a pH range of about 5.0 to about 5.2. In certain embodiments, the buffer system includes citric acid monohydrate, sodium citrate, disodium phosphate dihydrate, and/or sodium dihydrogen phosphate dihydrate. In certain embodiments, the buffer system includes about 1.3 mg/ml of citric acid (*e.g.*, 1.305 mg/ml), about 0.3 mg/ml of sodium citrate (*e.g.*, 0.305 mg/ml), about 1.5 mg/ml of disodium phosphate dihydrate (*e.g.*, 1.53 mg/ml), about 0.9 mg/ml of sodium dihydrogen phosphate dihydrate (*e.g.*, 0.86), and about 6.2 mg/ml of sodium chloride (*e.g.*, 6.165 mg/ml). In certain embodiments, the buffer system includes 1-1.5 mg/ml of citric acid, 0.25 to 0.5 mg/ml of sodium citrate, 1.25 to 1.75 mg/ml of disodium phosphate dihydrate, 0.7 to 1.1 mg/ml of sodium dihydrogen phosphate dihydrate, and 6.0 to 6.4 mg/ml of sodium chloride. In certain embodiments, the pH of the formulation is adjusted with sodium hydroxide.

**[00154]** A polyol, which acts as a tonicifier and may stabilize the antibody, may also be included in the formulation. The polyol is added to the formulation in an amount which may vary with respect to the desired isotonicity of the formulation. In certain embodiments, the aqueous formulation may be isotonic. The amount of polyol added may also be altered with respect to the molecular weight of the polyol. For example, a lower amount of a monosaccharide (*e.g.*, mannitol) may be added, compared to a disaccharide (such as trehalose). In certain embodiments, the polyol which may be used in the formulation as a tonicity agent is mannitol. In certain embodiments, the mannitol concentration may be about 5 to about 20 mg/ml. In certain embodiments, the concentration of mannitol may be about 7.5 to 15 mg/ml. In certain embodiments, the concentration of mannitol may be about 10-14 mg/ml. In certain embodiments, the concentration of mannitol may be about 12 mg/ml. In certain embodiments, the polyol sorbitol may be included in the formulation.

**[00155]** A detergent or surfactant may also be added to the formulation. Exemplary detergents include nonionic detergents such as polysorbates (*e.g.*, polysorbates 20, 80 etc.) or poloxamers (*e.g.*, poloxamer 188). The amount of detergent added is such that it reduces aggregation of the formulated antibody and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. In certain embodiments, the formulation may include a surfactant which is a polysorbate. In certain embodiments, the formulation may contain the detergent polysorbate 80 or Tween 80. Tween 80 is a term used to describe polyoxyethylene (20) sorbitanmonooleate (see Fiedler, Lexikon der Hifsstoffe, Editio Cantor Verlag Aulendorf, 4th edi., 1996). In certain embodiments, the formulation may contain between about 0.1 mg/mL and about 10 mg/mL of polysorbate 80, or between about 0.5 mg/mL and

about 5 mg/mL. In certain embodiments, about 0.1% polysorbate 80 may be added in the formulation.

**[00156]** In embodiments, the protein product of the present disclosure is formulated as a liquid formulation. The liquid formulation may be presented at a 10 mg/mL concentration in either a USP / Ph Eur type I 50R vial closed with a rubber stopper and sealed with an aluminum crimp seal closure. The stopper may be made of elastomer complying with USP and Ph Eur. In certain embodiments vials may be filled with 61.2 mL of the protein product solution in order to allow an extractable volume of 60 mL. In certain embodiments, the liquid formulation may be diluted with 0.9% saline solution.

**[00157]** In certain embodiments, the liquid formulation of the disclosure may be prepared as a 10 mg/mL concentration solution in combination with a sugar at stabilizing levels. In certain embodiments the liquid formulation may be prepared in an aqueous carrier. In certain embodiments, a stabilizer may be added in an amount no greater than that which may result in a viscosity undesirable or unsuitable for intravenous administration. In certain embodiments, the sugar may be disaccharides, *e.g.*, sucrose. In certain embodiments, the liquid formulation may also include one or more of a buffering agent, a surfactant, and a preservative.

**[00158]** In certain embodiments, the pH of the liquid formulation may be set by addition of a pharmaceutically acceptable acid and/or base. In certain embodiments, the pharmaceutically acceptable acid may be hydrochloric acid. In certain embodiments, the base may be sodium hydroxide.

**[00159]** In addition to aggregation, deamidation is a common product variant of peptides and proteins that may occur during fermentation, harvest/cell clarification, purification, drug substance/drug product storage and during sample analysis. Deamidation is the loss of $NH_3$ from a protein forming a succinimide intermediate that can undergo hydrolysis. The succinimide intermediate results in a 17 dalton mass decrease of the parent peptide. The subsequent hydrolysis results in an 18 dalton mass increase. Isolation of the succinimide intermediate is difficult due to instability under aqueous conditions. As such, deamidation is typically detectable as 1 dalton mass increase. Deamidation of an asparagine results in either aspartic or isoaspartic acid. The parameters affecting the rate of deamidation include pH, temperature, solvent dielectric constant, ionic strength, primary sequence, local polypeptide conformation and tertiary structure. The amino acid residues adjacent to Asn in the peptide chain affect deamidation rates. Gly and Ser following an Asn in protein sequences results in a higher susceptibility to deamidation.

**[00160]** In certain embodiments, the liquid formulation of the present disclosure may be preserved under conditions of pH and humidity to prevent deamination of the protein product.

**[00161]** The aqueous carrier of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

**[00162]** A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation.

**[00163]** Intravenous (IV) formulations may be the preferred administration route in particular instances, such as when a patient is in the hospital after transplantation receiving all drugs via the IV route. In certain embodiments, the liquid formulation is diluted with 0.9% Sodium Chloride solution before administration. In certain embodiments, the diluted drug product for injection is isotonic and suitable for administration by intravenous infusion.

**[00164]** In certain embodiments, a salt or buffer components may be added in an amount of 10 mM - 200 mM. The salts and/or buffers are pharmaceutically acceptable and are derived from various known acids (inorganic and organic) with "base forming" metals or amines. In certain embodiments, the buffer may be phosphate buffer. In certain embodiments, the buffer may be glycinate, carbonate, citrate buffers, in which case, sodium, potassium or ammonium ions can serve as counterion.

**[00165]** A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation.

**[00166]** The aqueous carrier of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

**[00167]** This present disclosure could exist in a lyophilized formulation including the proteins and a lyoprotectant. The lyoprotectant may be sugar, *e.g.*, disaccharides. In certain embodiments, the lyoprotectant may be sucrose or maltose. The lyophilized formulation may also include one or more of a buffering agent, a surfactant, a bulking agent, and/or a preservative.

**[00168]** The amount of sucrose or maltose useful for stabilization of the lyophilized drug product may be in a weight ratio of at least 1:2 protein to sucrose or maltose. In certain embodiments, the protein to sucrose or maltose weight ratio may be of from 1:2 to 1:5.

**[00169]** In certain embodiments, the pH of the formulation, prior to lyophilization, may be set by addition of a pharmaceutically acceptable acid and/or base. In certain embodiments the pharmaceutically acceptable acid may be hydrochloric acid. In certain embodiments, the pharmaceutically acceptable base may be sodium hydroxide.

**[00170]** Before lyophilization, the pH of the solution containing the protein of the present disclosure may be adjusted between 6 to 8. In certain embodiments, the pH range for the lyophilized drug product may be from 7 to 8.

**[00171]** In certain embodiments, a salt or buffer components may be added in an amount of 10 mM - 200 mM. The salts and/or buffers are pharmaceutically acceptable and are derived from various known acids (inorganic and organic) with "base forming" metals or amines. In certain embodiments, the buffer may be phosphate buffer. In certain embodiments, the buffer may be glycinate, carbonate, citrate buffers, in which case, sodium, potassium or ammonium ions can serve as counterion.

**[00172]** In certain embodiments, a "bulking agent" may be added. A "bulking agent" is a compound which adds mass to a lyophilized mixture and contributes to the physical structure of the lyophilized cake (*e.g.*, facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Illustrative bulking agents include mannitol, glycine, polyethylene glycol and sorbitol. The lyophilized formulations of the present invention may contain such bulking agents.

**[00173]** A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation.

**[00174]** In certain embodiments, the lyophilized drug product may be constituted with an aqueous carrier. The aqueous carrier of interest herein is one which is pharmaceutically acceptable (*e.g.*, safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation, after lyophilization. Illustrative diluents include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

**[00175]** In certain embodiments, the lyophilized drug product of the current disclosure is reconstituted with either Sterile Water for Injection, USP (SWFI) or 0.9% Sodium Chloride Injection, USP. During reconstitution, the lyophilized powder dissolves into a solution.

**[00176]** In certain embodiments, the lyophilized protein product of the instant disclosure is constituted to about 4.5 mL water for injection and diluted with 0.9% saline solution (sodium chloride solution).

**[00177]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

**[00178]** The specific dose can be a uniform dose for each patient, for example, 50-5000 mg of protein. Alternatively, a patient's dose can be tailored to the approximate body weight or surface area of the patient. Other factors in determining the appropriate dosage can include the disease or condition to be treated or prevented, the severity of the disease, the route of administration, and the age, sex and medical condition of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those skilled in the art, especially in light of the dosage information and assays disclosed herein. The dosage can also be determined through the use of known assays for determining dosages used in conjunction with appropriate dose-response data. An individual patient's dosage can be adjusted as the progress of the disease is monitored. Blood levels of the targetable construct or complex in a patient can be measured to see if the dosage needs to be adjusted to reach or maintain an effective concentration. Pharmacogenomics may be used to determine which targetable constructs and/or complexes, and dosages thereof, are most likely to be effective for a given individual (Schmitz *et al.*, *Clinica Chimica Acta* 308: 43-53, 2001; Steimer *et al.*, *Clinica Chimica Acta* 308: 33-41, 2001).

**[00179]** In general, dosages based on body weight are from about 0.01 μg to about 100 mg per kg of body weight, such as about 0.01 μg to about 100 mg/kg of body weight, about 0.01 μg to about 50 mg/kg of body weight, about 0.01 μg to about 10 mg/kg of body weight, about 0.01 μg to about 1 mg/kg of body weight, about 0.01 μg to about 100 μg/kg of body weight, about 0.01 μg to about 50 μg/kg of body weight, about 0.01 μg to about 10 μg/kg of body weight, about 0.01 μg to about 1 μg/kg of body weight, about 0.01 μg to about 0.1 μg/kg of body weight, about 0.1 μg to about 100 mg/kg of body weight, about 0.1 μg to about 50 mg/kg of body weight, about 0.1 μg to about 10 mg/kg of body weight, about 0.1 μg to about 1 mg/kg of body weight, about 0.1 μg to about 100 μg/kg of body weight, about 0.1 μg to

about 10 μg/kg of body weight, about 0.1 μg to about 1 μg/kg of body weight, about 1 μg to about 100 mg/kg of body weight, about 1 μg to about 50 mg/kg of body weight, about 1 μg to about 10 mg/kg of body weight, about 1 μg to about 1 mg/kg of body weight, about 1 μg to about 100 μg/kg of body weight, about 1 μg to about 50 μg/kg of body weight, about 1 μg to about 10 μg/kg of body weight, about 10 μg to about 100 mg/kg of body weight, about 10 μg to about 50 mg/kg of body weight, about 10 μg to about 10 mg/kg of body weight, about 10 μg to about 1 mg/kg of body weight, about 10 μg to about 100 μg/kg of body weight, about 10 μg to about 50 μg/kg of body weight, about 50 μg to about 100 mg/kg of body weight, about 50μg to about 50 mg/kg of body weight, about 50 μg to about 10 mg/kg of body weight, about 50 μg to about 1 mg/kg of body weight, about 50 μg to about 100 μg/kg of body weight, about 100 μg to about 100 mg/kg of body weight, about 100 μg to about 50 mg/kg of body weight, about 100 μg to about 10 mg/kg of body weight, about 100 μg to about 1 mg/kg of body weight, about 1 mg to about 100 mg/kg of body weight, about 1 mg to about 50 mg/kg of body weight, about 1 mg to about 10 mg/kg of body weight, about 10 mg to about 100 mg/kg of body weight, about 10 mg to about 50 mg/kg of body weight, about 50 mg to about 100 mg/kg of body weight.

**[00180]** Doses may be given once or more times daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the targetable construct or complex in bodily fluids or tissues. Administration of the present invention could be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, intracavitary, by perfusion through a catheter or by direct intralesional injection. This may be administered once or more times daily, once or more times weekly, once or more times monthly, and once or more times annually.

**[00181]** The description above describes multiple aspects and embodiments of the invention. The patent application specifically contemplates all combinations and permutations of the aspects and embodiments.

## EXAMPLES

**[00182]** The invention now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and is not intended to limit the invention.

**Example 1 – NKG2D-binding domains bind to NKG2D**

NKG2D-binding domains bind to purified recombinant NKG2D

**[00183]** The nucleic acid sequences of human, mouse or cynomolgus NKG2D ectodomains were fused with nucleic acid sequences encoding human IgG1 Fc domains and introduced into mammalian cells to be expressed. After purification, NKG2D-Fc fusion proteins were adsorbed to wells of microplates. After blocking the wells with bovine serum albumin to prevent non-specific binding, NKG2D-binding domains were titrated and added to the wells pre-adsorbed with NKG2D-Fc fusion proteins. Primary antibody binding was detected using a secondary antibody which was conjugated to horseradish peroxidase and specifically recognizes a human kappa light chain to avoid Fc cross-reactivity. 3,3',5,5'-Tetramethylbenzidine (TMB), a substrate for horseradish peroxidase, was added to the wells to visualize the binding signal, whose absorbance was measured at 450 nM and corrected at 540 nM. An NKG2D-binding domain clone, an isotype control or a positive control (selected from SEQ ID NOs:45-48, or anti-mouse NKG2D clones MI-6 and CX-5 available at eBioscience) was added to each well.

**[00184]** The isotype control showed minimal binding to recombinant NKG2D-Fc proteins, while the positive control bound strongest to the recombinant antigens. NKG2D-binding domains produced by all clones demonstrated binding across human, mouse, and cynomolgus recombinant NKG2D-Fc proteins, although with varying affinities from clone to clone. Generally, each anti-NKG2D clone bound to human (FIG. 3) and cynomolgus (FIG. 4) recombinant NKG2D-Fc with similar affinity, but with lower affinity to mouse (FIG. 5) recombinant NKG2D-Fc.

NKG2D-binding domains bind to cells expressing NKG2D

**[00185]** EL4 mouse lymphoma cell lines were engineered to express human or mouse NKG2D - CD3 zeta signaling domain chimeric antigen receptors. An NKG2D-binding clone, an isotype control or a positive control was used at a 100 nM concentration to stain extracellular NKG2D expressed on the EL4 cells. The antibody binding was detected using fluorophore-conjugated anti-human IgG secondary antibodies. Cells were analyzed by flow cytometry, and fold-over-background (FOB) was calculated using the mean fluorescence intensity (MFI) of NKG2D-expressing cells compared to parental EL4 cells.

**[00186]** NKG2D-binding domains produced by all clones bound to EL4 cells expressing human and mouse NKG2D. Positive control antibodies (selected from SEQ ID NO: 45-48, or anti-mouse NKG2D clones MI-6 and CX-5 available at eBioscience) gave the best FOB

binding signal. The NKG2D-binding affinity for each clone was similar between cells expressing human NKG2D (FIG. 6) and mouse (FIG. 7) NKG2D.

**Example 2 – NKG2D-binding domains block natural ligand binding to NKG2D**

Competition With ULBP-6

**[00187]** Recombinant human NKG2D-Fc proteins were adsorbed to wells of a microplate, and the wells were blocked with bovine serum albumin reduce non-specific binding. A saturating concentration of ULBP-6-His-biotin was added to the wells, followed by addition of the NKG2D-binding domain clones. After a 2-hour incubation, wells were washed and ULBP-6-His-biotin that remained bound to the NKG2D-Fc coated wells was detected by streptavidin-conjugated to horseradish peroxidase and TMB substrate. Absorbance was measured at 450 nM and corrected at 540 nM. After subtracting background, specific binding of NKG2D-binding domains to the NKG2D-Fc proteins was calculated from the percentage of ULBP-6-His-biotin that was blocked from binding to the NKG2D-Fc proteins in wells. The positive control antibody (selected from SEQ ID NOs:45-48) and various NKG2D-binding domains blocked ULBP-6 binding to NKG2D, while isotype control showed little competition with ULBP-6 (FIG. 8).

Competition With MICA

**[00188]** Recombinant human MICA-Fc proteins were adsorbed to wells of a microplate, and the wells were blocked with bovine serum albumin to reduce non-specific binding. NKG2D-Fc-biotin was added to wells followed by NKG2D-binding domains. After incubation and washing, NKG2D-Fc-biotin that remained bound to MICA-Fc coated wells was detected using streptavidin-HRP and TMB substrate. Absorbance was measured at 450 nM and corrected at 540 nM. After subtracting background, specific binding of NKG2D-binding domains to the NKG2D-Fc proteins was calculated from the percentage of NKG2D-Fc-biotin that was blocked from binding to the MICA-Fc coated wells. The positive control antibody (selected from SEQ ID NOs:45-48) and various NKG2D-binding domains blocked MICA binding to NKG2D, while isotype control showed little competition with MICA (FIG. 9).

Competition With Rae-1 delta

**[00189]** Recombinant mouse Rae-1delta-Fc (purchased from R&D Systems) was adsorbed to wells of a microplate, and the wells were blocked with bovine serum albumin to reduce non-specific binding. Mouse NKG2D-Fc-biotin was added to the wells followed by

NKG2D-binding domains. After incubation and washing, NKG2D-Fc-biotin that remained bound to Rae-1delta-Fc coated wells was detected using streptavidin-HRP and TMB substrate. Absorbance was measured at 450 nM and corrected at 540 nM. After subtracting background, specific binding of NKG2D-binding domains to the NKG2D-Fc proteins was calculated from the percentage of NKG2D-Fc-biotin that was blocked from binding to the Rae-1delta-Fc coated wells. The positive control (selected from SEQ ID NOs:45-48, or anti-mouse NKG2D clones MI-6 and CX-5 available at eBioscience) and various NKG2D-binding domain clones blocked Rae-1delta binding to mouse NKG2D, while the isotype control antibody showed little competition with Rae-1delta (FIG. 10).

**Example 3 – NKG2D-binding domain clones activate NKG2D**

**[00190]** Nucleic acid sequences of human and mouse NKG2D were fused to nucleic acid sequences encoding a CD3 zeta signaling domain to obtain chimeric antigen receptor (CAR) constructs. The NKG2D-CAR constructs were then cloned into a retrovirus vector using Gibson assembly and transfected into expi293 cells for retrovirus production. EL4 cells were infected with viruses containing NKG2D-CAR together with 8 μg/mL polybrene. 24 hours after infection, the expression levels of NKG2D-CAR in the EL4 cells were analyzed by flow cytometry, and clones which express high levels of the NKG2D-CAR on the cell surface were selected.

**[00191]** To determine whether NKG2D-binding domains activate NKG2D, they were adsorbed to wells of a microplate, and NKG2D-CAR EL4 cells were cultured on the antibody fragment-coated wells for 4 hours in the presence of brefeldin-A and monensin. Intracellular TNFα production, an indicator for NKG2D activation, was assayed by flow cytometry. The percentage of TNFα-positive cells was normalized to the cells treated with the positive control. All NKG2D-binding domains activated both human NKG2D (FIG. 11) and mouse NKG2D (FIG. 12).

**Example 4 – NKG2D-binding domains activate NK cells**

Primary human NK cells

**[00192]** Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. NK cells ($CD3^-CD56^+$) were isolated using negative selection with magnetic beads from PBMCs, and the purity of the isolated NK cells was typically >95%. Isolated NK cells were then cultured in media containing 100 ng/mL IL-2 for 24-48 hours before they were transferred to the wells of a

microplate to which the NKG2D-binding domains were adsorbed, and cultured in the media containing fluorophore-conjugated anti-CD107a antibody, brefeldin-A, and monensin. Following culture, NK cells were assayed by flow cytometry using fluorophore-conjugated antibodies against CD3, CD56 and IFN-γ. CD107a and IFN-γ staining were analyzed in $CD3^-$ $CD56^+$ cells to assess NK cell activation. The increase in CD107a/IFN-γ double-positive cells is indicative of better NK cell activation through engagement of two activating receptors rather than one receptor. NKG2D-binding domains and the positive control (selected from SEQ ID NOs:45-48) showed a higher percentage of NK cells becoming $CD107a^+$ and IFN-$\gamma^+$ than the isotype control (FIG. 13 & FIG. 14 represent data from two independent experiments, each using a different donor's PBMC for NK cell preparation).

Primary mouse NK cells

**[00193]** Spleens were obtained from C57Bl/6 mice and crushed through a 70 μm cell strainer to obtain single cell suspension. Cells were pelleted and resuspended in ACK lysis buffer (purchased from Thermo Fisher Scientific #A1049201; 155mM ammonium chloride, 10mM potassium bicarbonate, 0.01mM EDTA) to remove red blood cells. The remaining cells were cultured with 100 ng/mL hIL-2 for 72 hours before being harvested and prepared for NK cell isolation. NK cells ($CD3^-NK1.1^+$) were then isolated from spleen cells using a negative depletion technique with magnetic beads with typically >90% purity. Purified NK cells were cultured in media containing 100 ng/mL mIL-15 for 48 hours before they were transferred to the wells of a microplate to which the NKG2D-binding domains were adsorbed, and cultured in the media containing fluorophore-conjugated anti-CD107a antibody, brefeldin-A, and monensin. Following culture in NKG2D-binding domain-coated wells, NK cells were assayed by flow cytometry using fluorophore-conjugated antibodies against CD3, NK1.1 and IFN-γ. CD107a and IFN-γ staining were analyzed in $CD3^-$ $NK1.1^+$ cells to assess NK cell activation. The increase in CD107a/IFN-γ double-positive cells is indicative of better NK cell activation through engagement of two activating receptors rather than one receptor. NKG2D-binding domains and the positive control (selected from anti-mouse NKG2D clones MI-6 and CX-5 available at eBioscience) showed a higher percentage of NK cells becoming $CD107a^+$ and IFN-$\gamma^+$ than the isotype control (FIG. 15 & FIG. 16 represent data from two independent experiments, each using a different mouse for NK cell preparation).

**Example 5 – NKG2D-binding domains enable cytotoxicity of target tumor cells**

**[00194]** Human and mouse primary NK cell activation assays demonstrate increased cytotoxicity markers on NK cells after incubation with NKG2D-binding domains. To address whether this translates into increased tumor cell lysis, a cell-based assay was utilized where each NKG2D-binding domain was developed into a monospecific antibody. The Fc region was used as one targeting arm, while the Fab region (NKG2D-binding domain) acted as another targeting arm to activate NK cells. THP-1 cells, which are of human origin and express high levels of Fc receptors, were used as a tumor target and a Perkin Elmer DELFIA Cytotoxicity Kit was used. THP-1 cells were labeled with BATDA reagent, and resuspended at $10^5$/mL in culture media. Labeled THP-1 cells were then combined with NKG2D antibodies and isolated mouse NK cells in wells of a microtiter plate at 37 $^{o}$C for 3 hours. After incubation, 20 µl of the culture supernatant was removed, mixed with 200 µl of Europium solution and incubated with shaking for 15 minutes in the dark. Fluorescence was measured over time by a PheraStar plate reader equipped with a time-resolved fluorescence module (Excitation 337nm, Emission 620nm) and specific lysis was calculated according to the kit instructions.

**[00195]** The positive control, ULBP-6 - a natural ligand for NKG2D, showed increased specific lysis of THP-1 target cells by mouse NK cells. NKG2D antibodies also increased specific lysis of THP-1 target cells, while isotype control antibody showed reduced specific lysis. The dotted line indicates specific lysis of THP-1 cells by mouse NK cells without antibody added (FIG. 17).

**Example 6 – NKG2D antibodies show high thermostability**

**[00196]** Melting temperatures of NKG2D-binding domains were assayed using differential scanning fluorimetry. The extrapolated apparent melting temperatures are high relative to typical IgG1 antibodies (FIG. 18).

**Example 7 – Multi-specific binding proteins display enhanced ability to activate NK cells**

**[00197]** Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. NK cells ($CD3^-CD56^+$) were isolated using negative selection with magnetic beads from PBMCs, and the purity of the isolated NK cells was typically >95%. Isolated NK cells were then cultured in media containing 100 ng/mL IL-2 for 24-48 hours before they were transferred to the wells of a

microplate to which multi-specific and bispecific binding proteins were adsorbed respectively, and cultured in the media containing fluorophore-conjugated anti-CD107a antibody, brefeldin-A, and monensin. Following culture, NK cells were assayed by flow cytometry using fluorophore-conjugated antibodies against CD3, CD56 and IFN-γ. CD107a and IFN-γ staining were analyzed in $CD3^-CD56^+$ cells to assess NK cell activation. The increase in CD107a/IFN-γ double-positive cells is indicative of better NK cell activation. AL2.2 is a multi-specific binding protein containing HER2-binding domain (trastuzumab), NKG2D-binding domain (ULBP-6) and a human IgG1 Fc domain. It was made through a controlled Fab-arm exchange reaction (cFAE) starting from trastuzumab homodimer and ULBP-6-Fc homodimer (*see* Labrijn *et al.*, Nature Protocols 9, 2450-2463). SC2.2 is single chain protein including an scFv derived from trastuzumab, and ULBP-6 (SEQ ID NO:93).

SEQ ID NO:93
MAAAAIPALLLCLPLLFLLFGWSRARRDDPHSLCYDITVIPKFRPGPRWCAVQGQVD
EKTFLHYDCGNKTVTPVSPLGKKLNVTMAWKAQNPVLREVVDILTEQLLDIQLENY
TPKEPLTLQARMSCEQKAEGHSSGSWQFSIDGQTFLLFDSEKRMWTTVHPGARKMK
EKWENDKDVAMSFHYISMGDCIGWLEDFLMGMDSTLEPSAGAPLAMSSGTTQLRA
TATTLILCCLLIILPCFILPGI

**[00198]** Analysis of CD107a and IFN-γ staining indicated that isotype control IgG showed no activation of NK cells, while a higher percentage of NK cells becoming $CD107a^+$ and IFN-$\gamma^+$ after stimulation with a multi-specific binding protein compared with a bispecific protein, demonstrating stronger NK cell activation through engagement of two activating receptors (NKG2D and CD16) rather than just one (NKG2D) (FIG. 19). This increase in NK cell activation is expected to translate into more potent tumor cell killing.

**Example 8 – Multi-specific binding proteins display enhanced cytotoxicity towards target tumor cells**

Primary human NK cell cytotoxicity assay

**[00199]** Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. NK cells ($CD3^-CD56^+$) were isolated using negative selection with magnetic beads from PBMCs, and the purity of the isolated NK cells was typically >95%. NK cells were then cultured overnight in media containing 100ng/mL IL-2 before used in cytotoxicity assays. The following day NK cells were resuspended at $5x10^5$/mL in fresh culture media. Human breast cancer cell SkBr-3 cells

were labeled with BATDA reagent according to Perkin Elmer DELFIA Cytotoxicity Kit and resuspended at $5x10^4$/mL in culture media. Various dilution of the multi-specific binding proteins were made into culture media. NK cells, the labeled SkBr-3 cells and the multi-specific binding proteins were then combined in wells of a microtiter plate and incubated at 37°C for 3 hours. After incubation, 20 µl of the culture supernatant was removed, mixed with 200 µl of Europium solution and incubated with shaking for 15 minutes in the dark. Fluorescence was measured over time by a PheraStar plate reader equipped with a time-resolved fluorescence module (Excitation 337nm, Emission 620nm) and specific lysis was calculated according to the kit instructions. AL0.2 is a multi-specific binding protein containing HER2-binding domain (trastuzumab), NKG2D-binding domain (selected from SEQ ID NO: 1-44)) and a human IgG1 Fc domain. It was made through a controlled Fab-arm exchange reaction (cFAE) starting from trastuzumab homodimer and anti-NKG2D homodimer. AL0.2si is based on AL0.2 and contains an additional D265A mutation in Fc domain which abrogates CD16 binding. Trastuzumab-si is based on Trastuzumab and contains an additional D265A mutation in Fc domain which abrogates CD16 binding. AL2.2 is a multi-specific binding protein containing HER2-binding domain (trastuzumab), NKG2D-binding domain (ULBP-6) and a human IgG1 Fc domain. SC2.2 is single chain protein including an scFv derived from trastuzumab, and ULBP-6.

**[00200]** AL0.2 showed enhanced lysis of SkBr-3 target cells by human NK cells than trastuzumab in a does dependent manner, with a p value of 0.0311 in EC50 (FIG. 20). AL0.2si (FIG. 21) and trastuzumab-si (FIG. 22) showed reduction in both potency and maximum specific lysis of SkBr-3 cells compared to AL0.2, with a p-value of 0.0002, and 0.0001 in EC50, respectively (FIGs. 21-22). In addition, AL0.2 showed enhanced lysis of SkBr-3 cells than AL2.2 in a dose-dependent manner (FIG. 23). Isotype control IgG showed no increase in specific lysis at any of the concentrations tested. Together the data have demonstrated that multi-specific binding proteins engaging 2 activating receptors on NK cells and one tumor antigen, induce more potent killing of tumor cells by human NK cells compared to bispecific proteins engaging one activating receptor on NK cells and one tumor antigen.

Primary mouse NK cell cytotoxicity assay

**[00201]** Spleens were obtained from C57Bl/6 mice and crushed through a 70 µm cell strainer to obtain single cell suspension. Cells were pelleted and resuspended in ACK lysis buffer (purchased from Thermo Fisher Scientific #A1049201; 155mM ammonium chloride,

10mM potassium bicarbonate, 0.01mM EDTA) to remove red blood cells. The remaining cells were cultured with 100 ng/mL hIL-2 for 72 hours before being harvested and prepared for NK cell isolation. NK cells ($CD3^-NK1.1^+$) were then isolated from spleen cells using a negative depletion technique with magnetic beads with typically >90% purity. Purified NK cells were cultured in media containing 100 ng/mL mIL-15 for 48 hours before resuspended in culture media at $10^6$/mL for cytotoxic assays. RMA-HER2-dTomato, a mouse tumor cell line engineered to express HER2 and dTomato, and its control counterpart, RMA cells expressing zsGreen were used as targets. They were resuspended at $2x10^5$/mL in culture media and seeded into wells of a micro plate at 1:1 ratio. Dilutions of multi-specific protein were made into culture media, and added to the RMA cells together with the NK cells. After incubation overnight at 37 °C with 5% $CO_2$, the percentage of RMA-HER2-dTomato and RMA-zsGreen cells were determined by flow cytometry using the fluorescent reporter to identify the two cells types. Specific target cell death = (1- ((% RMA-Ca2T-dTomato cells in treatment group * % RMA-zsGreen cells in control group)/(% RMA-Ca2T-dTomato cells in control group * % RMA-zsGreen cells in treatment group))) * 100%.

**[00202]** AL2.2 is more potent in redirecting NK cell responses to tumor targets than SC2.2 (FIG. 25) and Trastuzumab (FIG. 24). Control protein showed little impact on specific target death. These data demonstrate the multi-specific binding proteins engaging 2 activating receptors on NK cells and one tumor antigen, induce more potent killing of tumor cells by mouse NK cells compared to bispecific proteins engaging one activating receptor on NK cells and one tumor antigen.

**Example 9 – Multi-specific binding proteins bind to NKG2D**

**[00203]** EL4 mouse lymphoma cell lines were engineered to express human NKG2D trispecific binding proteins (TriNKETs) that each contain an NKG2D-binding domain, a HER2-binding domain, and an Fc domain that binds to CD16 as shown in FIG. 1, were tested for their affinity to extracellular NKG2D expressed on EL4 cells. The binding of the multi-specific binding proteins to NKG2D was detected using fluorophore-conjugated anti-human IgG secondary antibodies. Cells were analyzed by flow cytometry, and fold-over-background (FOB) was calculated using the mean fluorescence intensity (MFI) of NKG2D-expressing cells compared to parental EL4 cells.

**[00204]** TriNKETs tested include HER2-TriNKET-C26 (ADI-28226 and a HER2-binding domain), and HER2-TriNKET-F04 (ADI-29404 and a HER2-binding domain). The HER2-

binding domain used in the tested molecules was composed of a heavy chain variable domain and a light chain variable domain of Trastuzumab.

**[00205]** The data show that a HER2 targeting TriNKETs of the present disclosure bind to NKG2D (FIG. 26).

**Example 10 – Multi-specific binding proteins bind to human tumor antigen**

Trispecific-binding proteins bind to HER2

**[00206]** Human cancer cell lines expressing HER2 were used to assay the binding of HER2 targeting TriNKETs to the tumor associated antigen. Renal cell carcinoma cell line 786-O expresses low levels of HER2. TriNKETs and optionally the parental anti-HER2 monoclonal antibody (Trastuzumab) were incubated with the cells, and the binding was detected using fluorophore-conjugated anti-human IgG secondary antibodies. Cells were analyzed by flow cytometry, and fold-over-background (FOB) was calculated using the mean fluorescence intensity (MFI) from TriNKETs and Trastuzumab normalized to secondary antibody controls. HER2-TriNKET-C26, and HER2-TriNKET-F04 show comparable levels of binding to HER2 expressed on 786-O cells as compared with Trastuzumab (FIG. 27A).

**[00207]** RMA cells transduced with human HER2 were used to test binding to cell expressed human HER2 by HER2 targeting TriNKETs. TriNKETs were diluted to 20 μg/mL, and binding was detected using a fluorophore conjugated anti-human IgG secondary antibody. Cells were analyzed by flow cytometry, binding to cell expressed HER2 was compared to isotype stained and unstained cell populations. FIG. 27B and FIG. 27C show binding profiles of TriNKETs containing two distinct NKG2D binding domains (the binding profile of C26.2 TriNKET with HER2-binding site shown in FIG. 27B; the binding profile of F04.2 TriNKET with HER2-binding site shown in FIG. 27C), but with the same HER-binding domain. Both TriNKETs show similar level of binding to cell surface HER2 on RMA cells.

**Example 11 – Multi-specific binding proteins activate NK cells**

**[00208]** Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. NK cells ($CD3^-CD56^+$) were isolated using negative selection with magnetic beads from PBMCs, and the purity of the isolated NK cells was typically >90%. Isolated NK cells were cultured in media

containing 100 ng/mL IL-2 for activation or rested overnight without cytokine. IL-2-activated NK cells were used within 24-48 hours after activation.

**[00209]** Human cancer cells expressing a tumor antigen were harvested and resuspended in culture media at $2x10^6$/mL. Monoclonal antibodies or TriNKETs targeting the tumor antigen were diluted in culture media. Activated NK cells were harvested, washed, and resuspended at $2x10^6$/mL in culture media. Cancer cells were then mixed with monoclonal antibodies/TriNKETs and activated NK cells in the presence of IL-2. Brefeldin-A and monensin were also added to the mixed culture to block protein transport out of the cell for intracellular cytokine staining. Fluorophore-conjugated anti-CD107a was added to the mixed culture and the culture was incubated for 4 hours before samples were prepared for FACS analysis using fluorophore-conjugated antibodies against CD3, CD56 and IFN-γ. CD107a and IFN-γ staining was analyzed in $CD3^-CD56^+$ cells to assess NK cell activation. The increase in CD107a/IFN-γ double-positive cells is indicative of better NK cell activation through engagement of two activating receptors rather than one receptor.

**[00210]** TriNKETs mediate activation of human NK cells co-cultured with HER2-expressing SkBr-3 cells (FIG. 28A), Colo201 cells (FIG. 28B), and HCC1954 cells (FIG. 28C) respectively as indicated by an increase of CD107a degranulation and IFN-γ production. SkBr-3 cells and HCC1954 cells have high levels of surface HER2 expression, and Colo201 has medium HER2 expression. Compared to the monoclonal antibody trastuzumab, TriNKETs show superior activation of human NK cells in the presence of human cancer cells. NK cells alone, NK cells plus SkBr-3 cells are used as negative controls.

**[00211]** TriNKETs (C26-TriNKET-HER2 and F04-TriNKET-HER2) mediate activation of human NK cells co-cultured with CD33-expressing human AML Mv4-11 cells showed an increase of CD107a degranulation and IFN-γ production. Compared to the monoclonal anti-CD33 antibody, TriNKETs (C26-TriNKET-HER2 and F04-TriNKET-HER2) showed superior activation of human NK cells in the presence of human cancer cells expressing HER2 (FIGs. 28A-28C).

**Example 12 – Trispecific-binding proteins enable cytotoxicity of target cancer cells**

**[00212]** Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. NK cells ($CD3^-CD56^+$) were isolated using negative selection with magnetic beads from PBMCs, and the purity of the isolated NK cells was typically >90%. Isolated NK cells were cultured in media

containing 100 ng/mL IL-2 for activation or rested overnight without cytokine. IL-2-activated or rested NK cells were used the following day in cytotoxicity assays.

**[00213]** In order to test the ability of human NK cells to lyse cancer cells in the presence of TriNKETs, a cyto Tox 96 non-radioactive cytotoxicity assay from Promega (G1780) was used according to the manufacturer's instructions. Briefly, human cancer cells expressing a tumor antigen were harvested, washed, and resuspended in culture media at 1-2x$10^5$/mL. Rested and/or activated NK cells were harvested, washed, and resuspended at $10^5$-2.0x$10^6$/mL in the same culture media as that of the cancer cells. In each well of a 96 well plate, 50 µl of the cancer cell suspension was mixed with 50 µl of NK cell suspension with or without TriNKETs targeting the tumor antigen expressed on the cancer cells. After incubation at 37 °C with 5% $CO_2$ for 3 hours and 15 minutes, 10x lysis buffer was added to wells containing only cancer cells, and to wells containing only media for the maximum lysis and negative reagent controls, respectively. The plate was then placed back into the incubator for an additional 45 minutes to reach a total of 4 hours incubation. Cells were then pelleted, and the culture supernatant was transferred to a new 96 well plate and mixed with a substrate for development. The new plate was incubated for 30 minutes at room temperature, and the absorbance was read at 492 nm on a SpectraMax i3x. Percentage of specific lysis of the cancer cells was calculated as follows: % Specific lysis = ((experimental lysis – spontaneous lysis from NK cells alone – spontaneous lysis from cancer cells alone) / (Maximum lysis – negative reagent control)) x 100%.

**[00214]** TriNKETs enhance NK cell cytotoxicity against targets with low surface expression compared to the cytotoxic activity of trastuzumab, an anti-HER2 monoclonal antibody. Rested human NK cells were mixed with high HER2-expressing SkBr tumor cells and low HER2-expressing 786-O cancer cells, and TriNKETs' ability to enhance the cytotoxic activity of rested human NK cells against the high and low HER2-expressing cancer cells in a dose-responsive manner was assayed. Dotted lines in FIG. 29A and FIG. 29B indicate the cytotoxic activity of rested NK cells against the cancer cells in the absence of TriNKETs. As shown in FIG. 29B, upon mixing activated human NK cells with low HER2-expressing 786-O cells and TriNKET (*e.g.*, CD26-TriNKET and F04-TriNKET, which includes a binding domain for HER2), dose-responsive cytotoxic activity of activated human NK cells against the cancer cells was observed.

**Example 13 – Synergistic activation of human NK cells by cross-linking NKG2D and CD16**

Primary human NK cell activation assay

**[00215]** Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral human blood buffy coats using density gradient centrifugation. NK cells were purified from PBMCs using negative magnetic beads (StemCell # 17955). NK cells were >90% $CD3^-$ $CD56^+$ as determined by flow cytometry. Cells were then expanded 48 hours in media containing 100 ng/mL hIL-2 (Peprotech #200-02) before use in activation assays. Antibodies were coated onto a 96-well flat-bottom plate at a concentration of 2 µg/ml (anti-CD16, Biolegend # 302013) and 5 µg/mL (anti-NKG2D, R&D #MAB139) in 100 µl sterile PBS overnight at 4 °C followed by washing the wells thoroughly to remove excess antibody. For the assessment of degranulation IL-2-activated NK cells were resuspended at $5\times10^5$ cells/ml in culture media supplemented with 100 ng/mL hIL2 and 1 µg/mL APC-conjugated anti-CD107a mAb (Biolegend # 328619). $1\times10^5$ cells/well were then added onto antibody coated plates. The protein transport inhibitors Brefeldin A (BFA, Biolegend # 420601) and Monensin (Biolegend # 420701) were added at a final dilution of 1:1000 and 1:270 respectively. Plated cells were incubated for 4 hours at 37 °C in 5% $CO_2$. For intracellular staining of IFN-γ NK cells were labeled with anti-CD3 (Biolegend #300452) and anti-CD56 mAb (Biolegend # 318328) and subsequently fixed and permeabilized and labeled with anti-IFN-γ mAb (Biolegend # 506507). NK cells were analyzed for expression of CD107a and IFN-γ by flow cytometry after gating on live $CD56^+CD3^-$cells.

**[00216]** To investigate the relative potency of receptor combination, crosslinking of NKG2D or CD16 and co-crosslinking of both receptors by plate-bound stimulation was performed. As shown in Figure 30 (FIGs. 30A-30C), combined stimulation of CD16 and NKG2D resulted in highly elevated levels of CD107a (degranulation) (FIG. 30A) and/or IFN-γ production (FIG. 30B). Dotted lines represent an additive effect of individual stimulations of each receptor.

**[00217]** CD107a levels and intracellular IFN-γ production of IL-2-activated NK cells were analyzed after 4 hours of plate-bound stimulation with anti-CD16, anti-NKG2D or a combination of both monoclonal antibodies. Graphs indicate the mean (n = 2) ± SD. FIG. 19A demonstrates levels of CD107a; FIG. 30B demonstrates levels of IFNγ; FIG. 30C demonstrates levels of CD107a and IFNγ. Data shown in FIGs. 30A-30C are representative of five independent experiments using five different healthy donors.

**[00218]** CD107a degranulation and intracellular IFN-γ production of IL-2-activated NK cells were analyzed after 4 hours of plate-bound stimulation with trastuzumab, anti-NKG2D, or a TriNKET derived from the binding domains of trastuzumab and the anti-NKG2D antibody (FIG. 31). In all cases antibodies tested were of the human IgG1 isotype. Graphs indicate the mean (n = 2) ± SD.

**Example 14 – Properties of the TriNKETs**

**Assessment of TriNKET binding to cell-expressed human NKG2D**

**[00219]** EL4 cells transduced with human NKG2D were used to test binding to cell-expressed human NKG2D. TriNKETs were diluted to 20 µg/mL, and then diluted serially. The mAb or TriNKET dilutions were used to stain cells, and binding of the TriNKET or mAb was detected using a fluorophore-conjugated anti-human IgG secondary antibody. Cells were analyzed by flow cytometry, binding MFI was normalized to secondary antibody controls to obtain fold over background values.

**Assessment of TriNKET binding to cell-expressed human cancer antigens**

**[00220]** Human cancer cell lines expressing HER2 were used to assess tumor antigen binding of TriNKETs derived from different NKG2D targeting clones. The human renal cell carcinoma cell line 786-O expresses low levels of HER2 and was used to assess TriNKET binding to cell-expressed HER2. TriNKETs were diluted to 20 µg/mL, and were incubated with the respective cells. Binding of the TriNKET was detected using a fluorophore-conjugated anti-human IgG secondary antibody. Cells were analyzed by flow cytometry, binding MFI to cell expressing HER2 was normalized to secondary antibody controls to obtain fold over background values.

**Determination of antibody binding capacity of human HER2-positive cancer cell lines**

**[00221]** Antibody binding capacity (ABC) of HER2-positive human cancer cell lines was measured. The Quantum Simply Cellular kit from Bangs Lab was used (#815), and the manufacturer instructions were followed for the preparation of antibody labeled beads. Briefly, each of the four populations of beads were stained with a saturating amount of anti-HER2 antibody, and the cell populations were also stained with a saturating amount of the same antibody. Sample data was acquired for each bead population, as well as the cell populations. The QuickCal worksheet, provided with the kit, was used for the generation of a standard curve and extrapolation of ABC values for each of the cell lines.

**Activation of primary NK cells by TriNKETs**

**[00222]** PBMCs were isolated from human peripheral blood buffy coats using density gradient centrifugation. Isolated PBMCs were washed and prepared for NK cell isolation. NK cells were isolated using a negative selection technique with magnetic beads; the purity of isolated NK cells was typically >90% CD3-CD56+. Isolated NK cells were cultured in media containing 100ng/mL IL-2 for activation or rested overnight without cytokine. IL-2-activated NK cells were used 24-48 hours later; rested NK cells were always used the day after purification.

**[00223]** Human cancer cell lines expressing a cancer target of interest were harvested from culture, and cells were adjusted to $2x10^6$/mL. Monoclonal antibodies or TriNKETs targeting the cancer target of interest were diluted in culture media. Rested and/or activated NK cells were harvested from culture, cells were washed, and were resuspended at $2x10^6$/mL in culture media. IL-2, and fluorophore-conjugated anti-CD107a were added to the NK cells for the activation culture. Brefeldin-A and monensin were diluted into culture media to block protein transport out of the cell for intracellular cytokine staining. Into a 96-well plate 50 µl of tumor targets, mAbs/TriNKETs, BFA/monensin, and NK cells were added for a total culture volume of 200 µl. The plate was cultured for 4 hours before samples were prepared for FACS analysis.

**[00224]** Following the 4 hour activation culture, cells were prepared for analysis by flow cytometry using fluorophore-conjugated antibodies against CD3, CD56 and IFNγ. CD107a and IFNγ staining was analyzed in CD3-CD56+ populations to assess NK cell activation.

**Primary human NK cell cytotoxicity assay**

**[00225]** PBMCs were isolated from human peripheral blood buffy coats using density gradient centrifugation. Isolated PBMCs were washed and prepared for NK cell isolation. NK cells were isolated using a negative selection technique with magnetic beads, purity of isolated NK cells was typically >90% CD3-CD56+. Isolated NK cells were cultured in media containing 100 ng/mL IL-2 or were rested overnight without cytokine. IL-2-activated or rested NK cells were used the following day in cytotoxicity assays.

*Cyto Tox 96 LHD release assay:*

**[00226]** The ability of human NK cells to lyse tumor cells was measured with or without the addition of TriNKETs using the cyto Tox 96 non-radioactive cytotoxicity assay from Promega (G1780). Human cancer cell lines expressing a cancer target of interest were

harvested from culture, cells were washed with PBS, and were resuspended in growth media at 1-2x$10^5$/mL for use as target cells. 50 µl of the target cell suspension were added to each well. Monoclonal antibodies or TriNKETs targeting a cancer antigen of interest were diluted in culture media, 50 µl of diluted mAb or TriNKET were added to each well. Rested and/or activated NK cells were harvested from culture, cells were washed, and were resuspended at $10^5$-2.0x$10^6$/mL in culture media depending on the desired E:T ratio. 50 µl of NK cells were added to each well of the plate to make a total of 150 µl culture volume. The plate was incubated at 37 °C with 5% CO2 for 3 hours and 15 minutes. After the incubation, 10x lysis buffer was added to wells of target cells alone, and to wells containing media alone, for maximum lysis and volume controls. The plate was then placed back into the incubator for an additional 45 minutes, to make to total of 4 hours of incubation before development.

**[00227]** After incubation, the plate was removed from the incubator and the cells were pelleted by centrifugation at 200g for 5 minutes. 50 µl of culture supernatant were transferred to a clean microplate and 50 µl of substrate solution were added to each well. The plate was protected from the light and incubated for 30 minutes at room temperature. 50 µl of stop solution were added to each well, and absorbance was read at 492nm on a SpectraMax i3x. % Specific lysis was calculated as follows: % Specific lysis = ((Experimental release – Spontaneous release from effector – Spontaneous release from target) / (Maximum release – Spontaneous release)) * 100%.

*DELFIA cytotoxicity assay:*

**[00228]** Human cancer cell lines expressing a target of interest were harvested from culture, cells were washed with PBS, and were resuspended in growth media at $10^6$/mL for labeling with BATDA reagent (Perkin Elmer AD0116). Manufacturer instructions were followed for labeling of the target cells. After labeling cells were washed 3x with PBS, and were resuspended at 0.5-1.0x$10^5$/mL in culture media. To prepare the background wells an aliquot of the labeled cells was put aside, and the cells were spun out of the media. 100 µl of the media were carefully added to wells in triplicate to avoid disturbing the pelleted cells. 100 µl of BATDA labeled cells were added to each well of the 96-well plate. Wells were saved for spontaneous release from target cells, and wells were prepared for max lysis of target cells by addition of 1% Triton-X. Monoclonal antibodies or TriNKETs against the tumor target of interest were diluted in culture media and 50 µl of diluted mAb or TriNKET were added to each well. Rested and/or activated NK cells were harvested from culture, cells were washed, and were resuspended at $10^5$-2.0x$10^6$/mL in culture media depending on the

desired E:T ratio. 50 µl of NK cells were added to each well of the plate to make a total of 200 µl culture volume. The plate was incubated at 37 °C with 5% CO2 for 2-3 hours before developing the assay.

**[00229]** After culturing for 2-3 hours, the plate was removed from the incubator and the cells were pelleted by centrifugation at 200g for 5 minutes. 20 µl of culture supernatant was transferred to a clean microplate provided from the manufacturer, 200 µl of room temperature europium solution was added to each well. The plate was protected from the light and incubated on a plate shaker at 250rpm for 15 minutes. Plate was read using either Victor 3 or SpectraMax i3X instruments. % Specific lysis was calculated as follows: % Specific lysis = ((Experimental release – Spontaneous release) / (Maximum release – Spontaneous release)) * 100%.

**Long term human PBMC cytotoxicity assay**

**[00230]** SkBr-3 target cells were labeled with BacMam 3.0 NucLight Green (#4622) to allow for tracking of the target cells. The manufacturer's protocol was followed for labeling of SkBr-3 target cells. Annexin V Red (Essen Bioscience #4641) was diluted and prepared according to the manufacturer's instructions. Monoclonal antibodies or TriNKETs were diluted into culture media. 50 µl of mAbs or TriNKETs, Annexin V, and rested NK cells were added to wells of a 96 well plate already containing labeled SkBr-3 cells; 50 µl of complete culture media was added for a total of 200 µl culture volume.

**[00231]** Image collection was setup on the IncuCyte S3. Images for the phase, green, and red channels were collected every hour, with 2 images per well. Image analysis was done using the IncuCyte S3 software. Masks for the green and red channels were created to count the number of tumor cells, and annexin V-positive cells respectively. To calculate the % annexin V-positive Mv4-11 target cells the following formula was used. % Annexin V-positive SkBr-3 cells = ((overlap object count) / (green object count)) * 100%.

**Comparing a TriNKET that targets HER+ cancer Cells with SC2.2**

**[00232]** A TriNKET targeting HER2 is more effective than Trastuzumab at reducing SkBr-3 cell number, and only 60% of the cells from time zero were left after 60 hours. A TriNKET of the present disclosure that targets HER2 expressing tumor/cancer cells is more effective than SC2.2 — a single chain bispecific molecule built from an scFv derived from trastuzumab linked to ULBP-6, a ligand for NKG2D. SC2.2 binds HER2+ cancer cells and NKG2D+ NK cells simultaneously. Therefore, effectiveness of SC2.2 in reducing HER2+

cancer cell number was investigated. *In vitro* activation and cytotoxity assays demonstrated that SC2.2 was effective in activating and killing NK cells. However, SC2.2 failed to demonstrate efficacy in the RMA/S-HER2 subcutaneous tumor model. The efficacy of SC2.2 was also tested *in vivo* using an RMA/S-HER2 overexpressing syngeneic mouse model. In this mouse model, SC2.2 failed to demonstrate control of tumor growth compared to vehicle control. Thus, although SC2.2 was able to activate and kill NK cells, and binds to HER2+ cancer cells, these properties were insufficient to effectively control HER2+ tumor growth.

**Assessment of SC2.2 serum half-life in C57Bl/6 mice**

**[00233]** To determine the serum half-life of SC2.2 in C57Bl/6 mice, SC2.2 was labeled with a fluorescent tag to track its concentration in vivo. SC2.2 was labeled with IRDye 800CW (Licor #929-70020). The labeled protein was injected intravenously into 3 C57Bl/6 mice, blood was taken from each mouse at the indicated time points. After collection blood was centrifuged at 1000g for 15 minutes and serum was collected from each sample and stored at 4C until all time points were collected.

**[00234]** Serum was imaged using an Odyssey CLx infrared imaging system, the fluorescent signal from the 800 channel was quantified using Image J software. Image intensities were normalized to the first time point, and the data was fit to a biphasic decay equation. In this experimental system the beta half-life of SC2.2 was calculated to be around 7 hours.

***In vivo* testing of SC2.2 against RMA/S-HER2 subcutaneous tumors**

**[00235]** An in vivo study was designed according to FIG. 37 to test the efficacy of SC2.2 against subcutaneous RMA/S-HER2 tumors. $10^6$ RMA/S cells transduced with human HER2 were injected subcutaneously into the flank of 20 C57Bl/6 mice. Starting day 2 after tumor innoculation SC2.2 was dosed daily via IP injection. SC2.2 was dosed at a high and a low concentrations along with a vehicle control. Starting day 4 after tumor innoculation tumors were measured Monday, Wednesday, and Friday for the duration of the study. Tumor volume was calculated using the following formula: Tumor volume = Length x width x height.

**Antibody binding capacity of human HER2-positive cancer cell lines**

**[00236]** Table 10 shows the results of HER2 surface quantification. SkBr-3 and HCC1954 cells were identified to have high (+++) levels of surface HER2. ZR-75-1 and Colo201

showed medium levels (++) of surface HER2, and 786-O showed the lowest level of HER2 (+).

**[00237]** Table 10: ABC of HER2-positive cancer cell lines

| Cell Line | HER2 expression | ABC |
|---|---|---|
| 786-0 | Low | 28,162 |
| Colo201 | Medium | 273,568 |
| ZR-75-1 | Medium | 281,026 |
| SkBr-3 | High | 6,820,532 |
| HCC1954 | High | 10,569,869 |

**Primary human NK cells are activated by TriNKETs in co-culture with human cancer lines expressing varying levels of HER2**

**[00238]** FIGs. 28A – 28C show that TriNKETs and trastuzumab were able to activate primary human NK cells in co-culture with HER2-positive human tumor cells, indicated by an increase in CD107a degranulation and IFNγ cytokine production. Compared to the monoclonal antibody trastuzumab, both TriNKETs (HER2-TriNKET-C26 and HER2-TriNKET-F04) showed superior activation of human NK cells with a variety of human HER2 cancer cells.

**[00239]** FIG. 28A shows that human NK cells are activated by TriNKETs when cultured with SkBr-3 cells. FIG. 28B shows that human NK cells are activated by TriNKETs when cultured with Colo201 cells. FIG. 28C shows that human NK cell are activated by TriNKETs when cultured with HCC1954 cells.

**TriNKETs enhance cytotoxicity of rested and IL-2-activated human NK cells**

**[00240]** FIGs. 32A – 32C show TriNKET enhancement of cytotoxic activity using IL-2-activated and rested human NK cells. FIG. 32A shows percent specific lysis of SkBr-3 tumor cells by rested human NK cells. FIG. 32B shows percent specific lysis of SkBr-3 tumor cells by IL-2-activated human NK cells. IL-2-activated and rested NK cell populations came from the same donor. Compared to trastuzumab, TriNKETs more potently direct responses against SkBr-3 cells by either activated or rested NK cell populations. FIG. 32C shows percent specific lysis of HER2-expressing NCI-H661 lung cancer cells by rested human NK cells.

Two TriNKETs with different NKG2D-binding domains are able to induce higher maximal lysis of NCI-H661 HER2+ cancer cells compared to the monoclonal antibody Trastuzumab.

**TriNKETs enhance NK cell cytotoxicity against targets with low surface expression**

**[00241]** Effects of TriNKETs against targets cells with low HER2 surface expression was investigated. FIGs. 29A-29B show TriNKETs provide a greater advantage against HER2-medium and low cancers compared to trastuzumab. FIG. 29A shows activated human NK cell killing of HER2-high SkBr-3 tumor cells. FIG. 29B shows human NK cell killing of HER2-low 786-O tumor cells. TriNKETs provide a greater advantage compared to trastuzumab against cancer cells with low HER2 expression..

**The advantage of TriNKETs in treating cancers with high expression of FcR, or in tumor microenvironments with high levels of FcR**

**[00242]** Monoclonal antibody therapy has been approved for the treatment of many cancer types, including both hematological and solid tumors. While the use of monoclonal antibodies in cancer treatment has improved patient outcomes, there are still limitations. Mechanistic studies have demonstrated monoclonal antibodies exert their effects on tumor growth through multiple mechanisms including ADCC, CDC, phagocytosis, and signal blockade amongst others.

**[00243]** Most notably, ADCC is thought to be a major mechanism through which monoclonal antibodies exert their effect. ADCC relies on antibody Fc engagement of the low-affinity FcγRIII (CD16) on the surface of natural killer cells, which mediate direct lysis of the tumor cell. Amongst FcγR, CD16 has the lowest affinity for IgG Fc, FcγRI (CD64) is the high-affinity FcR, and binds about 1000 times stronger to IgG Fc than CD16.

**[00244]** CD64 is normally expressed on many hematopoietic lineages such as the myeloid lineage, and can be expressed on tumors derived from these cell types, such as acute myeloid leukemia (AML). Immune cells infiltrating into the tumor, such as MDSCs and monocytes, also express CD64 and are known to infiltrate the tumor microenvironment. Expression of CD64 by the tumor or in the tumor microenvironment can have a detrimental effect on monoclonal antibody therapy. Expression of CD64 in the tumor microenvironment makes it difficult for these antibodies to engage CD16 on the surface of NK cells, as the antibodies prefer to bind the high-affinity receptor. Through targeting two activating receptors on the surface of NK cells, TriNKETs may be able to overcome the detrimental effect of CD64 expression on monoclonal antibody therapy.

**Killing of normal myeloid and normal B cells in PBMC cultures: TriNKETs provide better safety profile through less on-target off-tumor side effects**

**[00245]** Natural killer cells and CD8 T cells are both able to directly lyse tumor cells, although the mechanisms through which NK cells and CD8 T cell recognize normal self from tumor cells differ. The activity of NK cells is regulated by the balance of signals from activating (NCRs, NKG2D, CD16, *etc.*) and inhibitory (KIRs, NKG2A, *etc.*) receptors. The balance of these activating and inhibitory signals allow NK cells to determine healthy self-cells from stressed, virally infected, or transformed self-cells. This "built-in" mechanism of self-tolerance, will help protect normal heathy tissue from NK cell responses. To extend this principle, the self-tolerance of NK cells will allow TriNKETs to target antigens expressed both on self and tumor without off tumor side effects, or with an increased therapeutic window.

**[00246]** Unlike natural killer cells, T cells require recognition of a specific peptide presented by MHC molecules for activation and effector functions. T cells have been the primary target of immunotherapy, and many strategies have been developed to redirect T cell responses against the tumor. T cell bispecifics, checkpoint inhibitors, and CAR-T cells have all been approved by the FDA, but often suffer from dose-limiting toxicities. T cell bispecifics and CAR-T cells work around the TCR-MHC recognition system by using binding domains to target antigens on the surface of tumor cells, and using engineered signaling domains to transduce the activation signals into the effector cell. Although effective at eliciting an anti-tumor immune response these therapies are often coupled with cytokine release syndrome (CRS), and on-target off-tumor side effects. TriNKETs are unique in this context as they will not "override" the natural systems of NK cell activation and inhibition. Instead, TriNKETs are designed to sway the balance, and provide additional activation signals to the NK cells, while maintaining NK tolerance to healthy self.

**[00247]** PBMCs were isolated from whole blood by density gradient centrifugation. Any contaminating red blood cells were lysed by incubation in ACK lysis buffer. PBMCs were washed 3x in PBS, and total PBMCs were counted. PBMCs were adjusted to $10^6$/mL in primary cell culture media. 1mL of PBMCs were seeded into wells of a 24 well plate, the indicated TriNKETs or mAbs were added to the PBMC cultures at 10 µg/mL. Cells were cultured overnight at 37°C with 5% CO2. The following day (24 hours later) PBMCs were harvested from culture and prepared for FACS analysis. The percentage of CD45+; CD19+ B cells and CD45+; CD33+; CD11b+ myeloid cells was analyzed over the different treatment groups.

**[00248]** FIGs. 33A & 33B shows B cells from a health donor are sensitive to TriNKET mediated lysis, FIGs. 33C & 33D show that autologous myeloid cells are protected from TriNKET mediated NK cell responses, and, therefore, are resistant to TriNKET lysis. PBMCs treated with TriNKETs targeting CD20 showed reduced frequency of CD19+ B cells with the CD45+ lymphocyte population (FIG. 33A), but no effect in CD45+, CD3-, CD56- lymphocyte population (FIG. 33B). In these cultures the frequency of CD45+, CD33+, CD11b+ myeloid cells (FIG. 33C), or the frequency of CD45+, CD33+, CD11b+ myeloid cells (FIG. 33D) were unchanged.

**TriNKETs mediate hPBMC killing of SkBr-3 tumor cells in long-term co-cultures**

**Primary human PBMC cytotoxicity assay**

**[00249]** FIG. 34 shows long term killing of SkBr-3 cells in culture with human PBMCs. When cultured alone SkBr-3 cells proliferate and almost double in 60 hours. When human PBMCs are added to SkBr-3 cells in culture the rate of proliferation is slowed, and when an isotype control TriNKET targeting CD33 is added proliferation is also slowed, but to a lesser extent. When cultures are treated with Trastuzumab, SkBr-3 no longer proliferate and, after 60 hours, only 80% of the cells from time zero are left. As SkBr-3 cells are sensitive to HER2 signal blockade, the effect on SkBr-3 cell growth could be mediated by HER2 signal blockade or through Fc effector functions such as ADCC.

**Example 15 – Cytotoxic activity of rested human NK cells mediated by TriNKETs, monoclonal antibodies, or bispecific antibodies against HER2-positive cells**

**[00250]** PBMCs were isolated from human peripheral blood buffy coats using density gradient centrifugation. Isolated PBMCs were washed and prepared for NK cell isolation. NK cells were isolated using a negative selection technique with magnetic beads; the purity of the isolated NK cells was typically >90% CD3-CD56+. Isolated NK cells were cultured in media containing 100 ng/mL IL-2 or were rested overnight without cytokine. IL-2-activated or rested NK cells were used the following day in cytotoxicity assays.

*DELFIA cytotoxicity assay:*

**[00251]** Human cancer cell lines expressing a target of interest were harvested from culture, cells were washed with HBS, and were resuspended in growth media at $10^6$/mL for labeling with BATDA reagent (Perkin Elmer AD0116). Manufacturer instructions were

followed for labeling of the target cells. After labeling, cells were washed 3x with HBS, and were resuspended at 0.5-1.0x$10^5$/mL in culture media. To prepare the background wells an aliquot of the labeled cells was put aside, and the cells were spun out of the media. 100 µl of the media was carefully added to wells in triplicate to avoid disturbing the pelleted cells. 100 µl of BATDA labeled cells were added to each well of the 96-well plate. Wells were saved for spontaneous release from target cells, and wells were prepared for maximal lysis of target cells by addition of 1% Triton-X. Monoclonal antibodies or TriNKETs against the tumor target of interest were diluted in culture media and 50 µl of diluted mAb or TriNKET was added to each well. Rested and/or activated NK cells were harvested from culture, the cells were washed and were resuspended at $10^5$-2.0x$10^6$/mL in culture media depending on the desired E:T ratio. 50 µl of NK cells were added to each well of the plate to make a total 200 µl culture volume. The plate was incubated at 37°C with 5% CO2 for 2-3 hours before developing the assay.

**[00252]** After culturing for 2-3 hours, the plate was removed from the incubator and the cells were pelleted by centrifugation at 200g for 5 minutes. 20 µl of culture supernatant was transferred to a clean microplate provided from the manufacturer and 200 µl of room temperature europium solution was added to each well. The plate was protected from the light and incubated on a plate shaker at 250rpm for 15 minutes. The plate was read using either Victor 3 or SpectraMax i3X instruments. % Specific lysis was calculated as follows: % Specific lysis = ((Experimental release – Spontaneous release) / (Maximum release – Spontaneous release)) * 100%.

**Combination of monoclonal antibody and bispecifc NK cell engager does not recapitulate TriNKET activity**

**[00253]** FIG. 35 shows the cytotoxic activity of rested human NK cells mediated by TriNKETs, monoclonal antibodies, or bispecific antibodies against the HER2-positive Colo-201 cell line. A TriNKET (ADI-29404 (F04)) targeting HER2-induced maximum lysis of Colo-201 cells by rested human NK cells. The D265A mutation was introduced into the CH2 domain of the TriNKET to abrogate FcR binding. The HER2-TriNKET (ADI-29404 (F04))-D265A failed to mediate lysis of Colo-201 cells, demonstrating the importance of dual targeting of CD16 and NKG2D on NK cells. To further demonstrate the importance of dual targeting on NK cells, the monoclonal antibody Trastuzumab was used to target HER2 and mediate ADCC by NK cells, Trastuzumab alone was able to increase NK cell lysis of Colo-201 cells, but maximum lysis achieved by Trastuzumab alone was about 4x lower compared

to the TriNKET. To understand the importance of having CD16 and NKG2D targeting on the same molecule, TriNKET (ADI-29404 (F04)) activity was compared to the activity of a bispecific antibody targeting HER2 and NKG2D, combined with Trastuzumab. When used at equimolar concentrations the combination of bispecific and Trastuzumab was not able to mediate maximal lysis of Colo-201 cells by rested human NK cells. The failure of Trastuzumab + bispecific combination demonstrates the importance of containing the trispecific-binding of TriNKETs in one molecule.

**Example 16 – Bridging assay**

**[00254]** RMA cells transduced with human HER2 were used to test simultaneous binding to HER2 and NKG2D by HER2 targeting TriNKETs. The TriNKETs were used to stain surface HER2 at 20 μg/mL. Binding of the TriNKET was then detected using biotinylated recombinant human NKG2D-Fc. Bound NKG2D-Fc was then detected using streptavidin-APC. Cells were analyzed by flow cytometry, and TriNKET-bridging was compared to isotype stained and unstained cell populations. FIG. 38A shows that TriNKET-C26 that includes a binding domain for HER2, bridges hNKG2D-Fc to RMA-HER2 cells, and FIG. 38B shows TriNKET-F04 that includes a binding domain for HER2, bridges hNKG2D-Fc to RMA-HER2 cells.

INCORPORATION BY REFERENCE

**[00255]** The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

**[00256]** The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

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Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
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50 55 60

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Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
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50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 11
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 11
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Gly Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 12
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 12
Glu Leu Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
35 40 45

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

```
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Ser Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Asp Ile Pro Tyr
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210> 13
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 13
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20                  25                  30


Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45


Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60


Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80


Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95


Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115
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<210> 14
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 14
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Gly Ser Phe Pro Ile
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 15
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 15
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

```
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> 16
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 16
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

```
Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Lys Glu Val Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 17
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 17
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20                  25                  30


Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45


Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60


Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80


Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95


Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 18
<211> 106
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 18
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Phe Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 19
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 19
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 20
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Ile Tyr Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 21
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 21
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 22
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 22
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Ser Tyr Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 23
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 23
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 24
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 24
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Gly Ser Phe Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 25
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 25
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 26
<211> 106
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 26
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Gln Ser Phe Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 27
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 27
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

```
Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115
```

<210> 28
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 28

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Ser Phe Ser Thr
                85                  90                  95
```

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 29
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 29
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 30
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 30
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Glu Ser Tyr Ser Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 31
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 31
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 32
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 32
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Ser Phe Ile Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 33
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 33
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 34
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 34

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Gln Ser Tyr Pro Thr
                85                  90                  95


Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 35
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 35
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20                  25                  30


Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45


Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60
```

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 36
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 36
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr His Ser Phe Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 37
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 37
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 38
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 38
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Glu Leu Tyr Ser Tyr
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 39
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 39
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20                  25                  30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80
```

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 40
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 40
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Thr Phe Ile Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 41
<211> 125

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 41
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Asp Ser Ser Ile Arg His Ala Tyr Tyr Tyr Tyr Gly Met
100 105 110

Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 42
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 42
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
            20                  25                  30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Ser Thr Pro Ile Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
            100                 105                 110

Lys

<210> 43
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 43
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
            20                  25                  30

Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Gly Ser Asp Arg Phe His Pro Tyr Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 44
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 44
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Asp Thr Trp Pro Pro
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 45
<211> 121
<212> PRT
<213> Homo sapiens

<400> 45
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Asp Arg Gly Leu Gly Asp Gly Thr Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120

<210> 46
<211> 110
<212> PRT
<213> Homo sapiens

<400> 46
Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1               5                  10                  15

Ser Ile Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20                  25                  30

Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys Ala Pro Lys Leu Leu
        35                  40                  45

```
Ile Tyr Tyr Asp Asp Leu Leu Pro Ser Gly Val Ser Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Phe Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95

Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 47
<211> 115
<212> PRT
<213> Homo sapiens

<400> 47

```
Gln Val His Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Asp Asp Ser Ile Ser Ser Tyr
            20                  25                  30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly His Ile Ser Tyr Ser Gly Ser Ala Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Asn Trp Asp Asp Ala Phe Asn Ile Trp Gly Gln Gly Thr Met Val Thr
            100                 105                 110

Val Ser Ser
        115
```

<210> 48
<211> 108
<212> PRT
<213> Homo sapiens

<400> 48
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
            20                  25                  30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
                85                  90                  95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105

<210> 49
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 49
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

```
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110


Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> 50
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 50
Gly Phe Asn Ile Lys Asp Thr
1               5


<210> 51
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 51
Tyr Pro Thr Asn Gly Tyr
1               5


<210> 52
<211> 11
```

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 52
Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
1 5 10

<210> 53
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 53
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 54
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 54
Gln Asp Val Asn Thr Ala Val Ala
1 5

<210> 55
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 55
Ser Ala Ser Phe Leu Tyr Ser
1 5

<210> 56
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 56
Gln Gln His Tyr Thr Thr Pro Pro Thr
1 5

<210> 57
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 57
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Asp Tyr
20 25 30

```
Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr Asn Gln Arg Phe
    50                  55                  60

Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asn Leu Gly Pro Ser Phe Tyr Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala
        115                 120
```

<210> 58
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 58

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Ile Gly
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ile Tyr Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 59
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 59
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Asp Pro Lys Phe
50 55 60

Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Gln Val Ser Arg Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Ala Ser Val Thr Val Ser Ser Ala
115 120

<210> 60
<211> 108

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 60
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 61
<211> 1255
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 61
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
1 5 10 15

Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
20 25 30

```
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
            100                 105                 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
        115                 120                 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145                 150                 155                 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
                165                 170                 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255
```

```
His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
        275                 280                 285

Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
    290                 295                 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305                 310                 315                 320

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
                325                 330                 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460
```

```
Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
                485                 490                 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
        515                 520                 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
    530                 535                 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545                 550                 555                 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
                565                 570                 575

Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
            580                 585                 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
        595                 600                 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
    610                 615                 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625                 630                 635                 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
                645                 650                 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
            660                 665                 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
        675                 680                 685
```

```
Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
    690                 695                 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705                 710                 715                 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
                725                 730                 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
            740                 745                 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785                 790                 795                 800

Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
                805                 810                 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
            820                 825                 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
        835                 840                 845

Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
    850                 855                 860

Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865                 870                 875                 880

Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
                885                 890                 895
```

```
Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
            900                 905                 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
        915                 920                 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
    930                 935                 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945                 950                 955                 960

Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
                965                 970                 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
            980                 985                 990

Asp Leu Gly Pro Ala Ser Pro Leu  Asp Ser Thr Phe Tyr  Arg Ser Leu
        995                 1000                1005

Leu Glu  Asp Asp Asp Met Gly  Asp Leu Val Asp Ala  Glu Glu Tyr
    1010                1015                1020

Leu Val  Pro Gln Gln Gly Phe  Phe Cys Pro Asp Pro  Ala Pro Gly
    1025                1030                1035

Ala Gly  Gly Met Val His His  Arg His Arg Ser Ser  Ser Thr Arg
    1040                1045                1050

Ser Gly  Gly Gly Asp Leu Thr  Leu Gly Leu Glu Pro  Ser Glu Glu
    1055                1060                1065

Glu Ala  Pro Arg Ser Pro Leu  Ala Pro Ser Glu Gly  Ala Gly Ser
    1070                1075                1080

Asp Val  Phe Asp Gly Asp Leu  Gly Met Gly Ala Ala  Lys Gly Leu
    1085                1090                1095

Gln Ser  Leu Pro Thr His Asp  Pro Ser Pro Leu Gln  Arg Tyr Ser
    1100                1105                1110
```

Glu Asp  Pro Thr Val Pro Leu  Pro Ser Glu Thr Asp  Gly Tyr Val
    1115                1120                1125

Ala Pro  Leu Thr Cys Ser Pro  Gln Pro Glu Tyr Val  Asn Gln Pro
    1130                1135                1140

Asp Val  Arg Pro Gln Pro Pro  Ser Pro Arg Glu Gly  Pro Leu Pro
    1145                1150                1155

Ala Ala  Arg Pro Ala Gly Ala  Thr Leu Glu Arg Pro  Lys Thr Leu
    1160                1165                1170

Ser Pro  Gly Lys Asn Gly Val  Val Lys Asp Val Phe  Ala Phe Gly
    1175                1180                1185

Gly Ala  Val Glu Asn Pro Glu  Tyr Leu Thr Pro Gln  Gly Gly Ala
    1190                1195                1200

Ala Pro  Gln Pro His Pro Pro  Pro Ala Phe Ser Pro  Ala Phe Asp
    1205                1210                1215

Asn Leu  Tyr Tyr Trp Asp Gln  Asp Pro Pro Glu Arg  Gly Ala Pro
    1220                1225                1230

Pro Ser  Thr Phe Lys Gly Thr  Pro Thr Ala Glu Asn  Pro Glu Tyr
    1235                1240                1245

Leu Gly  Leu Asp Val Pro Val
    1250                1255

<210> 62
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 62
Gly Ser Phe Ser Gly Tyr Tyr Trp Ser
1               5

<210> 63
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 63
Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 64
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 64
Ala Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro
1 5 10

<210> 65
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 65
Gly Thr Phe Ser Ser Tyr Ala Ile Ser
1 5

<210> 66
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 66

Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 67
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 67
Ala Arg Gly Asp Ser Ser Ile Arg His Ala Tyr Tyr Tyr Tyr Gly Met
1 5 10 15

Asp Val

<210> 68
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 68
Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Asn Lys Asn Tyr Leu
1 5 10 15

Ala

<210> 69
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 69

Trp Ala Ser Thr Arg Glu Ser
1 5

<210> 70
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 70
Gln Gln Tyr Tyr Ser Thr Pro Ile Thr
1 5

<210> 71
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 71
Gly Ser Ile Ser Ser Ser Ser Tyr Tyr Trp Gly
1 5 10

<210> 72
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 72
Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 73
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 73
Ala Arg Gly Ser Asp Arg Phe His Pro Tyr Phe Asp Tyr
1               5                   10


<210> 74
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 74
Arg Ala Ser Gln Ser Val Ser Arg Tyr Leu Ala
1               5                   10


<210> 75
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 75
Asp Ala Ser Asn Arg Ala Thr
1               5


<210> 76
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<400> 76
Gln Gln Phe Asp Thr Trp Pro Pro Thr
1               5


<210> 77
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
peptide

<400> 77
Gly Phe Thr Phe Thr Asp Tyr
1 5

<210> 78
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 78
Asn Pro Asn Ser Gly Gly
1 5

<210> 79
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 79
Asn Leu Gly Pro Ser Phe Tyr Phe Asp Tyr
1 5 10

<210> 80
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 80
Gln Asp Val Ser Ile Gly Val Ala
1 5

<210> 81
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 81
Ser Ala Ser Tyr Arg Tyr Thr
1 5

<210> 82
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 82
Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr
1 5

<210> 83
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 83
Gly Phe Asn Ile Lys Asp Thr
1 5

<210> 84
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 84
Tyr Pro Thr Asn Gly Tyr
1 5

<210> 85
<211> 11

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 85
Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
1 5 10

<210> 86
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 86
Gln Asp Val Asn Thr Ala Val Ala
1 5

<210> 87
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 87
Ser Ala Ser Phe Arg Tyr Thr
1 5

<210> 88
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 88
Gln Gln His Tyr Thr Thr Pro Pro Thr
1 5

<210> 89
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 89
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Ala Arg Gly Pro Trp Ser Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 90
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 90
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Lys Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Asp Asp Phe Ala Thr Tyr Tyr Cys Glu Gln Tyr Asp Ser Tyr Pro Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 91
<211> 126
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 91

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Gly Arg Lys Ala Ser Gly Ser Phe Tyr Tyr Tyr Tyr Gly
100 105 110

Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 92
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 92
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Glu Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Pro Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 93
<211> 246
<212> PRT
<213> Homo sapiens

<400> 93
Met Ala Ala Ala Ala Ile Pro Ala Leu Leu Leu Cys Leu Pro Leu Leu
1               5                  10                  15

Phe Leu Leu Phe Gly Trp Ser Arg Ala Arg Arg Asp Asp Pro His Ser
            20                  25                  30

Leu Cys Tyr Asp Ile Thr Val Ile Pro Lys Phe Arg Pro Gly Pro Arg
        35                  40                  45

Trp Cys Ala Val Gln Gly Gln Val Asp Glu Lys Thr Phe Leu His Tyr
    50                  55                  60

Asp Cys Gly Asn Lys Thr Val Thr Pro Val Ser Pro Leu Gly Lys Lys
65                  70                  75                  80

Leu Asn Val Thr Met Ala Trp Lys Ala Gln Asn Pro Val Leu Arg Glu
                85                  90                  95

Val Val Asp Ile Leu Thr Glu Gln Leu Leu Asp Ile Gln Leu Glu Asn
            100                 105                 110

Tyr Thr Pro Lys Glu Pro Leu Thr Leu Gln Ala Arg Met Ser Cys Glu
        115                 120                 125

Gln Lys Ala Glu Gly His Ser Ser Gly Ser Trp Gln Phe Ser Ile Asp
    130                 135                 140

Gly Gln Thr Phe Leu Leu Phe Asp Ser Glu Lys Arg Met Trp Thr Thr
145                 150                 155                 160

Val His Pro Gly Ala Arg Lys Met Lys Glu Lys Trp Glu Asn Asp Lys
                165                 170                 175

Asp Val Ala Met Ser Phe His Tyr Ile Ser Met Gly Asp Cys Ile Gly
            180                 185                 190

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Trp Leu Glu Asp Phe Leu Met Gly Met Asp Ser Thr Leu Glu Pro Ser
        195                 200                 205


Ala Gly Ala Pro Leu Ala Met Ser Ser Gly Thr Thr Gln Leu Arg Ala
    210                 215                 220


Thr Ala Thr Thr Leu Ile Leu Cys Cys Leu Leu Ile Ile Leu Pro Cys
225                 230                 235                 240


Phe Ile Leu Pro Gly Ile
                245


<210> 94
<211> 126
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 94
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80


Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Gly Ala Pro Asn Tyr Gly Asp Thr Thr His Asp Tyr Tyr Tyr
            100                 105                 110
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Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 95
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 95
Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asp Asp Trp Pro Phe
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 96
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 96

Tyr Thr Phe Thr Ser Tyr Tyr Met His
1 5

<210> 97
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 97
Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 98
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 98
Ala Arg Gly Ala Pro Asn Tyr Gly Asp Thr Thr His Asp Tyr Tyr Tyr
1 5 10 15

Met Asp Val

<210> 99
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 99
Arg Ala Ser Gln Ser Val Ser Ser Asn Leu Ala
1 5 10

<210> 100
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 100
Gly Ala Ser Thr Arg Ala Thr
1 5

<210> 101
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 101
Gln Gln Tyr Asp Asp Trp Pro Phe Thr
1 5

<210> 102
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 102
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Ala Pro Met Gly Ala Ala Ala Gly Trp Phe Asp Pro Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 103
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 103
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Val Ser Phe Pro Arg
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 104
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 104
Phe Thr Phe Ser Ser Tyr Ser Met Asn
1 5

<210> 105
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 105
Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 106
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 106
Ala Arg Gly Ala Pro Met Gly Ala Ala Ala Gly Trp Phe Asp Pro
1 5 10 15

<210> 107
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 107
Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
1 5 10

<210> 108
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 108
Ala Ala Ser Ser Leu Gln Ser
1 5

<210> 109
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 109
Gln Gln Gly Val Ser Phe Pro Arg Thr
1 5

## CLAIMS

WHAT IS CLAIMED IS:

1. A protein comprising:

(a) a first antigen-binding site that binds NKG2D;

(b) a second antigen-binding site that binds HER2; and

(c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

2. The protein of claim 1, wherein the first antigen-binding site binds to NKG2D in humans, non-human primates, and rodents.

3. The protein of claim 1 or 2, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

4. A protein according to claim 3, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.

5. The protein according to any one of claims 3-4, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

6. The protein according to claim 5, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

7. The protein according to claim 5 or 6, wherein the light chain variable domain of the first antigen-binding site has an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.

8. The protein according to any one of the preceding claims, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:1.

9. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:41 and a light chain variable domain at least 90% identical to SEQ ID NO:42.

10. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:43 and a light chain variable domain at least 90% identical to SEQ ID NO:44.

11. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:45 and a light chain variable domain at least 90% identical to SEQ ID NO:46.

12. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:47 and a light chain variable domain at least 90% identical to SEQ ID NO:48.

13. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:94 and a light chain variable domain at least 90% identical to SEQ ID NO:95.

14. The protein according to any of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:102 and a light chain variable domain at least 90% identical to SEQ ID NO:103.

15. The protein of claim 1 or 2, wherein the first antigen-binding site is a single-domain antibody.

16. The protein of claim 15, wherein the single-domain antibody is a $V_HH$ fragment or a $V_{NAR}$ fragment.

17. The protein according to any one of claims 1-2 or 15-16, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

18. The protein according to claim 17, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

19. A protein according to any of the preceding claims, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:49 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:53.

20. The protein according to any of the preceding claims, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:50;

a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:51; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:52.

21. The protein according to claim 20, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:54;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:55; and

a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:56.

22. A protein according to any one of claims 1-18, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:57 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:58.

23. The protein according to any one of claims 1-18 or 22, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:77;

a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:78; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:79.

24. The protein according to claim 23, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:80;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:81; and

a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:82.

25. A protein according to any one of claims 1-18, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:59 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:60.

26. The protein according to any one of claims 1-18 or 25, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:83;

a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:84; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:85.

27. The protein according to any one of claim 26, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:86;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:87; and

a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:88.

28. The protein according to any one of claims 1-4 or 8-16, wherein the second antigen-binding site is a single-domain antibody.

29. The protein of claim 28, wherein the second antigen-binding site is a $V_HH$ fragment or a $V_{NAR}$ fragment.

30. A protein according to any one of the preceding claims, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains.

31. The protein according to claim 30, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.

32. The protein according to claim 30 or 31, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

33. The protein according to any one of claims 30-32, wherein the Fc domain comprises amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, T350, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, K439.

34. A formulation comprising a protein according to any one of the preceding claims and a pharmaceutically acceptable carrier.

35. A cell comprising one or more nucleic acids expressing a protein according to any one of claims 1-33.

36. A method of directly and/or indirectly enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to a protein according to any one of claims 1-33.

37. A method of treating cancer, wherein the method comprises administering a protein according to any one of claims 1-33 or a formulation according to claim 34 to a patient.

38. The method of claim 37, wherein the cancer is selected from the group consisting of breast, ovarian, esophageal, bladder and gastric cancer, salivary duct carcinoma,

adenocarcinoma of the lung and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.