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(54) Title: BISPECIFIC BIVALENT SCFV-FC MOLECULES

(57) Abstract: Described herein is a bispecific molecule containing an Fc polypeptide chain and immunoglobulin variable regions. Also provided are pharmaceutical formulations comprising such molecules, nucleic acids encoding such molecules, host cells containing such nucleic acids, methods of making such molecules, and methods of using such molecules.

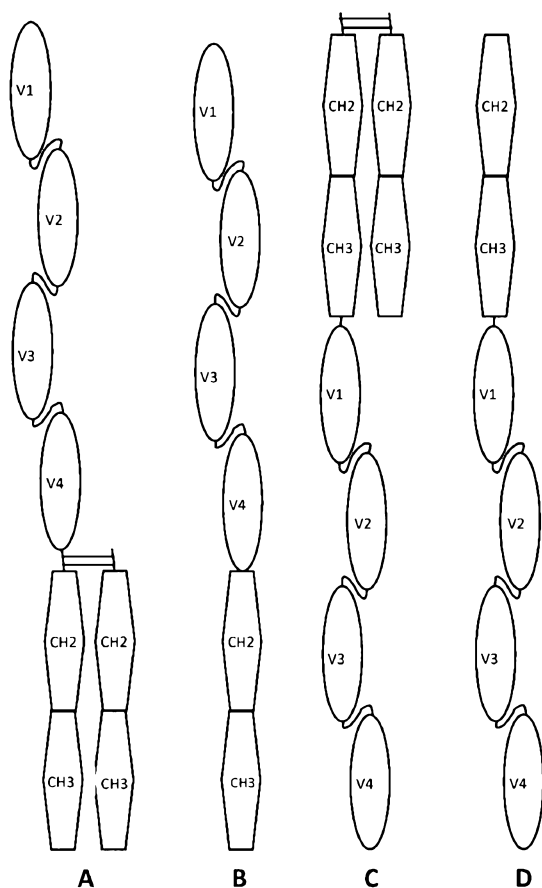


Figure 1



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## BISPECIFIC-Fc MOLECULES

### Cross Reference to Related Applications

This application claims the benefit of US Provisional Application 61/791,424,  
5 filed March 15, 2013, the content of which is incorporated herein by reference in its entirety.

### Field

This invention is in the field of protein engineering.

### Background

Bispecific antibodies have promise as therapeutics in a variety of indications. Bispecific antibodies having a standard IgG format can be challenging to produce because they include four different polypeptide chains. The efficacy of a smaller,  
15 more easily-produced bispecific molecule has been clinically demonstrated in non-Hodgkin's lymphoma. *See, e.g.,* Bargou et al. (2008), Science 321(5891): 974-977. Prolonged administration by continuous intravenous infusion was used to achieve these results because of the short *in vivo* half life of this small, single chain molecule. *Id.* Hence, there is a need in the art for bispecific therapeutics that retain similar  
20 therapeutic efficacy, that have a format that is straightforward to produce, and that have favorable pharmacokinetic properties, including a longer half-life.

### Summary

A Bispecific-Fc (Bi-Fc) as described herein can bind to two different proteins  
25 and contains an Fc region of an antibody or a portion thereof. A Bi-Fc can have favorable pharmacokinetic properties relative to a bispecific single chain molecule lacking an Fc region. One protein bound by a Bi-Fc can be expressed on an immune effector cell such as a T cell, an NK cell, a neutrophil, or a macrophage, and the other protein can be expressed on a target cell, for example, a cancer cell, a cell infected by  
30 a pathogen, or a cell mediating a disease, such as a fibroblast causing fibrosis. The Bi-Fc molecules described herein can elicit activation of an immune effector cell in

the presence of a target cell and/or killing of a target cell in the presence of an immune effector cell.

In one aspect, provided herein is a Bi-Fc, which can comprise: (a) (i) a first polypeptide chain having the formula V1-L1-V2-L2-V3-L3-V4-L4-Fc, wherein Fc is an Fc polypeptide chain, wherein V1, V2, V3, and V4 are each immunoglobulin variable regions that have different amino acid sequences, wherein L1, L2, L3, and L4 are linkers, and wherein L2 and/or L4 can be present or absent, and (ii) a second polypeptide chain that comprises an Fc polypeptide chain; or (b) (i) a first polypeptide chain having the formula Fc-L4-V1-L1-V2-L2-V3-L3-V4, wherein Fc is an Fc polypeptide chain, wherein V1, V2, V3, and V4 are each immunoglobulin variable regions that have different amino acid sequences, wherein L1, L2, L3, and L4 are linkers, and wherein L2 and/or L4 can be present or absent, and (ii) a second polypeptide chain that comprises an Fc polypeptide chain; wherein the Bi-Fc mediates cytolysis of a target cell displaying a target cell protein by an immune effector cell, and does not mediate cytolysis of a cell not displaying the target cell protein by the immune effector cell and/or wherein the Bi-Fc can bind to a target cell and to an immune effector cell. The Fc polypeptide chains in the first and second polypeptide chains can be human IgG Fc polypeptide chains. V1 can be a heavy chain variable (VH) region, and V2 can be a light chain variable (VL) region. In an alternate embodiment, V1 can be a VL region and V2 can be a VH region. V3 and V4 can be a VH and a VL region, respectively, or V3 and V4 can be a VL and a VH region, respectively. L1 and L3 can be at least 15 amino acids long, and L2, when present, can be less than 12 amino acids long. V1 and V2 can bind to a target cell or an immune effector cell when they are part of an IgG and/or an scFv antibody, and V3 and V4 can bind to a target cell or an immune effector cell when they are part of an IgG and/or an scFv antibody. The Fc polypeptide chain in the first polypeptide chain can comprise a heterodimerizing alteration, and the Fc polypeptide chain in the second polypeptide chain can comprise another heterodimerizing alteration. The heterodimerizing alteration in the first polypeptide chain can be a charge pair substitution, and the heterodimerizing alteration in the second polypeptide chain can be a charge pair substitution. The first polypeptide chain can comprise the charge pair substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D or K392E, and the second polypeptide chain can comprise the charge pair

substitutions D399K or D399R and E356K, E356R, D356K, or D356R; or the second polypeptide chain can comprise the charge pair substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D or K392E, and the first polypeptide chain can comprise the charge pair substitutions D399K or D399R and E356K, E356R, D356K, or D356R. The Fc polypeptide chains of the first and second polypeptide chains can be human IgG Fc polypeptide chains, such as IgG1, IgG2, IgG3, or IgG4 Fc polypeptide chains. The Fc polypeptide chains of the first and second polypeptide chains can comprise one or more alterations that inhibit(s) Fc gamma receptor (FcγR) binding or enhance(s) ADCC. The Fc polypeptide chains of the first and second polypeptide chains comprise, for example, L234A, L235A, and any substitution at N297.

In a further aspect, described herein is a Bi-Fc, which is monomeric and comprises: (a) a polypeptide chain comprising an amino acid sequence having the following formula: V1-L1-V2-L2-V3-L3-V4-L4-Fc; wherein Fc is a human IgG Fc polypeptide chain which is C-terminal relative to the four immunoglobulin variable regions; wherein two of V1, V2, V3, and V4 are immunoglobulin heavy chain variable (VH) regions and the other two are immunoglobulin light chain variable (VL) regions; wherein either V1 is a VH region and V2 is a VL region or vice versa and either V3 is a VH region and V4 is a VL region or vice versa; wherein L1, L2, L3, and L4 are linkers; and wherein L2 is present and wherein L2 is not more than 12 amino acids long; wherein L1 and L3 are each at least 15 amino acids long; and wherein L4 can be present or absent; or (b) a polypeptide chain comprising an amino acid sequence having the following formula: Fc-L4-V1-L1-V2-L2-V3-L3-V4; wherein Fc is a human IgG Fc polypeptide chain which is N-terminal relative to the four immunoglobulin variable regions; wherein either V1 is a VH region and V2 is a VL region or vice versa and either V3 is a VH region and V4 is a VL region or vice versa; wherein two of V1, V2, V3, and V4 are VH regions and the other two are VL regions; wherein L1, L2, L3, and L4 are linkers; and wherein L2 is present and wherein L2 is not more than 12 amino acids long; wherein L1 and L3 are each at least 15 amino acids long; and wherein L4 can be present or absent; wherein the Bi-Fc binds to a target cell and an immune effector cell

and/or mediates cytolysis of a target cell by an immune effector cell, wherein the Bi-Fc is a monomer, wherein the polypeptide chain targeting the effector cell binds to human and/or cynomolgus CD3ε; and wherein Fc polypeptide chain of (a) or (b) comprises one or more the following alterations: K392D, K392E, N392D, N392E, R409D, R409E, K409D, K409E, D399K, D399R, E356R, E356K, D356R, D356K, Y349T, L351T, L368T, L398T, F405T, Y407T, and Y407R.

In another aspect, described herein is a Bi-Fc, which is heteromeric, comprising: (a) (i) a first polypeptide chain comprising an amino acid sequence having the following formula: V1-L1-V2-L2-V3-L3-V4-L4-Fc; wherein Fc is a human IgG Fc polypeptide chain which is C-terminal relative to the four immunoglobulin variable regions; wherein V1, V2, V3, and V4 are each immunoglobulin variable regions; wherein L1, L2, L3, and L4 are linkers; and wherein L4 can be present or absent; and (ii) a second polypeptide chain that comprises a human IgG Fc polypeptide chain; or (b) (i) a first polypeptide chain having the following formula: Fc-L4-V1-L1-V2-L2-V3-L3-V4; wherein Fc is a human IgG Fc polypeptide chain which is N-terminal relative to the four immunoglobulin variable regions; wherein V1, V2, V3, and V4 are each immunoglobulin variable regions; wherein L1, L2, L3, and L4 are linkers; and wherein L4 can be present or absent; and (ii) a second polypeptide chain that comprises a human IgG Fc polypeptide chain; wherein the Bi-Fc binds to a target cell and an immune effector cell and/or mediates cytolysis of a target cell by an immune effector cell, wherein L1 and L3 are at least 15 amino acids long, wherein L2 is less than 12 amino acids long, wherein either V1 is a VH region and V2 is a VL region or vice versa, wherein either V3 is a VH region and V4 is a VL region or vice versa, wherein the Bi-Fc binds to human and/or cynomolgus CD3ε, wherein the first polypeptide chain comprises the charge pair substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D, or K392E, and the second polypeptide chain comprises the charge pair substitutions D399K or D399R and E356K, E356E, D356K, or D356R; or the second polypeptide chain comprises the charge pair substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D, or K392E, and the first

polypeptide chain comprises the charge pair substitutions D399K or D399R and E356K, E356E, D356K, or D356R.

In a further aspect, described herein is a Bi-Fc, which can comprise: (i) a first polypeptide chain having following formula: V1-L1-V2-L2-V3-L3-V4-L4-Fc, wherein Fc is an Fc polypeptide chain, wherein V1, V2, V3, and V4 are each immunoglobulin variable regions that have different amino acid sequences, wherein L1, L2, L3, and L4 are linkers, and wherein L2 and/or L4 can be present or absent; and (ii) a second polypeptide chain comprising an Fc polypeptide chain; wherein L1 and L3 are at least 15 amino acids long and L2 is less than 12 amino acids long; wherein either V1 is a VH region and V2 is a VL region or V1 is a VL region and V2 is a VH region; wherein either V3 is a VH region and V4 is a VL region or V3 is a VL region and V4 is a VH region; wherein the Fc polypeptide chains of each of the first and second polypeptide chains each contain a heterodimerizing alteration; and wherein the Bi-Fc mediates cytolysis of a target cell displaying a target cell protein by an immune effector cell, and does not mediate cytolysis of a cell not displaying the target cell protein by the immune effector cell, and/or the Bi-Fc can bind to a target cell and to an immune effector cell. The Fc polypeptide chains can be human IgG Fc polypeptide chains, such as IgG1, IgG2, IgG3, or IgG4 Fc polypeptide chains. The Fc polypeptide chains of the first and second polypeptide chains can comprise one or more alteration that inhibits FcγR binding, such as one or more of L234A, L235A, and any substitution at N297.

In a further aspect, a Bi-Fc can comprise: (a) a first polypeptide chain having the formula V1-L1-V2-L2-V3-L3-V4-L4-Fc, wherein Fc is an Fc polypeptide chain, wherein V1, V2, V3, and V4 are each immunoglobulin variable regions that have different amino acid sequences, wherein L1, L2, L3, and L4 are linkers, and wherein L2



and/or L4 can be present or absent; or (b) a first polypeptide chain having the following formula: Fc-L4-V1-L1-V2-L2-V3-L3-V4, wherein Fc is an Fc polypeptide chain, wherein V1, V2, V3, and V4 are each immunoglobulin variable regions that have different amino acid sequences, wherein L1, L2, L3, and L4 are linkers, and  
5 wherein L2 and/or L4 can be present or absent; wherein the Bi-Fc is a monomer; and wherein the Bi-Fc mediates cytolysis of a target cell displaying a target cell protein by an immune effector cell, and does not mediate cytolysis of a cell not displaying the target cell protein by the immune effector cell, and/or the Bi-Fc can bind to a target cell and to an immune effector cell. The Fc polypeptide chain can be a human  
10 IgG Fc polypeptide chain, such as IgG1, IgG2, IgG3, or IgG4 Fc polypeptide chain. The Fc polypeptide chain of (a) or (b) can comprise one or more the following alterations: K392D, K392E, N392D, N392E, R409E, R409E, K409D, K409E, Y349T, L351T, L368T, L398T, F405T, Y407T, Y407R, D399K, D399R, D356K, and/or D356R. The Fc polypeptide chain of (a) or (b) can comprise one or more alteration that inhibits  
15 FcγR binding, such as one or more of L234A, L235A, and any substitution at N297.

The immune effector cell of any Bi-Fc described herein can be a human T cell and/or a cynomolgus monkey T cell. The effector cell protein of any Bi-Fc described herein can be part of the human and/or cynomolgus monkey TCR-CD3 complex. The effector cell protein of any Bi-Fc described herein can be the human and/or  
20 cynomolgus monkey TCRα, TCRβ, TCRγ, TCRδ, CD3β chain, CD3γ chain, CD3δ chain, CD3ε chain, or CD3ζ chain. In some embodiments, the effector cell protein is CD3ε. In such embodiments, one VH region of the Bi-Fc can have a CDR1 having the amino acid sequence of SEQ ID NO:48, a CDR2 having the amino acid sequence of SEQ ID NO:49, and a CDR3 having the amino acid sequence of SEQ ID NO:50, and on VL  
25 region of the Bi-Fc can have a CDR1 having the amino acid sequence of SEQ ID NO:51, a CDR2 having the amino acid sequence of SEQ ID NO:52, and a CDR3 having the amino acid sequence of SEQ ID NO:53. In another such embodiment, one VH region of the Bi-Fc can have a CDR1 having the amino acid sequence of SEQ ID NO:54, a CDR2 having the amino acid sequence of SEQ ID NO:55, and a CDR3 having  
30 the amino acid sequence of SEQ ID NO:56, and on VL region of the Bi-Fc can have a CDR1 having the amino acid sequence of SEQ ID NO:57, a CDR2 having the amino acid sequence of SEQ ID NO:58, and a CDR3 having the amino acid sequence of SEQ ID NO:59.

If the effector cell protein is the CD3 $\epsilon$  chain, the Bi-Fc can comprise a VH region and a VL comprising the amino acid sequences of SEQ ID NOs:7 and 8, respectively, or comprising the amino acid sequences of SEQ ID NOs:29 and 31, respectively. Alternatively such a Bi-Fc can comprise a VH region comprising an amino acid sequence at least 95% identical to SEQ ID NO:7 or SEQ ID NO:29 and a VL region comprising an amino acid sequence at least 95% identical to SEQ ID NO:8 or SEQ ID NO:31, wherein the identity region is at least 50, 60, 70, 80, 90, or 100 amino acids long.

The target cell of any Bi-Fc can be a cancer cell, a cell infected by a pathogen, or a cell that mediates disease. If the target cell is a cancer cell, the cancer can be a hematologic malignancy or a solid tumor malignancy. If the target cell is a cancer cell, the Bi-Fc can bind to a cancer cell antigen such as epidermal growth factor receptor (EGFR), EGFRvIII (a mutant form of EGFR), melanoma-associated chondroitin sulfate proteoglycan (MCSP), mesothelin (MSLN), folate receptor 1 (FOLR1), CD133, CDH19, and human epidermal growth factor 2 (HER2), among many others. If the target cell is a cell infected by a pathogen, the pathogen can be virus, including human immunodeficiency virus, hepatitis virus, human papilloma virus, or cytomegalovirus, or a bacterium of the genus *Listeria*, *Mycobacterium*, *Staphylococcus*, or *Streptococcus*. If the target cell is a cell that mediates a disease, the target cell can be a cell that mediates a fibrotic disease or an autoimmune or inflammatory disease.

Provided herein are pharmaceutical formulations comprising any of the Bi-Fc molecules described herein and a physiologically acceptable excipient.

Further provided herein are nucleic acids encoding any of the Bi-Fc described herein and vectors containing such nucleic acids, as well as host cell containing such nucleic acids and/or vectors. In another aspect, described herein is a method for making a Bi-Fc comprising culturing the host cell containing the nucleic acids or vector under conditions such that the nucleic acids are expressed, and recovering the Bi-Fc from the cell mass or the culture medium.

In another aspect, provided herein is a method for treating a cancer patient comprising administering to the patient a therapeutically effective dose of any of the Bi-Fc molecules described herein, wherein the target cell of the Bi-Fc is a cancer cell. This method can further comprise administering radiation, a chemotherapeutic

agent, or a non-chemotherapeutic, anti-neoplastic agent before, after, or concurrently with the administration of the Bi-Fc. The patient can have a hematologic malignancy or a solid tumor malignancy.

In a further embodiment, described herein is a method for treating a patient having a fibrotic disease comprising administering to the patient a therapeutically effective dose of any of the Bi-Fc molecules described herein, wherein the target cell of the Bi-Fc is a fibrotic cell. The Bi-Fc can be administered concurrently with, before, or after the administration of other therapeutics used to treat the disease. The fibrotic disease can be atherosclerosis, chronic obstructive pulmonary disease (COPD), cirrhosis, scleroderma, kidney transplant fibrosis, kidney allograft nephropathy, or a pulmonary fibrosis, including idiopathic pulmonary fibrosis.

In still another aspect, described herein is a method for treating a patient having a disease mediated by a pathogen comprising administering to the patient a therapeutically effective dose of any of the Bi-Fc molecules described herein. The pathogen can be a virus, a bacterium, or a protozoan. The Bi-Fc can be administered concurrently with, before, or after the administration of other therapeutics used to treat the pathogen-mediated disease.

Also provided herein are pharmaceutical compositions comprising any of the Bi-Fc molecules described herein plus a physiologically acceptable excipient. Such compositions can be for the treatment of a cancer, an infectious disease, an autoimmune or inflammatory disease, or a fibrotic disease.

### Brief Description of the Figures

**Figure 1:** Diagrams of exemplary heterodimeric and monomeric Bi-Fc molecules. Four immunoglobulin variable regions are indicated by ovals and labeled V1, V2, V3, and V4. CH2 and CH3 regions are labeled as such and diagramed as elongated hexagons. Lines between these regions indicate linkers or a hinge region. Exemplary disulfide bridges are indicated by horizontal lines. Panels A and C depict heterodimeric Bi-Fc's, and panels B and D depict monomeric Be-Fc's.

**Figure 2:** Binding of a heterodimeric Bi-Fc to target cells and immune effector cells. Methods are described in Example 2. Mean fluorescence intensity (MFI) is indicated on the x axis, and the number of cells is indicated on the y axis. The unfilled profiles represent data from cells in the absence of one of the bispecific molecules, and the

solidly filled profiles represent data from cells in the presence of one of the bispecific molecules. As indicated in the figure, panels at left represent data from samples containing the heterodimeric anti-HER2/CD3 $\epsilon$  Bi-Fc, and panels at right represent data from samples containing the single chain anti-HER2/CD3 $\epsilon$ . Top two panels represent data from samples containing JIMT-1 cells (which express the target cell protein HER2), and bottom two panels represent data from samples containing T cells (which express the effector cell protein CD3 $\epsilon$ ).

**Figure 3:** Cytolytic activity of a heterodimeric anti-FOLR1/CD3 $\epsilon$  Bi-Fc and a single chain anti-FOLR1/CD3 $\epsilon$  molecule. Methods are described in Example 3. The x axis in each panel indicates the concentration of the Bi-Fc or single chain molecule (pM) in each sample. The y axis in each panel indicates the percent specific lysis calculated as described in Example 3. Open circles connected by a dashed line indicate data from samples containing the single chain molecule, and filled circles connected by a solid line indicate data from the Bi-Fc molecule. The top, middle, and bottom panels, as indicated, show data from Cal-51 cells (which express FOLR1), T47D cells (which express FOLR1), and BT474 cells (which do not express FOLR1), respectively.

**Figure 4:** Cytolytic activity of a heterodimeric anti-HER2/CD3 $\epsilon$  Bi-Fc and a single chain anti-HER2/CD3 $\epsilon$  molecule. Methods are described in Example 3. The x axis in each panel indicates the concentration of the Bi-Fc or single chain molecule (pM) in each sample. The y axis in each panel indicates the percent specific lysis calculated as described in Example 3. Open circles connected by a dashed line indicate data from samples containing the single chain molecule, and filled circles connected by a solid line indicate data from the Bi-Fc molecule. The top, middle, and bottom panels, as indicated, show data from JIMT-1 cells (which express HER2), T47D cells (which express HER2), and SHP77 cells (which do not express HER2), respectively.

**Figure 5:** Cytokine production by T cells in the presence of a heterodimeric anti-FOLR1/CD3 $\epsilon$  Bi-Fc or single chain molecule. Methods are described in Example 4. Open circles connected by dashed lines indicate data from assays containing the heterodimeric anti-FOLR1/CD3 $\epsilon$  Bi-Fc, and solidly filled circles connected by solid lines indicate data from the single chain anti-FOLR1/CD3 $\epsilon$  molecule. The x axis in each panel indicates the concentration of the Bi-Fc or single chain molecule (pM) in each assay. The y axis indicates the concentration and identity of the cytokine

detected (pg/mL). Figure 5A shows data for interferon gamma (IFN $\gamma$ , top), tumor necrosis factor alpha (TNF $\alpha$ , middle), and interleukin-10 (IL-10, bottom), and Figure 5B shows data for interleukin-2 (IL-2, top) and interleukin-13 (IL-13, bottom), as indicated. As indicated, panels on the left show data from samples containing T47D  
 5 cells (which express FOLR1), and panels on the right show data from samples containing BT474 cells (which do not express FOLR1).

**Figure 6:** Cytokine production by T cells in the presence of an anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc or single chain molecule. Methods are described in Example 4. Open circles connected by dashed lines indicate data from assays containing the  
 10 heterodimeric anti-HER2/CD3 $\epsilon$  Bi-Fc, and solidly filled circles connected by solid lines indicate data from the single chain anti-HER2/CD3 $\epsilon$  molecule. The x axis in each panel indicates the concentration of the Bi-Fc or single chain molecule (pM) in each assay. The y axis indicates the concentration and identity of the cytokine detected (pg/mL). Figure 6A data for IFN $\gamma$  (top), TNF $\alpha$  (middle), and IL-10 (bottom),  
 15 and Figure 6B shows data for IL-2 (top) and IL-13 (bottom), as indicated. As indicated, panels on the left show data from samples containing JIMT-1 cells (which express HER2), and panels on the right show data from samples containing SHP77 cells (which do not express HER2).

**Figure 7:** Percentage of CD25 $^{+}$  and CD69 $^{+}$  cells in the presence of an anti-  
 20 HER2/CD3 $\epsilon$  heterodimeric Bi-Fc or single chain molecule. Methods are described in Example 5. The x axis indicates the concentration (pM) of the anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc or single chain molecule. The y axis indicates the percent of CD3 $^{+}$  T cells that are also CD25 $^{+}$  (left panel) or CD69 $^{+}$  (right panel) cells. Symbols indicate as follows: open squares connected by dashed line, the single chain  
 25 molecule plus JIMT-1 target cells; solidly filled, downward pointing triangles connected by a solid line, the Bi-Fc molecule plus JIMT-1 target cells; open circles connected by a dashed line, the single chain molecule without JIMT-1 target cells; and solidly filled, upward pointing triangles connected by a solid line, the Bi-Fc without JIMT-1 target cells.

**Figure 8:** Pharmacokinetic properties of a heterodimeric Bi-Fc and a single chain bispecific molecule in mice. Methods are described in Example 6. In the top panel, a pharmacokinetic profile following an intravenous injection is shown, and below is shown the profile following a subcutaneous injection. Solidly filled circles connected  
 30

by a solid line indicate data from the anti-HER2/CD3 $\epsilon$  single chain molecule, and asterisks connected by a solid line indicate data from the heterodimeric anti-HER2/CD3 $\epsilon$  Bi-Fc molecule.

**Figure 9:** Binding of anti-CD33/CD3 $\epsilon$  molecules to various cell types. Experimental procedures are described in Example 8. Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc. As indicated, panels A, B, C, D, and E show data on binding to Molm-13 cells, Namalwa cells, human pan T cells, human peripheral blood mononuclear cells (PBMCs), and cynomolgus monkey PBMCs, respectively.

**Figure 10:** Lysis of Molm-13 cells, but not Namalwa cells, in the presence of PBMCs from cynomolgus monkey and a bispecific anti-CD33/CD3 $\epsilon$  molecule. Experimental procedures are described in Example 9. Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc. Cultures contained PBMCs, a bispecific anti-CD33/CD3 $\epsilon$  molecule, and either Molm-13 cells (panel A) or Namalwa cells (panel B).

**Figure 11:** Lysis of Molm-13 cells, but not Namalwa cells, in the presence of pan T cells and a bispecific anti-CD33/CD3 $\epsilon$  molecule. Experimental procedures are described in Example 9. Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc. Cultures contained pan T cells, a bispecific anti-CD33/CD3 $\epsilon$  molecule, and either Molm-13 cells (panel A) or Namalwa cells (panel B).

**Figure 12:** Lysis of CD33-expressing tumor cells in the presence of PBMCs and either the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the single chain anti-CD33/CD3 $\epsilon$ . Experimental procedures are described in Example 10. The graphs show data from cultures containing an anti-CD33/CD3 $\epsilon$  molecule and CD33-expressing Molm-13 cells, plus either human PBMCs (panel A) or cynomolgus monkey PBMCs (panel B). Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc.

**Figure 13:** Release of interferon gamma (IFN- $\gamma$ ) by PBMCs in the presence of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and CD33-expressing tumor cells. Experimental procedures are described in Example 10. The graphs show data from cultures containing an anti-CD33/CD3 $\epsilon$  molecule plus CD33-expressing Molm-13 cells plus either human PBMCs (panel A) or cynomolgus monkey PBMCs (panel B). Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc.

**Figure 14:** Proliferation and CD25 expression by T cells. Experimental procedures are described in Example 11. As indicated, graphs in the left column represent data from cell cultures containing Molm-13 cells (which express CD33) and pan T cells, and graphs in the right column represent data from cell cultures containing Namalwa cells (which do not express CD33) and pan T cells. As indicated, panel A shows the percent of proliferating T cells in the cultures, and panel B shows the percent of CD25 positive T cells in the culture. Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc.

**Figure 15:** Cytokine release by T cells in the presence of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and CD33-expressing tumor cells. Experimental procedures are described in Example 11. As indicated, graphs in the left column represent data from cell cultures containing Molm-13 cells (which express CD33) and pan T cells, and graphs in the right column represent data from cell cultures containing Namalwa cells (which do not express CD33) and pan T cells. Open circles with dotted lines represent data from cultures containing the single chain anti-CD33/CD3 $\epsilon$ , and filled circles with solid lines represent data from cultures containing the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc. The cytokine assayed is indicated at left of each panel.

**Figure 16:** *In vivo* inhibition of tumor growth by a heterodimeric anti-FOLR1/CD3 $\epsilon$  Bi-Fc. Methods are described in Example 13. The x axis show the time (days) elapsed since three million FOLR1-expressing, NCI-N87-luc tumor cells were implanted into the mice. The y axis shows tumor volume (mm<sup>3</sup>). Symbols signify the treatment used for each group of mice as follows: vehicle (25 mM lysine-

hydrochloride, 0.002% Tween 80 in 0.9% NaCl, pH 7.0), solidly filled triangle; single chain anti-FOLR1/CD3 $\epsilon$  bispecific, solidly filled circles; and heterodimeric anti-FOLR1/CD3 $\epsilon$  Bi-Fc, open circles.

**Figure 17:** *In vivo* inhibition of tumor growth by a heterodimeric anti-CD33/CD3 $\epsilon$

5 Bi-Fc and a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc. Methods are described in Example 14. The x axis shows the time (days) elapsed since one million tumor cells were implanted subcutaneously into the right flank of each mouse. The y axis shows bioluminescence, which reflects the number of tumor cells present. The vertical dotted line indicates the time at which 20 x 10<sup>6</sup> human T cells were injected into the  
10 mice. Symbols signify the treatment used for each group of mice as follows: vehicle (25 mM lysine-hydrochloride, 0.002% Tween 80 in 0.9% NaCl, pH 7.0), solidly filled triangle; single chain anti-MEC/CD3 $\epsilon$  bispecific, open triangle; single chain anti-CD33/CD3 $\epsilon$  bispecific, open squares; heterodimeric anti-CD33/CD3 $\epsilon$  Bi-Fc, open circles; monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc, solidly filled squares; and naïve animals,  
15 solidly filled circles.

**Figure 18:** *In vivo* inhibition of tumor growth by a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc. Methods are described in Example 15. The x axis shows the time (days) elapsed since one million tumor cells were implanted subcutaneously into the right flank of each mouse. The y axis shows tumor bioluminescence. The vertical dotted line

20 indicates the time at which 20 x 10<sup>6</sup> human T cells were injected into the mice. Symbols signify the treatment used for each group of mice as follows: vehicle (25 mM lysine-hydrochloride, 0.002% Tween 80 in 0.9% NaCl, pH 7.0), solidly filled triangle; a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc (N297G), solidly filled square; a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc (N297 wild type), open squares; and naïve animals,  
25 filled circles.

### Brief Description of the Sequences

SEQ ID NO	Description
SEQ ID NO:1	Amino acid sequence preceding VH CDR1
SEQ ID NO:2	Amino acid sequence preceding VH CDR2
SEQ ID NO:3	Amino acid sequence following VH CDR3
SEQ ID NO:4	Amino acid sequence following light chain CDR3
SEQ ID NO:5	Amino acid sequence of anti-HER2 VH region
SEQ ID NO:6	Amino acid sequence of anti-HER2 VL region



SEQ ID NO	Description
SEQ ID NO:7	Amino acid sequence of anti CD3 $\epsilon$ VH region
SEQ ID NO:8	Amino acid sequence of anti-CD3 $\epsilon$ VL region
SEQ ID NO:9	Amino acid sequence of a single chain anti-HER2/CD3 $\epsilon$ (P136629.3)
SEQ ID NO:10	Amino acid sequence of a first polypeptide chain of a heterodimeric anti-HER2/CD3 $\epsilon$ of a Bi-Fc
SEQ ID NO:11	Nucleic acid sequence encoding SEQ ID NO:10
SEQ ID NO:12	Amino acid sequence of a human IgG1 Fc polypeptide containing alterations D356K and D399K
SEQ ID NO:13	Nucleic acid sequence encoding SEQ ID NO:12
SEQ ID NO:14	Amino acid sequence of a single chain anti-FOLR1/CD3 $\epsilon$ molecule
SEQ ID NO:15	Amino acid sequence of a first polypeptide chain of a heterodimeric anti-FOLR1/CD3 $\epsilon$ molecule
SEQ ID NO:16	Nucleic acid sequence encoding SEQ ID NO:15
SEQ ID NO:17	Amino acid sequence of a linker
SEQ ID NO:18	Amino acid sequence of a linker
SEQ ID NO:19	Amino acid sequence of a linker
SEQ ID NO:20	Amino acid sequence of a linker
SEQ ID NO:21	Amino acid sequence of a linker
SEQ ID NO:22	Mature amino acid sequence of CD3 epsilon chain of <i>Homo sapiens</i>
SEQ ID NO:23	Mature amino acid sequence of CD3 epsilon chain of <i>Macaca fascicularis</i>
SEQ ID NO:24	A portion of an epitope that is part of CD3 epsilon
SEQ ID NO:25	Amino acid sequence of human IgG1 Fc region
SEQ ID NO:26	Amino acid sequence of human IgG2 Fc region
SEQ ID NO:27	Amino acid sequence of human IgG3 Fc region
SEQ ID NO:28	Amino acid sequence of human IgG4 Fc region
SEQ ID NO:29	Amino acid sequence of an anti-CD3 $\epsilon$ VH region
SEQ ID NO:30	Nucleic acid sequence encoding SEQ ID NO:29
SEQ ID NO:31	Amino acid sequence of an anti-CD3 $\epsilon$ VL region
SEQ ID NO:32	Nucleic acid sequence encoding SEQ ID NO:31
SEQ ID NO:33	Amino acid sequence of an anti-CD33/CD3 $\epsilon$ single chain molecule
SEQ ID NO:34	Amino acid sequence of monomeric anti-CD33/CD3 $\epsilon$ Bi-Fc
SEQ ID NO:35	Nucleic acid sequence encoding SEQ ID NO:34
SEQ ID NO:36	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:37	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:38	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:39	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:40	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:41	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:42	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:43	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:44	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:45	Amino acid sequence of an insertion that prolongs half life

SEQ ID NO	Description
SEQ ID NO:46	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:47	Amino acid sequence of an insertion that prolongs half life
SEQ ID NO:48	Amino acid sequence of a VH region CDR1 of SEQ ID NO:7
SEQ ID NO:49	Amino acid sequence of a VH region CDR2 of SEQ ID NO:7
SEQ ID NO:50	Amino acid sequence of a VH region CDR3 of SEQ ID NO:7
SEQ ID NO:51	Amino acid sequence of a VL region CDR1 of SEQ ID NO:8
SEQ ID NO:52	Amino acid sequence of a VL region CDR2 of SEQ ID NO:8
SEQ ID NO:53	Amino acid sequence of a VL region CDR3 of SEQ ID NO:8
SEQ ID NO:54	Amino acid sequence of a VH region CDR1 of SEQ ID NO:29
SEQ ID NO:55	Amino acid sequence of a VH region CDR2 of SEQ ID NO:29
SEQ ID NO:56	Amino acid sequence of a VH region CDR3 of SEQ ID NO:29
SEQ ID NO:57	Amino acid sequence of a VL region CDR1 of SEQ ID NO:31
SEQ ID NO:58	Amino acid sequence of a VL region CDR2 of SEQ ID NO:31
SEQ ID NO:59	Amino acid sequence of a VL region CDR3 of SEQ ID NO:31
SEQ ID NO:60	Amino acid sequence of a VH region CDR1 of SEQ ID NO:5
SEQ ID NO:61	Amino acid sequence of a VH region CDR2 of SEQ ID NO:5
SEQ ID NO:62	Amino acid sequence of a VH region CDR3 of SEQ ID NO:5
SEQ ID NO:63	Amino acid sequence of a VL region CDR1 of SEQ ID NO:6
SEQ ID NO:64	Amino acid sequence of a VL region CDR2 of SEQ ID NO:6
SEQ ID NO:65	Amino acid sequence of a VL region CDR3 of SEQ ID NO:6
SEQ ID NO:66	Amino acid sequence of a VH region CDR1 of SEQ ID NO:15
SEQ ID NO:67	Amino acid sequence of a VH region CDR2 of SEQ ID NO:15
SEQ ID NO:68	Amino acid sequence of a VH region CDR3 of SEQ ID NO:15
SEQ ID NO:69	Amino acid sequence of a VL region CDR1 of SEQ ID NO:15
SEQ ID NO:70	Amino acid sequence of a VL region CDR2 of SEQ ID NO:15
SEQ ID NO:71	Amino acid sequence of a VL region CDR3 of SEQ ID NO:15
SEQ ID NO:72	Amino acid sequence of a VH region CDR1 of SEQ ID NO:34
SEQ ID NO:73	Amino acid sequence of a VH region CDR2 of SEQ ID NO:34
SEQ ID NO:74	Amino acid sequence of a VH region CDR3 of SEQ ID NO:34
SEQ ID NO:75	Amino acid sequence of a VL region CDR1 of SEQ ID NO:34
SEQ ID NO:76	Amino acid sequence of a VL region CDR2 of SEQ ID NO:34
SEQ ID NO:77	Amino acid sequence of a VL region CDR3 of SEQ ID NO:34
SEQ ID NO:78	Amino acid sequence of an anti-Mec/CD3 $\epsilon$ single chain molecule
SEQ ID NO:79	Nucleic acid sequence encoding SEQ ID NO:78
SEQ ID NO:80	Amino acid sequence of the first polypeptide chain of a heterodimeric anti-CD33/CD3 $\epsilon$ Bi-Fc
SEQ ID NO:81	Nucleic acid sequence encoding SEQ ID NO:80
SEQ ID NO:82	Amino acid sequence of the second polypeptide chain of a heterodimeric anti-CD33/CD3 $\epsilon$ Bi-Fc, which comprises an Fc polypeptide chain
SEQ ID NO:83	Nucleic acid sequence encoding SEQ ID NO:82
SEQ ID NO:84	Amino acid sequence of a monomeric anti-CD33/CD3 $\epsilon$ Bi-Fc
SEQ ID NO:85	Nucleic acids sequence encoding SEQ ID NO:84
SEQ ID NO:86	Amino acid sequence of a first polypeptide chain of a heterodimeric anti-FOLR1/CD3 $\epsilon$ Bi-Fc molecule
SEQ ID NO:87	Nucleic acid sequence encoding SEQ ID NO:86

SEQ ID NO	Description
SEQ ID NO:88	Amino acid sequence of the second polypeptide chain of the heterodimeric anti-FOLR1/CD3 $\epsilon$ Bi-Fc molecule where SEQ ID NO:86 is the amino acid sequence of the first polypeptide chain
SEQ ID NO:89	Nucleic acid sequence encoding SEQ ID NO:88
SEQ ID NO:90	Amino acid sequence of a single chain anti-FOLR1/CD3 $\epsilon$ bispecific
SEQ ID NO:91	Nucleic acid sequence encoding SEQ ID NO:90

### Detailed Description

Described is a new form of bispecific antibody, called herein a Bi-Fc, which contains one polypeptide chain or two different polypeptide chains. One chain comprises two heavy chain variable (VH) regions, two light chain variable (VL) regions, and an Fc polypeptide chain, and an optional second polypeptide chain comprises an Fc polypeptide chain. In some embodiments, one of the proteins to which the Bi-Fc binds is expressed on the surface of an immune effector cell, such as a T cell, an NK cell, a macrophage, or a neutrophil, and the other protein to which the Bi-Fc binds is expressed on the surface of a target cell, for example a cancer cell, a cell infected by a pathogen, or a cell that mediates a disease, such as, for example, a fibrotic disease. Since a Bi-Fc has only one binding site for each of these proteins (*i.e.*, it binds each protein "monovalently," as meant herein), its binding, by itself, will not oligomerize the proteins it binds to on a cell surface. For example, if it binds to CD3 on the surface of a T cell, CD3 will not be oligomerized on the T cell surface in the absence of a target cell. Oligomerization of CD3 can cause a generalized activation of a T cell or downmodulation of CD3 on the T cell, which can be undesirable. The Bi-Fc tethers an immune effector cell to a target cell, thereby eliciting specific cytolytic activity against the target cell, rather than a generalized inflammatory response. Further, the Bi-Fc molecules have favorable pharmacokinetic properties and are not unduly complex to manufacture since they contain only one or only two different polypeptide chains.

### Definitions

Throughout the specification and claims, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be

understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

An “**antibody**,” as meant herein, is a protein containing at least one VH or VL region, in many cases both a heavy and a light chain variable region. Thus, the term

5 “antibody” encompasses molecules having a variety of formats, including single chain Fv antibodies (scFv, which contain VH and VL regions joined by a linker), Fab,

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**[Text continued on page 15]**

F(ab)<sub>2</sub>', Fab', scFv:Fc antibodies (as described in Carayannopoulos and Capra, Ch. 9 *in* FUNDAMENTAL IMMUNOLOGY, 3<sup>rd</sup> ed., Paul, ed., Raven Press, New York, 1993, pp. 284-286) or full length antibodies containing two full length heavy and two full length light chains, such as naturally-occurring IgG antibodies found in mammals. *Id.* Such

5 IgG antibodies can be of the IgG1, IgG2, IgG3, or IgG4 isotype and can be human or humanized antibodies. The portions of Carayannopoulos and Capra that describe the structure of antibodies are incorporated herein by reference. Further, the term "antibody" includes dimeric antibodies containing two heavy chains and no light chains such as the naturally-occurring antibodies found in camels and other

10 dromedary species and sharks. *See, e.g.*, Muldermans *et al.*, 2001, J. Biotechnol. 74:277-302; Desmyter *et al.*, 2001, J. Biol. Chem. 276:26285-90; Streltsov *et al.* (2005), Protein Science 14: 2901-2909. An antibody can be "**monospecific**" (that is, binding to only one kind of antigen), "**bispecific**" (that is, binding to two different antigens), or "**multispecific**" (that is, binding to more than one different antigen).

15 Further, an antibody can be monovalent, bivalent, or multivalent, meaning that it can bind to one, two, or multiple antigen molecules at once, respectively. An antibody binds "**monovalently**" to a particular protein when one molecule of the antibody binds to only one molecule of the protein, even though the antibody may also bind to a different protein as well. That is, an antibody binds "monovalently," as meant

20 herein, to two different proteins when it binds to only one molecule of each protein. Such an antibody is "bispecific" and binds to each of two different proteins "monovalently." An antibody can be "**monomeric**," *i.e.*, comprising a single polypeptide chain. An antibody can comprise multiple polypeptide chains ("**multimeric**") or can comprise two ("**dimeric**"), three ("**trimeric**"), or four

25 ("**tetrameric**") polypeptide chains. If multimeric, an antibody can be a homomultimer, *i.e.*, containing more than one molecule of only one kind of polypeptide chain, including homodimers, homotrimer, or homotetramers. Alternatively, a multimeric antibody can be a heteromultimer, *i.e.*, containing more than one different kind of polypeptide chain, including heterodimers, heterotrimers,

30 or heterotetramers. An antibody can have a variety of possible formats including, for example, monospecific monovalent antibodies (as described in International Application W0 2009/089004 and US Publication 2007/0105199, the relevant portions of which are incorporated herein by reference) that may inhibit or activate

the molecule to which they bind, bivalent monospecific or bispecific dimeric Fv-Fc, scFv-Fc, or diabody Fc, monospecific monovalent scFv-Fc/Fc's, the multispecific binding proteins and dual variable domain immunoglobulins described in US Publication 2009/0311253 (the relevant portions of which are incorporated herein by reference), the heterodimeric bispecific antibodies described herein, and the many formats for bispecific antibodies described in Chapters 1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 of BISPECIFIC ANTIBODIES, Kontermann, ed., Springer, 2011 (which chapters are incorporated herein by reference), among many other possible antibody formats.

A "**Bi-Fc**," as meant herein, comprises a first polypeptide chain and, optionally, a second polypeptide chain. In many embodiments, a Bi-Fc comprises both a first and a second polypeptide chain. In some embodiments, a Bi-Fc is a monomer comprising only the first polypeptide chain. The first polypeptide chain comprises two VH regions and two VL regions that can be separated by linkers and an Fc polypeptide chain. The Fc polypeptide chain can be N-terminal or C-terminal relative to the four immunoglobulin variable regions, and it can be joined to the variable regions via a linker. This linker can be present or absent. The second polypeptide chain, if present, comprises an Fc polypeptide chain. Thus, a Bi-Fc can be a monomer or a heterodimer. A Bi-Fc can bind to an immune effector cell via an effector cell protein and to a target cell via a target cell protein and can mediate cytolysis of a target cell by an immune effector cell.

Monomeric Fc polypeptides are described in detail in United States Patent Application Publication 2012/244578, the relevant portions of which are incorporated herein by reference. A monomeric Bi-Fc can comprise an altered Fc polypeptide chain that is more stable as a monomer than a naturally-occurring Fc polypeptide chain. Briefly, such monomers can comprise an altered human IgG Fc polypeptide comprising the following alterations: (1) K409D, K409E, R409D, or R409E; (2) K392D, K392E, N392D or N392E; and (3) F405T or Y349T. In alternate embodiments, positions 409 and 392 are not altered, and other alterations are present, which can include one or more of the alterations described in the definition of "heterodimerizing alterations" below, including, *e.g.*, D399K, D399R, E356K, E356R, D356K, and/or D356R. Such "heterodimerizing alterations" are described below and in US Patent 8,592,562, the relevant portions of which are incorporated herein by reference. Alterations of amino acids within an Fc polypeptide chain are denoted as

follows. The amino acid normally present at a given position is named in one letter code, followed by the position numbered according to EU numbering (as shown in Table 2 below), followed by the amino acid replacing the amino acid normally present at that position. For example, the designation "N297G" means that the asparagine normally present at position 297 has been changed to glycine. Further, the Fc polypeptide chain portion of a monomeric Bi-Fc may lack a hinge region (as defined in connection with Table 2 below) or may have deleted or altered cysteine residues in its hinge region.

A "**cancer cell antigen**," as meant herein, is a protein expressed on the surface of a cancer cell. Some cancer cell antigens are also expressed on some normal cells, and some are specific to cancer cells. Cancer cell antigens can be highly expressed on the surface of a cancer cell. There are a wide variety of cancer cell antigens. Examples of cancer cell antigens include, without limitation, the following human proteins: epidermal growth factor receptor (EGFR), EGFRvIII (a mutant form of EGFR), melanoma-associated chondroitin sulfate proteoglycan (MCSP), mesothelin (MSLN), folate receptor 1 (FOLR1), CD33, CDH19, and human epidermal growth factor 2 (HER2), among many others.

"**Chemotherapy**," as used herein, means the treatment of a cancer patient with a "**chemotherapeutic agent**" that has cytotoxic or cytostatic effects on cancer cells. A "**chemotherapeutic agent**" specifically targets cells engaged in cell division and not cells that are not engaged in cell division. Chemotherapeutic agents directly interfere with processes that are intimately tied to cell division such as, for example, DNA replication, RNA synthesis, protein synthesis, the assembly, disassembly, or function of the mitotic spindle, and/or the synthesis or stability of molecules that play a role in these processes, such as nucleotides or amino acids. A chemotherapeutic agent therefore has cytotoxic or cytostatic effects on both cancer cells and other cells that are engaged in cell division. Chemotherapeutic agents are well-known in the art and include, for example: alkylating agents (*e.g.* busulfan, temozolomide, cyclophosphamide, lomustine (CCNU), methyllomustine, streptozotocin, *cis*-diamminedi-chloroplatinum, aziridinylbenzo-quinone, and thiotepa); inorganic ions (*e.g.* cisplatin and carboplatin); nitrogen mustards (*e.g.* melphalan hydrochloride, ifosfamide, chlorambucil, and mechlorethamine HCl); nitrosoureas (*e.g.* carmustine (BCNU)); anti-neoplastic antibiotics (*e.g.* adriamycin

(doxorubicin), daunomycin, mitomycin C, daunorubicin, idarubicin, mithramycin, and bleomycin); plant derivatives (*e.g.* vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, vindesine, VP-16, and VM-26); antimetabolites (*e.g.* methotrexate with or without leucovorin, 5-fluorouracil with or without leucovorin, 5-fluorodeoxyuridine, 5 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, gemcitabine, and fludarabine); podophyllotoxins (*e.g.* etoposide, irinotecan, and topotecan); as well as actinomycin D, dacarbazine (DTIC), mAMSA, procarbazine, hexamethylmelamine, pentamethylmelamine, L-asparaginase, and mitoxantrone, among many known in the art. *See e.g.* Cancer: Principles and Practice of Oncology, 4<sup>th</sup> Edition, DeVita *et al.*, eds., J.B. Lippincott Co., Philadelphia, PA 10 (1993), the relevant portions of which are incorporated herein by reference. Alkylating agents and nitrogen mustard act by alkylating DNA, which restricts uncoiling and replication of strands. Methotrexate, cytarabine, 6-mercaptopurine, 5-fluorouracil, and gemcitabine interfere with nucleotide synthesis. Plant derivatives 15 such a paclitaxel and vinblastine are mitotic spindle poisons. The podophyllotoxins inhibit topoisomerases, thus interfering with DNA replication. Antibiotics doxorubicin, bleomycin, and mitomycin interfere with DNA synthesis by intercalating between the bases of DNA (inhibiting uncoiling), causing strand breakage, and alkylating DNA, respectively. Other mechanisms of action include carbamoylation of 20 amino acids (lomustine, carmustine), and depletion of asparagine pools (asparaginase). Merck Manual of Diagnosis and Therapy, 17<sup>th</sup> Edition, Section 11, Hematology and Oncology, 144. Principles of Cancer Therapy, Table 144-2 (1999). Specifically included among chemotherapeutic agents are those that directly affect the same cellular processes that are directly affected by the chemotherapeutic 25 agents listed above.

A drug or treatment is “**concurrently**” administered with a Bi-Fc if it is administered in the same general time frame as the Bi-Fc, optionally, on an ongoing basis. For example, if a patient is taking Drug A once a week on an ongoing basis and a Bi-Fc once every six months on an ongoing basis, Drug A and the Bi-Fc are 30 concurrently administered, whether or not they are ever administered on the same day. Similarly, if the Bi-Fc is taken once per week on an ongoing basis and Drug A is administered only once or a few times on a daily basis, Drug A and the Bi-Fc are concurrently administered as meant herein. Similarly, if both Drug A and the Bi-Fc



are administered for short periods of time either once or multiple times within a one month period, they are administered concurrently as meant herein as long as both drugs are administered within the same month.

A “**conservative amino acid substitution**,” as meant herein, is a substitution of an amino acid with another amino acid with similar properties. Properties considered include chemical properties such as charge and hydrophobicity. Table 1 below lists substitutions for each amino acid that are considered to be conservative substitutions as meant herein.

**Table 1: Conservative Amino Acid Substitutions**

Original Residue	Conservative Substitutions
Ala	Val, Leu, Ile
Arg	Lys, Gln, Asn
Asn	Gln
Asp	Glu
Cys	Ser, Ala
Gln	Asn
Glu	Asp
Gly	Pro, Ala
His	Asn, Gln, Lys, Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine
Leu	Norleucine, Ile, Val, Met, Ala, Phe
Lys	Arg, Gln, Asn
Met	Leu, Phe, Ile
Phe	Leu, Val, Ile, Ala, Tyr
Pro	Ala
Ser	Thr, Ala, Cys
Thr	Ser
Trp	Tyr, Phe
Tyr	Trp, Phe, Thr, Ser
Val	Ile, Met, Leu, Phe, Ala, Norleucine

As meant herein, an “**Fc region**” is a dimer consisting of two polypeptide chains joined by one or more disulfide bonds, each chain comprising part or all of a hinge domain plus a CH2 and a CH3 domain. Each of the polypeptide chains is referred to as an “**Fc polypeptide chain**.” In some embodiments an “Fc polypeptide chain” may lack a hinge region, especially where the Fc polypeptide chain is intended to be monomeric as in a monomeric Bi-Fc. To distinguish the two Fc polypeptide chains in an Fc region, in some instances one is referred to herein as

an **"A chain"** and the other is referred to as a **"B chain."** More specifically, the Fc regions (or Fc polypeptide chain in monomeric Bi-Fc's) contemplated for use with the present invention are IgG Fc regions (or Fc polypeptide chains), which can be mammalian, for example human, IgG1, IgG2, IgG3, or IgG4 Fc regions. Among  
5 human IgG1 Fc regions, at least two allelic types are known. In other embodiments, the amino acid sequences of the two Fc polypeptide chains can vary from those of a mammalian Fc polypeptide by no more than 10 amino acid substitutions, insertions, and/or deletions of a single amino acid per 100 amino acids relative to the sequence of a mammalian Fc polypeptide amino acid sequence. In some embodiments, such  
10 variations can be "heterodimerizing alterations" that facilitate the formation of heterodimers over homodimers and/or inhibit the formation of homodimers, an Fc alteration that extends half life, an alteration that inhibits Fc gamma receptor (FcγR) binding, and/or an alteration that enhances ADCC.

An **"Fc alteration that extends half life,"** as meant herein is an alteration  
15 within an Fc polypeptide chain that lengthens the *in vivo* half life of a protein that contains the altered Fc polypeptide chain as compared to the half life of a similar protein containing the same Fc polypeptide, except that it does not contain the alteration. Such alterations can be included in an Fc polypeptide chain that is part of a Bi-Fc. The alterations M252Y, S254T, and T256E (methionine at position 252  
20 changed to tyrosine; serine at position 254 changed to threonine; and threonine at position 256 changed to glutamic acid; numbering according to EU numbering as shown in Table 2) are Fc alterations that extend half life and can be used together, separately or in any combination. These alterations and a number of others are described in detail in U.S. Patent 7,083,784. The portions of U.S. Patent 7,083,784  
25 that describe such alterations are incorporated herein by reference. Similarly, M428L and N434S are Fc alterations that extend half life and can be used together, separately or in any combination. These alterations and a number of others are described in detail in U.S. Patent Application Publication 2010/0234575 and U.S. Patent 7,670,600. The portions of U.S. Patent Application Publication 2010/0234575  
30 and U.S. Patent 7,670,600 that describe such alterations are incorporated herein by reference. In addition, any substitution at one of the following sites can be considered an Fc alteration that extends half life as meant here: 250, 251, 252, 259, 307, 308, 332, 378, 380, 428, 430, 434, 436. Each of these alterations or combinations

of these alterations can be used to extend the half life of a heterodimeric or monomeric Bi-Fc antibody as described herein. Other alterations that can be used to extend half life are described in detail in International Application

PCT/US2012/070146 filed December 17, 2012 (published as WO 2013/096221). The

portions of this application that describe such alterations are incorporated herein by reference. Some specific embodiments described in this application include

insertions between positions 384 and 385 (EU numbering as shown in Table 2) that

extend half life, including the following amino acid sequences: GGCVFNMFNCGG

(SEQ ID NO:36), GGCHLPFAVCGG (SEQ ID NO:37), GGC GHEYMWCGG (SEQ ID NO:38),

GGCWPLQDYCGG (SEQ ID NO:39), GGCMQMKNKWC GG (SEQ ID NO:40),

GGCDGRTKYCGG (SEQ ID NO:41), GG CALYPTNCGG (SEQ ID NO:42),

GGCGKHWHQCGG (SEQ ID NO:43), GGCHSFKHF CGG (SEQ ID NO:44),

GGCQGMWTWCGG (SEQ ID NO:45), GGCAQQWHHEYCGG (SEQ ID NO:46), and

GGCERFHHACGG (SEQ ID NO:47), among others. Heterodimeric or monomeric Bi-Fc

antibodies containing such insertions are contemplated.

An **"Fc alteration that is unfavorable to homodimer formation,"** includes any alteration in an Fc polypeptide chain such that the Fc polypeptide chain has decreased ability to form homodimers compared to a wild type Fc polypeptide chain. Such alterations are described in detail in U.S. Patent Application Publication

US2012/0244578. The portions of this publication that described such alteration are incorporated herein by reference. Examples of such alterations include, without limitation, the following, which can be used individually or in any combination:

R409D, R409E, D399K, D399R, N392D, N392E, K392D, K392E, K439D, K439E, D356K,

D356R, E356K, E356R, K370D, K370E, E357K, and E357R. Such alterations can be

included in an Fc polypeptide chain that is part of a Bi-Fc, especially in embodiments where the Bi-Fc is a monomer. In some embodiments, such alterations occur in the

CH3 region of the Fc polypeptide chain and comprise an alteration such that one or

more charged amino acids in the wild type amino acid sequence are replaced with amino acids electrostatically unfavorable to CH3 homodimer formation, and/or one

or more hydrophobic interface residues are replaced with a small polar amino acid,

such as, for example, asparagine, cysteine, glutamine, serine, or threonine. More

specifically, for example, a charged amino acid, *e.g.*, lysine at position 392 and/or

position 409, can be replaced with a neutral or oppositely charged amino acid, for

example aspartate or glutamate. This can also occur at any other charged amino acid within the Fc polypeptide chain. Alternatively or in addition, one or more hydrophobic interface residues selected from the group consisting of Y349, L351, L368, V397, L398, F405, and Y407 can be replaced with a small polar amino acid.

Further, the Fc polypeptide chain can have one or more mutated cysteine residues to prevent di-sulfide bond formation. Particularly useful cysteine mutations in this regard are those in the hinge region of the Fc polypeptide chain. Such cysteines can be deleted or substituted with other amino acids. For monomeric Bi-Fc's, the hinge region can be entirely absent.

**"Heterodimerizing alterations"** generally refer to alterations in the A and B chains of an Fc region that facilitate the formation of heterodimeric Fc regions, that is, Fc regions in which the A chain and the B chain of the Fc region do not have identical amino acid sequences. Such alterations can be included in an Fc polypeptide chain that is part of a Bi-Fc. Heterodimerizing alterations can be asymmetric, that is, an A chain having a certain alteration can pair with a B chain having a different alteration. These alterations facilitate heterodimerization and disfavor homodimerization. Whether hetero- or homo-dimers have formed can be assessed by size differences as determined by polyacrylamide gel electrophoresis in some situations or by other appropriate means (such as molecular tags or binding by antibodies that recognize certain portions of the heterodimer) in situations where size is not a distinguishing characteristic. One example of such paired heterodimerizing alterations are the so-called "knobs and holes" substitutions. *See, e.g.,* US Patent 7,695,936 and US Patent Application Publication 2003/0078385, the portions of which describe such mutations are incorporated herein by reference. As meant herein, an Fc region that contains one pair of knobs and holes substitutions, contains one substitution in the A chain and another in the B chain. For example, the following knobs and holes substitutions in the A and B chains of an IgG1 Fc region have been found to increase heterodimer formation as compared with that found with unmodified A and B chains: 1) Y407T in one chain and T366Y in the other; 2) Y407A in one chain and T366W in the other; 3) F405A in one chain and T394W in the other; 4) F405W in one chain and T394S in the other; 5) Y407T in one chain and T366Y in the other; 6) T366Y and F405A in one chain and T394W and Y407T in the other; 7) T366W and F405W in one chain and T394S and Y407A in the other; 8)

F405W and Y407A in one chain and T366W and T394S in the other; and 9) T366W in one polypeptide of the Fc and T366S, L368A, and Y407V in the other. As explained above, this way of notating mutations can be explained as follows. The amino acid (using the one letter code) normally present at a given position in the CH3 region using the EU numbering system (which is presented in Edelman *et al.* (1969), Proc. Natl. Acad. Sci. 63: 78-85; *see also* Table 2 below) is followed by the EU position, which is followed by the alternate amino acid that is present at that position. For example, Y407T means that the tyrosine normally present at EU position 407 is replaced by a threonine. Alternatively or in addition to such alterations, substitutions creating new disulfide bridges can facilitate heterodimer formation. *See, e.g.*, US Patent Application Publication 2003/0078385, the portions of which describe such mutations are incorporated herein by reference. Such alterations in an IgG1 Fc region include, for example, the following substitutions: Y349C in one Fc polypeptide chain and S354C in the other; Y349C in one Fc polypeptide chain and E356C in the other; Y349C in one Fc polypeptide chain and E357C in the other; L351C in one Fc polypeptide chain and S354C in the other; T394C in one Fc polypeptide chain and E397C in the other; or D399C in one Fc polypeptide chain and K392C in the other. Similarly, substitutions changing the charge of a one or more residue, for example, in the C<sub>H</sub>3-C<sub>H</sub>3 interface, can enhance heterodimer formation as explained in WO 2009/089004, the portions of which describe such substitutions are incorporated herein by reference. Such substitutions are referred to herein as "charge pair substitutions," and an Fc region containing one pair of charge pair substitutions contains one substitution in the A chain and a different substitution in the B chain. General examples of charge pair substitutions include the following: 1) K409D or K409E in one chain plus D399K or D399R in the other; 2) K392D or K392E in one chain plus D399K or D399R in the other; 3) K439D or K439E in one chain plus E356K or E356R in the other; and 4) K370D or K370E in one chain plus E357K or E357R in the other. In addition, the substitutions R355D, R355E, K360D, or K360R in both chains can stabilize heterodimers when used with other heterodimerizing alterations. Specific charge pair substitutions can be used either alone or with other charge pair substitutions. Specific examples of single pairs of charge pair substitutions and combinations thereof include the following: 1) K409E in one chain plus D399K in the other; 2) K409E in one chain plus D399R in the other;

3) K409D in one chain plus D399K in the other; 4) K409D in one chain plus D399R in the other; 5) K392E in one chain plus D399R in the other; 6) K392E in one chain plus D399K in the other; 7) K392D in one chain plus D399R in the other; 8) K392D in one chain plus D399K in the other; 9) K409D and K360D in one chain plus D399K and  
 5 E356K in the other; 10) K409D and K370D in one chain plus D399K and E357K in the other; 11) K409D and K392D in one chain plus D399K, E356K, and E357K in the other; 12) K409D and K392D on one chain and D399K on the other; 13) K409D and K392D on one chain plus D399K and E356K on the other; 14) K409D and K392D on one chain plus D399K and D357K on the other; 15) K409D and K370D on one chain  
 10 plus D399K and D357K on the other; 16) D399K on one chain plus K409D and K360D on the other; and 17) K409D and K439D on one chain plus D399K and E356K on the other. Any of the these heterodimerizing alterations can be used in the Fc regions of the heterodimeric bispecific antibodies described herein.

An **"alteration that inhibits FcγR binding,"** as meant herein, is one or more  
 15 insertions, deletions, or substitutions within an Fc polypeptide chain that inhibits the binding of FcγRIIA, FcγRIIB, and/or FcγRIIIA as measured, for example, by an ALPHALISA<sup>®</sup>-based competition binding assay (PerkinElmer, Waltham, MA). Such alterations can be included in an Fc polypeptide chain that is part of a Bi-Fc. More specifically, alterations that inhibit Fc gamma receptor (FcγR) binding include L234A,  
 20 L235A, or any alteration that inhibits glycosylation at N297, including any substitution at N297. In addition, along with alterations that inhibit glycosylation at N297, additional alterations that stabilize a dimeric Fc region by creating additional disulfide bridges are also contemplated. Further examples of alterations that inhibit FcγR binding include a D265A alteration in one Fc polypeptide chain and an A327Q  
 25 alteration in the other Fc polypeptide chain. Some such mutations are described in, *e.g.*, Xu et al. (2000), Cellular Immunol. 200: 16-26, the portions of which describe such mutations and how their activity is assessed are incorporated herein by reference.

An **"alteration that enhances ADCC,"** as meant herein is one or more  
 30 insertions, deletions, or substitutions within an Fc polypeptide chain that enhances antibody dependent cell-mediated cytotoxicity (ADCC). Such alterations can be included in an Fc polypeptide chain that is part of a Bi-Fc. Many such alterations are described in International Patent Application Publication WO 2012/125850. Portions

of this application that describe such alterations are incorporated herein by reference. Such alterations can be included in an Fc polypeptide chain that is part of a heterodimeric bispecific antibody as described herein. ADCC assays can be performed as follows. Cell lines that express high and lower amounts of a cancer cell antigen on the cell surface can be used as target cells. These target cells can be labeled with carboxyfluorescein succinimidyl ester (CFSE) and then washed once with phosphate buffered saline (PBS) before being deposited into 96-well microtiter plates with V-shaped wells. Purified immune effector cells, for example T cells, NK cells, macrophages, neutrophils can be added to each well. A monospecific antibody that binds to the cancer antigen and contains the alteration(s) being tested and an isotype-matched control antibody can be diluted in a 1:3 series and added to the wells. The cells can be incubated at 37 °C with 5% CO<sub>2</sub> for 3.5 hrs. The cells can be spun down and re-suspended in 1x FACS buffer (1x phosphate buffered saline (PBS) containing 0.5% fetal bovine serum (FBS)) with the dye TO-PRO<sup>®</sup>-3 iodide (Molecular Probes, Inc. Corporation, Oregon, USA), which stains dead cells, before analysis by fluorescence activated cell sorting (FACS). The percentage of cell killing can be calculated using the follow formula:

$$\frac{(\text{percent tumor cell lysis with bispecific} - \text{percent tumor cell lysis without bispecific})}{(\text{percent total cell lysis} - \text{percent tumor cell lysis without bispecific})}$$

Total cell lysis is determined by lysing samples containing effector cells and labeled target cells without a bispecific molecule with cold 80% methanol. Exemplary alterations that enhance ADCC include the following alterations in the A and B chains of an Fc region: (a) the A chain comprises Q311M and K334V substitutions and the B chain comprises L234Y, E294L, and Y296W substitutions or vice versa; (b) the A chain comprises E233L, Q311M, and K334V substitutions and the B chain comprises L234Y, E294L, and Y296W substitutions or vice versa; (c) the A chain comprises L234I, Q311M, and K334V substitutions and the B chain comprises L234Y, E294L, and Y296W substitutions or vice versa; (d) the A chain comprises S298T and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (e) the A chain comprises A330M and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (f) the A chain comprises A330F and K334V substitutions and the B chain comprises L234Y, K290Y,

and Y296W substitutions or vice versa; (g) the A chain comprises Q311M, A330M, and K334V substitutions and the B chain comprises L234Y, E294L, and Y296W substitutions or vice versa; (h) the A chain comprises Q311M, A330F, and K334V substitutions and the B chain comprises L234Y, E294L, and Y296W substitutions or vice versa; (i) the A chain comprises S298T, A330M, and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (j) the A chain comprises S298T, A330F, and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (k) the A chain comprises S239D, A330M, and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (l) the A chain comprises S239D, S298T, and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (m) the A chain comprises a K334V substitution and the B chain comprises Y296W and S298C substitutions or vice versa; (n) the A chain comprises a K334V substitution and the B chain comprises L234Y, Y296W, and S298C substitutions or vice versa; (o) the A chain comprises L235S, S239D, and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W, substitutions or vice versa; (p) the A chain comprises L235S, S239D, and K334V substitutions and the B chain comprises L234Y, Y296W, and S298C substitutions or vice versa; (q) the A chain comprises Q311M and K334V substitutions and the B chain comprises L234Y, F243V, and Y296W substitutions or vice versa; (r) the A chain comprises Q311M and K334V substitutions and the B chain comprises L234Y, K296W, and S298C substitutions or vice versa; (s) the A chain comprises S239D and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (t) the A chain comprises S239D and K334V substitutions and the B chain comprises L234Y, Y296W, and S298C substitutions or vice versa; (u) the A chain comprises F243V and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W, substitutions or vice versa; (v) the A chain comprises F243V and K334V substitutions and the B chain comprises L234Y, Y296W, and S298C substitutions or vice versa; (w) the A chain comprises E294L and K334V substitutions and the B chain comprises L234Y, K290Y, and Y296W substitutions or vice versa; (x) the A chain comprises E294L and K334V substitutions and the B chain comprises L234Y, Y296W, and S298C substitutions or vice versa; (y) the A chain comprises A330M and K334V substitutions and the B chain comprises L234Y and Y296W substitutions or vice



versa; or (z) the A chain comprises A330M and K334V substitutions and the B chain comprises K290Y and Y296W substitutions or vice versa.

An **"IgG antibody,"** as meant herein, is an antibody consisting essentially of two immunoglobulin IgG heavy chains and two immunoglobulin light chains, which  
5 can be kappa or lambda light chains. More specifically, the heavy chains contain a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region in that order, while the light chains contain a VL region followed by a CL region. Numerous sequences of such immunoglobulin regions are known in the art. *See, e.g.,* Kabat *et al.* in SEQUENCES OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD,  
10 1991. Sequences of regions from IgG antibodies disclosed in Kabat *et al.* are incorporated herein by reference. Close variants of a known and/or naturally-occurring IgG antibody comprising no more than 10 amino acid substitutions, insertions, and/or deletions of a single amino acid per 100 amino acids relative to a known or naturally occurring sequence of an immunoglobulin IgG heavy and/or light  
15 chain are encompassed within what is meant by an IgG antibody.

An **"immune effector cell,"** as meant herein, is a cell that is involved in the mediation of a cytolytic immune response, including, for example, T cells, NK cells, macrophages, or neutrophils. The heterodimeric or monomeric Bi-Fc antibodies described herein bind to an antigen that is part of a protein expressed on the surface  
20 of an immune effector cell. Such proteins are referred to herein as **"effector cell proteins."**

An **"immunoglobulin heavy chain,"** as meant herein, consists essentially of a VH region, a CH1 region, a hinge region, a CH2 region, a CH3 region in that order, and, optionally, a region downstream of the CH3 region in some isotypes. Close  
25 variants of an immunoglobulin heavy chain containing no more than 10 amino acid substitutions, insertions, and/or deletions of a single amino acid per 100 amino acids relative to a known or naturally occurring immunoglobulin heavy chain amino acid sequence are encompassed within what is meant by an immunoglobulin heavy chain.

A **"immunoglobulin light chain,"** as meant herein, consists essentially of a  
30 light chain variable region (VL) and a light chain constant domain (CL). Close variants of an immunoglobulin light chain containing no more than 10 amino acid substitutions, insertions, and/or deletions of a single amino acid per 100 amino acids

relative to a known or naturally occurring immunoglobulin light chain amino acid sequence are encompassed within what is meant by an immunoglobulin light chain.

An **"immunoglobulin variable region,"** as meant herein, is a VH region, a VL region, or a variant thereof. Close variants of an immunoglobulin variable region containing no more than 10 amino acid substitutions, insertions, and/or deletions of a single amino acid per 100 amino acids relative to a known or naturally occurring immunoglobulin variable region amino acid sequence are encompassed within what is meant by an immunoglobulin variable region. Many examples of VH and VL regions are known in the art, such as, for example, those disclosed by Kabat *et al.* in SEQUENCES OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD, 1991. Based on the extensive sequence commonalities in the less variable portions of the VH and VL regions, the position within a sequence of more variable regions, and the predicted tertiary structure, one of skill in the art can recognize an immunoglobulin variable region by its sequence. *See, e.g.,* Honegger and Plückthun (2001), J. Mol. Biol. 309: 657-670.

An immunoglobulin variable region contains three hypervariable regions, known as complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3). These regions form the antigen binding site of an antibody. The CDRs are embedded within the less variable framework regions (FR1-FR4). The order of these subregions within an immunoglobulin variable region is as follows: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. Numerous sequences of immunoglobulin variable regions are known in the art. *See, e.g.,* Kabat *et al.*, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD, 1991.

CDRs can be located in a VH region sequence in the following way. CDR1 starts at approximately residue 31 of the mature VH region and is usually about 5-7 amino acids long, and it is almost always preceded by a Cys-Xxx-Xxx-Xxx-Xxx-Xxx-Xxx-Xxx (SEQ ID NO:1) (where "Xxx" is any amino acid). The residue following the heavy chain CDR1 is almost always a tryptophan, often a Trp-Val, a Trp-Ile, or a Trp-Ala. Fourteen amino acids are almost always between the last residue in CDR1 and the first in CDR2, and CDR2 typically contains 16 to 19 amino acids. CDR2 may be immediately preceded by Leu-Glu-Trp-Ile-Gly (SEQ ID NO:2) and may be immediately followed by Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala. Other

amino acids may precede or follow CDR2. Thirty two amino acids are almost always between the last residue in CDR2 and the first in CDR3, and CDR3 can be from about 3 to 25 residues long. A Cys-Xxx-Xxx almost always immediately precedes CDR3, and a Trp-Gly-Xxx-Gly (SEQ ID NO:3) almost always follows CDR3.

5           Light chain CDRs can be located in a VL region in the following way. CDR1 starts at approximately residue 24 of the mature antibody and is usually about 10 to 17 residues long. It is almost always preceded by a Cys. There are almost always 15 amino acids between the last residue of CDR1 and the first residue of CDR2, and CDR2 is almost always 7 residues long. CDR2 is typically preceded by Ile-Tyr, Val-Tyr,  
10   Ile-Lys, or Ile-Phe. There are almost always 32 residues between CDR2 and CDR3, and CDR3 is usually about 7 to 10 amino acids long. CDR3 is almost always preceded by Cys and usually followed by Phe-Gly-Xxx-Gly (SEQ ID NO:4).

          A **"linker,"** as meant herein, is a peptide that links two polypeptides, which can be two immunoglobulin variable regions or a variable region and an Fc  
15   polypeptide chain in the context of a Bi-Fc antibody. A linker can be from 2-30 or 2-40 amino acids in length. In some embodiments, a linker can be 2-25, 2-20, or 3-18 amino acids long. In some embodiments, a linker can be a peptide no more than 14, 13, 12, 11, 10, 9, 8, 7, 6, or 5 amino acids long. In other embodiments, a linker can be 5-25, 5-15, 4-11, 10-20, 20-30, or 30-40 amino acids long. In other embodiments, a  
20   linker can be about, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids long. Exemplary linkers include, for example, the amino acid sequences TVAAP (SEQ ID NO:17), ASTKGP (SEQ ID NO:18), GGGGSGGGGS (SEQ ID NO:19), GGGGSAAA (SEQ ID NO:20), GGGGSGGGGSGGGGS (SEQ ID NO:21), and AAA, among many others.

25           A Bi-Fc **"mediates cytolysis of a target cell by an immune effector cell,"** as meant herein, when addition of an amount from 0.001 pM to 20000 pM of the Bi-Fc to a cell cytolysis assay as described in the section below entitled "Target Cell Cytolysis Assays" and in Example 3 effectively elicits cytolysis of the target cells.

**"Non-chemotherapeutic anti-neoplastic agents"** are chemical agents,  
30   compounds, or molecules having cytotoxic or cytostatic effects on cancer cells other than chemotherapeutic agents. Non-chemotherapeutic antineoplastic agents may, however, be targeted to interact directly with molecules that indirectly affect cell division such as cell surface receptors, including receptors for hormones or growth

factors. However, non-chemotherapeutic antineoplastic agents do not interfere directly with processes that are intimately linked to cell division such as, for example, DNA replication, RNA synthesis, protein synthesis, or mitotic spindle function, assembly, or disassembly. Examples of non-chemotherapeutic anti-neoplastic agents include inhibitors of Bcl2, inhibitors of farnesyltransferase, anti-estrogenic agents such as tamoxifen, anti-androgenic compounds, interferon, arsenic, retinoic acid, retinoic acid derivatives, antibodies targeted to tumor-specific antigens, and inhibitors of the Bcr-Abl tyrosine kinase (*e.g.*, the small molecule STI-571 marketed under the trade name GLEEVEC<sup>TM</sup> by Novartis, New York and New Jersey, USA and Basel, Switzerland), among many possible non-chemotherapeutic anti-neoplastic agents.

**"Percent identity"** of one amino acid or nucleic acid sequence with another can be determined using a computer program. An exemplary computer program is the Genetics Computer Group (GCG; Madison, WI) Wisconsin package version 10.0 program, GAP (Devereux *et al.* (1984), *Nucleic Acids Res.* 12: 387-95). The preferred default parameters for the GAP program includes: (1) The GCG implementation of an unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted amino acid comparison matrix of Gribskov and Burgess, ((1986) *Nucleic Acids Res.* 14: 6745) as described in *Atlas of Polypeptide Sequence and Structure*, Schwartz and Dayhoff, eds., National Biomedical Research Foundation, pp. 353-358 (1979) or other comparable comparison matrices; (2) a penalty of 8 for each gap and an additional penalty of 2 for each symbol in each gap for amino acid sequences, or a penalty of 50 for each gap and an additional penalty of 3 for each symbol in each gap for nucleotide sequences; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps. Other programs used by those skilled in the art of sequence comparison can also be used.

In connection with comparisons to determine sequence identity of polynucleotides or polypeptides, an **"identity region"** is the portion of the polynucleotide or polypeptide sequence that is matched, partially or exactly, with another polynucleotide or polypeptide by the computer program GAP (Devereux *et al.* (1984), *Nucleic Acids Res.* 12: 387-95) using the parameters stated below. For example, when a polypeptide of 20 amino acids is aligned with a considerably longer

protein, the first 10 amino acids match the longer protein exactly, and the last 10 amino acids do not match the longer protein at all, the identity region is 10 amino acids. If, on the other hand, the first and last amino acids of the 20 amino acid polypeptide match the longer protein, and eight other matches are scattered  
5 between, the identity region is 20 amino acids long. However, long stretches in either aligned strand without identical or conservatively substituted amino acids or identical nucleotides of at least, for example, 20 amino acids or 60 nucleotides constitute an endpoint of an identity region, as meant herein.

A **"target cell"** is a cell that a Bi-Fc binds to and that is involved in mediating  
10 a disease. In some cases, a target cell can be a cell that is ordinarily involved in mediating an immune response, but is also involved in the mediation of a disease. For example in B cell lymphoma, a B cell, which is ordinarily involved in mediating immune response, can be a target cell. In some embodiments, a target cell is a cancer cell, a cell infected with a pathogen, or a cell involved in mediating an  
15 autoimmune or inflammatory disease, for example a fibrotic disease. The Bi-Fc can bind to the target cell via binding to an antigen on a **"target cell protein,"** which is a protein that is displayed on the surface of the target cell, possibly a highly expressed protein.

**"Tumor burden"** refers to the number of viable cancer cells, the number of  
20 tumor sites, and/or the size of the tumor(s) in a patient suffering from a cancer. A reduction in tumor burden can be observed, for example, as a reduction in the amount of a tumor-associated antigen or protein in a patient's blood or urine, a reduction in the number of tumor cells or tumor sites, and/or a reduction in the size of one or more tumors.

A **"therapeutically effective amount"** of a Bi-Fc or any other drug is an  
25 amount that has the effect of, for example, reducing or eliminating the tumor burden of a cancer patient or reducing or eliminating the symptoms of any disease condition that the protein is used to treat. A therapeutically effective amount need not completely eliminate all symptoms of the condition, but may reduce severity of one  
30 or more symptoms or delay the onset of more serious symptoms or a more serious disease that can occur with some frequency following the treated condition.

**"Treatment"** of any disease mentioned herein encompasses an alleviation of at least one symptom of the disease, a reduction in the severity of the disease, or the

delay or prevention of disease progression to more serious symptoms that may, in some cases, accompany the disease or lead to at least one other disease. Treatment need not mean that the disease is totally cured. A useful therapeutic agent needs only to reduce the severity of a disease, reduce the severity of one or more symptoms associated with the disease or its treatment, or delay the onset of more serious symptoms or a more serious disease that can occur with some frequency following the treated condition.

When it is said that a named VH/VL pair of immunoglobulin variable regions can bind to a target cell or and/or an immune effector cell **"when they are part of an IgG and/or scFv antibody,"** it is meant that an IgG antibody that contains the named VH region in both heavy chains and the named VL region in both light chains and/or an scFv antibody containing these VH and VL regions can bind to the target cell and/or the immune effector cell. The binding assay described in Example 2 can be used to assess binding.

### ***Bi-Fc Molecules***

In the most general sense, a Bi-Fc can bind monovalently to two different antigens and comprises one polypeptide chain or two different polypeptide chains having different amino acid sequences. In addition, it can bind to the neonatal Fc receptor (FcRn) at slightly acidic pH (about pH 5.5-6.0) via its Fc region. This interaction with FcRn can lengthen the half life of a molecule *in vivo*. The first polypeptide chain (which, in some cases, is the only polypeptide chain) comprises an Fc polypeptide chain and two VH regions plus two VL regions that can be separated by linkers. The Fc polypeptide chain can be N-terminal or C-terminal relative to the four immunoglobulin variable regions, and it can be joined to the variable regions via a linker. The second polypeptide chain, when present, comprises an Fc polypeptide chain. A Bi-Fc can bind to an immune effector cell and a target cell and/or can mediate cytotoxicity of a target cell by an immune effector cell. The general structure of a Bi-Fc is diagrammed in Figure 1, which shows embodiments where the Fc polypeptide chain is C-terminal (panels A and B) and embodiments where the Fc polypeptide chain is N-terminal (panels C and D).

More particular embodiments specify the order of immunoglobulin variable regions and the length of the linkers and specify which immunoglobulin variable

regions can associate to form a binding site for an effector cell protein or a target cell protein. Generally, the antigen-binding portion of an antibody includes both a VH and a VL region, referred to herein as a **"VH/VL pair,"** although in some cases a VH or a VL region can bind to an antigen without a partner. *See, e.g.,* US Application Publication 2003/0114659.

In one group of embodiments, the four variable regions can be arranged in the following order: VH1-linker1-VL1-linker2-VH2-linker3-VL2, where VH1/VL1 is an antigen-binding pair and VH2/VL2 is another antigen-binding pair. In this group of embodiments, linker1 and linker3 can be at least 15 amino acids long, and linker2 can be less than 12 amino acids long or, in some cases, absent. In some embodiments, the VH1/VL1 pair can bind to a target cell protein, and the VH2/VL2 pair can bind to an effector cell protein. In other embodiments, the VH1/VL1 pair can bind to an effector cell protein, and the VH2/VL2 pair can bind to a target cell protein.

In another group of embodiments the four variable regions can be arranged in the following order: VL1-linker1-VH1-linker2-VL2-linker3-VH2, where VH1/VL1 is an antigen-binding pair and VH2/VL2 is an antigen-binding pair. In these embodiments, linker2 can be less than 12 amino acids long or absent, and linker1 and linker3 can be at least 15 amino acids long. In some embodiments, the VH1/VL1 pair can bind to a target cell protein, and the VH2/VL2 pair can bind to an effector cell protein. In other embodiments, the VH1/VL1 pair can bind to an effector cell protein, and the VH2/VL2 pair can bind to a target cell protein.

In another group of embodiments the four variable regions can be arranged in the following order: VH1-linker1-VL1-linker2-VL2-linker3-VH2, where VH1/VL1 is an antigen-binding pair and VH2/VL2 is an antigen-binding pair. In these embodiments, linker2 can be less than 12 amino acids long or absent, and linker1 and linker3 can be at least 15 amino acids long. In some embodiments, the VH1/VL1 pair can bind to a target cell protein, and the VH2/VL2 pair can bind to an effector cell protein. In other embodiments, the VH1/VL1 pair can bind to an effector cell protein, and the VH2/VL2 pair can bind to a target cell protein.

In further group of embodiments the four variable regions can be arranged in the following order: VL1-linker1-VH1-linker2-VH2-linker3-VL2, where VH1/VL1 is an antigen-binding pair and VH2/VL2 is an antigen-binding pair. In these

embodiments, linker2 can be less than 12 amino acids long or absent, and linker1 and linker3 can be at least 15 amino acids long. In some embodiments, the VH1/VL1 pair can bind to a target cell protein, and the VH2/VL2 pair can bind to an effector cell protein. In other embodiments, the VH1/VL1 pair can bind to an effector cell protein, and the VH2/VL2 pair can bind to a target cell protein.

A Bi-Fc can comprise an Fc polypeptide chain of an antibody. The Fc polypeptide chain can be of mammalian (for example, human, mouse, rat, rabbit, dromedary, or new or old world monkey), avian, or shark origin. For example, the Fc polypeptide chain can be a human IgG1, IgG2, IgG3, or IgG4 Fc polypeptide chain. In addition, as explained above, an Fc polypeptide chain can comprise a limited number of alterations. More particularly, an Fc polypeptide chain can contain no more than 10 insertions, deletions, and/or substitutions of a single amino acid per 100 amino acids relative to a known or naturally-occurring sequence. In some embodiments, the two Fc polypeptide chains of a heterodimeric Bi-Fc contain heterodimerizing alterations, which can be, for example, charge pair substitutions. For example, the first polypeptide chain of the Bi-Fc can comprise the substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D, or K392E and the second polypeptide chain of the Bi-Fc can comprise D399K or D399R and E356K, E356R, D356K, or D356R. Alternatively, the first polypeptide chain of the Bi-Fc can comprise D399K or D399R and E356K, E356R, D356K or D356R, and the second polypeptide chain of the Bi-Fc can comprise R409E, R409E, K409D, or K409E and N392E, N392D, K392D or K392E. An Fc polypeptide chain can also comprise one or more "Fc alterations unfavorable to homodimer formation" and/or one or more "Fc alterations that extend half life," as meant herein.

In monomeric embodiments of the Bi-Fc, the Bi-Fc can comprise one or more "Fc alterations that are unfavorable to homodimer formation," as defined above.

Other kinds of alterations can also be part of an Fc polypeptide chain that is part of a Bi-Fc. In one aspect, an Fc region included in a Bi-Fc can comprise one or more "alterations that inhibit the binding of an Fc gamma receptor (FcγR)" to the Fc region as defined above. In another aspect, an Fc region included in a Bi-Fc can comprise one or more "Fc alteration that extends half life," as defined above. In still another aspect, one or more "alterations that enhance ADCC" can be included in an Fc region that is part of a Bi-Fc.



In some embodiments the amino acid sequences of the Fc polypeptides can be mammalian, for example a human, amino acid sequences or variants thereof that comprise not more than 10 deletions, insertions, or substitutions of a single amino acid per 100 amino acids of sequence relative to a human amino acid sequence.

Alternatively, an Fc polypeptide that is part of a Bi-Fc can be 90% or 95% identical to a human IgG Fc polypeptide chain and the identity region can be at least about 50, 60, 70, 80, 90, or 100 amino acids long. The isotype of the Fc polypeptide can be IgA, IgD, IgE, IgM, or IgG, such as IgG1, IgG2, IgG3, or IgG4,. Table 2 below shows an alignment of the amino acid sequences of human IgG1, IgG2, IgG3, and IgG4 Fc polypeptide chain sequences.

**Table 2: Amino acid sequences of human IgG Fc regions**

	IgG1	-----
	IgG2	-----
15	IgG3	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRC
	IgG4	-----
		225 235 245 255 265 275
		* * * * *
20	IgG1	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
	IgG2	ERKCCVE---CPPCPAPPVA-GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF
	IgG3	EPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF
	IgG4	ESKYG---PPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQF
25		285 295 305 315 325 335
		* * * * *
	IgG1	NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
	IgG2	NWYVDGMEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
	IgG3	KWYVDGVEVHNAKTKPREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
30	IgG4	NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIET
		345 355 365 375 385 395
		* * * * *
35	IgG1	ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
	IgG2	ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
	IgG3	ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTP
	IgG4	ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
40		405 415 425 435 445
		* * * * *
	IgG1	PVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID NO:25)
	IgG2	PMLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID NO:26)
	IgG3	PMLDS DGSFFLYSKLTVDKSRWQQGN I FSCSV MHEALHN RFTQKSLSLSPGK (SEQ ID NO:27)
45	IgG4	PVLDS DGSFFLYSRLTVDKSRWQEGNV FSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:28)

The numbering shown in Table 2 is according to the EU system of numbering, which is based on the sequential numbering of the constant region of a human IgG1 antibody. Edelman *et al.* (1969), Proc. Natl. Acad. Sci. 63: 78-85. Thus, it does not accommodate the additional length of the IgG3 hinge well. It is nonetheless used herein to designate positions in an Fc region because it is still commonly used in the

art to refer to positions in Fc regions. The hinge regions of the IgG1, IgG2, and IgG4 Fc polypeptides extend from about position 216 to about 230. It is clear from the alignment that the IgG2 and IgG4 hinge regions are each three amino acids shorter than the IgG1 hinge. The IgG3 hinge is much longer, extending for an additional 47 amino acids upstream. The CH2 region extends from about position 231 to 340, and the CH3 region extends from about position 341 to 447.

Naturally occurring amino acid sequences of Fc polypeptides can be varied slightly. Such variations can include no more than 10 insertions, deletions, and/or substitutions of one amino acid per 100 amino acids of sequence in a known or naturally-occurring amino acid sequence of an Fc polypeptide. If there are substitutions, they can be conservative amino acid substitutions, as defined above. The Fc polypeptides on the first and second polypeptide chains of a heterodimeric Bi-Fc can differ in amino acid sequence. In some embodiments, they can include one or more "heterodimerizing alterations," "alterations that enhance ADCC," "alterations that inhibit FcγR binding," "Fc alterations that are unfavorable to homodimer formation," and/or "Fc alterations that extend half life," as defined above.

A Bi-Fc can bind to an immune effector cell through an antigen that is part of an effector cell protein and can bind to a target cell through an antigen that is part of a target cell protein. A number of possible effector cell proteins are described in detail below. Similarly, a number of possible target cell proteins is also described below. A Bi-Fc can bind to any combination of an effector cell protein and a target cell protein.

Exemplary amino acid sequences of Bi-Fc's include the following amino acid sequences: SEQ ID NOs:10 and 12 (a heterodimeric Bi-Fc); SEQ ID NOs:15 and 12 (a heterodimeric Bi-Fc); and SEQ ID NO:34 (a monomeric Bi-Fc that includes the alterations Y349T, K392D, and K409D (EU numbering) in its Fc polypeptide chain portion).

### ***Nucleic Acids Encoding Bi-Fc Molecules***

Provided are nucleic acids encoding Bi-Fc's. Numerous nucleic acid sequences encoding immunoglobulin regions including VH, VL, hinge, CH1, CH2, CH3, and CH4 regions are known in the art. *See, e.g.,* Kabat *et al.* in SEQUENCES OF IMMUNOLOGICAL INTEREST, Public Health Service N.I.H., Bethesda, MD, 1991. Using the

guidance provided herein, one of skill in the art could combine such nucleic acid sequences and/or other nucleic acid sequences known in the art to create nucleic acid sequences encoding Bi-Fc's. Exemplary nucleic acids encoding Bi-Fc's include (1) SEQ ID NOs:11 and 13, (2) SEQ ID NOs:16 and 13, and (3) SEQ ID NO:35.

5 In addition, nucleic acid sequences encoding Bi-Fc's can be determined by one of skill in the art based on the amino acid sequences provided herein and elsewhere and knowledge in the art. Besides more traditional methods of producing cloned DNA segments encoding a particular amino acid sequence, companies such as DNA 2.0 (Menlo Park, CA, USA) and BlueHeron (Bothell, WA, USA), among others,  
10 now routinely produce chemically synthesized, gene-sized DNAs of any desired sequence to order, thus streamlining the process of producing such DNAs.

### ***Methods of Making Bi-Fc Molecules***

Bi-Fc's can be made using methods well known in the art. For example,  
15 nucleic acids encoding the one or two polypeptide chains of a Bi-Fc can be introduced into a cultured host cell by a variety of known methods, such as, for example, transformation, transfection, electroporation, bombardment with nucleic acid-coated microprojectiles, etc. In some embodiments the nucleic acids encoding a Bi-Fc can be inserted into a vector appropriate for expression in the host cells  
20 before being introduced into the host cells. Typically such vectors can contain sequence elements enabling expression of the inserted nucleic acids at the RNA and protein levels. Such vectors are well known in the art, and many are commercially available. The host cells containing the nucleic acids can be cultured under conditions so as to enable the cells to express the nucleic acids, and the resulting Bi-  
25 Fc's can be collected from the cell mass or the culture medium. Alternatively, a Bi-Fc can be produced *in vivo*, for example in plant leaves (*see, e.g.*, Scheller et al. (2001), Nature Biotechnol. 19: 573-577 and references cited therein), bird eggs (*see, e.g.*, Zhu et al. (2005), Nature Biotechnol. 23: 1159-1169 and references cited therein), or mammalian milk (*see, e.g.*, Laible et al. (2012), Reprod. Fertil. Dev. 25(1): 315).

30 A variety of cultured host cells can be used including, for example, bacterial cells such as *Escherichia coli* or *Bacillus stearothermophilus*, fungal cells such as *Saccharomyces cerevisiae* or *Pichia pastoris*, insect cells such as lepidopteran insect cells including *Spodoptera frugiperda* cells, or mammalian cells such as Chinese

hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, monkey kidney cells, HeLa cells, human hepatocellular carcinoma cells, or 293 cells, among many others.

### ***Immune Effector Cells and Effector Cell Proteins***

5 A Bi-Fc can bind to a molecule expressed on the surface of an immune effector cell (called "**effector cell protein**" herein) and to another molecule expressed on the surface of a target cell (called a "**target cell protein**" herein). The immune effector cell can be a T cell, an NK cell, a macrophage, or a neutrophil. In some embodiments the effector cell protein is a protein included in the T cell  
10 receptor (TCR)-CD3 complex. The TCR-CD3 complex is a heteromultimer comprising a heterodimer comprising TCR $\alpha$  and TCR $\beta$  or TCR $\gamma$  and TCR $\delta$  plus various CD3 chains from among the CD3 zeta (CD3 $\zeta$ ) chain, CD3 epsilon (CD3 $\epsilon$ ) chain, CD3 gamma (CD3 $\gamma$ ) chain, and CD3 delta (CD3 $\delta$ ) chain. In some embodiments the effector cell protein can be the human CD3 epsilon (CD3 $\epsilon$ ) chain (the mature amino acid  
15 sequence of which is disclosed in SEQ ID NO:22), which can be part of a multimeric protein. Alternatively, the effector cell protein can be human and/or cynomolgus monkey TCR $\alpha$ , TCR $\beta$ , TCR $\delta$ , TCR $\gamma$ , CD3 $\beta$ , CD3 $\gamma$ , CD3 $\delta$ , or CD3 $\zeta$ .

Moreover, in some embodiments, a Bi-Fc can also bind to a CD3 $\epsilon$  chain from a non-human species, such as mouse, rat, rabbit, new world monkey, and/or old world  
20 monkey species. Such species include, without limitation, the following mammalian species: *Mus musculus*; *Rattus rattus*; *Rattus norvegicus*; the cynomolgus monkey, *Macaca fascicularis*; the hamadryas baboon, *Papio hamadryas*; the Guinea baboon, *Papio papio*; the olive baboon, *Papio anubis*; the yellow baboon, *Papio cynocephalus*; the Chacma baboon, *Papio ursinus*; *Callithrix jacchus*; *Saguinus*  
25 *Oedipus*, and *Saimiri sciureus*. The mature amino acid sequence of the CD3 $\epsilon$  chain of cynomolgus monkey is provided in SEQ ID NO:23. As is known in the art of development of protein therapeutics, having a therapeutic that can have comparable activity in humans and species commonly used for preclinical testing, such as mice and monkeys, can simplify and speed drug development. In the long and expensive  
30 process of bringing a drug to market, such advantages can be critical.

In more particular embodiments, the heterodimeric bispecific antibody can bind to an epitope within the first 27 amino acids of the CD3 $\epsilon$  chain, which may be a

human CD3 $\epsilon$  chain or a CD3 $\epsilon$  chain from different species, particularly one of the mammalian species listed above. The epitope can contain the amino acid sequence Gln-Asp-Gly-Asn-Glu (SEQ ID NO:24). The advantages of an antibody that binds such an epitope are explained in detail in U.S. Patent Application Publication 2010/183615, the relevant portions of which are incorporated herein by reference. The epitope to which an antibody binds can be determined by alanine scanning, which is described in, *e.g.*, U.S. Patent Application Publication 2010/183615, the relevant portions of which are incorporated herein by reference.

Briefly, alanine scanning can be performed as follows. In a control, DNA encoding wild type CD3 $\epsilon$  is inserted into an expression vector appropriate for the host cells, preferably a mammalian T cell line, and transfected into the host cells where it can be expressed as part of a TCR-CD3 complex. In test samples, the DNA encodes CD3 $\epsilon$  where a single one of amino acids of CD3 $\epsilon$  is changed to alanine. DNA constructs are made to generate a whole series of molecules in which one amino acid at a time is changed to alanine. Only one amino acid is varied from construct to construct to scan all possible amino acid positions in the extracellular domain of CD3 $\epsilon$  involved in binding to the Bi-Fc. The Bi-Fc to be tested is made by transfecting mammalian host cells with DNA encoding the Bi-Fc and recovering the antibody from the cell culture. Binding of the Bi-Fc to the cells expressing CD3 $\epsilon$ , either wild type or with an alanine replacement, can be assessed by standard fluorescence-activated cell sorting (FACS) methods. Samples where the CD3 $\epsilon$  has an alanine replacement at a particular position and the binding detected is reduced or eliminated compared to that detected with a wild type CD3 $\epsilon$  indicate that the amino acid at the altered position is normally involved in the binding of the Bi-Fc to CD3 $\epsilon$ .

Where a T cell is the immune effector cell, effector cell proteins to which a Bi-Fc can bind include, without limitation, CD3 $\epsilon$ , CD3 $\gamma$ , CD3 $\delta$ , CD3 $\zeta$ , TCR $\alpha$ , TCR $\beta$ , TCR $\gamma$ , and TCR $\delta$ . Where an NK cell or a cytotoxic T cell is an immune effector cell, NKG2D, CD352, NKp46, or CD16a can, for example, be an effector cell protein. Where a CD8<sup>+</sup> T cell is an immune effector cell, 4-1BB or NKG2D, for example, can be an effector cell protein. Alternatively, a Bi-Fc could bind to other effector cell proteins expressed on T cells, NK cells, macrophages, or neutrophils.

***Target Cells and Target cell proteins Expressed on Target Cells***

As explained above, a Bi-Fc can bind to an effector cell protein and a target cell protein. The target cell protein can, for example, be expressed on the surface of a cancer cell, a cell infected with a pathogen, or a cell that mediates a disease, for example an inflammatory, autoimmune, and/or fibrotic condition. In some  
5       embodiments, the target cell protein can be highly expressed on the target cell, although high levels of expression are not necessarily required.

Where the target cell is a cancer cell, a heterodimeric bispecific antibody as described herein can bind to a cancer cell antigen as described above. A cancer cell  
10       antigen can be a human protein or a protein from another species. For example, a heterodimeric bispecific antibody may bind to a target cell protein from a mouse, rat, rabbit, new world monkey, and/or old world monkey species, among many others. Such species include, without limitation, the following species: *Mus musculus*;  
*Rattus rattus*; *Rattus norvegicus*; cynomolgus monkey, *Macaca fascicularis*; the  
15       hamadryas baboon, *Papio hamadryas*; the Guinea baboon, *Papio papio*; the olive baboon, *Papio anubis*; the yellow baboon, *Papio cynocephalus*; the Chacma baboon, *Papio ursinus*, *Callithrix jacchus*, *Saguinus oedipus*, and *Saimiri sciureus*.

In some examples, the target cell protein can be a protein selectively expressed on an infected cell. For example, in the case of a hepatitis B virus (HBV) or  
20       a hepatitis C virus (HCV) infection, the target cell protein can be an envelope protein of HBV or HCV that is expressed on the surface of an infected cell. In other embodiments, the target cell protein can be gp120 encoded by human immunodeficiency virus (HIV) on HIV-infected cells.

In other aspects, a target cell can be a cell that mediates an autoimmune or  
25       inflammatory disease. For example, human eosinophils in asthma can be target cells, in which case, EGF-like module-containing mucin-like hormone receptor (EMR1), for example, can be a target cell protein. Alternatively, excess human B cells in a systemic lupus erythematosus patient can be target cells, in which case CD19 or CD20, for example, can be a target cell protein. In other autoimmune conditions,  
30       excess human Th2 T cells can be target cells, in which case CCR4 can, for example, be a target cell protein. Similarly, a target cell can be a fibrotic cell that mediates a disease such as atherosclerosis, chronic obstructive pulmonary disease (COPD), cirrhosis, scleroderma, kidney transplant fibrosis, kidney allograft nephropathy, or a

pulmonary fibrosis, including idiopathic pulmonary fibrosis and/or idiopathic pulmonary hypertension. For such fibrotic conditions, fibroblast activation protein alpha (FAP alpha) can, for example, be a target cell protein.

### ***Target Cell Cytolysis Assays***

In the Examples below, an assay for determining whether a Bi-Fc antibody as described herein can induce cytolysis of a target cell by an immune effector cell *in vitro* is described. In this assay, the immune effector cell is a T cell. The following very similar assay can be used where the immune effector cells are NK cells.

A target cell line expressing the target cell protein of interest can be labeled with 2  $\mu$ M carboxyfluorescein succinimidyl ester (CFSE) for 15 minutes at 37 °C and then washed. An appropriate number of labeled target cells can then be incubated in one or more 96 well flat bottom culture plates for 40 minutes at 4 °C, with or without a bispecific protein, a control protein, or no added protein at varying concentrations. NK cells isolated from healthy human donors can be isolated using the Miltenyi NK Cell Isolation Kit II (Miltenyi Biotec, Auburn, CA) and then added to the target cells at an Effector:Target ratio of 10:1. The NK cells, which are the immune effector cells in this assay, can be used immediately post-isolation or after overnight culture at 37 °C. Plates containing tumor target cells, bispecific proteins, and immune effector cells can be cultured for 18-24 hours at 37 °C with 5% CO<sub>2</sub>. Appropriate control wells can also be set up. After the 18-24 hour assay period, all cells can be removed from the wells. A volume of a 7-AAD solution equal to the volume of the content of the wells can be added to each sample. Samples can then be assayed to determine the percentage of live versus dead target cells via flow cytometry as described in the Examples below.

### ***Therapeutic Methods and Compositions***

Bi-Fc's can be used to treat a wide variety of conditions including, for example, various forms of cancer, infections, autoimmune or inflammatory conditions, and/or fibrotic conditions.

Provided herein are pharmaceutical compositions comprising Bi-Fc's. Such pharmaceutical compositions comprise a therapeutically effective amount of a Bi-Fc plus one or more additional components such as a physiologically acceptable carrier,

excipient, or diluent. Such additional components can include buffers, carbohydrates, polyols, amino acids, chelating agents, stabilizers, and/or preservatives, among many possibilities.

In some embodiments, a Bi-Fc can be used to treat cell proliferative diseases, including cancer, which involve the unregulated and/or inappropriate proliferation of cells, sometimes accompanied by destruction of adjacent tissue and growth of new blood vessels, which can allow invasion of cancer cells into new areas, *i.e.* metastasis. Included within conditions treatable with a Bi-Fc are non-malignant conditions that involve inappropriate cell growth, including colorectal polyps, cerebral ischemia, gross cystic disease, polycystic kidney disease, benign prostatic hyperplasia, and endometriosis. A Bi-Fc can be used to treat a hematologic or solid tumor malignancy. More specifically, cell proliferative diseases that can be treated using a Bi-Fc are, for example, cancers including mesotheliomas, squamous cell carcinomas, myelomas, osteosarcomas, glioblastomas, gliomas, carcinomas, adenocarcinomas, melanomas, sarcomas, acute and chronic leukemias, lymphomas, and meningiomas, Hodgkin's disease, Sézary syndrome, multiple myeloma, and lung, non-small cell lung, small cell lung, laryngeal, breast, head and neck, bladder, ovarian, skin, prostate, cervical, vaginal, gastric, renal cell, kidney, pancreatic, colorectal, endometrial, esophageal, hepatobiliary, bone, skin, and hematologic cancers, as well as cancers of the nasal cavity and paranasal sinuses, the nasopharynx, the oral cavity, the oropharynx, the larynx, the hypolarynx, the salivary glands, the mediastinum, the stomach, the small intestine, the colon, the rectum and anal region, the ureter, the urethra, the penis, the testis, the vulva, the endocrine system, the central nervous system, and plasma cells.

Among the texts providing guidance for cancer therapy is *Cancer, Principles and Practice of Oncology*, 4th Edition, DeVita *et al.*, Eds. J. B. Lippincott Co., Philadelphia, PA (1993). An appropriate therapeutic approach is chosen according to the particular type of cancer, and other factors such as the general condition of the patient, as is recognized in the pertinent field. A Bi-Fc can be used by itself or can be added to a therapy regimen using other anti-neoplastic agents in treating a cancer patient.

In some embodiments, a Bi-Fc can be administered concurrently with, before, or after a variety of drugs and treatments widely employed in cancer treatment such



as, for example, chemotherapeutic agents, non-chemotherapeutic, anti-neoplastic agents, and/or radiation. For example, chemotherapy and/or radiation can occur before, during, and/or after any of the treatments described herein. Examples of chemotherapeutic agents are discussed above and include, but are not limited to, cisplatin, taxol, etoposide, mitoxantrone (Novantrone<sup>®</sup>), actinomycin D, cycloheximide, camptothecin (or water soluble derivatives thereof), methotrexate, mitomycin (e.g., mitomycin C), dacarbazine (DTIC), anti-neoplastic antibiotics such as adriamycin (doxorubicin) and daunomycin, and all the chemotherapeutic agents mentioned above.

A Bi-Fc can also be used to treat infectious disease, for example a chronic HBV infection, an HCV infection, an HIV infection, an Epstein-Barr virus (EBV) infection, or a cytomegalovirus (CMV) infection, among many others. A Bi-Fc can be administered by itself or can be administered concurrently with, before, or after administration of other therapeutics used to treat such infectious diseases.

A Bi-Fc can find further use in other kinds of conditions where it is beneficial to deplete certain cell types. For example, depletion of human eosinophils in asthma, excess human B cells in systemic lupus erythematosus, excess human Th2 T cells in autoimmune conditions, or pathogen-infected cells in infectious diseases can be beneficial. In a fibrotic condition, it can be useful to deplete cells forming fibrotic tissue. A Bi-Fc can be administered by itself or can be administered concurrently with, before, or after administration of other therapeutics used to treat such diseases.

Therapeutically effective doses of a Bi-Fc can be administered. The amount of Bi-Fc that constitutes a therapeutically dose may vary with the indication treated, the weight of the patient, the calculated skin surface area of the patient. Dosing of a Bi-Fc can be adjusted to achieve the desired effects. In many cases, repeated dosing may be required. For example, a Bi-Fc can be dosed twice per week, once per week, once every two, three, four, five, six, seven, eight, nine, or ten weeks, or once every two, three, four, five, or six months. The amount of a Bi-Fc administered on each day can be from about 0.0036 mg to about 450 mg. Alternatively, the dose can be calibrated according to the estimated skin surface of a patient, and each dose can be from about 0.002 mg/m<sup>2</sup> to about 250 mg/m<sup>2</sup>. In another alternative, the dose can be calibrated according to a patient's weight, and each dose can be from about 0.000051 mg/kg to about 6.4 mg/kg.

A Bi-Fc, or a pharmaceutical composition containing such a molecule, can be administered by any feasible method. Protein therapeutics will ordinarily be administered by a parenteral route, for example by injection, since oral administration, in the absence of some special formulation or circumstance, would lead to fragmentation and/or hydrolysis of the protein in the acid environment of the stomach. Subcutaneous, intramuscular, intravenous, intraarterial, intralesional, or peritoneal bolus injection are possible routes of administration. A Bi-Fc can also be administered via infusion, for example intravenous or subcutaneous infusion. Topical administration is also possible, especially for diseases involving the skin.

Alternatively, a Bi-Fc can be administered through contact with a mucus membrane, for example by intra-nasal, sublingual, vaginal, or rectal administration or administration as an inhalant. Alternatively, certain appropriate pharmaceutical compositions comprising a Bi-Fc can be administered orally.

Having described the invention in general terms above, the following examples are offered by way of illustration and not limitation.

## Examples

### ***Example 1: Construction of anti-HER2 CD3 $\epsilon$ and anti-FOLR1/ CD3 $\epsilon$ Bi-Fc molecules and single chain bispecific molecules***

Bi-Fc molecules were generated using methods essentially described previously. Löffler *et al.* (2000), *Blood* 95(6): 2098-2103. In more detail, a construct encoding a heterodimeric anti-HER2/CD3 $\epsilon$  Bi-Fc was made as follows. DNA fragments encoding the VH region (SEQ ID NO:5) and the VL region (SEQ ID NO:6) of an anti-HER2 IgG antibody and the VH region (SEQ ID NO:7) and VL region (SEQ ID NO:8) of anti-human CD3 $\epsilon$  IgG antibody were amplified by PCR using forward and reverse primers and spliced together with flexible linkers. The resulting DNA fragment, which encodes a linear fusion DNA encoding two scFv's joined by a linker is referred to herein as the single chain anti-HER2/CD3 $\epsilon$  (SEQ ID NO:9). This construct was subcloned into a mammalian expression vector for antibody production.

A heterodimeric anti-HER2/CD3 $\epsilon$  Bi-Fc (SEQ ID NO:10) was constructed by fusing DNA encoding the single chain anti-HER2/CD3 $\epsilon$  to DNA encoding one of the

two chains of an engineered human IgG1 Fc region. Specifically, DNA encoding an Fc polypeptide chain containing two positively charged mutations (D356K/D399K, EU numbering) plus alterations that inhibit FcγR binding (L234A and L235A) was fused to the DNA encoding the single chain anti-HER2/CD3ε at the 3' end. The amino acid sequence of this anti-HER/CD3ε Bi-Fc and the nucleic acid sequence encoding it are shown in SEQ ID NO:10 and 11, respectively. The second polypeptide chain that was part of the anti-HER2/CD3ε Bi-Fc was a human IgG1 Fc polypeptide chain containing two negatively charged mutations (K392D/K409D, EU numbering) plus L234A and L235A, as shown in SEQ ID NO:12. DNA encoding this polypeptide (SEQ ID NO:13) was amplified and inserted into an appropriate vector for expression. Using similar methods, a single chain anti-FOLR1/CD3ε (SEQ ID NO: 14) and a heterodimeric anti-FOLR1/CD3ε Bi-Fc (SEQ ID NO:15) were constructed by replacing DNA encoding the anti-HER2 scFv fragment with DNA encoding an scFv fragment derived from an anti-human FOLR1 IgG antibody.

All single chain and heterodimeric Bi-Fc molecules described above were produced by transient transfection in human HEK 293-6E cells. The culture media was harvested after 6 days. The single chain anti-HER2/CD3ε and anti-FOLR1/CD3ε molecules were purified by nickel HISTRAP® (GE Healthcare Bio-Sciences, L.L.C., Uppsala, Sweden) column chromatography and eluted with a 25 to 300 mM imidazole gradient. The elution pools were further purified by size exchange chromatography (SEC) using a preparative SUPERDEX® 200 (GE Healthcare Bio-Sciences, L.L.C., Uppsala, Sweden) column, concentrated to > 1 mg/mL, and stored at -70 °C. Heterodimeric anti-HER2/CD3ε Bi-Fc and anti-FOLR1/CD3ε Bi-Fc molecules were purified using MABSELECT SURE™ (GE Healthcare Bio-Sciences, L.L.C., Uppsala, Sweden) affinity chromatography, eluting with 50 mM citrate, 1M L-Arginine, pH 3.5. The eluate was buffer-exchanged into formulation buffer by a preparative SEC with 10 mM potassium phosphate, 161 mM L-Arginine, pH 7.6 or with a solution containing acetate and sucrose with 150 mM NaCl, 161 mM L-Arginine, pH 5.2

***Example 2: Testing BiTE:Fc molecules for binding to target cells and immune effector cells***

Binding of the heterodimeric anti-HER2/CD3ε Bi-Fc and single chain anti-HER2/CD3ε to T cells expressing CD3 and JIMT-1 cells expressing HER2 was assessed

as follows. Human pan-T cells (purified using Pan T Cell Isolation Kit II, human, Miltenyi Biotec, Auburn, CA) or purified JIMT-1 cells were incubated for 16 hrs at 4 °C in the absence or presence of 10 µg/mL of the heterodimeric anti-HER2/CD3ε Bi-Fc or the single chain anti-HER2/CD3ε. Cell binding of the heterodimeric anti-

5 HER2/CD3ε Bi-Fc was detected using an allophycocyanin (APC)-labeled anti-human Fc secondary antibody. The single chain anti-HER2/CD3ε, which includes a FLAG<sup>®</sup> tag, was detected using a mouse anti-FLAG<sup>®</sup> antibody followed by an APC-labeled mouse Ig-specific antibody.

10 In the fluorescence-activated cell sorting (FACS) histograms shown in Figure 2, the unfilled profiles represent data from cells in the absence of one of the bispecific molecules, and the solidly filled profiles represent data from cells in the presence of one of the bispecific molecules, as indicated in Figure 2 and its description. These results indicate that the heterodimeric anti-HER2/CD3ε Bi-Fc, as well as the single chain anti-HER2/CD3ε, binds to both T cells (expressing CD3ε) and to JIMT-1 cells  
15 expressing HER2.

### ***Example 3: Lysis of tumor cell lines in the presence of Bi-Fc's and T cells***

The heterodimeric anti-HER2/CD3ε and anti-FOLR1/CD3ε Bi-Fc's and the single chain anti-HER2/CD3ε and anti-FOLR1/CD3ε molecules described above were  
20 assayed to determine their activity in a T cell-dependent cell cytotoxicity (TDCC) assay using tumor cells expressing HER2 or FOLR1 as target cells. Briefly, pan T cells were isolated from healthy human donors using the Pan T Cell Isolation Kit II, human (Miltenyi Biotec, Auburn, CA). The T cells were incubated with CFSE-labeled tumor target cells at a ratio of 10:1 in the presence or absence of the heterodimeric anti-  
25 HER2/CD3ε or anti- FOLR1/CD3ε Bi-Fc or the single chain anti-HER2/CD3ε or anti-FOR1/CD3ε described in Example 1 at the varying concentrations as indicated in Figures 3 and 4. As a control, some samples contained T cells and tumor target cells, but no Bi-Fc or single chain molecule.

30 The target cells for the anti-FOLR1/CD3ε heterodimeric Bi-Fc and single chain molecule were either Cal-51 cells (expressing about 148,000 molecules of FOLR1 per cell), T47D cells (expressing about 101,000 molecules of FOLR1 per cell), or the control cell line BT474 (which did not express detectable levels of FOLR1).

The target cells for the anti-HER2/ CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecules were JIMT-1 cells (expressing about 181,000 molecules of HER2 per cell), T47D cells (expressing about 61,000 molecules of HER2 per cell), or the control cell line SHP77 (which did not express detectable amounts of HER2).

After 39 to 48 hours of incubation, cells were harvested, and the percent of tumor cell lysis was monitored by uptake of 7-amino-actinomycin D (7-AAD), which stains double-stranded nucleic acids. Intact cells exclude 7-AAD, whereas 7-AAD can penetrate the membranes of dead or dying cells and stain the double-stranded nucleic acids inside these cells. Percent specific lysis was calculated according to the following formula:

$$\% \text{ specific lysis} = \left[ \frac{\% \text{ tumor lysis with Bi-Fc} - \% \text{ tumor cell lysis without bispecific}}{\% \text{ of total cell lysis} - \% \text{ tumor cell lysis without bispecific}} \right] \times 100$$

To determine percent total cell lysis, samples containing immune effector and labeled target cells without a Bi-Fc or single chain molecule were lysed with cold 80% methanol.

Results for the anti-FOLR1/CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecule are shown in Figure 3. Both the anti-FOLR1/CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecule exhibited dose dependent lysis of both the Cal-51 and the T47D target cells. The EC<sub>50</sub> for each of these molecules in each of these cell lines is shown in Table 3 below.

**Table 3: EC<sub>50</sub> of Bi-Fc and single chain anti-FOLR1/CD3 $\epsilon$  molecules**

Molecule	EC <sub>50</sub> (pM)		
	Cell Line		
	Cal-51	T47D	B7474
<b>Anti-FOLR1/CD3<math>\epsilon</math> Bi-Fc</b>	1.27	1.35	NA*
<b>Anti-FOLR1/CD3<math>\epsilon</math> single chain</b>	0.087	0.19	NA*

\*NA means that there was little or no cell lysis detected.

These data indicate that both the anti-FOLR1/CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecule can mediate lysis of cells expressing FOLR1 in the presence of T cells, but do not mediate lysis of cells not expressing FOLR1. The EC<sub>50</sub>'s of the Bi-Fc are about 7 to 15 fold higher than those of the single chain molecule, but they are still in the pM range. Thus, both the Bi-Fc and the single chain molecule are highly potent in this assay.

Results for the anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecule are shown in Figure 4. Both the anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecule exhibited dose dependent lysis of both the JIMT-1 and the T47D target cells, but no lysis of the control SHP77 cell line (which does not express HER2).

- 5 The EC<sub>50</sub> for each of these molecules in each of these cell lines is shown in Table 4 below.

**Table 4: EC<sub>50</sub> of Bi-Fc and single chain anti-HER2/CD3 $\epsilon$  molecules**

Molecule	EC <sub>50</sub> (pM)		
	Cell Line		
	JIMT-1	T47D	SHP77
<b>Anti-HER2/CD3<math>\epsilon</math> Bi-Fc</b>	11.52	1.03	NA*
<b>Anti-HER2/CD3<math>\epsilon</math> single chain</b>	1.12	0.12	NA*

\*NA means that there was little or no cell lysis detected.

- 10 These data indicate that both the anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc and single chain molecules can mediate lysis of cells expressing HER2 in the presence of T cells, but do not mediate lysis of cells not expressing HER2. The EC<sub>50</sub>'s of the Bi-Fc's are about 8.6 to 10.3 fold higher than those of the single chain molecule.

15 ***Example 4: Release of cytokines by T cells in the presence of Bi-Fc and target cells***

- The anti-HER2/CD3 $\epsilon$  single chain and heterodimeric Bi-Fc and the anti-FOLR1/CD3 $\epsilon$  single chain and heterodimeric Bi-Fc described above were assayed to determine whether they could stimulate the production of inflammatory cytokines by T cells. Briefly, twenty four hour cell culture supernatants from the TDCC assays like those described in Example 3 were assessed for cytokine concentrations using the Human TH1/TH2 7-Plex and Human Proinflammatory 14-Plex ultra Sensitive Kits from Meso Scale Diagnostics, L.L.C. Assays were performed according to the manufacturer's directions.

- 25 These results are shown in Figures 5A, 5B, 6A, and 6B. As shown in Figures 5A and 5B, the T cells secreted cytokines in the presence of the anti-FOLR1/CD3 $\epsilon$  heterodimeric Bi-Fc or single chain in the presence of cells expressing FOLR1 (T47D, left panels), but not in the presence of cells that did not express FOLR1 (BT474, right panels). Similarly, as shown in Figure 6A and 6B, T cells secreted cytokines in the

presence of the anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc or single chain in the presence of cells expressing HER2 (JIMT-1, left panels), but not in the presence of cells that did not express HER2 (SHP77). Thus, the secretion of interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-10 (IL-10), interleukin-2 (IL-2), and interleukin-13 (IL-13) by T cells in the presence of a heterodimeric Bi-Fc or a single chain molecule was dependent on the presence of cells expressing a target cell protein. Hence, activation of the T cells by the Bi-Fc's and single chain molecules was specific in the sense that it occurred only in the presence of target cells expressing a target cell protein.

In addition, the Bi-Fc's had very potent activity in the assay, exhibiting EC<sub>50</sub>'s in the pM range as shown in the table below.

**Table 5: EC<sub>50</sub>'s for eliciting cytokine secretion**

Cytokine	EC <sub>50</sub> (pM)			
	JIMT-1 cells		T47D cells	
	anti-HER2/CD3 $\epsilon$ Bi-Fc	anti-HER2/CD3 $\epsilon$ single chain	anti-FOLR1/CD3 $\epsilon$ Bi-Fc	anti-FOLR1/CD3 $\epsilon$ single chain
IFN- $\gamma$	32.9	2.1	48.6	7.5
TNF- $\alpha$	19.5	1.8	41.2	8.8
IL-10	9.6	0.9	110.1	18.4
IL-2	22.3	1.2	67.3	12.9
IL-13	16.4	1.8	126.9	28.1

Thus, even though the heterodimeric Bi-Fc is almost twice the size of the single chain molecule, it remains a very potent activator of cytokine secretion by T cells in the presence, but not in the absence of, target cells. In addition, the heterodimeric Bi-Fc and the single chain molecule show a very similar cytokine profile. The EC<sub>50</sub>'s for cytokine secretion induced by the anti-HER2/CD3 $\epsilon$  heterodimeric Bi-Fc were about 9 to 19 fold higher than those induced by the anti-HER2/CD3 $\epsilon$  single chain. The EC<sub>50</sub>'s for cytokine secretion induced by the anti-FOLR1/CD3 $\epsilon$  Bi-Fc were about 4.5 to 6.5 fold higher than those induced the anti-FOLR1/CD3 $\epsilon$  single chain.

***Example 5: Upregulation of T cell activation markers in the presence of Bi-Fc and target cells***

The following experiment was done to determine whether a heterodimeric Bi-Fc could activate T cells in the presence of peripheral blood mononuclear cells (PBMCs) and in the presence or absence of target cells. PBMCs from healthy donors

were purified on a FICOLL™ gradient from human leukocytes purchased from Biological Specialty Corporation of Colmar, Pennsylvania. These PBMCs were incubated with the heterodimeric anti-HER2/ CD3ε Bi-Fc or the single chain anti-HER2/CD3ε bispecific molecule described above in the presence or absence of JIMT-1 cells at a 10:1 ratio. After 48 hours of incubation, non-adherent cells were removed from the wells and divided into two equal samples. All samples were stained with fluorescein isothiocyanate (FITC)-conjugated anti-human CD3 antibody plus an allophycocyanin (APC)-conjugated anti-CD25 or anti-CD69 antibody. CD25 and CD69 are markers of activation of T cells.

Up-regulation of CD25 and CD69 (Figure 7) activation markers by CD3<sup>+</sup> peripheral T cells was observed with the heterodimeric anti-HER2/CD3ε Bi-Fc and the anti-HER2/CD3ε single chain in the presence, but not in the absence, of HER2-expressing JIMT-1 tumor target cells. These observations suggest that T cell activation by the Bi-Fc is dependent on the presence of tumor target cells expressing the target cell protein. An alternate potential path to T cell activation, that is, cross-linking by FcγR's in the presence of a Bi-Fc such as the anti-HER2/CD3ε Bi-Fc, likely is not responsible for the observed effects because the Fc region of the anti-HER2/CD3ε Bi-Fc contains alterations that inhibit binding to FcγRs and because activation of T cells is not observed in the absence of target cells expressing HER2.

#### ***Example 6: Pharmacokinetic properties of Bi-Fc's***

In the following experiment, the single dose pharmacokinetic profiles of a heterodimeric anti-HER2/CD3ε Bi-Fc (comprising the amino acid sequences of SEQ ID NOs:10 and 12) and an anti-HER2/CD3ε single chain (comprising the amino acid sequence of SEQ ID NO:9) was assessed by intravenous and subcutaneous bolus administration in male NOD.SCID mice (Harlan, Livermore, CA). These test molecules were injected as a bolus at 1 mg/kg intravenously via the lateral tail vein in some mice or subcutaneously under the skin over the shoulders in others. Serial bleeds of approximately 0.1 mL of whole blood were collected at each time point via retro-orbital sinus puncture. Upon clotting of whole blood the samples were processed to obtain serum (~0.040 mL per sample). Serum samples were analyzed by immunoassay using the technology Gyros AB (Warren, NJ) to determine the serum concentrations of the anti-HER2/CD3ε single chain and Bi-Fc. Serum samples were



collected at 0, 0.5, 2, 8, 24, 72, 120, 168, 240, 312, 384, and 480 hours. Serum samples were maintained at -70°C ( $\pm 10^\circ\text{C}$ ) prior to analysis. Pharmacokinetic parameters were estimated from serum concentrations using non-compartmental analysis using Phoenix<sup>®</sup> 6.3 software (Pharsight, Sunnyvale, CA).

5        The single dose pharmacokinetic profiles of the heterodimeric Bi-Fc and the single chain molecule are shown in Figure 8. The Bi-Fc showed an extended serum half life (219 hours) compared to the single chain molecule, which was rapidly eliminated and had a half life of only 5 hours. Exposure of the Bi-Fc was characterized by an area under the curve (AUC) of 524 hr\* $\mu\text{g/mL}$ , as compared to 19  
10 hr\* $\mu\text{g/mL}$  for the single chain molecule. The subcutaneous bioavailability of the Bi-Fc was 83%, while that of the single chain molecule was 29%. Thus, the heterodimeric Bi-Fc showed favorable single dose pharmacokinetic properties as compared to the single chain molecule.

15    ***Example 7: Construction of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc***

A monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc was constructed, the overall structure of which is represented by the second diagram from the left in Figure 1. Monomeric Fc polypeptide chains, containing specific alterations relative to a naturally occurring Fc polypeptide chain, are described in US Patent Application Publication 2012/0244578,  
20 the relevant portions of which are incorporated herein by reference. Starting with a vector encoding a human monomeric IgG1 Fc polypeptide chain (which lacked a hinge region, *i.e.*, started at position 231 in the EU numbering system, and had a carboxyterminal hexa-histidine tag plus the alterations Y349T, K392D, and K409D), further mutations were introduced using Agilent's Quikchange Site-Directed  
25 Mutagenesis Kit (catalog number 200518-5) that specified the alteration N297G. Thus, the final Fc polypeptide chain began with the alanine at position 231 and continued through to the lysine at position 447, which was followed by a hexa-histidine tag (SEQ ID NO:92). It contained the following alterations: N297G, Y349T, K392D, and K409D.

30        DNA encoding an anti-CD33/CD3 $\epsilon$  single chain molecule was amplified by PCR from a second vector encoding a single chain molecule containing heavy and light chain variable regions binding to CD33 followed by heavy and light chain variable regions binding to CD3 $\epsilon$ . The amino acid sequence of this anti-CD33/CD3 $\epsilon$

single chain is given in SEQ ID NO:33, and it is described in detail in US Patent Application 2012/244162, the relevant portions of which are incorporated herein by reference. This DNA was attached to DNA encoding the altered Fc polypeptide chain described above using splice overhang extension by polymerase chain reaction (SOE  
5 by PCR). *See, e.g.,* Warrens *et al.* (1997), Gene 186: 29-35, the portions of which describe this method are incorporated herein by reference.

The amino acid sequence of the resulting monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc is provided in SEQ NO NO:34, and the nucleic acid sequence encoding it is provided in SEQ ID NO:35. DNA encoding the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc was introduced into  
10 mammalian cells, which were cultured under conditions suitable for expression. The protein was recovered from the cell supernatant.

***Example 8: Binding of anti-CD33/CD3 $\epsilon$  Bi-Fc to cells expressing CD3 $\epsilon$  or CD33***

Binding of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$   
15 single chain to cells expressing CD3 $\epsilon$ , CD33, or neither was assessed. Molm-13 cells (expressing CD33), Namalwa cells (expressing neither CD33 nor CD3 $\epsilon$ ), purified human pan-T cells (expressing CD3 $\epsilon$ ), human PBMCs (expressing CD3 $\epsilon$ ), and cynomolgus PBMCs (expressing CD3 $\epsilon$ ) were tested. Cells were incubated for 2 hours at 4 °C in the absence or presence of the bispecific molecules. Cell binding of  
20 the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$  single chain were then detected by incubating the cells with a mouse antibody that binds to the CD3-binding regions of the bispecific molecules at 10  $\mu$ g/mL overnight at 4 °C, followed by an APC-labeled anti-mouse Fc secondary antibody (Jackson 115-136-071) at 5  $\mu$ g/mL for 2 hrs at 4 °C. The cells were analyzed by FACS, and the mean fluorescent  
25 intensity (MFI) of the signal was determined.

Figure 9 shows the MFI detected for the various cell types in the presence of various concentrations of the bispecific molecules as follows: panel A, Molm-13 cells (expressing CD33); panel B, Namalwa cells (expressing neither CD33 nor CD3 $\epsilon$ ); panel C, human pan-T cells (expressing CD3 $\epsilon$ ); panel D, human PBMCs (expressing  
30 CD3 $\epsilon$ ); and panel E, cynomolgus PBMCs (expressing CD3 $\epsilon$ ). The results demonstrate that these two bispecific molecules bind to these cells types in a similar manner, indicating that the addition of an Fc polypeptide chain to the anti-

CD33/CD3 $\epsilon$  single chain did not detectably affect its ability to bind to CD33 and CD3 $\epsilon$  as measured by this assay.

**Example 9: Lysis of CD33-expressing tumor cells in the presence of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc**

The following experiments were done to determine whether the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc described above could induce lysis of CD33-expressing tumor cells in the presence of peripheral blood mononuclear cells (PBMCs). PBMC effector cells from cynomolgus monkeys were obtained from SNBL USA (a subsidiary of Shin Nippon Biomedical Laboratories). In this preparation of PBMCs, 61% were CD3 $^{+}$  T cells (data not shown). These PBMCs were incubated with carboxyfluorescein succinimidyl ester (CFSE)-labeled tumor target cells at a ratio of 10:1 in the presence and absence of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain at the concentrations indicated in Figure 10. Following 40-48 hours of incubation at 37 °C, cells were harvested, and live and dead tumor cells were monitored by 7AAD uptake using flow cytometry. Percent specific lysis was calculated according to the following formula:

$$\% \text{ specific lysis} = 1 - (\text{live cell counts (with bispecific)} / \text{live cell counts (without bispecific)}) \times 100$$

These results are shown in Figure 10. Data shown in Figure 10, panel A indicate that Molm-13 cells, which express about 33,000 molecules of CD33 per cell, were lysed with both the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$  single chain. The concentrations for half maximal lysis (EC $_{50}$ 's) were in the pM range, that is. 1.45 pM and 0.96 pM for the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$  single chain, respectively. Thus, the EC $_{50}$  of the monomeric Bi-Fc was less than two fold higher than that of the single chain molecule. Data shown in Figure 10, panel B indicate that there was no lysis of Namalwa cells, which do not express detectable levels of CD33. These observations suggest that the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc is a highly specific and potent reagent capable of inducing tumor cell lysis by cynomolgus monkey PBMCs.

In a second experiment, pan T effector cells isolated from human healthy donors were incubated with CFSE-labeled Molm-13 or Namalwa cells at a ratio of 10:1 in the presence and absence of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain at the concentrations indicated in Figure 11.

- 5 Following 40-48 hours of incubation at 37 °C, cells were harvested, and live and dead tumor cells were monitored by 7AAD uptake using flow cytometry. Percent specific lysis was calculated according to the formula given above in this example. Results are shown in Figure 11.

Specific lysis of Molm-13 cells was observed with both the monomeric anti-  
10 CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$  single chain. Figure 11, panel A. The EC<sub>50</sub>'s were in the pM range, that is, 0.65 pM and 0.12 pM for the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$  single chain, respectively. Hence, the EC<sub>50</sub> for the Bi-Fc is 5 to 6 fold higher than that of the single chain molecule. There was  
15 no lysis of Namalwa cells detected with either bispecific molecule except for a small amount of lysis detected at the highest concentration of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc tested. Figure 11, panel. B. No lysis of Molm-13 cells occurred in the absence of T cells (data not shown). These observations suggest that the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc, like the heterodimeric Bi-Fc's described above, is a highly specific and potent reagent capable of inducing tumor cell lysis by T cells.

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***Example 10: Lysis of CD33-expressing tumor cells and release of interferon gamma from PBMCs in the presence of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc***

In another experiment, PBMCs isolated from healthy human donors or cynomolgus monkeys (obtained from SNBL USA) were tested for their ability to lyse  
25 tumor target cells expressing CD33. In these preparations, the PBMCs were 42% CD3<sup>+</sup> T cells (human) and 30% CD3<sup>+</sup> T cells (cynomolgus monkey). PBMCs were incubated at 37°C with CFSE-labeled tumor target cells at a ratio of 5:1 in the presence and absence of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain at the concentrations indicated in Figure 12. Following  
30 hours of incubation at 37 °C, cells were harvested, and live and dead tumor cells were monitored by 7AAD uptake using flow cytometry. Percent specific lysis was calculated according to the formula described above. Results are shown in Figure 12.

Specific lysis of Molm-13 cells was observed with both the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and the anti-CD33/CD3 $\epsilon$  single chain using human PBMCs (Figure 12, panel A) or cynomolgus PBMCs (Figure 12, panel B). The concentrations for half maximal lysis (EC<sub>50</sub>'s) were in the pM range, as shown in Table 6 below.

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**Table 6: EC<sub>50</sub>'s for lysis of Molm-13 cells by PBMCs in the presence of anti-CD33/CD3 $\epsilon$  bispecific molecules**

Anti-CD33/CD3 $\epsilon$ bispecific	EC <sub>50</sub> (pM) for lysis of Molm-13 cells	
	Human PBMCs	Cynomolgus monkey PBMCs
Monomeric Bi-Fc	0.68	3.55
Single chain	0.14	1.39

Molm-13 cells were not lysed in the presence of either of the bispecifics in the absence of T cells (data not shown). These data show that the EC<sub>50</sub>'s for the monomeric Bi-Fc are in the sub-picomolar to low picomolar range and are very close to the EC<sub>50</sub>'s of the single chain molecule using both human and cynomolgus monkey PBMCs as the effector cells.

Twenty four hour cell culture supernatants from the cell lysis assays described immediately above were assessed for cytokine concentrations using the commercially available BD OptEIA™ Human IFN- $\gamma$  ELISA Kit II (BD Biosciences) and the Monkey Interferon gamma ELISA Kit (Cell Sciences). The assays were performed according to the manufacturer's directions. In the presence of Molm-13 cells, IFN- $\gamma$  was released from human (Figure 13, panel A) and cynomolgus monkey (Figure 13, panel B) PBMCs treated with the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain. These results suggest that the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc, like the anti-CD33/CD3 $\epsilon$  single chain, is a highly specific and potent reagent capable of mediating release of interferon gamma from PBMCs.

**Example 11: Induction of T cell proliferation, CD25 expression, and cytokine release by monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc**

Pan T effector cells isolated from human healthy donors were labeled with CFSE and incubated with tumor target cells, either Molm-13 or Namalwa cells, at a ratio of 10:1 in the presence and absence of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain at the concentrations indicated in Figures 14 and

15. Following 72 hours of incubation at 37 °C, cells were harvested, and T cell proliferation and expression of CD25, a marker for activation, were analyzed by flow cytometry.

Proliferation was assessed by monitoring the numbers of cells with a  
5 decreased fluorescent signal from the CFSE dye. With each cell division following labeling of the T cells with CFSE, the intensity of the fluorescent signal from the CFSE for each individual dividing cell decreases. The percent proliferating T cells was determined by gating on CFSE-labeled T cells and comparing the number of mitotic T cells, *i.e.*, cells having a diminished fluorescent signal, with the total number of T  
10 cells. The percent CD25 positive T cells was determined by staining the cells in the co-culture with an allophycocyanin (APC)-labeled anti-human CD25 antibody and measuring the APC levels of CFSE-labeled cells using two-color flow cytometry.

Proliferation of T cells was observed in the presence of the CD33-expressing tumor cell line, Molm-13, plus either the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the  
15 anti-CD33/CD3 $\epsilon$  single chain. Figure 14, panel A. The EC<sub>50</sub>'s were in the single digit pM range, that is, 4.27 pM in the presence of the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc and 1.09 pM in the presence of the anti-CD33/CD3 $\epsilon$  single chain. No proliferation of T cells was observed in the presence Namalwa cells, which do not express detectable levels of CD33. Figure 14, panel A.

20 T cells in the presence of Molm-13 cells and either the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain expressed the activation marker, CD25. Figure 14, panel B. T cells in the presence of Namalwa cells (which do not express detectable levels of CD33) and either of the bispecifics did not express CD25. Figure 14, panel B. These observations suggest that the monomeric anti-  
25 CD33/CD3 $\epsilon$  Bi-Fc is capable of specifically inducing T activation and proliferation.

Twenty four hour cell culture supernatants from assays described immediately above were assessed for cytokine concentrations using the commercially available Human TH1/TH2 7-Plex and Human Proinflammatory 14-Plex Ultra-Sensitive Kits from Meso Scale Diagnostics, LLC. The assays were performed according to the  
30 manufacturer's directions. Results are shown in Figure 15.

In the presence of the CD33-expressing Molm-13 tumor cell line, interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-10 (IL-10), interleukin-2 (IL-2), and interleukin-13 (IL-13) were released from T cells treated with

the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the anti-CD33/CD3 $\epsilon$  single chain as shown in Figure 15, panels A, B, C, D, and E, respectively. The highest cytokine concentrations were seen with IFN- $\gamma$ , TNF- $\alpha$ , IL-2 and IL-10 (greater than 400 pg/mL). Moderate levels of IL-13 were also observed. Cytokine secretion was not observed in the presence of the CD33-negative cell line, Namalwa. In Table 7 below, the EC<sub>50</sub>'s for the production of the various cytokines by Molm-13 cells in the presence of either the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc or the single chain anti-CD33/CD3 $\epsilon$  are shown.

**Table 7: EC<sub>50</sub>'s for cytokine production**

	EC <sub>50</sub> (pM) Molm-13 tumor cell line	
	Monomeric anti-CD33/CD3 $\epsilon$ Bi-Fc	Single chain anti-CD33/CD3 $\epsilon$
IFN- $\gamma$	9.5	2.6
TNF- $\alpha$	6.1	1.7
IL-10	6.4	1.1
IL-2	10.3	5.2
IL-13	8.4	1.3

These results suggest that the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc is a highly specific and potent scaffold capable of mediating cytokine release by T cells.

***Example 12: Cytolytic synapse formation in the presence of an anti-HER2/CD3 $\epsilon$  single chain bispecific or an anti-HER2/CD3 $\epsilon$  Bi-Fc***

The anti-HER2/CD3 $\epsilon$  single chain bispecific and HER2/CD3 $\epsilon$  Bi-Fc described in Example 1 were assayed to determine their ability to induce cytolytic synapse formation between T cells and HER2-expressing JIMT-1 tumor cells. JIMT-1 cells were distributed into 24-well poly-L-lysine-coated glass bottom culture plates (0.5 x 10<sup>6</sup> cells/well in RPMI medium with 1% FCS and 2 g/L glucose). Following 1 hr incubation at 37 °C, JIMT-1 cells adhering to the glass wells were gently washed with warm DPBS. Freshly isolated CD8<sup>+</sup> T cells (1 x 10<sup>6</sup> cells per well from healthy donors) with or without 1 nM anti-HER2/CD3 $\epsilon$  single chain bispecific or anti-HER2/CD3 $\epsilon$  Bi-Fc were added to the tumor cells and allowed to incubate for an additional 20 minutes at 37 °C to generate cytolytic synapses. Cells adhering to the plate were washed with pre-warmed DPBS and immediately fixed with 3.7% paraformaldehyde for 10 minutes.

The cells were then washed with DPBS and permeabilized with 0.1% titron X-100 for 5 minutes at room temperature. A mixture of primary antibodies (5 µg/mL anti-PKCθ and 0.4 µg/mL anti-CD45) was incubated with cells overnight at 4 °C and then washed 3 times. A mixture of 8 µg/mL secondary antibodies (green for anti-CD45 and red for anti- PKCθ) were added for 3 hours at room temperature, and the plates were then washed 2X with DPBS. PCKθ is known to localize to immune synapses, while CD45 is expressed on the surface of T cells. SLOWFADE® Gold antifade reagent with DAPI (nuclear stain) (Life Technologies #536939) was added directly to glass wells and plates stored at -70 °C protected from light.

Immunofluorescence confocal microscopy showed that CD45 (green staining) was present on the surface of T cells (identified as the smaller cell type with green CD45 staining), while PKCθ (red staining) gave a focused signal at the site of synapse formation between tumor cells (identified as the larger cell type) and T cells. Cytolytic synapses between the T cells and tumor targets were observed with the anti-HER2/CD3ε single chain bispecific and anti-HER2/CD3ε Bi-Fc, but were not observed in the absence of a bispecific (data not shown). These observations suggest that cytolytic synapse formation is dependent on the presence a bispecific molecule, and the Bi-Fc can form synapses similar to those seen with the single chain bispecific molecule.

***Example 13: In vivo effects of a heterodimeric anti-FOLR1/CD3ε Bi-Fc on tumor growth***

The experiment described below demonstrates the activity of heterodimeric Bi-Fc bispecific antibody in an *in vivo* cancer model system, using FOLR1-expressing NCI-N87-luc, human gastric carcinoma cells. Although these cells do express luciferase, which can enable tumor detection by luminescence, tumor growth was monitored by physical measurement of the tumors in this experiment. NCI-N87-luc cells ( $3 \times 10^6$ ) in 50% matrigel were implanted subcutaneously into 8 week old female NOD scid gamma (NSG) mice (day 0). On day 10,  $20 \times 10^6$  activated human Pan-T cells were administered by intraperitoneal injection into each mouse. The human Pan-T cells engrafted into the mice were pre-activated and expanded using anti-CD3/CD28/CD2 antibodies on days 0 and 14 of an 18 day culture using Miltenyi T cell activation/expansion kit according to the manufacturer's directions. On day 11



and day 18, an FcγR block consisting of 10 mg/mouse GAMMAGARD [Immune Globulin Infusion (Human)] 10% (Baxter) plus 0.2 mg/mouse anti-mu FcγRII/III (clone 2.4G2) was administered IP. One hour following the first FcγR block, animals (N=10/group) received either (1) daily intraperitoneal injections of 0.05 mg/kg of an anti-FOLR1/anti-CD3ε single chain molecule (comprising the amino acid sequence of SEQ ID NO:90) or (2) two intraperitoneal injections, spaced 5 days apart of 1 mg/kg of a heterodimeric anti-FOLR1/anti-CD3ε Bi-Fc (comprising the amino acid sequences of SEQ ID NOs:86 and 88), or 25 mM Lysine-hydrochloride, 0.002% Tween 80 in 0.9% NaCl, pH 7.0 (vehicle control). Tumor volumes were measured, and animals were euthanized when their tumor reached 2000 mm<sup>3</sup> or at the end of the study (day 27).

In vehicle-treated mice, tumors grew in all the animals tested. See Figure 16. In contrast, tumor growth was significantly inhibited in the mice that were treated with the single chain anti-FOLR1/CD3ε bispecific or the heterodimeric anti-FOLR1/CD3ε Bi-Fc (p<0.0001 when compared to vehicle-treated mice). Throughout the experiment, there were no significant changes in body weight of treated or untreated mice (data not shown). These data indicate that the anti-FOLR1/anti-CD3ε heterodimeric Bi-Fc can induce T cell-mediated killing of target cells *in vivo*.

**Example 14: Comparison of the *in vivo* effects of monomeric and heterodimeric anti-CD33/CD3 Bi-Fc's on tumor growth**

The following experiment was aimed at determining whether a monomeric Bi-Fc could kill tumor cells *in vivo*. Human pan-T cells were pre-activated and expanded in culture for use in this experiment by addition of anti-CD3/CD28/CD2 antibodies on days 0 and 14 of an 18-day culture period using a Miltenyi T cell activation/expansion kit according to the manufacturer's directions. Molm-13-luc cells (1 × 10<sup>6</sup>), which are CD33-expressing tumor cells that luminesce in the presence of D-luciferin, were injected subcutaneously (SC) into the right flank of 10 week old female NSG mice (day 0). On the third day following tumor cell inoculation, 20 × 10<sup>6</sup> of the activated human pan-T cells were administered to each mouse by IP injection. On days 4 and 11, an FcγR block as described in Example 13 was administered by IP injection. One hour following the day 4 FcγR block, the mice (N=8/group) received

one of the following treatments: (1) daily intraperitoneal injections of either 0.05 mg/kg of the anti-CD33/CD3 $\epsilon$  single chain bispecific (having the amino acid sequence of SEQ ID NO:33), 0.05 mg/kg of an anti-MEC/CD3 $\epsilon$  single chain bispecific (having the amino acid sequence of SEQ ID NO:78; a negative control), 0.05 mg/kg of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc (having the amino acid sequence of SEQ ID NO:34), or 25 mM lysine-hydrochloride, 0.002% Tween 80 in 0.9% NaCl, pH 7.0 (a vehicle control) for 10 days; or (2) two IP injections, spaced 5 days apart of 1 mg/kg anti-CD33/CD3 $\epsilon$  heterodimeric Bi-Fc (comprising the amino acid sequences of SEQ ID NOs:80 and 82).

Bioluminescent imaging was performed on Monday, Wednesday, and Friday for two weeks after dosing began with an IVIS<sup>®</sup>-200 In Vivo Imaging System (Perkin Elmer). Nine minutes before imaging, mice were given 150 mg/kg D-luciferin by IP injection. Images were collected and analyzed using LIVING IMAGE<sup>®</sup> software 2.5 (Caliper Life Sciences). Naïve animals (animals not inoculated with Molm-13-luc or human pan-T cells) were used as to measure baseline bioluminescence.

Mice that received vehicle or the anti-MEC/CD3 $\epsilon$  single chain experienced tumor growth throughout the study, and naïve control mice that received no tumor cells did not exhibit appreciable tumor cell growth. Figure 17. Mice that received either the anti-CD33/CD3 $\epsilon$  heterodimeric Bi-Fc or the single chain molecule initially experienced tumor growth, although tumor cell luminescence approximately equal to levels seen in naïve mice were observed in these groups by the end of the study. Mice that received the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc also experienced initial tumor growth followed by tumor cell luminescence that was intermediate between that observed in vehicle-treated mice and that observed in naïve mice by the end of the study. Figure 17. The difference between tumor growth in mice treated with vehicle versus those treated with the monomeric Bi-Fc was statistically significant ( $p < .0001$ ). Thus, the monomeric Bi-Fc did elicit tumor cell killing *in vivo*.

***Example 15: Effect of the Fc alteration N297G on the *in vivo* anti-tumor efficacy of a monomeric anti-CD33/CD3 Bi-Fc***

The following experiment compared the *in vivo* activity of a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc that had the N297G alteration in the Fc polypeptide chain portion of the Bi-Fc to the activity of one that had the wild type N297. Methods are

essentially the same as those described in Example 14. Molm-13-luc cells ( $1 \times 10^6$ ) were injected subcutaneously (SC) into the right flank of 10 week old female NSG mice (day 0), and  $20 \times 10^6$  pre-activated human pan-T cells were administered to each mouse by IP injection on day 3. On days 4 and 11, an Fc $\gamma$ R block as described in Example 13 was administered. One hour following the day 4 Fc $\gamma$ R block, the mice (N=10/group) received daily IP injections of one of the following: vehicle (25 mM lysine-hydrochloride, 0.002% Tween 80 in 0.9% NaCl, pH 7.0); a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc comprising the amino acid sequence of SEQ ID NO:34 (which has the N297G alteration); or a monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc comprising the amino acid sequence of SEQ ID NO:84 (which has the wild type N297). Naïve control mice did not receive an injection of tumor cells and received no treatment injections. Results are shown in Figure 18.

Vehicle-treated mice exhibited tumor growth. Small but significant ( $p < 0.0005$ ) differences in tumor growth existed between vehicle-treated mice and mice treated with the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc having the N297G alteration. Figure 18. Mice treated with the monomeric anti-CD33/CD3 $\epsilon$  Bi-Fc with the wild type N297 had significantly ( $p < 0.0001$ ) lower levels of tumor bioluminescence by the end of the study than vehicle-treated mice. Naïve mice had, as expected, low levels of bioluminescence. Figure 18. At the least, these results suggest that a monomeric Bi-Fc having the wild type N297 is as active, if not more active, than one having N297G in an *in vivo* tumor cell killing assay.

What is claimed is:

1. A Bi-Fc, which is monomeric and comprises

(a) a polypeptide chain comprising an amino acid sequence having the following formula: V1-L1-V2-L2-V3-L3-V4-L4-Fc; wherein Fc is a human IgG Fc polypeptide chain which is C-terminal relative to the four immunoglobulin variable regions;

wherein two of V1, V2, V3, and V4 are immunoglobulin heavy chain variable (VH) regions and the other two are immunoglobulin light chain variable (VL) regions;

wherein either V1 is a VH region and V2 is a VL region or vice versa and either V3 is a VH region and V4 is a VL region or vice versa;

wherein L1, L2, L3, and L4 are linkers; and wherein L2 is present and wherein L2 is not more than 12 amino acids long;

wherein L1 and L3 are each at least 15 amino acids long; and

wherein L4 can be present or absent; or

(b) a polypeptide chain comprising an amino acid sequence having the following formula: Fc-L4-V1-L1-V2-L2-V3-L3-V4; wherein Fc is a human IgG Fc polypeptide chain which is N-terminal relative to the four immunoglobulin variable regions;

wherein either V1 is a VH region and V2 is a VL region or vice versa and either V3 is a VH region and V4 is a VL region or vice versa;

wherein two of V1, V2, V3, and V4 are VH regions and the other two are VL regions; wherein L1, L2, L3, and L4 are linkers; and wherein L2 is present and wherein L2 is not more than 12 amino acids long;

wherein L1 and L3 are each at least 15 amino acids long; and

wherein L4 can be present or absent;

wherein the Bi-Fc binds to a target cell and an immune effector cell and/or mediates cytotoxicity of a target cell by an immune effector cell,

wherein the Bi-Fc is a monomer, wherein the polypeptide chain targeting the effector cell binds to human and/or cynomolgus CD3ε; and wherein Fc polypeptide chain of (a) or (b) comprises one or more the following alterations: K392D, K392E, N392D, N392E, R409D, R409E, K409D, K409E, D399K, D399R, E356R, E356K, D356R, D356K, Y349T, L351T, L368T, L398T, F405T, Y407T, and Y407R.

2. The Bi-Fc of claim 1, wherein the Fc polypeptide chain of (a) or (b) is an IgG1, IgG2, or IgG4 Fc polypeptide chain and comprises the alterations K392D, K409D, and Y349T.

3. The Bi-Fc of claims 1 or 2, which binds to a cell expressing human HER2, human FOLR1 or human CD33.
4. The Bi-Fc of any one of claims 1 to 3, wherein the Fc polypeptide chain of the Bi-Fc comprises an insertion of the amino acid sequence of any of SEQ ID NOs: 36-47 between positions 384 and 385, wherein these position numbers are assigned according to the EU numbering scheme.
5. A Bi-Fc, which is heteromeric, comprising:
  - (a) (i) a first polypeptide chain comprising an amino acid sequence having the following formula: V1-L1-V2-L2-V3-L3-V4-L4-Fc; wherein Fc is a human IgG Fc polypeptide chain which is C-terminal relative to the four immunoglobulin variable regions; wherein V1, V2, V3, and V4 are each immunoglobulin variable regions; wherein L1, L2, L3, and L4 are linkers; and wherein L4 can be present or absent; and  
(ii) a second polypeptide chain that comprises a human IgG Fc polypeptide chain; or  
(b) (i) a first polypeptide chain having the following formula: Fc-L4-V1-L1-V2-L2-V3-L3-V4; wherein Fc is a human IgG Fc polypeptide chain which is N-terminal relative to the four immunoglobulin variable regions; wherein V1, V2, V3, and V4 are each immunoglobulin variable regions; wherein L1, L2, L3, and L4 are linkers; and wherein L4 can be present or absent; and  
(ii) a second polypeptide chain that comprises a human IgG Fc polypeptide chain;wherein the Bi-Fc binds to a target cell and an immune effector cell and/or mediates cytotoxicity of a target cell by an immune effector cell,  
wherein L1 and L3 are at least 15 amino acids long,  
wherein L2 is less than 12 amino acids long,  
wherein either V1 is a VH region and V2 is a VL region or vice versa,  
wherein either V3 is a VH region and V4 is a VL region or vice versa,  
wherein the Bi-Fc binds to human and/or cynomolgus CD3ε,  
wherein the first polypeptide chain comprises the charge pair substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D, or K392E, and the second polypeptide chain comprises the charge pair substitutions D399K or D399R and E356K, E356E, D356K, or D356R; or the second polypeptide chain comprises the charge pair substitutions R409D, R409E, K409D, or K409E and N392D, N392E, K392D, or K392E, and the first polypeptide chain comprises the charge pair substitutions D399K or D399R and E356K, E356E, D356K, or D356R.

6. The Bi-Fc of claim 5, which binds to a cell expressing human CD33, human FOLR1, or human HER2.
7. The Bi-Fc of claim 1 or 5, which comprises:
  - (a) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:60,  
a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:61,  
a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:62,  
a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:63,  
a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:64, and  
a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:65;
  - (b) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:66,  
a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:67,  
a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:68,  
a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:69,  
a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:70, and  
a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:71; or
  - (c) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:72;  
a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:73;  
a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:74;  
a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:75;  
a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:76; and  
a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:77.
8. The Bi-Fc of any one of claims 5 to 7, wherein the Fc polypeptide chains of the first and second polypeptide chains comprise one or more alteration that inhibits FcγR binding selected from the group consisting of: L234A, L235A, and any substitution at N297.
9. The Bi-Fc of any one of claims 5 to 8, wherein the Fc polypeptide chain(s) comprise(s) an insertion of the amino acid sequence of any of SEQ ID NOs:36-47 between positions 384 and 385 of each Fc polypeptide chain, wherein positions 384 and 385 are positions assigned according to the EU numbering scheme.
10. The Bi-Fc of claim 5, wherein V3 comprises the amino acid sequence of SEQ ID NO:7 or 29 and V4 comprises the amino acid sequence of SEQ ID NO:8 or 31.

11. The Bi-Fc of any one of claims 1 to 10, wherein the target cell is a cancer cell.
12. The Bi-Fc of any one of claims 1, 2, 4, 5 and 7-10, wherein the target cell is a cell infected by a pathogen or a cell that mediates disease.
13. The Bi-Fc of claim 12, wherein
  - (a) the pathogen is virus including human immunodeficiency virus, hepatitis virus, human papilloma virus, or cytomegalovirus, or a bacterium of the genus *Listeria*, *Mycobacterium*, *Staphylococcus*, or *Streptococcus*; or
  - (b) the cell that mediates disease is a fibrotic cell.
14. The Bi-Fc of any one of claims 1 to 13, wherein the Bi-Fc binds to an amino acid sequence within the first 27 amino acids of human or cynomolgus monkey CD3ε as determined by alanine scanning.
15. The Bi-Fc of any one of claims 1 to 14, wherein the Bi-Fc comprises
  - (1) a VH region comprising a CDR1, CDR2 and CR3 comprising, respectively, the amino acid sequence of SEQ ID NO:48, SEQ ID NO:49 and SEQ ID NO:50; and
  - (2) a VL region comprising a CDR1, CDR2 and CDR3 comprising, respectively the amino acid sequence of SEQ ID NO:51, SEQ ID NO:52; and SEQ ID NO:53.
16. A pharmaceutical formulation comprising the Bi-Fc of any one of claims 1 to 15 and a physiologically acceptable excipient.
17. One or more nucleic acid(s) encoding the Bi-Fc of any one of claims 1 to 15.
18. A host cell containing the nucleic acid(s) of claim 17.
19. A method of making a Bi-Fc comprising culturing the host cell of claim 18 under conditions such that the nucleic acid is expressed, and recovering the Bi-Fc from the cell mass or the culture medium.
20. A method when used in treating cancer, an infectious disease, an autoimmune or inflammatory disease or a fibrotic disease comprising administering a therapeutically effective dose of the Bi-Fc of any one of claims 1 to 15 to a patient.

21. Use of the Bi-Fc according to any one of claims 1-15 in the preparation of a medicament for the treatment of cancer, an infectious disease, an autoimmune or inflammatory disease or a fibrotic disease.



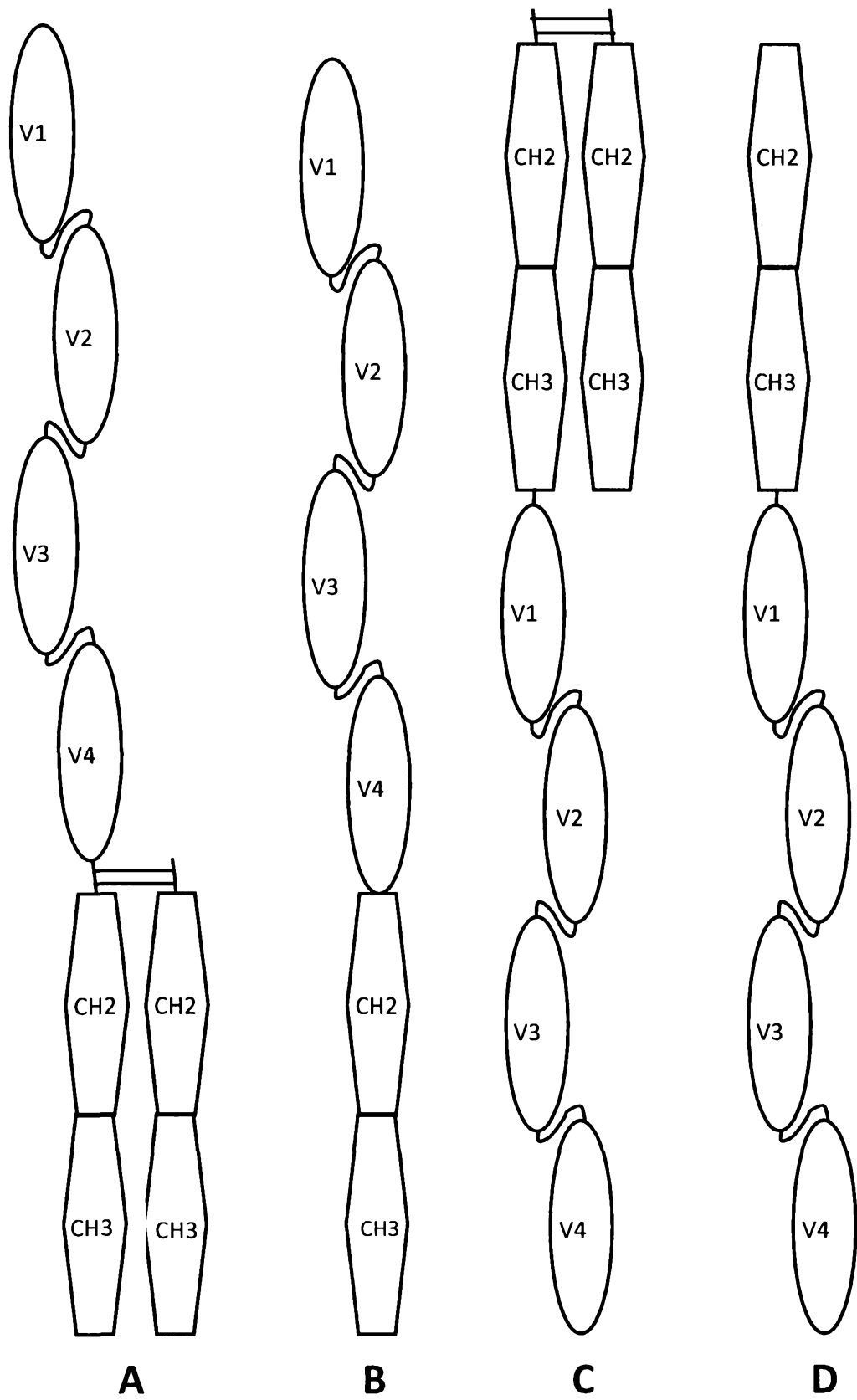
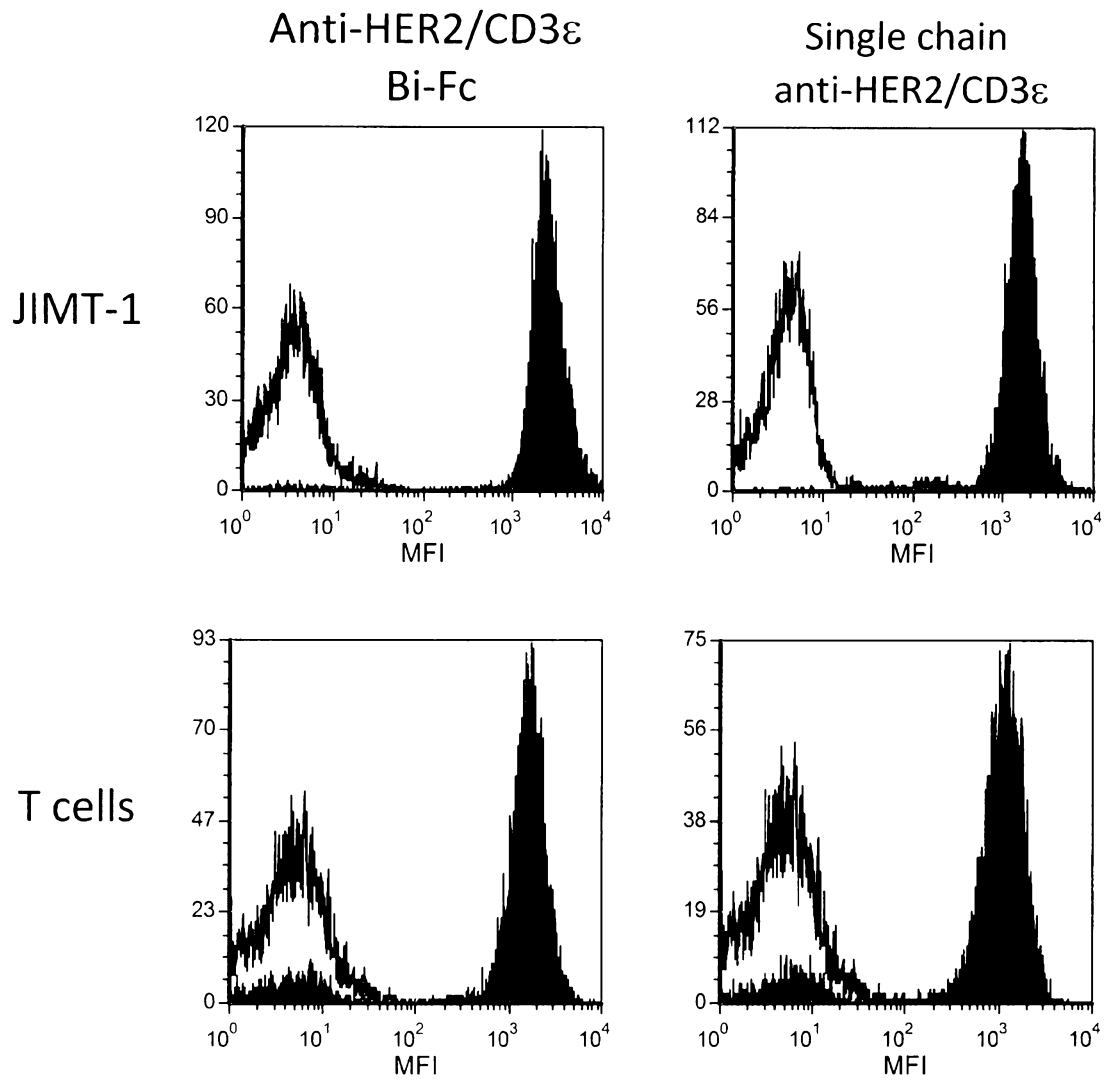


Figure 1

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**Figure 2**

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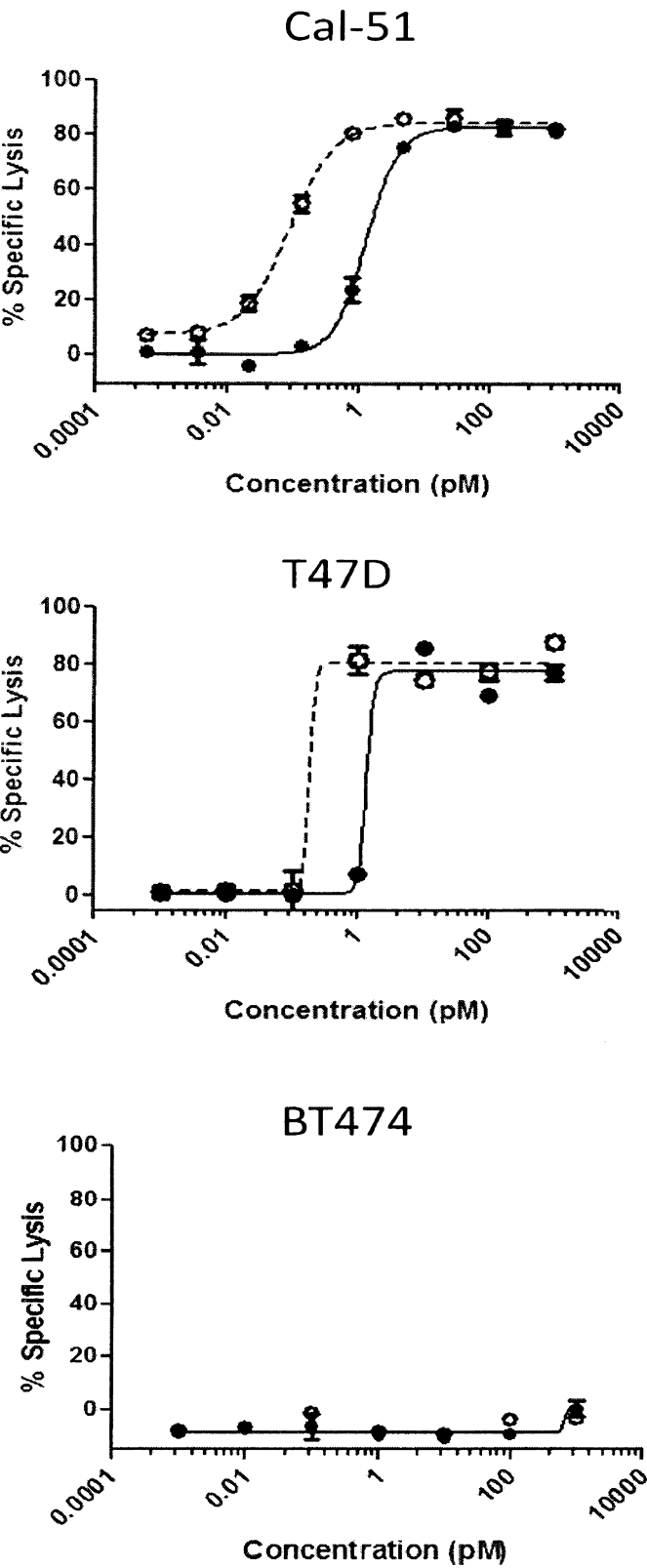


Figure 3

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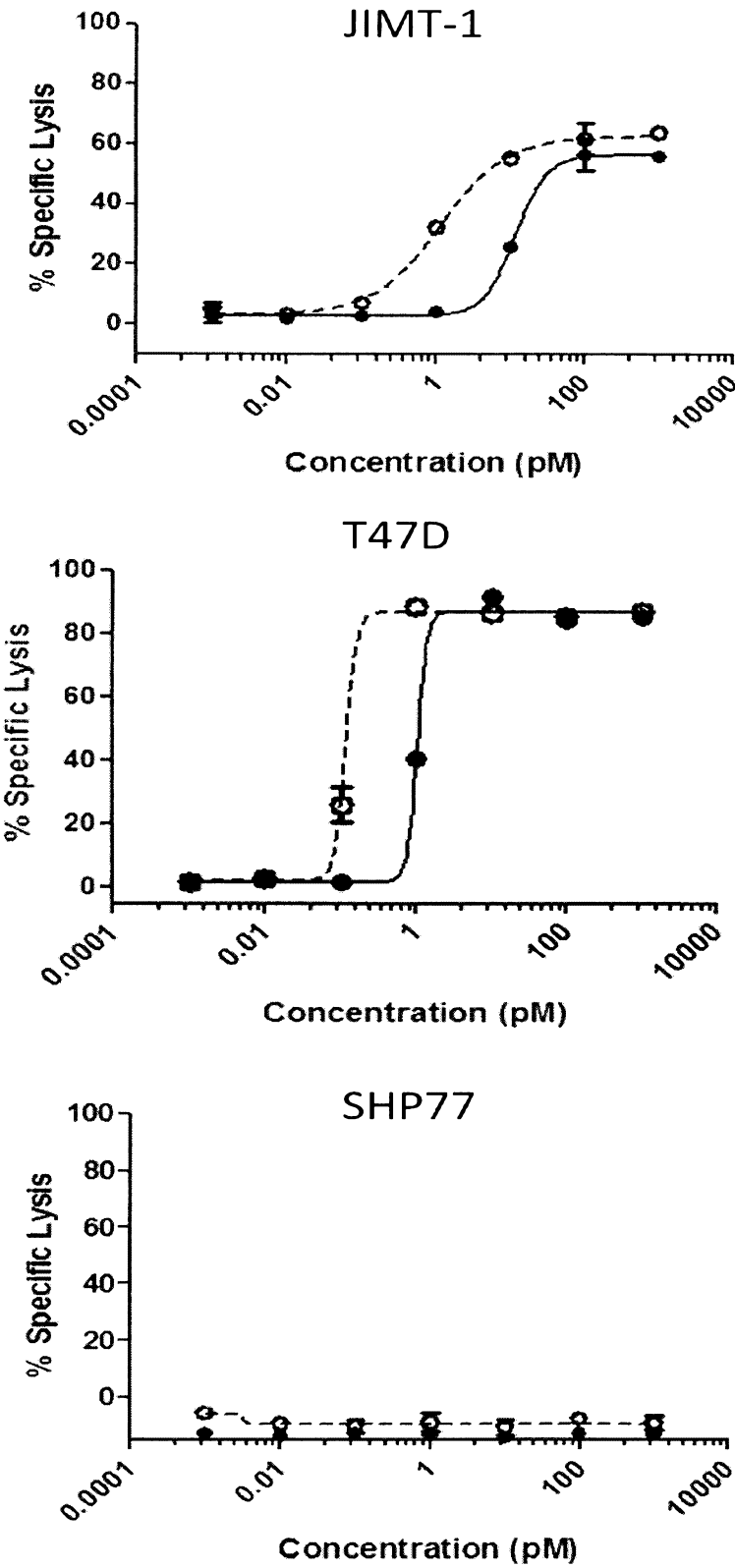


Figure 4

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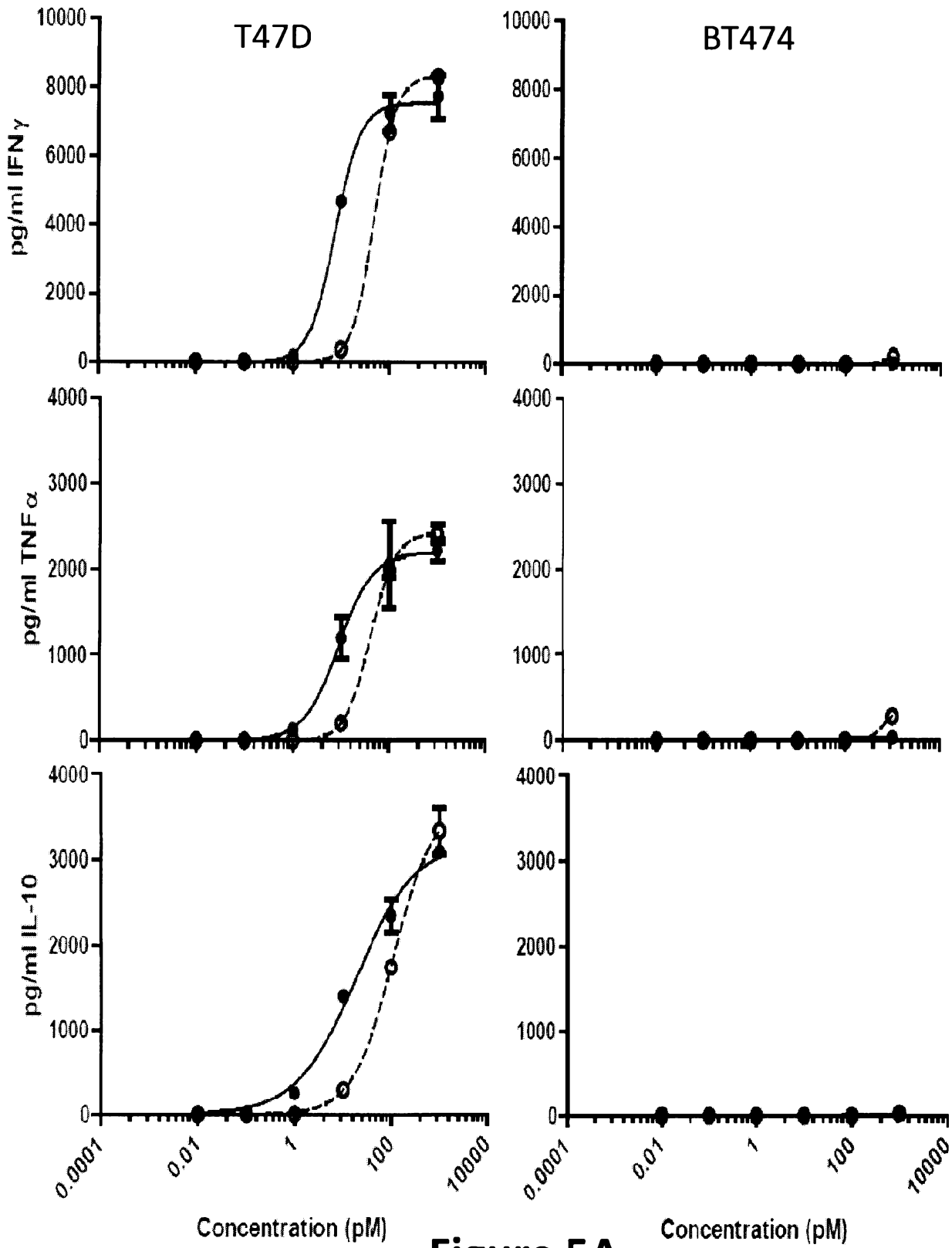


Figure 5A

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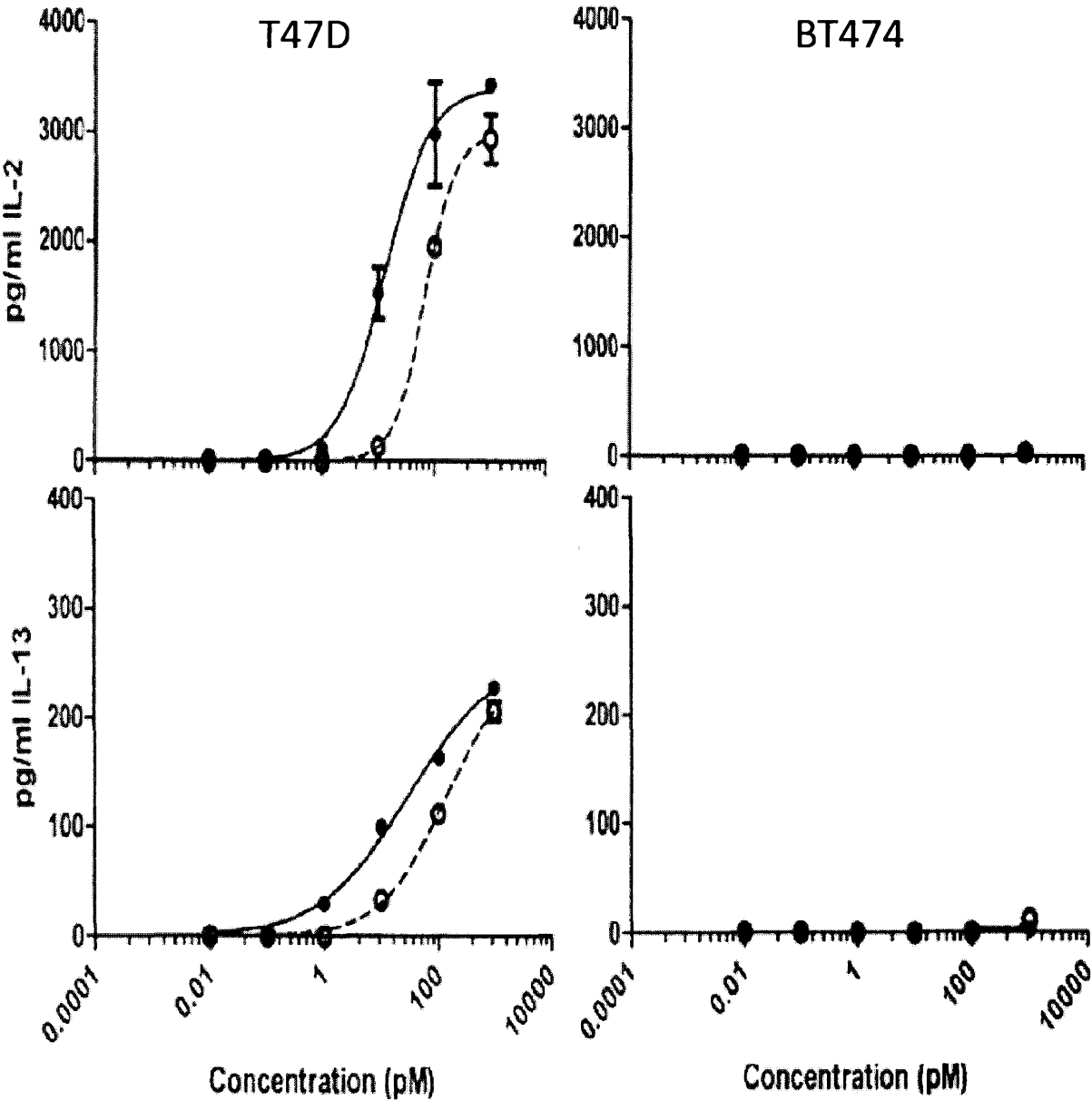


Figure 5B

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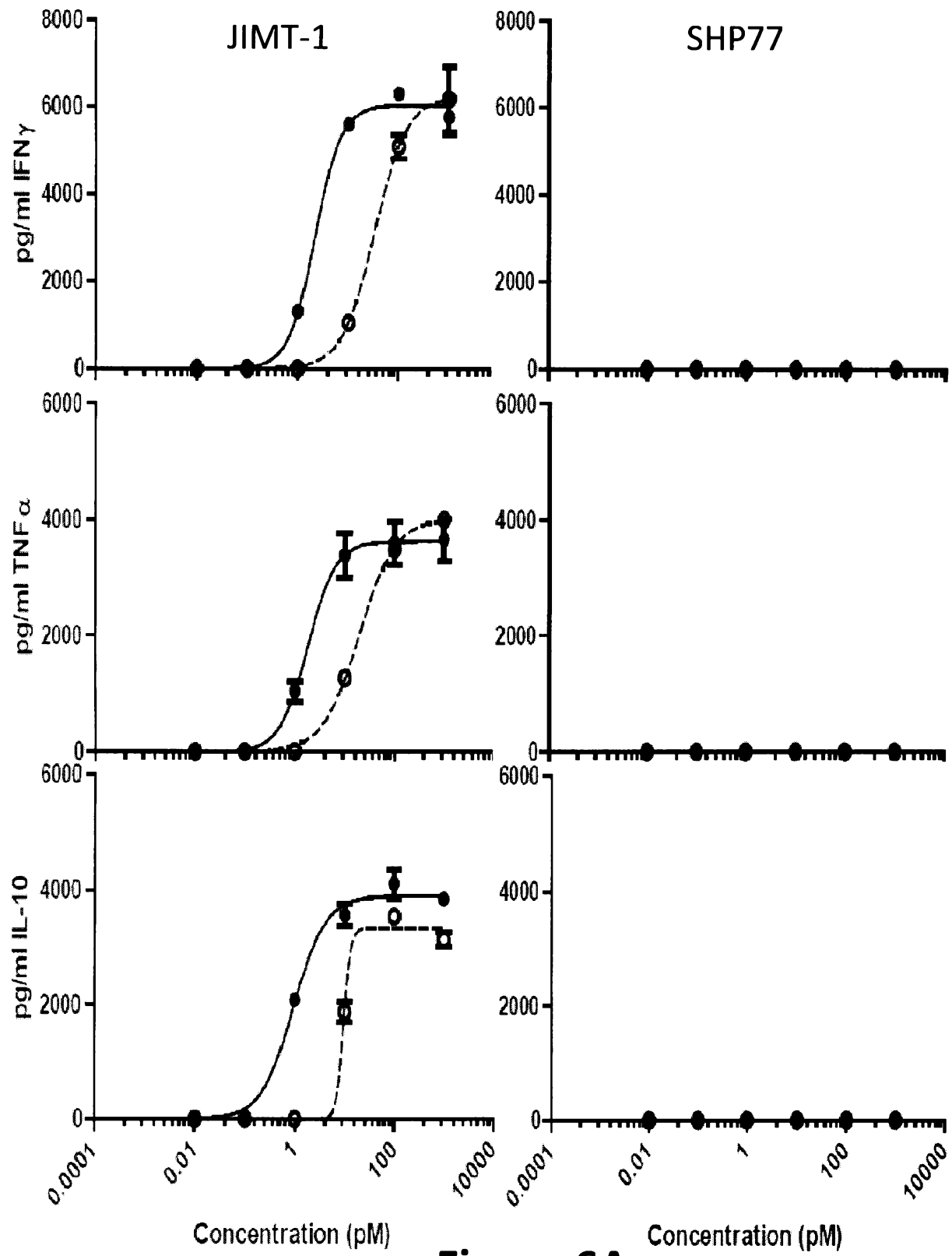


Figure 6A

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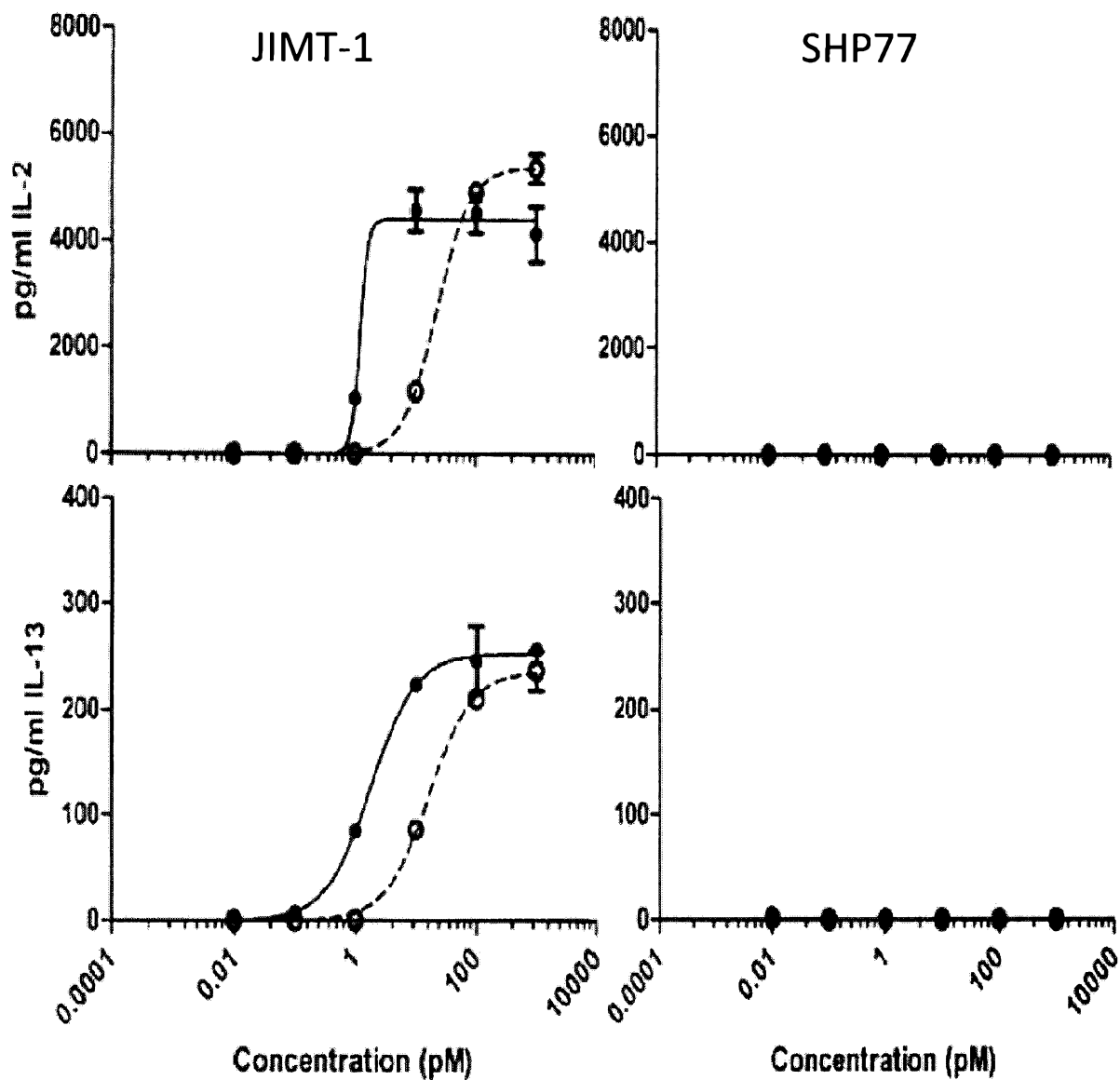


Figure 6B



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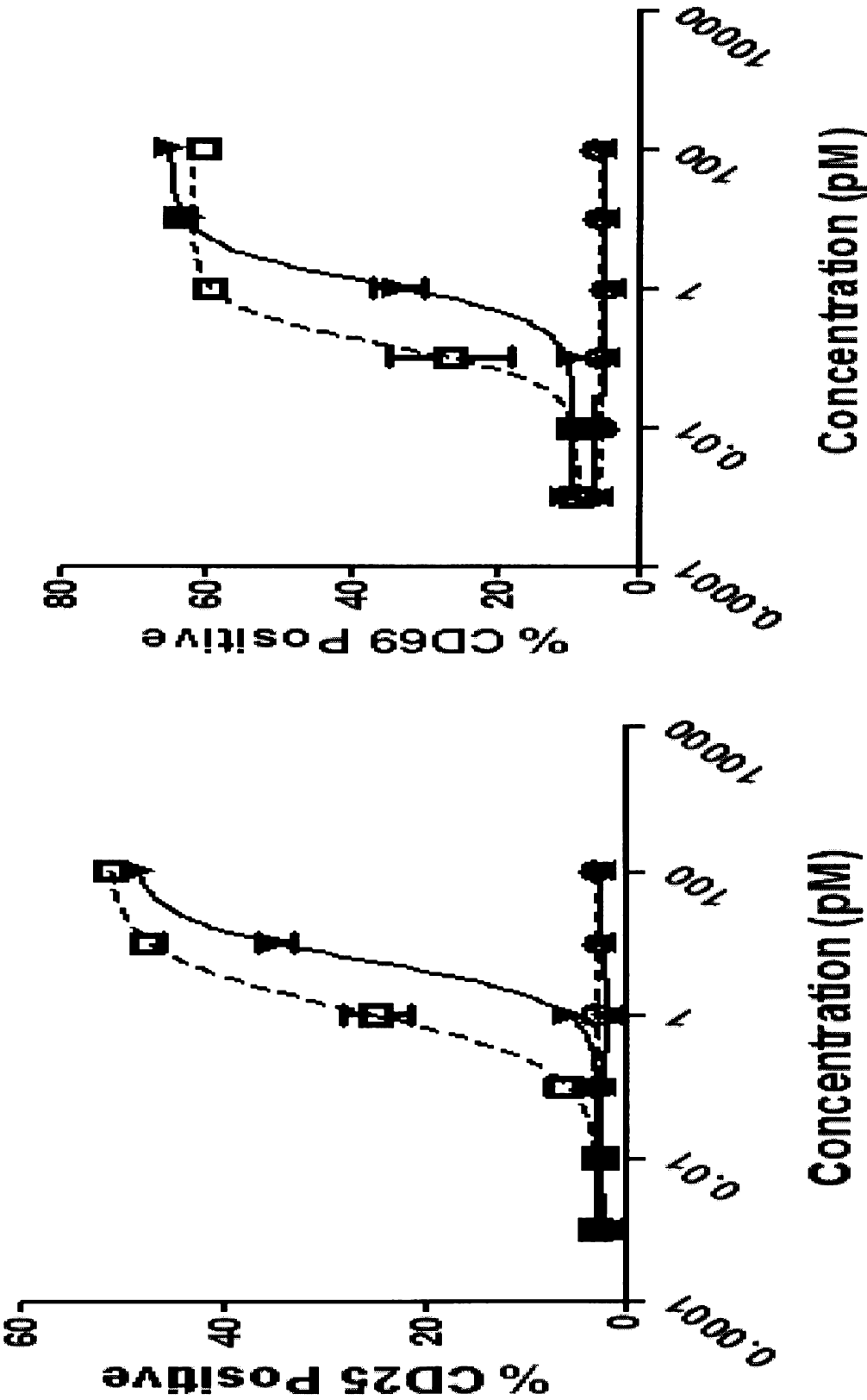


Figure 7

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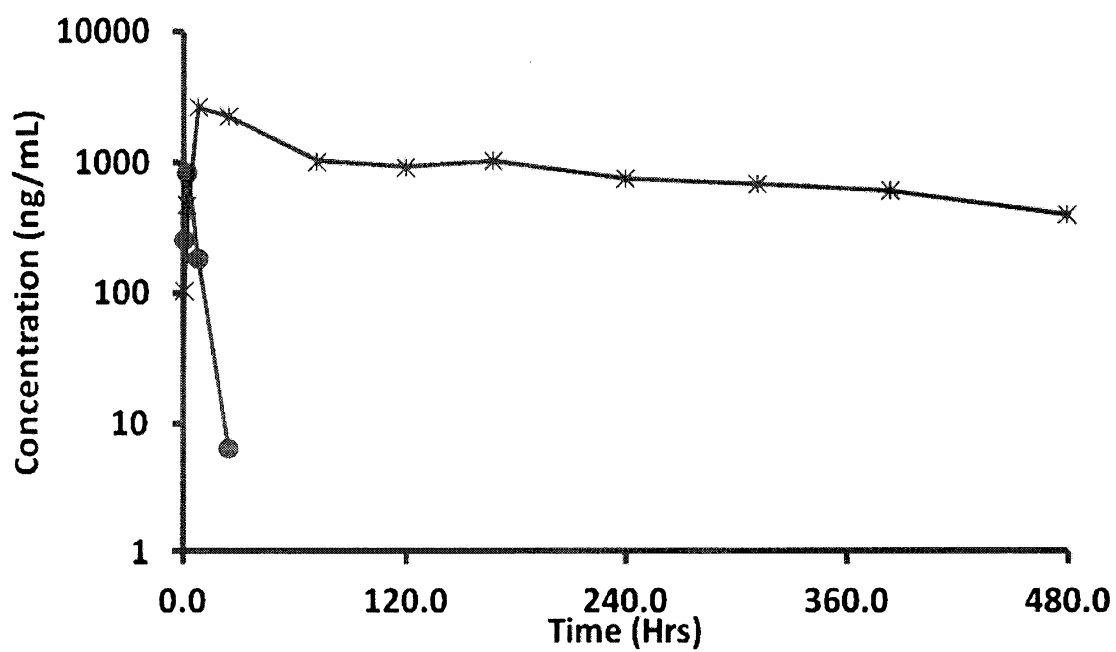
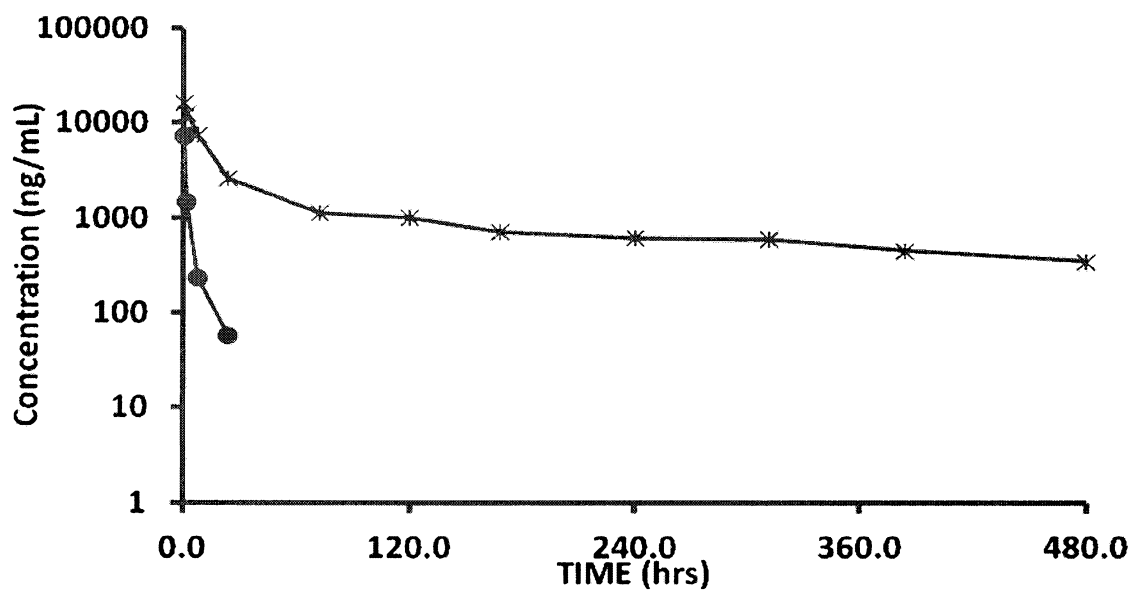


Figure 8

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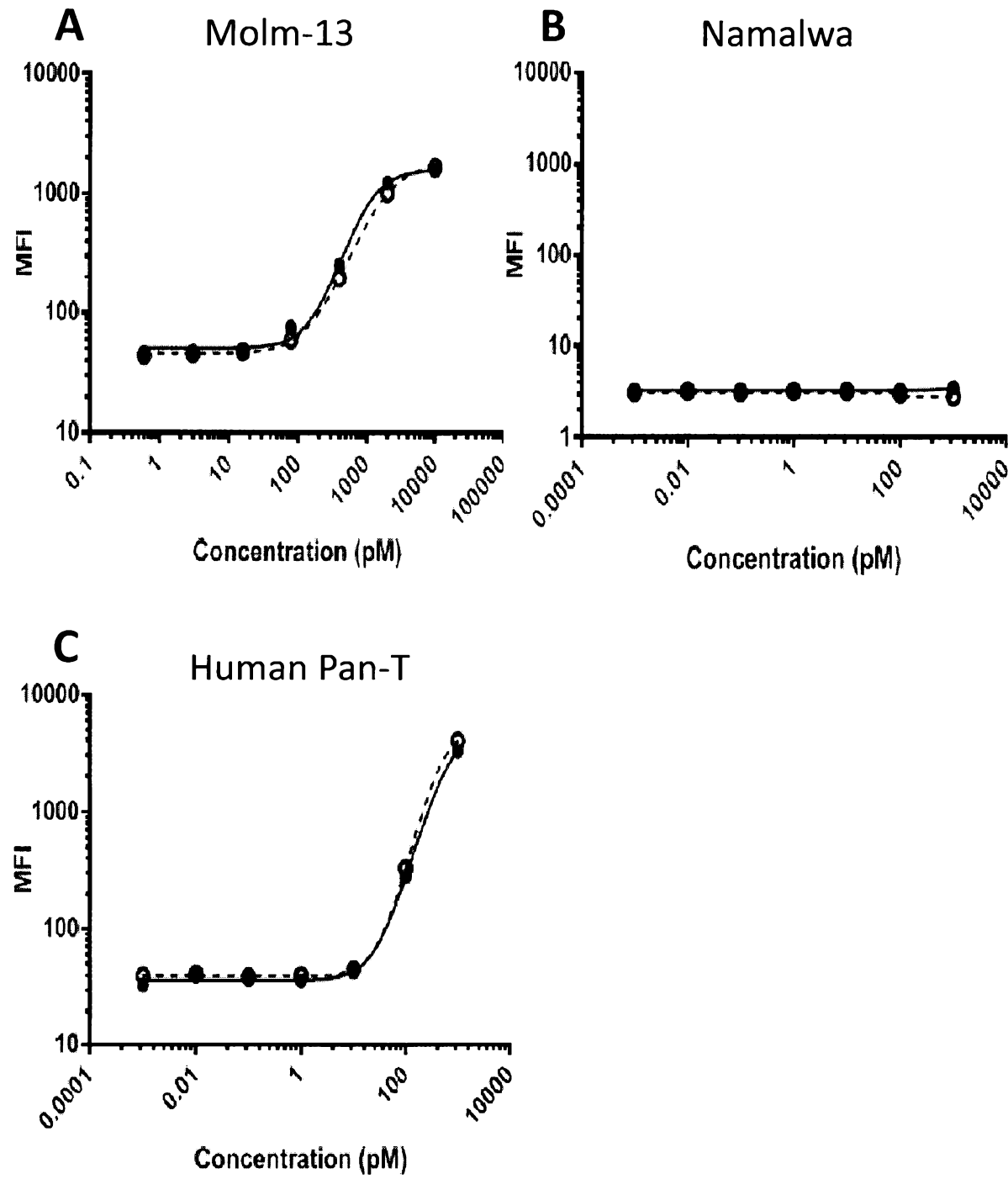
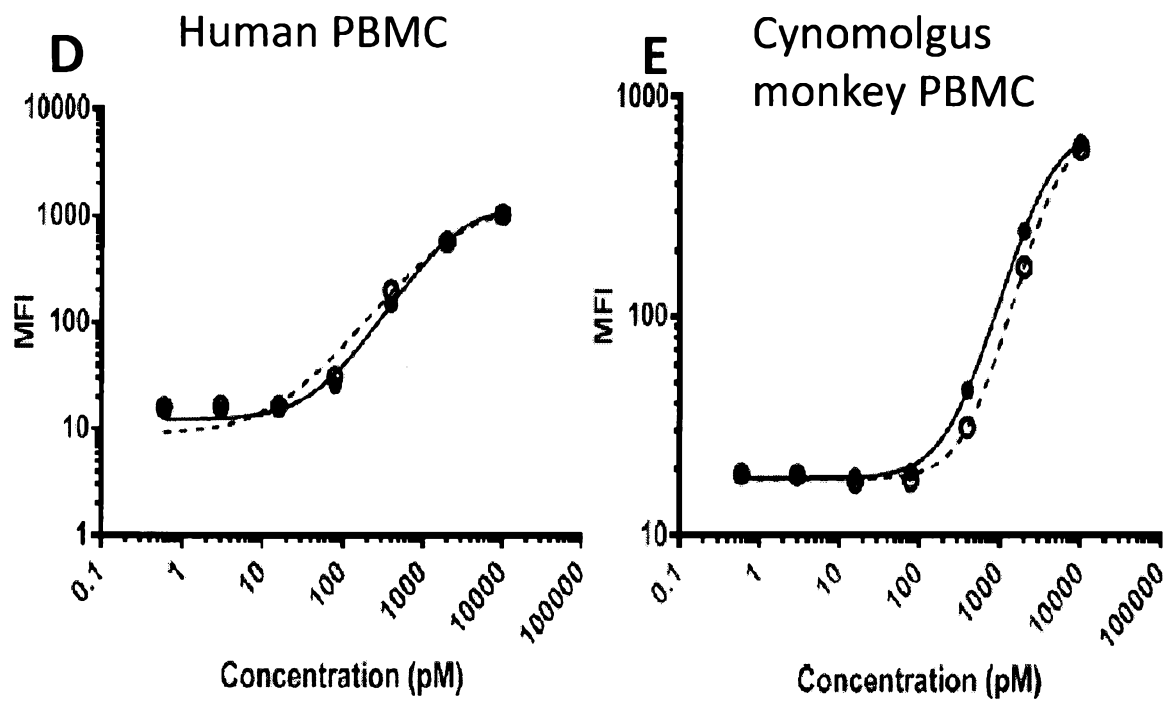
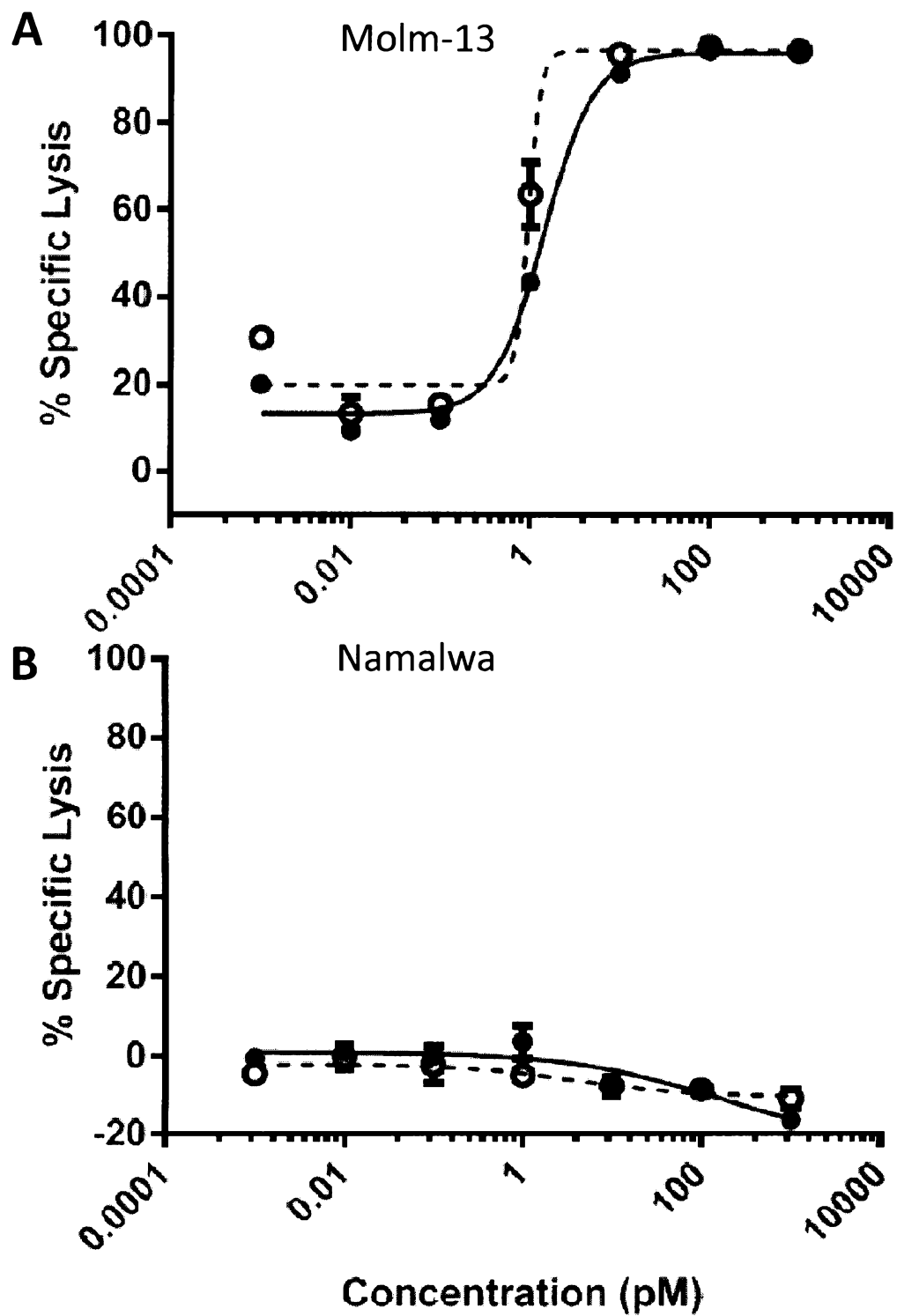


Figure 9

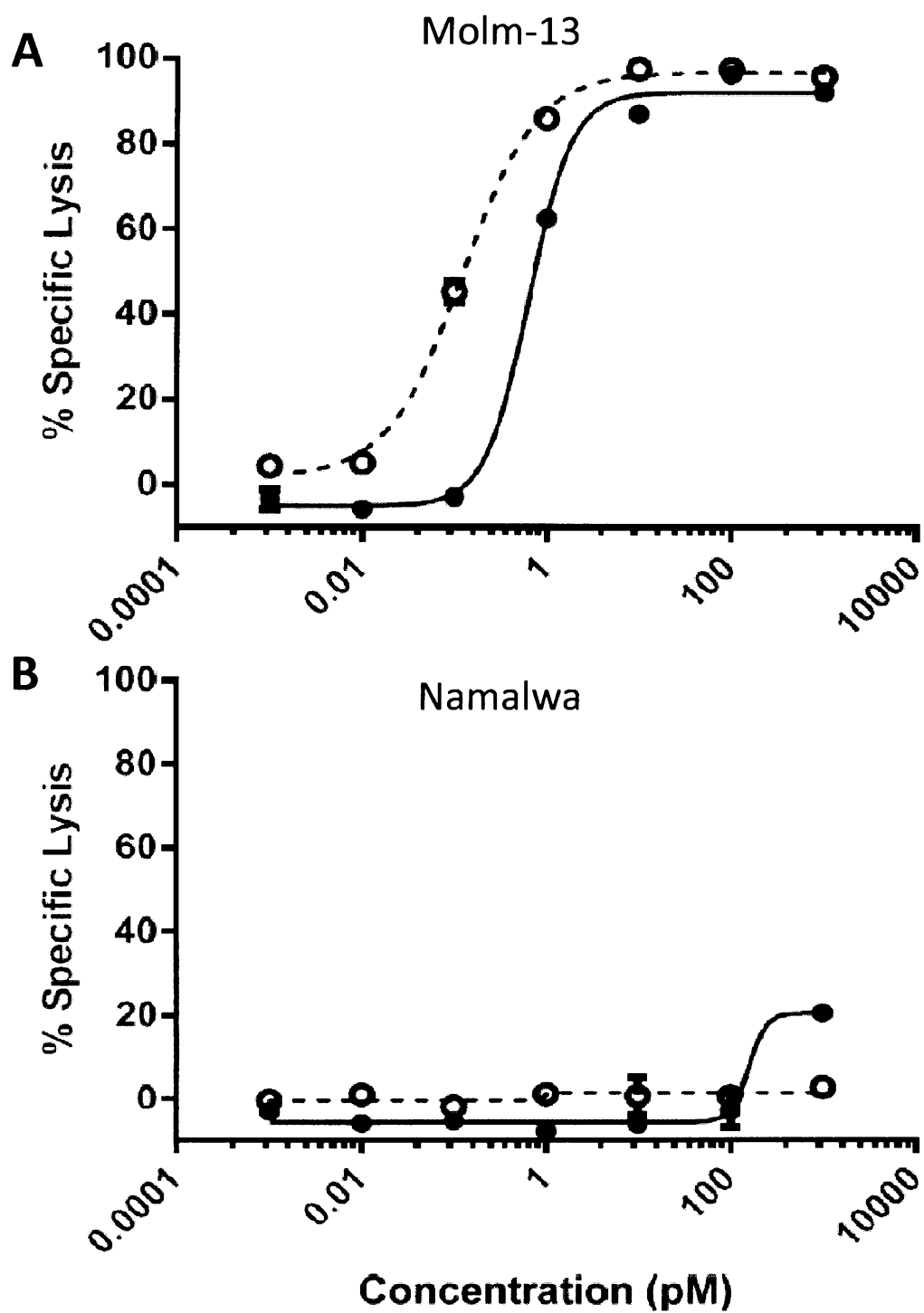
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**Figure 9** (cont.)

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**Figure 10**

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**Figure 11**

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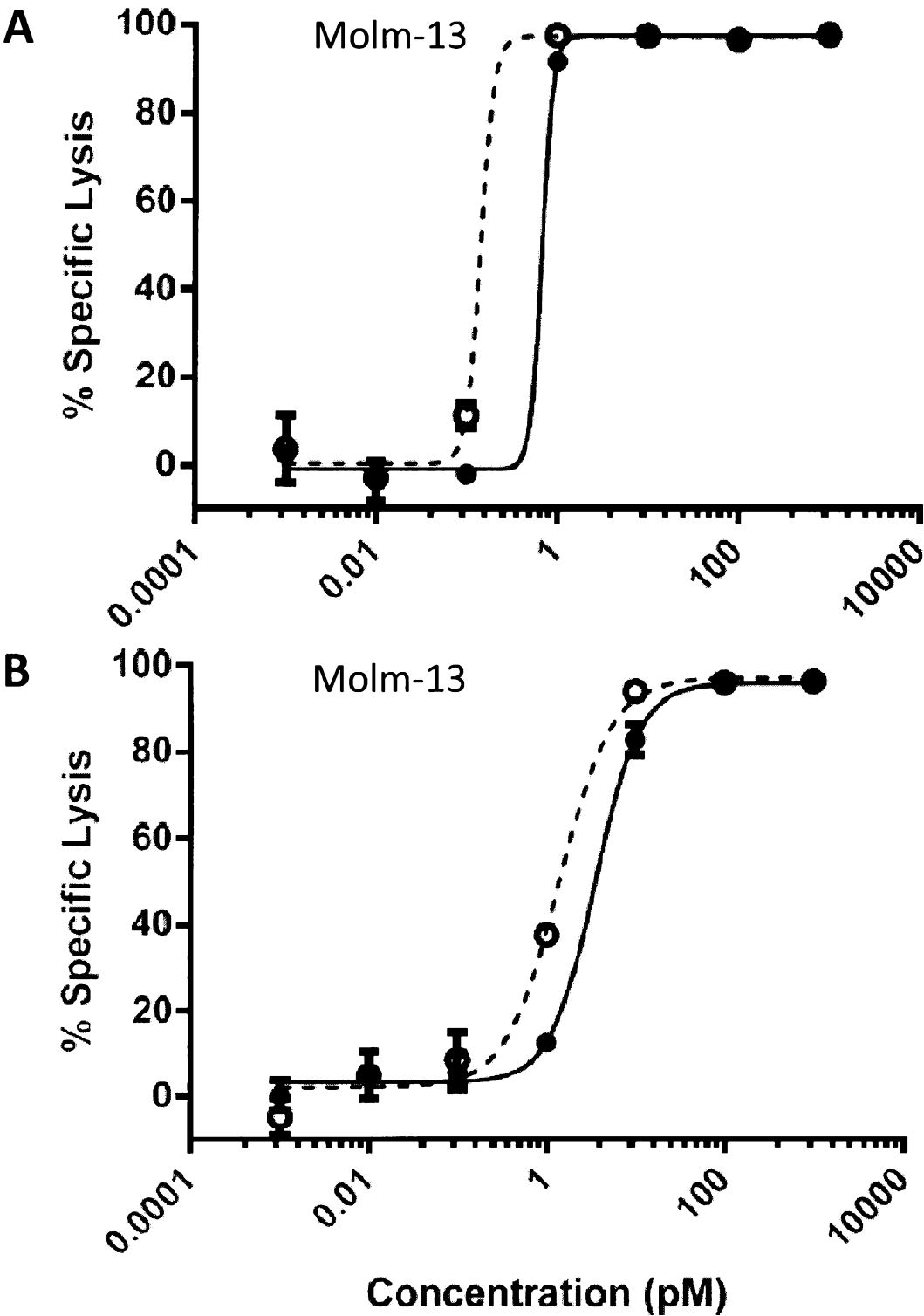


Figure 12

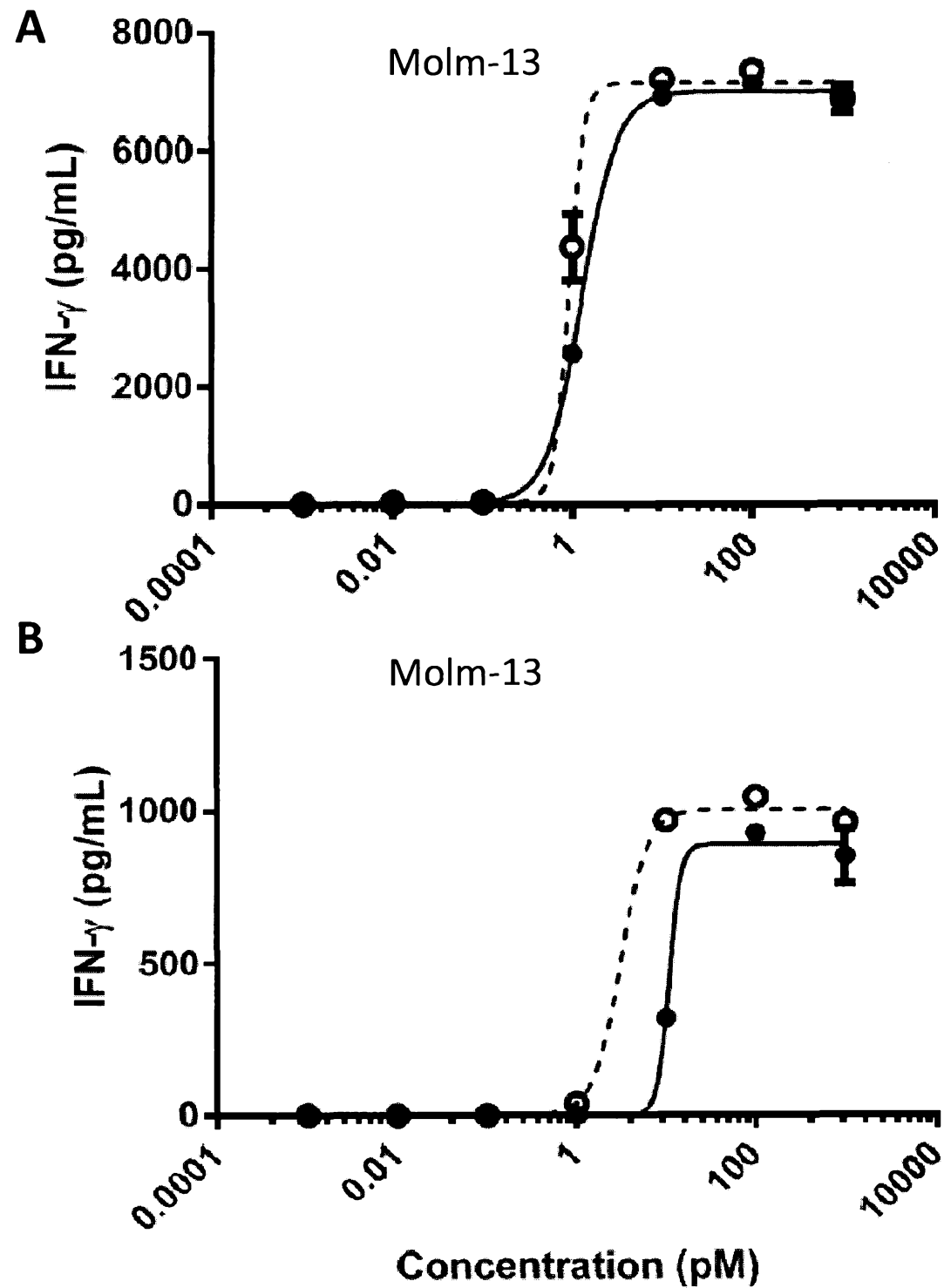


Figure 13



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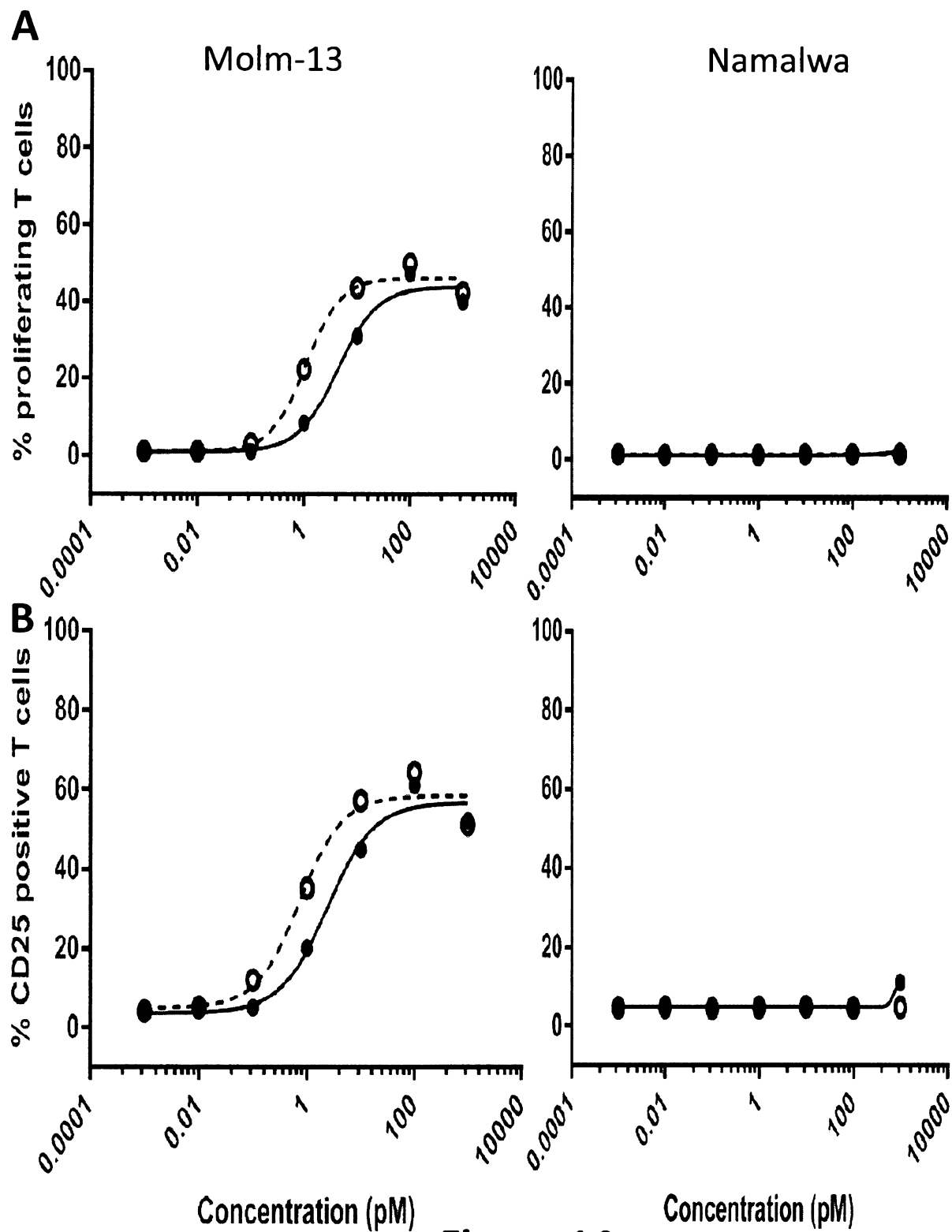


Figure 14

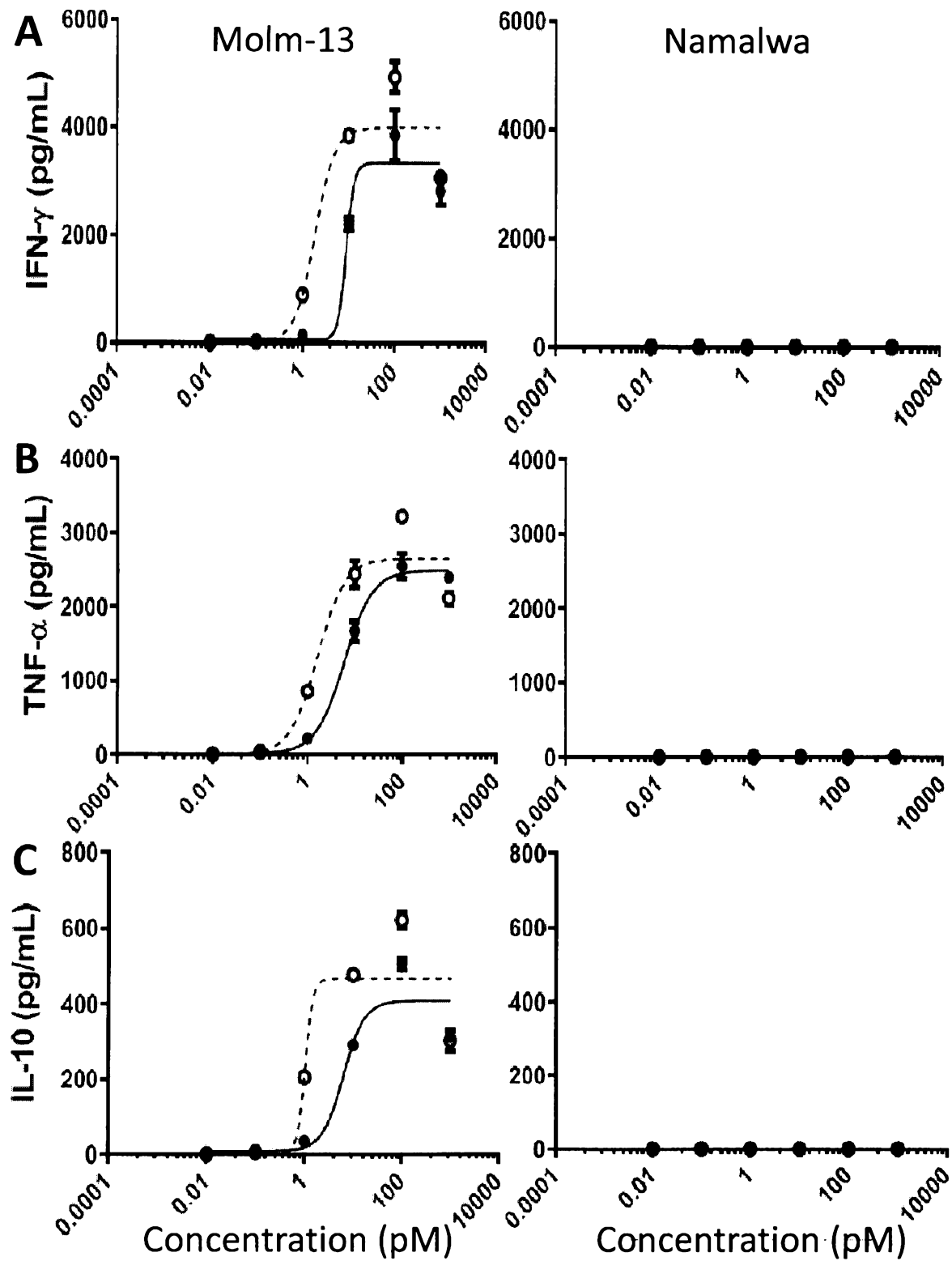


Figure 15

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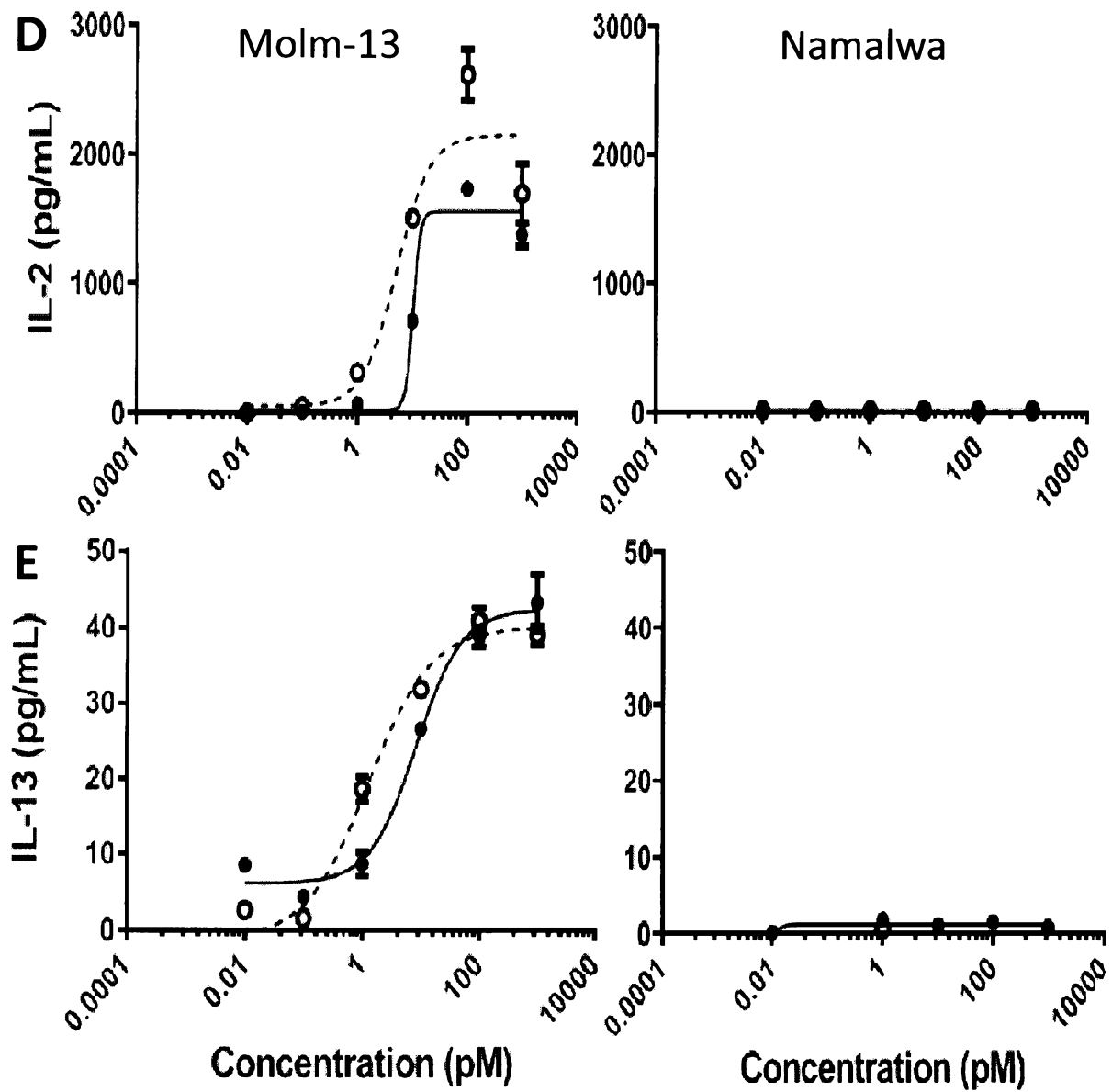
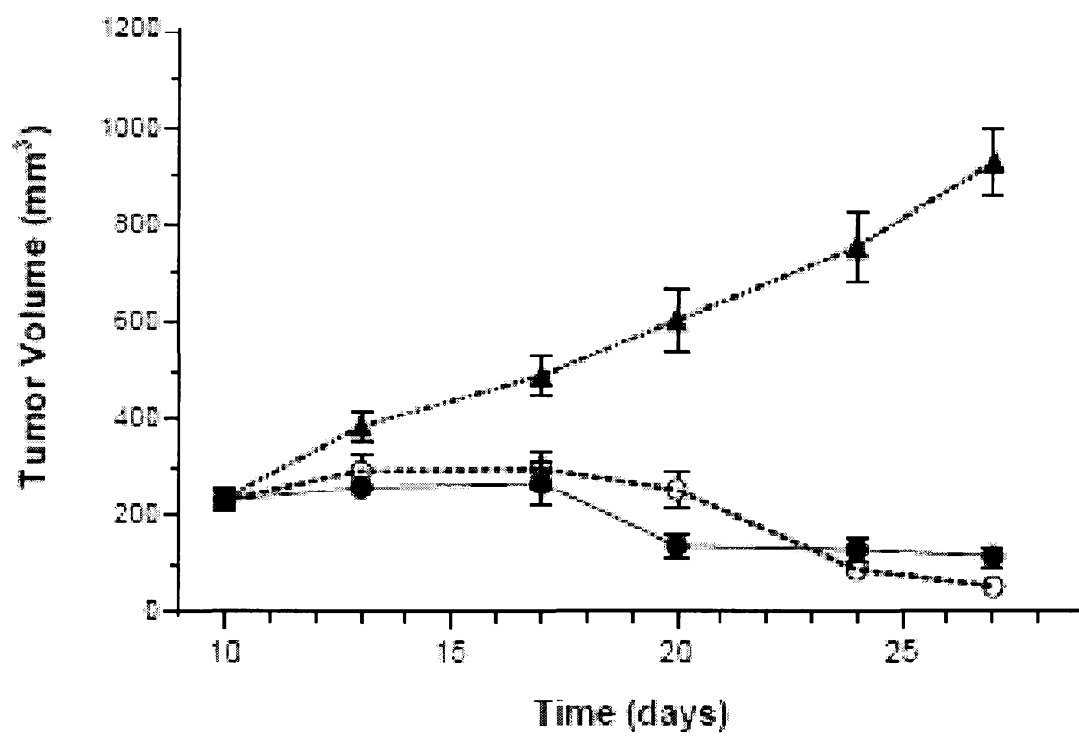


Figure 15 (cont.)

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**Figure 16**

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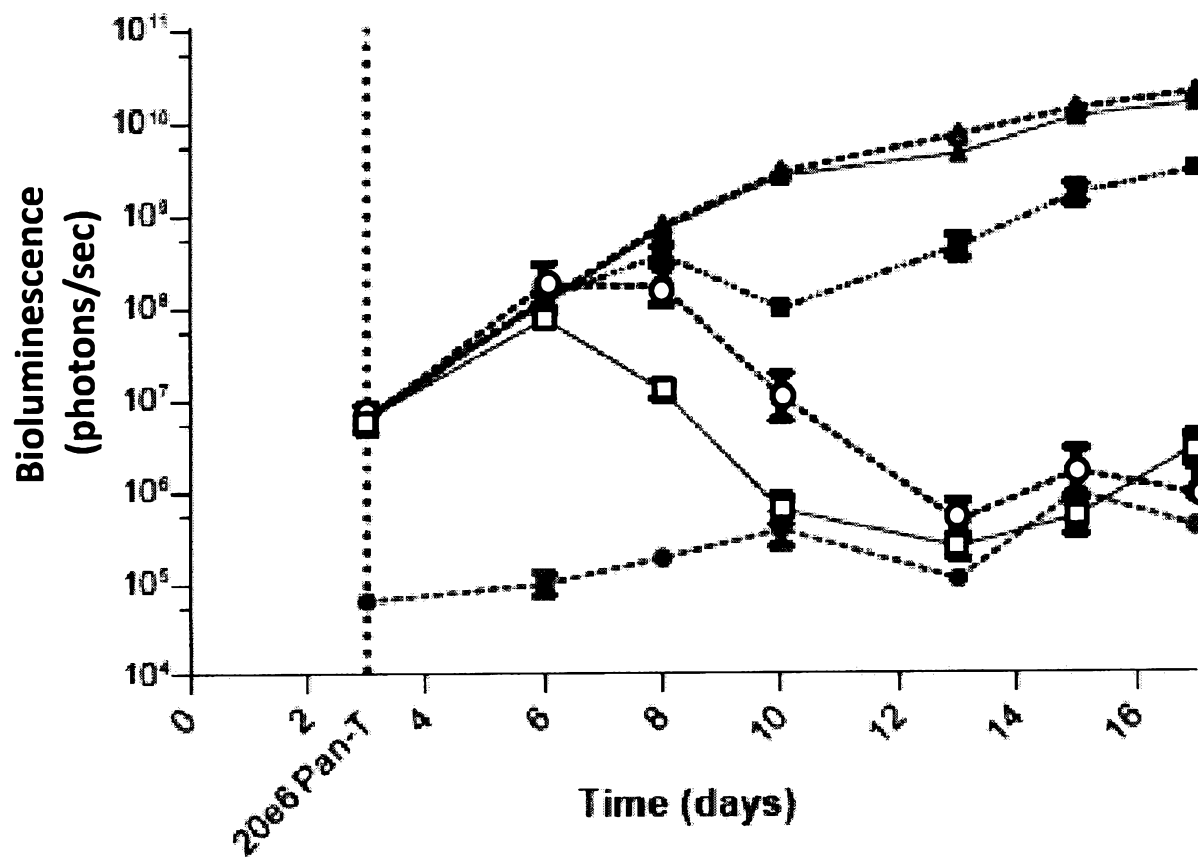


Figure 17

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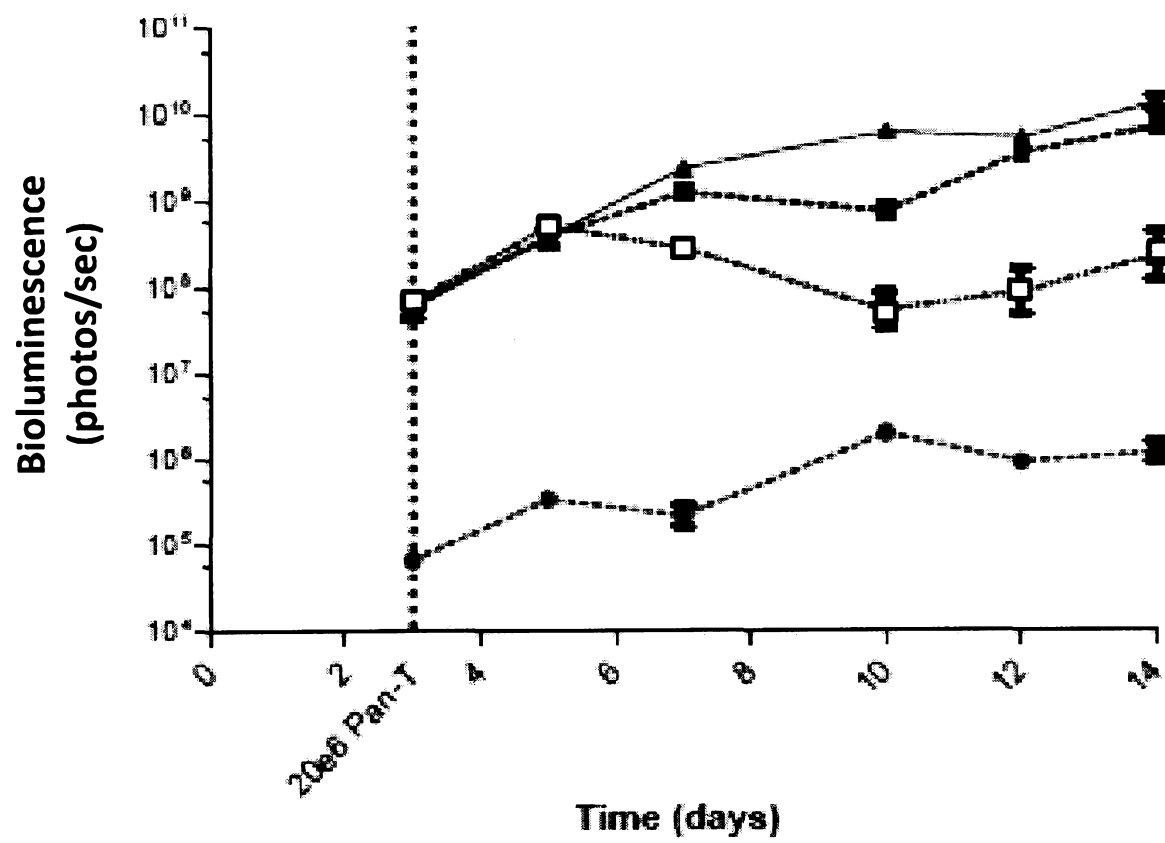


Figure 18

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<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence preceding HC CDR2"  
  
<400> 2  
Leu Glu Trp Ile Gly  
1 5  
  
<210> 3  
<211> 4  
<212> PRT  
<213> Artificial Sequence  
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence following HC CDR3"  
  
<220>  
<221> MOD\_RES  
<222> (3)..(3)

<223> Any amino acid

<400> 3

Trp Gly Xaa Gly

1

<210> 4

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence following LC CDR3"

<220>

<221> MOD\_RES

<222> (3)..(3)

<223> Any amino acid

<400> 4

Phe Gly Xaa Gly

1

<210> 5

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of anti-HER2 VH region"

<400> 5

Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Arg Pro Gly  
1 5 10 15

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

Tyr Phe Val Asn Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp  
35 40 45

Ile Gly Trp Ile Asp Pro Glu Asn Asp Asn Ser Leu Tyr Gly Pro Asn  
50 55 60

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

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

Cys Ala Leu Tyr Tyr Gly Ser Arg Gly Asp Ala Met Asp Tyr Trp Gly  
100 105 110

Gln Gly Thr Thr Val Thr Val



115

<210> 6  
 <211> 107  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of anti-HER2 VL region"

<400> 6  
 Glu Leu Val Met Thr Gln Thr Pro Ser Ser Leu Ser Ala Ser Leu Gly  
 1 5 10 15

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

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

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln  
 65 70 75 80

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

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

<210> 7  
 <211> 125  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of anti-CD3 VH region"

<400> 7  
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

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

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

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
 50 55 60

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

Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Trp  
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120 125

<210> 8  
<211> 109  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of anti-CD3 VL region"

<400> 8  
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

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

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

Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val  
65 70 75 80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn  
85 90 95

Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
100 105

<210> 9  
<211> 518  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a single chain anti-HER2/CD3  
(P136629.3) P136629.3 aHer2(D3)scFv-aCD3(F12Q)scFv"

<400> 9

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Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Arg Pro Gly  
 1 5 10 15  
 Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Lys Ile Lys Asp  
 20 25 30  
 Tyr Phe Val Asn Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp  
 35 40 45  
 Ile Gly Trp Ile Asp Pro Glu Asn Asp Asn Ser Leu Tyr Gly Pro Asn  
 50 55 60  
 Phe Gln Asp Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn Thr Gly  
 65 70 75 80  
 Tyr Leu Gln Leu Ser Gly Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr  
 85 90 95  
 Cys Ala Leu Tyr Tyr Gly Ser Arg Gly Asp Ala Met Asp Tyr Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly  
 115 120 125  
 Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro  
 130 135 140  
 Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg  
 145 150 155 160  
 Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro  
 165 170 175  
 Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser  
 180 185 190  
 Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser  
 195 200 205  
 Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys  
 210 215 220  
 Gln Gln Gly Asn Thr Leu Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 225 230 235 240  
 Glu Ile Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser  
 245 250 255  
 Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala  
 260 265 270

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Ala Ser Gly Phe Thr Phe Asn Ser Tyr Ala Met Asn Trp Val Arg Gln  
275 280 285

Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys Tyr  
290 295 300

Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr  
305 310 315 320

Ile Ser Arg Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Asn  
325 330 335

Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn  
340 345 350

Phe Gly Asn Ser Tyr Val Ser Trp Trp Ala Tyr Trp Gly Gln Gly Thr  
355 360 365

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
370 375 380

Gly Gly Gly Gly Ser Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr  
385 390 395 400

Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly  
405 410 415

Ala Val Thr Ser Gly Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly  
420 425 430

Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly  
435 440 445

Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu  
450 455 460

Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val  
465 470 475 480

Leu Trp Tyr Ser Asn Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr  
485 490 495

Val Leu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Gly Ser Ser  
500 505 510

His His His His His His  
515

<210> 10  
<211> 742  
<212> PRT  
<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of an anti-HER2/CD3 Bi-Fc (P136632.3) D356K, D399K, L234A, L235A P136632.3 (aHer2(D3)scFv-aCD3 (F12Q)scFv-FCAAKK)"

<400> 10

Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Arg Pro Gly  
1 5 10 15

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

Tyr Phe Val Asn Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp  
35 40 45

Ile Gly Trp Ile Asp Pro Glu Asn Asp Asn Ser Leu Tyr Gly Pro Asn  
50 55 60

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

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

Cys Ala Leu Tyr Tyr Gly Ser Arg Gly Asp Ala Met Asp Tyr Trp Gly  
100 105 110

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

Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro  
130 135 140

Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg  
145 150 155 160

Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro  
165 170 175

Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser  
180 185 190

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser  
195 200 205

Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys  
210 215 220

Gln Gln Gly Asn Thr Leu Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
225 230 235 240

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Glu Ile Lys Ser Gly<sub>245</sub> Gly Gly Gly Ser Glu<sub>250</sub> Val Gln Leu Val Glu<sub>255</sub> Ser  
 Gly Gly Gly Leu<sub>260</sub> Val Gln Pro Gly Gly<sub>265</sub> Ser Leu Lys Leu Ser<sub>270</sub> Cys Ala  
 Ala Ser Gly<sub>275</sub> Phe Thr Phe Asn Ser<sub>280</sub> Tyr Ala Met Asn Trp<sub>285</sub> Val Arg Gln  
 Ala Pro Gly<sub>290</sub> Lys Gly Leu Glu<sub>295</sub> Trp Val Ala Arg Ile<sub>300</sub> Arg Ser Lys Tyr  
 Asn<sub>305</sub> Asn Tyr Ala Thr Tyr<sub>310</sub> Tyr Ala Asp Ser Val<sub>315</sub> Lys Gly Arg Phe Thr<sub>320</sub>  
 Ile Ser Arg Asp Asp<sub>325</sub> Ser Lys Asn Thr Ala<sub>330</sub> Tyr Leu Gln Met Asn<sub>335</sub> Asn  
 Leu Lys Thr Glu<sub>340</sub> Asp Thr Ala Val Tyr<sub>345</sub> Tyr Cys Val Arg His Gly Asn  
 Phe Gly Asn<sub>355</sub> Ser Tyr Val Ser Trp<sub>360</sub> Trp Ala Tyr Trp Gly<sub>365</sub> Gln Gly Thr  
 Leu Val<sub>370</sub> Thr Val Ser Ser Gly<sub>375</sub> Gly Gly Gly Ser Gly<sub>380</sub> Gly Gly Gly Ser  
 Gly<sub>385</sub> Gly Gly Gly Ser Gln<sub>390</sub> Thr Val Val Thr Gln<sub>395</sub> Glu Pro Ser Leu Thr<sub>400</sub>  
 Val Ser Pro Gly Gly<sub>405</sub> Thr Val Thr Leu Thr<sub>410</sub> Cys Gly Ser Ser Thr<sub>415</sub> Gly  
 Ala Val Thr Ser<sub>420</sub> Gly Asn Tyr Pro Asn<sub>425</sub> Trp Val Gln Gln Lys<sub>430</sub> Pro Gly  
 Gln Ala Pro<sub>435</sub> Arg Gly Leu Ile Gly<sub>440</sub> Gly Thr Lys Phe Leu<sub>445</sub> Ala Pro Gly  
 Thr Pro<sub>450</sub> Ala Arg Phe Ser Gly<sub>455</sub> Ser Leu Leu Gly Gly<sub>460</sub> Lys Ala Ala Leu  
 Thr<sub>465</sub> Leu Ser Gly Val Gln<sub>470</sub> Pro Glu Asp Glu Ala<sub>475</sub> Glu Tyr Tyr Cys Val<sub>480</sub>  
 Leu Trp Tyr Ser Asn<sub>485</sub> Arg Trp Val Phe Gly<sub>490</sub> Gly Gly Thr Lys Leu<sub>495</sub> Thr  
 Val Leu Ala Ala<sub>500</sub> Ala Glu Pro Lys Ser<sub>505</sub> Ser Asp Lys Thr His<sub>510</sub> Thr Cys

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Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu  
515 520 525

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu  
530 535 540

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys  
545 550 555 560

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys  
565 570 575

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu  
580 585 590

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys  
595 600 605

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys  
610 615 620

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
625 630 635 640

Arg Lys Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys  
645 650 655

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
660 665 670

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Lys Ser Asp Gly  
675 680 685

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln  
690 695 700

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn  
705 710 715 720

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ala Ala Ala  
725 730 735

His His His His His His  
740

<210> 11  
<211> 2226  
<212> DNA  
<213> Artificial sequence

<220>  
<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:10 aHer2(D3)  
scFv-aCD3(F12Q)scFv-FcAAKK)"

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<400> 11
gaggtgcagc tgctcgagca gtctggagct gagcttgtga ggccaggggc cttagtcaag      60
ttgtcctgca aagcttctgg cttcaaaatt aaagactact ttgtgaactg ggtgaagcag      120
aggcctgaac agggcctgga gtggattgga tggattgatc ctgagaatga taatagttta      180
tatggcccga acttccagga caaggccagt atcacagcag acacatcctc caacacaggc      240
tacctgcagc tcagcggcct gacatctgag gacactgccg tctattactg tgctctttat      300
tacggaagta ggggggatgc tatggactac tggggccaag ggaccacggt caccgtctcc      360
tcaggtggtg gtggttctgg cggcggcggc tccggtggtg gtggttctga gtcctgatg      420
accagactc catcctcctt gtctgcctct ctgggagaca gagtcacat cagttgcagg      480
gcaagtcagg acattagcaa ttatttaaac tggatcagc agaaaccaga tggaactggt      540
aaactcctga tctactacac atcaagatta cactcaggag tcccatcaag gttcagtggc      600
agtgggtctg gaacagatta ttctctcacc attagcaacc tggagcaaga agatattgcc      660
acttactttt gccaacaggg taatacgctt ccgctcacgt tcggtgctgg gaccaagctt      720
gagatcaaat ccggaggtgg tggatccgag gtgcagctgg tcgagtctgg aggaggattg      780
gtgcagcctg gagggtcatt gaaactctca tgtgcagcct ctggattcac cttcaatagc      840
tacgccatga actgggtccg ccaggctcca ggaaagggtt tggaatgggt tgctcgcata      900
agaagtaaata ataataatta tgcaacatat tatgccgatt cagtgaagg caggttcacc      960
atctccagag atgattcaaa aaacactgcc tatctacaaa tgaacaactt gaaaactgag     1020
gacactgccg tgtactactg tgtgagacat gggaacttcg gtaatagcta cgtttcctgg     1080
tgggcttact ggggccaagg gactctggtc accgtctcct cagggtggtg tggttctggc     1140
ggcggcggct ccggtggtgg tggttctcag actggtgtga ctcaggaacc ttcactcacc     1200
gtatcacctg gtggaacagt cacactcact tgtggctcct cgactggggc tgttacatct     1260
ggcaactacc caaactgggt ccaacaaaaa ccaggtcagg caccctgtgg tctaataagg     1320
gggactaagt tcctcgcccc cgggtactcct gccagattct caggctccct gcttgagggc     1380
aaggctgccc tcacctctc aggggtacag ccagaggatg aggcagaata ttactgtggt     1440
ctatggtaca gcaaccgctg ggtgttcggt ggaggaacca aactgactgt cctagcggcc     1500
gcagagccca aatcttctga caaaactcac acatgcccc cgtgcccagc acctgaagca     1560
gctgggggac cgtcagtctt cctcttcccc caaaaccca aggacaccct catgatctcc     1620
cggacccttg aggtcacatg cgtggtggtg gacgtgagcc acgaagacc tgaggtaag     1680
ttcaactggt acgtggacgg cgtggagggt cataatgcca agacaaagcc gcgagaggag     1740
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg     1800
aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa     1860
accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc     1920

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cggaaggaga tgaccaagaa ccaggtcagc ctgacctgcc tgggtcaaagg cttctatccc 1980  
 agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 2040  
 cctcccgtgc tgaagtccga cggctccttc ttcctctata gcaagctcac cgtggacaag 2100  
 agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac 2160  
 cactacacgc agaagagcct ctccctgtct ccgggtaaag ctgcagcgca tcaccaccac 2220  
 catcac 2226

<210> 12  
 <211> 243  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence encoding a human IgG1 Fc polypeptide  
 containing alterations K392D and K409D, plus L234A and  
 L235A"

<400> 12  
 His Met Ser Ser Val Ser Ala Gln Ala Ala Ala Glu Pro Lys Ser Ser  
 1 5 10 15

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly  
 20 25 30

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 35 40 45

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 50 55 60

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 65 70 75 80

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 85 90 95

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 100 105 110

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 115 120 125

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

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 145 150 155 160

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

165

170

175

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asp Thr Thr Pro Pro  
                   180                  185                  190

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Asp Leu Thr Val  
           195                  200                  205

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
       210                  215                  220

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
   225                  230                  235                  240

Pro Gly Lys

<210> 13  
 <211> 729  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
           nucleic acid sequence encoding SEQ ID NO:12"

<400> 13  
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 acatgccac cgtgcccagc acctgaagca gctgggggac cgtcagtctt cctcttcccc 120  
 ccaaaacca aggacacct catgatctcc cggacccttg aggtcacatg cgtgggtggtg 180  
 gacgtgagcc acgaagacct tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 240  
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 300  
 gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 360  
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 420  
 gaaccacagg tgtacacct gccccatcc cgggaggaga tgaccaagaa ccaggtcagc 480  
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 540  
 gggcagccgg agaacaacta cgacaccacg cctcccgtgc tggactccga cggctccttc 600  
 ttctctata gcgacctac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 660  
 tgctccgtga tgcattaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 720  
 ccgggtaaa 729

<210> 14  
 <211> 518  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of a single chain anti-FOLR1/CD3 molecule P136637.3 aFOLR1(5G1)scFv-aCD3(F12Q)scFv"

<400> 14

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

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

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

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

Leu Lys Ser Arg Val Ser Ile Ser Ile 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 Ser Ser Trp Phe Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
115 120 125

Gly Gly Gly Gly Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser  
130 135 140

Glu Ala Pro Arg Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser  
145 150 155 160

Asn Ile Gly Asn Asn Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys  
165 170 175

Ala Pro Lys Leu Leu Ile Tyr Tyr Asp Asp Met Leu Ser Ser Gly Val  
180 185 190

Ser Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala  
195 200 205

Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala  
210 215 220

Trp Asp Asp Ser Leu Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu  
225 230 235 240

Thr Val Leu Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser  
245 250 255

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Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala  
260 265 270

Ala Ser Gly Phe Thr Phe Asn Ser Tyr Ala Met Asn Trp Val Arg Gln  
275 280 285

Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys Tyr  
290 295 300

Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr  
305 310 315 320

Ile Ser Arg Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Asn  
325 330 335

Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn  
340 345 350

Phe Gly Asn Ser Tyr Val Ser Trp Trp Ala Tyr Trp Gly Gln Gly Thr  
355 360 365

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
370 375 380

Gly Gly Gly Gly Ser Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr  
385 390 395 400

Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly  
405 410 415

Ala Val Thr Ser Gly Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly  
420 425 430

Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly  
435 440 445

Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu  
450 455 460

Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val  
465 470 475 480

Leu Trp Tyr Ser Asn Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr  
485 490 495

Val Leu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Gly Ser Ser  
500 505 510

His His His His His  
515

<210> 15  
 <211> 742  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of an anti-FOLR1/CD3 Bi-Fc P136635.4  
 (aFOLR1(5G1)scFv-aCD3(F12Q)scFv-FcAAKK)"

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

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

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

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

Leu Lys Ser Arg Val Ser Ile Ser Ile 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 Ser Ser Trp Phe Asp Tyr Trp Gly Gln Gly Thr  
 100 105 110

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 115 120 125

Gly Gly Gly Gly Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser  
 130 135 140

Glu Ala Pro Arg Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser  
 145 150 155 160

Asn Ile Gly Asn Asn Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys  
 165 170 175

Ala Pro Lys Leu Leu Ile Tyr Tyr Asp Asp Met Leu Ser Ser Gly Val  
 180 185 190

Ser Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala  
 195 200 205

Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala  
 210 215 220

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Trp Asp Asp Ser Leu Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu  
 225 230 235  
 Thr Val Leu Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser  
 245 250 255  
 Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala  
 260 265 270  
 Ala Ser Gly Phe Thr Phe Asn Ser Tyr Ala Met Asn Trp Val Arg Gln  
 275 280 285  
 Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys Tyr  
 290 295 300  
 Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr  
 305 310 315 320  
 Ile Ser Arg Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Asn  
 325 330 335  
 Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn  
 340 345 350  
 Phe Gly Asn Ser Tyr Val Ser Trp Trp Ala Tyr Trp Gly Gln Gly Thr  
 355 360 365  
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 370 375 380  
 Gly Gly Gly Gly Ser Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr  
 385 390 395 400  
 Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly  
 405 410 415  
 Ala Val Thr Ser Gly Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly  
 420 425 430  
 Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly  
 435 440 445  
 Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu  
 450 455 460  
 Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val  
 465 470 475 480  
 Leu Trp Tyr Ser Asn Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr  
 485 490 495

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Val Leu Ala Ala Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys  
500 505 510

Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu  
515 520 525

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu  
530 535 540

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys  
545 550 555 560

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys  
565 570 575

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu  
580 585 590

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys  
595 600 605

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys  
610 615 620

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
625 630 635 640

Arg Lys Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys  
645 650 655

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
660 665 670

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Lys Ser Asp Gly  
675 680 685

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln  
690 695 700

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn  
705 710 715 720

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ala Ala Ala  
725 730 735

His His His His His His  
740

<210> 16  
<211> 2226  
<212> DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic nucleic acid sequence encoding SEQ ID NO:15 (aFOLR1(5G1) scFV-aCD3(F12Q)scFV-FcAAKK)"

&lt;400&gt; 16

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc	60
acctgcactg tctctggtgg ctccatcagc agtgggtgctt actactggac ctggatccgc	120
cagcaccag ggaagggcct ggagtggatt gggtagatct attacagtgg gagcacctac	180
tacaacccgt ccctcaagag tcgagttagc atatcaatag acacgtctaa gaaccagttc	240
tccctgaagc tgagctctgt gactgccgcg gacacggccg tgtattactg tgcgcgagggc	300
agcagcagct ggttcgacta ctggggccag ggaaccctgg tcaccgtctc ctcaggaggc	360
ggcggttcag gcggaggtgg ctctggcggg ggcggaagtc agtctgtgct gactcagcca	420
ccctcgggtg ctgaagcccc caggcagagg gtcaccatct cctgttcttg aagcagctcc	480
aacatcgga ataatgctgt aaactggtag cagcagctcc caggaaaggc tcccaaactc	540
ctcatctatt atgatgatat gttgtcttca ggggtctcgg accgattttc tggctccaag	600
tctggcacct cagcctccct ggccatcagt gggctccagt ctgaggatga ggctgattat	660
tactgtgcag catgggatga cagcctgaat ggtgtggtat tcggcgaggg gaccaagctg	720
accgtcctat ccggaggtgg tggatccgag gtgcagctgg tcgagctctg aggaggattg	780
gtgcagcctg gagggtcatt gaaactctca tgtgcagcct ctggattcac cttcaatagc	840
tacgccatga actgggtccg ccagggtcca ggaaaggggt tggaatgggt tgctcgcata	900
agaagtaaata ataataatta tgcaacatat tatgccgatt cagtgaaggg caggttcacc	960
atctccagag atgattcaaa aaactgctc tatctacaaa tgaacaactt gaaaactgag	1020
gacactgccg tgtactactg tgtgagacat gggaaacttc gtaatagcta cgtttccttg	1080
tgggcttact ggggccaagg gactctggtc accgtctcct cagggtggtg tggttctggc	1140
ggcggcggct ccgggtggtg tggttctcag actgttgtga ctcaggaacc ttcactcacc	1200
gtatcacctg gtggaacagt cacactcact tgtggctcct cgactggggc tgttacatct	1260
ggcaactacc caaactgggt ccaacaaaaa ccaggtcagg caccctctgg tctaataagg	1320
gggactaagt tcctcgcccc cggtactcct gccagattct caggctccct gcttggaggc	1380
aaggctgccc tcaccctctc aggggtacag ccagaggatg aggcagaata ttactgtgtt	1440
ctatggtaca gcaaccgctg ggtgttcggg ggaggaacca aactgactgt cctagcggcc	1500
gcagagccca aatcttctga caaaactcac acatgcccc cgtgcccagc acctgaagca	1560
gctggggggc cgtcagctct cctcttcccc ccaaaaccca aggacaccct catgatctcc	1620
cggacccttg aggtcacatg cgtgggtggtg gacgtgagcc acgaagacc tgaggtcaag	1680
ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgagaggag	1740
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg	1800



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aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa	1860
accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc	1920
cggaaggaga tgaccaagaa ccaggtcagc ctgacctgcc tgggtcaaagg cttctatccc	1980
agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg	2040
cctcccgtgc tgaagtccga cggctccttc ttctctata gcaagctcac cgtggacaag	2100
agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac	2160
cactacacgc agaagagcct ctccctgtct ccgggtaaag ctgcagcgca tcaccaccac	2220
catcac	2226

<210> 17  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic linker peptide"

<400> 17  
 Thr Val Ala Ala Pro  
 1 5

<210> 18  
 <211> 6  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic linker peptide"

<400> 18  
 Ala Ser Thr Lys Gly Pro  
 1 5

<210> 19  
 <211> 10  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic linker peptide"

<400> 19  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 1 5 10

<210> 20  
 <211> 8  
 <212> PRT  
 <213> Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic linker peptide"

&lt;400&gt; 20

Gly Gly Gly Gly Ser Ala Ala Ala  
1 5

&lt;210&gt; 21

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Description of Artificial Sequence: Synthetic linker peptide"

&lt;400&gt; 21

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

&lt;210&gt; 22

&lt;211&gt; 186

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; source

&lt;223&gt; /note="Mature amino acid sequence of CD3 epsilon chain of Homo sapiens"

&lt;400&gt; 22

Gln Asp Gly Asn Glu Glu Met Gly Gly Ile Thr Gln Thr Pro Tyr Lys  
1 5 10 15Val Ser Ile Ser Gly Thr Thr Val Ile Leu Thr Cys Pro Gln Tyr Pro  
20 25 30Gly Ser Glu Ile Leu Trp Gln His Asn Asp Lys Asn Ile Gly Gly Asp  
35 40 45Glu Asp Asp Lys Asn Ile Gly Ser Asp Glu Asp His Leu Ser Leu Lys  
50 55 60Glu Phe Ser Glu Leu Glu Gln Ser Gly Tyr Tyr Val Cys Tyr Pro Arg  
65 70 75 80Gly Ser Lys Pro Glu Asp Ala Asn Phe Tyr Leu Tyr Leu Arg Ala Arg  
85 90 95Val Cys Glu Asn Cys Met Glu Met Asp Val Met Ser Val Ala Thr Ile  
100 105 110Val Ile Val Asp Ile Cys Ile Thr Gly Gly Leu Leu Leu Leu Val Tyr  
115 120 125

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Tyr Trp Ser Lys Asn Arg Lys Ala Lys Ala Lys Pro Val Thr Arg Gly  
130 135 140

Ala Gly Ala Gly Gly Arg Gln Arg Gly Gln Asn Lys Glu Arg Pro Pro  
145 150 155 160

Pro Val Pro Asn Pro Asp Tyr Glu Pro Ile Arg Lys Gly Gln Arg Asp  
165 170 175

Leu Tyr Ser Gly Leu Asn Gln Arg Arg Ile  
180 185

<210> 23

<211> 177

<212> PRT

<213> Macaca fascicularis

<220>

<221> source

<223> /note="Mature amino acid sequence of CD3 epsilon chain of  
Macaca fascicularis"

<400> 23

Gln Asp Gly Asn Glu Glu Met Gly Ser Ile Thr Gln Thr Pro Tyr Gln  
1 5 10 15

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

Gly Ser Glu Ala Gln Trp Gln His Asn Gly Lys Asn Lys Gly Asp Ser  
35 40 45

Gly Asp Gln Leu Phe Leu Pro Glu Phe Ser Glu Met Glu Gln Ser Gly  
50 55 60

Tyr Tyr Val Cys Tyr Pro Arg Gly Ser Asn Pro Glu Asp Ala Ser His  
65 70 75 80

His Leu Tyr Leu Lys Ala Arg Val Cys Glu Asn Cys Met Glu Met Asp  
85 90 95

Val Met Ala Val Ala Thr Ile Val Ile Val Asp Ile Cys Ile Thr Leu  
100 105 110

Gly Leu Leu Leu Leu Val Tyr Tyr Trp Ser Lys Asn Arg Lys Ala Lys  
115 120 125

Ala Lys Pro Val Thr Arg Gly Ala Gly Ala Gly Gly Arg Gln Arg Gly  
130 135 140

Gln Asn Lys Glu Arg Pro Pro Pro Val Pro Asn Pro Asp Tyr Glu Pro  
145 150 155 160

Ile Arg Lys Gly Gln Gln Asp Leu Tyr Ser Gly Leu Asn Gln Arg Arg

Ile

<210> 24  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic peptide which is a portion of an epitope of CD3 epsilon"

<400> 24  
 Gln Asp Gly Asn Glu  
 1 5

<210> 25  
 <211> 232  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> source  
 <223> /note="amino acid sequence of human IgG1 Fc region"

<400> 25  
 Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
 1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
 20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
 35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
 50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
 65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
 85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
 100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
 115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
 130 135 140

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Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys  
225 230

<210> 26  
<211> 228  
<212> PRT  
<213> Homo sapiens

<220>  
<221> source  
<223> /note="amino acid sequence of human IgG2 Fc region"

<400> 26  
Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val  
1 5 10 15

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
35 40 45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu  
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr  
65 70 75 80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn  
85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro  
100 105 110

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln  
115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val  
130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
165 170 175

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
210 215 220

Ser Pro Gly Lys  
225

<210> 27  
<211> 279  
<212> PRT  
<213> Homo sapiens

<220>  
<221> source  
<223> /note="amino acid sequence of human IgG3 Fc region"

<400> 27  
Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys  
1 5 10 15

Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro  
20 25 30

Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu  
35 40 45

Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala Pro  
50 55 60

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys  
65 70 75 80

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
85 90 95

Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp  
100 105 110

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr  
115 120 125

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Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp  
130 135 140

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu  
145 150 155 160

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg  
165 170 175

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys  
180 185 190

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp  
195 200 205

Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn  
210 215 220

Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser  
225 230 235 240

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser  
245 250 255

Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser  
260 265 270

Leu Ser Leu Ser Pro Gly Lys  
275

<210> 28  
<211> 229  
<212> PRT  
<213> Homo sapiens

<220>  
<221> source  
<223> /note="amino acid sequence of human IgG4 Fc region"

<400> 28  
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe  
1 5 10 15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
35 40 45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val  
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser  
65 70 75 80

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Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser  
100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro  
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln  
130 135 140

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala  
145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu  
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser  
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
210 215 220

Leu Ser Leu Gly Lys  
225

<210> 29  
<211> 125  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of an anti-CD3-epsilon VH region  
(12C)"

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

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

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

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
50 55 60



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

Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp  
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120 125

<210> 30  
<211> 375  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:29"

<400> 30  
gaggtgcagc tggtcgagtc tggaggagga ttggtgcagc ctggagggtc attgaaactc 60  
tcatgtgcag cctctggatt caccttcaat aagtacgcca tgaactgggt ccgccaggct 120  
ccaggaaagg gtttggaatg ggttgctcgc ataagaagta aatataataa ttatgcaaca 180  
tattatgccg attcagtga agacagggtc accatctcca gagatgattc aaaaaacact 240  
gcctatctac aaatgaacaa cttgaaaact gaggacactg ccgtgtacta ctgtgtgaga 300  
catgggaact tcggaatag ctacatatcc tactgggctt actggggcca agggactctg 360  
gtcaccgtct cctca 375

<210> 31  
<211> 109  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of an anti-CD3-epsilon VL region  
(12C)"

<400> 31  
Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

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

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

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Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val  
65 70 75 80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn  
85 90 95

Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
100 105

<210> 32  
<211> 327  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:31"

<400> 32  
cagactgttg tgactcagga accttcactc accgtatcac ctggtggaac agtcacactc 60  
acttgtggct cctcgactgg ggctgttaca tctggcaact acccaaactg ggtccaacaa 120  
aaaccaggtc aggcaccccg tggctctaata ggtgggacta agttcctcgc ccccggtact 180  
cctgccagat tctcaggctc cctgcttgga ggcaaggctg ccctcaccct ctcaggggta 240  
cagccagagg atgaggcaga atattactgt gttctatggt acagcaaccg ctgggtgttc 300  
ggtggaggaa ccaaactgac tgtccta 327

<210> 33  
<211> 511  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of an anti-CD33/CD3 bispecific single  
chain molecule"

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

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

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Lys Phe  
50 55 60

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

Met Glu Ile Arg Asn Leu Gly Gly Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Trp Ser Trp Ser Asp Gly Tyr Tyr Val Tyr Phe Asp Tyr Trp  
100 105 110

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

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser  
130 135 140

Pro Asp Ser Leu Thr Val Ser Leu Gly Glu Arg Thr Thr Ile Asn Cys  
145 150 155 160

Lys Ser Ser Gln Ser Val Leu Asp Ser Ser Thr Asn Lys Asn Ser Leu  
165 170 175

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Leu Ser  
180 185 190

Trp Ala Ser Thr Arg Glu Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser  
195 200 205

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Ser Pro Gln Pro Glu  
210 215 220

Asp Ser Ala Thr Tyr Tyr Cys Gln Gln Ser Ala His Phe Pro Ile Thr  
225 230 235 240

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Ser Gly Gly Gly Gly Ser  
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
260 265 270

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr  
275 280 285

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
290 295 300

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
305 310 315 320

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
325 330 335

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Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr  
340 345 350

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp  
355 360 365

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly  
370 375 380

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val  
385 390 395 400

Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu  
405 410 415

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn  
420 425 430

Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly  
435 440 445

Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu  
450 455 460

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp  
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Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn Arg Trp Val Phe  
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<210> 34

<211> 728

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of monomeric anti-CD33/CD3 Bi-Fc"

<400> 34

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

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

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Lys Phe

50

55

60

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

Met Glu Ile Arg Asn Leu Gly Gly Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Trp Ser Trp Ser Asp Gly Tyr Tyr Val Tyr Phe Asp Tyr Trp  
100 105 110

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

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser  
130 135 140

Pro Asp Ser Leu Thr Val Ser Leu Gly Glu Arg Thr Thr Ile Asn Cys  
145 150 155 160

Lys Ser Ser Gln Ser Val Leu Asp Ser Ser Thr Asn Lys Asn Ser Leu  
165 170 175

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Leu Ser  
180 185 190

Trp Ala Ser Thr Arg Glu Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser  
195 200 205

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Ser Pro Gln Pro Glu  
210 215 220

Asp Ser Ala Thr Tyr Tyr Cys Gln Gln Ser Ala His Phe Pro Ile Thr  
225 230 235 240

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Ser Gly Gly Gly Gly Ser  
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
260 265 270

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr  
275 280 285

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
290 295 300

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
305 310 315 320

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
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335

Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 340 345 350

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp  
 355 360 365

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly  
 370 375 380

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val  
 385 390 395 400

Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu  
 405 410 415

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn  
 420 425 430

Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly  
 435 440 445

Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu  
 450 455 460

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp  
 465 470 475 480

Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn Arg Trp Val Phe  
 485 490 495

Gly Gly Gly Thr Lys Leu Thr Val Leu Ala Pro Glu Leu Leu Gly Gly  
 500 505 510

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 515 520 525

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 530 535 540

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 545 550 555 560

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Gly Ser Thr Tyr Arg  
 565 570 575

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 580 585 590

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
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Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Thr  
610 615 620

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
625 630 635 640

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
645 650 655

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asp Thr Thr Pro Pro Val  
660 665 670

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Asp Leu Thr Val Asp  
675 680 685

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
690 695 700

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
705 710 715 720

Gly Lys His His His His His His  
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<210> 35  
<211> 1643  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:34"

<400> 35  
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tggtccgacg gctactacgt gtacttcgac tactggggcc agggcacctc cgtgacagtg 360  
tccagcggag ggggaggaag tggcggaggg ggctctggag gtggcggctc cgacatcgtg 420  
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aagtcctccc agtccgtgct ggactcctcc accaacaaga actcctggc ctggtatcag 540  
cagaagcctg gccagcctcc taagctgctg ctctcttggg cttccaccag agagagcggg 600  
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gtgagccacg aagaccctga ggt 1643
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<210> 36

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of an insertion that prolongs half life"

<400> 36

Gly Gly Cys Val Phe Asn Met Phe Asn Cys Gly Gly  
1 5 10

<210> 37

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of an insertion that prolongs half life"

<400> 37

Gly Gly Cys His Leu Pro Phe Ala Val Cys Gly Gly  
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<210> 38



<211> 12  
<212> PRT  
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<220>  
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<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of an insertion that prolongs half life"

<400> 38  
Gly Gly Cys Gly His Glu Tyr Met Trp Cys Gly Gly  
1 5 10

<210> 39  
<211> 12  
<212> PRT  
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<220>  
<221> source  
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<400> 39  
Gly Gly Cys Trp Pro Leu Gln Asp Tyr Cys Gly Gly  
1 5 10

<210> 40  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
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1 5 10

<210> 41  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of an insertion that prolongs half life"

<400> 41  
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1 5 10

<210> 42  
<211> 12  
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<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of an insertion that prolongs half  
 life"

<400> 42  
 Gly Gly Cys Ala Leu Tyr Pro Thr Asn Cys Gly Gly  
 1 5 10

<210> 43  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of an insertion that prolongs half  
 life"

<400> 43  
 Gly Gly Cys Gly Lys His Trp His Gln Cys Gly Gly  
 1 5 10

<210> 44  
 <211> 12  
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 Gly Gly Cys His Ser Phe Lys His Phe Cys Gly Gly  
 1 5 10

<210> 45  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

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 amino acid sequence of an insertion that prolongs half  
 life"

<400> 45  
 Gly Gly Cys Gln Gly Met Trp Thr Trp Cys Gly Gly  
 1 5 10

<210> 46  
 <211> 14  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of an insertion that prolongs half  
 life"

life"

&lt;400&gt; 46

Gly	Gly	Cys	Ala	Gln	Gln	Trp	His	His	Glu	Tyr	Cys	Gly	Gly
1				5					10				

&lt;210&gt; 47

&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of an insertion that prolongs half  
life"

&lt;400&gt; 47

Gly	Gly	Cys	Glu	Arg	Phe	His	His	Ala	Cys	Gly	Gly
1				5					10		

&lt;210&gt; 48

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a VH region CDR1 which is part  
of SEQ ID NO:7"

&lt;400&gt; 48

Ser	Tyr	Ala	Met	Asn
1				5

&lt;210&gt; 49

&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a VH region CDR2 which is part  
of SEQ ID NO:7"

&lt;400&gt; 49

Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp	Ser
1				5					10					15	

Val Lys Gly

&lt;210&gt; 50

&lt;211&gt; 14

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a VH region CDR3 which is part

of SEQ ID NO:7"

<400> 50

His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Trp Ala Tyr  
1 5 10

<210> 51

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of a VL region CDR1 which is part of SEQ ID NO:8"

<400> 51

Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn  
1 5 10

<210> 52

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of a VL region CDR2 which is part of SEQ ID NO:8"

<400> 52

Gly Thr Lys Phe Leu Ala Pro  
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<210> 53

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of a VL region CDR3 which is part of SEQ ID NO:8"

<400> 53

Val Leu Trp Tyr Ser Asn Arg Trp Val  
1 5

<210> 54

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of a VH region CDR1 which is part of SEQ ID NO:29"

<400> 54

Lys Tyr Ala Met Asn

1

5

<210> 55  
 <211> 19  
 <212> PRT  
 <213> Artificial Sequence

<220>  
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 amino acid sequence of a VH region CDR2 which is part  
 of SEQ ID NO:29"

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 Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser  
 1 5 10 15

Val Lys Asp

<210> 56  
 <211> 14  
 <212> PRT  
 <213> Artificial Sequence

<220>  
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 amino acid sequence of a VH region CDR3 which is part  
 of SEQ ID NO:29"

<400> 56  
 His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp Ala Tyr  
 1 5 10

<210> 57  
 <211> 14  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VL region CDR1 which is part  
 of SEQ ID NO:31"

<400> 57  
 Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn  
 1 5 10

<210> 58  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VL region CDR2 which is part  
 of SEQ ID NO:31"

<400> 58  
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5

<210> 59  
 <211> 9  
 <212> PRT  
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<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VL region CDR3 which is part  
 of SEQ ID NO:31"

<400> 59  
 Val Leu Trp Tyr Ser Asn Arg Trp Val  
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 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
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 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VH CDR1 of SEQ ID NO:5"

<400> 60  
 Asp Tyr Phe Val Asn  
 1 5

<210> 61  
 <211> 17  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VH CDR2 of SEQ ID NO:5"

<400> 61  
 Trp Ile Asp Pro Glu Asn Asp Asn Ser Leu Tyr Gly Pro Asn Phe Gln  
 1 5 10 15

Asp

<210> 62  
 <211> 11  
 <212> PRT  
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 amino acid sequence of a VH CDR3 of SEQ ID NO:5"

<400> 62  
 Tyr Tyr Gly Ser Arg Gly Asp Ala Met Asp Tyr  
 1 5 10

<210> 63  
 <211> 11  
 <212> PRT  
 <213> Artificial Sequence

<220>  
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 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VL CDR1 of SEQ ID NO:6"

<400> 63  
 Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn  
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<210> 64  
 <211> 7  
 <212> PRT  
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 Tyr Thr Ser Arg Leu His Ser  
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<210> 65  
 <211> 9  
 <212> PRT  
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<220>  
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 <223> /note="Description of Artificial Sequence: Synthetic  
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<400> 65  
 Gln Gln Gly Asn Thr Leu Pro Leu Thr  
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<210> 66  
 <211> 5  
 <212> PRT  
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<220>  
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 <223> /note="Description of Artificial Sequence: Synthetic  
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<400> 66  
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<210> 67  
 <211> 16  
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<220>  
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 <223> /note="Description of Artificial Sequence: Synthetic

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amino acid sequence of a VH region CDR2 of SEQ ID NO:15"

<400> 67  
Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser  
1 5 10 15

<210> 68  
<211> 8  
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<220>  
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<223> /note="Description of Artificial Sequence: Synthetic  
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<210> 69  
<211> 13  
<212> PRT  
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<220>  
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<223> /note="Description of Artificial Sequence: Synthetic  
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<400> 69  
Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Ala Val Asn  
1 5 10

<210> 70  
<211> 7  
<212> PRT  
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<220>  
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<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a VL region CDR2 of SEQ ID NO:15"

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Tyr Asp Asp Met Leu Ser Ser  
1 5

<210> 71  
<211> 11  
<212> PRT  
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<223> /note="Description of Artificial Sequence: Synthetic  
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<400> 71  
Ala Ala Trp Asp Asp Ser Leu Asn Gly Val Val  
1 5 10

<210> 72



<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
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<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a VH region CDR1 of SEQ ID NO:34"

<400> 72  
Asn Tyr Gly Met Asn  
1 5

<210> 73  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
amino acid sequence of a VH region CDR2 of SEQ ID NO:34"

<400> 73  
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1 5 10 15

Gly

<210> 74  
<211> 13  
<212> PRT  
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<223> /note="Description of Artificial Sequence: Synthetic  
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<400> 74  
Trp Ser Trp Ser Asp Gly Tyr Tyr Val Tyr Phe Asp Tyr  
1 5 10

<210> 75  
<211> 17  
<212> PRT  
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<223> /note="Description of Artificial Sequence: Synthetic  
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Ala

<210> 76

<211> 7  
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 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
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 <220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of a VL region CDR3 of SEQ ID NO:34"  
  
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 <210> 78  
 <211> 508  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
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 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of an anti-Mec/CD3-epsilon single  
 chain bispecific (P137424.7)"  
  
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 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile  
 35 40 45  
  
 Tyr Lys Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60  
  
 Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser Asp Val Gln Ser  
 65 70 75 80  
  
 Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Ser His Asp Thr Asn Pro Gly  
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 Ser Ser Thr Tyr Gly Ala Pro Phe Gly Gly Gly Thr Glu Val Val Val  
 100 105 110

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115 120 125

Glu Val Gln Leu Met Glu Ser Gly Gly Gly Leu Val Lys Pro Glu Gly  
130 135 140

Ser Leu Thr Leu Thr Cys Lys Val Ser Gly Phe Ser Phe Ser Thr Ser  
145 150 155 160

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Pro Glu Trp Ile  
165 170 175

Ala Gly Ile Tyr Asn Gly Gly Gly Ser Thr Tyr Tyr Ala Ser Trp Val  
180 185 190

Asn Gly Arg Phe Ser Ile Ser Arg Ser Thr Ser Leu Asn Thr Val Thr  
195 200 205

Leu Gln Met Thr Arg Leu Thr Ala Ala Asp Thr Ala Thr Tyr Phe Cys  
210 215 220

Ala Arg Trp Ile Arg Ile His Tyr Ser Phe Asp Leu Trp Gly Pro Gly  
225 230 235 240

Thr Leu Val Thr Val Ser Ser Ser Gly Gly Gly Gly Ser Glu Val Gln  
245 250 255

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys  
260 265 270

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr Ala Met Asn  
275 280 285

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile  
290 295 300

Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys  
305 310 315 320

Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ala Tyr Leu  
325 330 335

Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val  
340 345 350

Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp Ala Tyr Trp  
355 360 365

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly  
370 375 380

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Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val Thr Gln Glu  
385 390 395 400

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Gly  
405 410 415

Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn Trp Val Gln  
420 425 430

Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Lys Phe  
435 440 445

Leu Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly  
450 455 460

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu  
465 470 475 480

Tyr Tyr Cys Val Leu Trp Tyr Ser Asn Arg Trp Val Phe Gly Gly Gly  
485 490 495

Thr Lys Leu Thr Val Leu His His His His His His  
500 505

<210> 79  
<211> 1527  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
Polynucleotide sequence encoding the amino acid sequence  
of the anti-Mec/CD3-epsilon single chain bispecific  
(P137424.7)"

<400> 79	gtcctcactc agacccttc tccggtctcc gcggccgtcg gcggaactgt cactatcaat	60
	tgccagagtt cccaagtct gatctatgac agtagattag cctggtatca gcaaaaacca	120
	gggcaaccac ctaagctcct gatctataaa gcatccactt tggccagtgg ggtcccatca	180
	aggttcagcg gcagtggatc tgggacacag ttcactctca caatcagcga cgtccagtct	240
	gacgatgccg caacttatta ctgtcaaagt catgacacta atcctgggtc tagtacttat	300
	ggcgccccat tcggaggagg cactgaagtc gtagtccagg gtggtggtgg ttctggcggc	360
	ggcgggtcca gtggtggtgg ttctgaggtg cagctgatgg agtctggggg aggcttggtg	420
	aagcccagag ggtccctgac cctcacctgc aaggtctctg gattctcctt ttcaacgagt	480
	gccattagct ggggtccgcca ggctccaggg aagagaccag agtggatcgc cgggatttat	540
	aacggaggtg gtagcacata ctacgcatcc tgggtgaacg gccggttcag catctccaga	600
	tccacgtcct tgaacacggt gacgctgcaa atgaccagac tgacggccgc tgacacggcc	660

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```

acatatttct gtgcgagatg gattcggatc cattattcct tcgacctgtg gggacctggc      720
acgttgggtca ccgtctcctc atccggaggt ggtgggtccg aggtgcagct ggtcgagtct      780
ggaggaggat tgggtgcagcc tggagggtca ttgaaactct catgtgcagc ctctggattc      840
accttcaata agtacgcat gaactgggtc cgccaggctc caggaaaggg tttggaatgg      900
gttgctcgca taagaagtaa atataataat tatgcaacat attatgccga ttcagtgaaa      960
gacaggttca ccatctccag agatgattca aaaaacactg cctatctaca aatgaacaac     1020
ttgaaaactg aggacactgc cgtgtactac tgtgtgagac atgggaactt cggtaatagc     1080
tacatatcct actgggctta ctggggccaa gggactctgg tcaccgtctc ctcaggtggg     1140
ggtggttctg gcggcggcgg ctccggtggg ggtggttctc agactgttgt gactcaggaa     1200
ccttcactca ccgtatcacc tgggtgaaca gtcacactca cttgtggctc ctcgactggg     1260
gctgttacat ctggcaacta cccaactgg gtccaacaaa aaccagggtca ggcaccccg     1320
ggtctaatag gtgggactaa gttcctcgcc cccggtactc ctgccagatt ctcaggctcc     1380
ctgcttggag gcaaggctgc cctcacctc tcaggggtac agccagagga tgaggcagaa     1440
tattactgtg ttctatggta cagcaaccgc tgggtgttcg gtggaggaac caaactgact     1500
gtcctacatc atcacatca tcattag                                           1527

```

<210> 80

<211> 743

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of the first polypeptide chain of a heterodimeric anti-CD33/CD3-epsilon Bi-Fc aCD33(AMG330)-scFv-aCD3(I2C)-scFv-Fc(L234A, L235A, E356K, D399K (PL-31769))"

<400> 80

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

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

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Lys Phe  
50 55 60

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

Met Glu Ile Arg Asn Leu Gly Gly Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

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Ala Arg Trp Ser Trp Ser Asp Gly Tyr Tyr Val Tyr Phe Asp Tyr Trp  
100 105 110

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

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser  
130 135 140

Pro Asp Ser Leu Thr Val Ser Leu Gly Glu Arg Thr Thr Ile Asn Cys  
145 150 155 160

Lys Ser Ser Gln Ser Val Leu Asp Ser Ser Thr Asn Lys Asn Ser Leu  
165 170 175

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Leu Ser  
180 185 190

Trp Ala Ser Thr Arg Glu Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser  
195 200 205

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Ser Pro Gln Pro Glu  
210 215 220

Asp Ser Ala Thr Tyr Tyr Cys Gln Gln Ser Ala His Phe Pro Ile Thr  
225 230 235 240

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Ser Gly Gly Gly Gly Ser  
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
260 265 270

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr  
275 280 285

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
290 295 300

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
305 310 315 320

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
325 330 335

Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr  
340 345 350

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp  
355 360 365

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly  
 370 375 380

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val  
 385 390 395 400

Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu  
 405 410 415

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly Asn Tyr Pro Asn  
 420 425 430

Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly  
 435 440 445

Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu  
 450 455 460

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp  
 465 470 475 480

Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn Arg Trp Val Phe  
 485 490 495

Gly Gly Gly Thr Lys Leu Thr Val Leu Glu Pro Lys Ser Ser Asp Lys  
 500 505 510

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro  
 515 520 525

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser  
 530 535 540

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 545 550 555 560

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
 565 570 575

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
 580 585 590

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
 595 600 605

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys  
 610 615 620

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 625 630 635 640

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Leu Pro Pro Ser Arg Lys Glu Met Thr Lys Asn Gln Val Ser Leu Thr  
645 650 655

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
660 665 670

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
675 680 685

Lys Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
690 695 700

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
705 710 715 720

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
725 730 735

Lys His His His His His His  
740

<210> 81  
<211> 2232  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:80"

<400> 81  
caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagagtc tgtcaaagtc 60  
agctgcaagg cctccggcta caccttcacc aactacggca tgaactgggt gaaacaggct 120  
ccaggacagg gactcgagtg gatgggctgg atcaacacct acaccggcga gcctacctac 180  
gccgacaagt tccagggcag agtgaccatg accaccgaca catctaccag cacagcttac 240  
atggaaatcc ggaacctggg cggcgacgac accgccgtgt actactgcdc ccggtggtct 300  
tgggtccgacg gctactacgt gtacttcgac tactggggcc agggcacctc cgtgacagtg 360  
tccagcggag ggggaggaag tggcggaggg ggctctggag gtggcggctc cgacatcgtg 420  
atgaccagcgt ccccccgactc cctgaccgtg tccttgggag agcggaccac catcaactgc 480  
aagtcctccc agtccgtgct ggactcctcc accaacaaga actccctggc ctggtatcag 540  
cagaagcctg gccagcctcc taagctgctg ctctcttggg cttccaccag agagagcggg 600  
attcccagata ggttctccgg ctctggctcc ggcaccgact tcaccctgac catcgactcc 660  
cctcagcctg aggactccgc cacctactac tgccagcagt ccgcccactt ccctatcacc 720  
ttcggccagg gaaccggct ggaaatcaag tctggcggcg gtggctctga agtgcagctc 780  
gtggagagtg gcggaggact ggtgcagcca ggcggctccc tgaagctgtc ttgcgccgcc 840  
agcggcttca ctttaataa gtacgctatg aattgggtcc ggcaggcacc tggaaaaggg 900



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ctcgaatggg tgcgaaggat taggtctaag tacaacaact acgccaccta ttacgccgac      960
tctgtgaagg accggttcac catctcccgg gacgactcta agaacaccgc ttacctgcag      1020
atgaacaacc tgaaaaccga ggataccgct gtgtactatt gtgtgcggca cggcaacttc      1080
ggcaactcct acatctccta ctgggcctat tggggacagg gcacactggg caccgtgtcc      1140
tctggcgggtg gaggatctgg tggcggcgga tctggcggcg gaggttccca gaccgtggtc      1200
acccaggaac cttctctgac cgtcagtcctt ggcggaaccg tgaccctgac ctgtggctcc      1260
tctaccggcg ctgtgacctc cggcaactac cctaactggg tgcagcagaa acccggccag      1320
gctcccagag gactcatcgg cggcaccaag tttctggccc ctggcacccc tgccagattc      1380
tctggctccc tgctgggcgg caaggctgct ctgaccctga gcggagtcca gccagaggac      1440
gaggccgagt actactgtgt gctgtggtac tccaacagat ggggtgttcg cggtggcacc      1500
aagctgaccg tgctggagcc caaatcttct gacaaaactc acacatgccc cccgtgcccc      1560
gcacctgaag cagctggggg accgtcagtc ttctcttctc ccccaaaacc caaggacacc      1620
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac      1680
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag      1740
ccgcgagagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac      1800
caggactggc tgaatggcaa ggagtacaag tgcaaggctt ccaacaaagc cctcccagcc      1860
cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc      1920
ctgcccccat cccggaagga gatgaccaag aaccagggtca gcctgacctg cctgggtcaaa      1980
ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac      2040
tacaagacca cgctctccgt gctgaagtcc gacggctcct tcttctctta tagcaagctc      2100
accgtggaca agagcagggt gcagcagggg aacgtcttct catgctccgt gatgcatgag      2160
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa acatcatcat      2220
catcatcatt aa                                                                2232
```

<210> 82

<211> 243

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of an Fc polypeptide chain dummyFc-AADD"

<400> 82

His Met Ser Ser Val Ser Ala Gln Ala Ala Ala Glu Pro Lys Ser Ser  
1 5 10 15

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly  
20 25 30

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met

35

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
50 55 60

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
65 70 75 80

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
85 90 95

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
100 105 110

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
115 120 125

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

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
145 150 155 160

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
165 170 175

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asp Thr Thr Pro Pro  
180 185 190

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Asp Leu Thr Val  
195 200 205

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
210 215 220

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
225 230 235 240

Pro Gly Lys

<210> 83

<211> 729

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:82"

<400> 83

cacatgtctt cggttaagtc acaggcggcc gcagagccca aatcttctga caaaactcac

60

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```

acatgcccac cgtgcccagc acctgaagca gctggggggac cgtcagtcctt cctctttcccc 120
ccaaaaccca aggacaccct catgatctcc cggacccttg aggtcacatg cgtgggtggtg 180
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 240
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 300
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 360
aacaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 420
gaaccacagg tgtacaccct gccccatcc cgggaggaga tgaccaagaa ccaggtcagc 480
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 540
gggcagccgg agaacaacta cgacaccacg cctcccgtgc tggactccga cggctccttc 600
ttcctctata gcgacctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 660
tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 720
ccgggtaaa 729

```

<210> 84  
 <211> 728  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic amino acid sequence of a monomeric anti-CD33/CD3 Bi-Fc having the wild type sequence at residue N297 in the Fc portion of the molecule aCD33(AMF330)scFv-aCD3(I2C)scFv-Fc-mono"

<400> 84  
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu  
 1 5 10 15

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

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Lys Phe  
 50 55 60

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

Met Glu Ile Arg Asn Leu Gly Gly Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Trp Ser Trp Ser Asp Gly Tyr Tyr Val Tyr Phe Asp Tyr Trp  
 100 105 110

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

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser  
 130 135 140  
 Pro Asp Ser Leu Thr Val Ser Leu Gly Glu Arg Thr Thr Ile Asn Cys  
 145 150 155 160  
 Lys Ser Ser Gln Ser Val Leu Asp Ser Ser Thr Asn Lys Asn Ser Leu  
 165 170 175  
 Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Leu Ser  
 180 185 190  
 Trp Ala Ser Thr Arg Glu Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser  
 195 200 205  
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Ser Pro Gln Pro Glu  
 210 215 220  
 Asp Ser Ala Thr Tyr Tyr Cys Gln Gln Ser Ala His Phe Pro Ile Thr  
 225 230 235 240  
 Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Ser Gly Gly Gly Gly Ser  
 245 250 255  
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 260 265 270  
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr  
 275 280 285  
 Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 290 295 300  
 Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
 305 310 315 320  
 Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 325 330 335  
 Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 340 345 350  
 Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp  
 355 360 365  
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly  
 370 375 380  
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Thr Val Val  
 385 390 395 400

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Thr Gln Glu Pro Ser<sub>405</sub> Leu Thr Val Ser<sub>410</sub> Gly Gly Thr Val<sub>415</sub> Leu

Thr Cys Gly Ser<sub>420</sub> Ser Thr Gly Ala Val<sub>425</sub> Thr Ser Gly Asn<sub>430</sub> Tyr Pro Asn

Trp Val Gln<sub>435</sub> Gln Lys Pro Gly Gln<sub>440</sub> Ala Pro Arg Gly Leu<sub>445</sub> Ile Gly Gly

Thr Lys<sub>450</sub> Phe Leu Ala Pro Gly<sub>455</sub> Thr Pro Ala Arg Phe<sub>460</sub> Ser Gly Ser Leu

Leu Gly Gly Lys Ala<sub>470</sub> Ala Leu Thr Leu Ser Gly<sub>475</sub> Val Gln Pro Glu Asp<sub>480</sub>

Glu Ala Glu Tyr Tyr<sub>485</sub> Cys Val Leu Trp Tyr<sub>490</sub> Ser Asn Arg Trp Val<sub>495</sub> Phe

Gly Gly Gly Thr<sub>500</sub> Lys Leu Thr Val Leu<sub>505</sub> Ala Pro Glu Leu<sub>510</sub> Leu Gly Gly

Pro Ser Val<sub>515</sub> Phe Leu Phe Pro Pro<sub>520</sub> Lys Pro Lys Asp Thr<sub>525</sub> Leu Met Ile

Ser Arg<sub>530</sub> Thr Pro Glu Val Thr<sub>535</sub> Cys Val Val Val Asp<sub>540</sub> Val Ser His Glu

Asp<sub>545</sub> Pro Glu Val Lys Phe<sub>550</sub> Asn Trp Tyr Val Asp<sub>555</sub> Gly Val Glu Val His<sub>560</sub>

Asn Ala Lys Thr Lys<sub>565</sub> Pro Arg Glu Glu Gln<sub>570</sub> Tyr Asn Ser Thr Tyr<sub>575</sub> Arg

Val Val Ser Val<sub>580</sub> Leu Thr Val Leu His<sub>585</sub> Gln Asp Trp Leu Asn<sub>590</sub> Gly Lys

Glu Tyr Lys<sub>595</sub> Cys Lys Val Ser Asn<sub>600</sub> Lys Ala Leu Pro Ala<sub>605</sub> Pro Ile Glu

Lys Thr<sub>610</sub> Ile Ser Lys Ala Lys<sub>615</sub> Gly Gln Pro Arg Glu<sub>620</sub> Pro Gln Val Thr

Thr<sub>625</sub> Leu Pro Pro Ser Arg<sub>630</sub> Glu Glu Met Thr Lys<sub>635</sub> Asn Gln Val Ser Leu<sub>640</sub>

Thr Cys Leu Val Lys<sub>645</sub> Gly Phe Tyr Pro Ser<sub>650</sub> Asp Ile Ala Val Glu<sub>655</sub> Trp

Glu Ser Asn Gly<sub>660</sub> Gln Pro Glu Asn Asn<sub>665</sub> Tyr Asp Thr Thr Pro<sub>670</sub> Pro Val

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Asp Leu Thr Val Asp  
 675 680 685

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 690 695 700

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 705 710 715 720

Gly Lys His His His His His His  
 725

<210> 85

<211> 2187

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
 nucleic acid sequence encoding SEQ ID NO:84"

<400> 85

caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagagtc tgtcaaagtc	60
agctgcaagg cctccggcta caccttcacc aactacggca tgaactgggt gaaacaggct	120
ccaggacagg gactcgagtg gatgggctgg atcaacacct acaccggcga gcctacctac	180
gccgacaagt tccagggcag agtgaccatg accaccgaca catctaccag cacagcttac	240
atggaaatcc ggaacctggg cggcgacgac accgccgtgt actactgcg cgggtggtct	300
tgggtccgacg gctactacgt gtacttcgac tactggggcc agggcacctc cgtgacagtg	360
tccagcggag ggggaggaag tggcggaggg ggctctggag gtggcggctc cgacatcgtg	420
atgaccaggt ccccgactc cctgaccgtg tccctgggag agcggaccac catcaactgc	480
aagtcctccc agtccgtgct ggactcctcc accaacaaga actccctggc ctggtatcag	540
cagaagcctg gccagcctcc taagctgctg ctctcttggg cttccaccag agagagcggg	600
attcccgata ggttctccgg ctctggctcc ggcaccgact tcaccctgac catcgactcc	660
cctcagcctg aggactccgc cacctactac tgccagcagt ccgcccactt ccctatcacc	720
ttcggccagg gaaccggct ggaaatcaag tctggcggcg gtggctctga agtgcagctc	780
gtggagagtg gcggaggact ggtgcagcca ggcggctccc tgaagctgtc ttgcgccgcc	840
agcggcttca cttcaataa gtacgctatg aattgggtcc ggcaggcacc tggaaaagg	900
ctcgaatggg tcgcaaggat taggtctaag tacaacaact acgccaccta ttacgccgac	960
tctgtgaagg accggttcac catctcccgg gacgactcta agaacaccgc ttacctgcag	1020
atgaacaacc tgaaaaccga ggataccgct gtgtactatt gtgtgcggca cggcaacttc	1080
ggcaactcct acatctccta ctgggcctat tggggacagg gcacactggt caccgtgtcc	1140
tctggcggtg gaggatctgg tggcggcgga tctggcggcg gaggttccca gaccgtggtc	1200

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accaggaac cttctctgac cgtcagtcgc ggcggaaccg tgaccctgac ctgtggctcc 1260
tctaccggcg ctgtgacctc cggcaactac cctaactggg tgcagcagaa acccgccag 1320
gctcccagag gactcatcgg cggcaccaag tttctggccc ctggcacccc tgccagattc 1380
tctggctccc tgctgggcgg caaggctgct ctgacctga gcggagtcca gccagaggac 1440
gaggccgagt actactgtgt gctgtggtac tccaacagat ggggtgttcgg cgggtggcacc 1500
aagctgaccg tgctggcacc tgaactcctg gggggaccgt cagtcttcct cttcccccca 1560
aaaccaagg acaccctcat gatctcccgg acccctgagg tcacatgcgt ggtggtggac 1620
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aatgccaaga caaagccgcg ggaggagcag tacaacagca cgtaccgtgt ggtcagcgtc 1740
ctcaccgtcc tgcaccagga ctggctgaat ggcaaggagt acaagtcaa ggtctccaac 1800
aaagccctcc cagcccccat cgagaaaacc atctccaaag ccaaagggca gccccgagaa 1860
ccacaggtga ctaccctgcc cccatcccgg gaggagatga ccaagaacca ggtcagcctg 1920
acctgcctgg tcaaaggctt ctatcccagc gacatcgccg tggagtggga gagcaatggg 1980
cagccggaga acaactacga caccacgcct cccgtgctgg actccgacgg ctctttcttc 2040
ctctatagcg acctaccgt ggacaagagc aggtggcagc aggggaacgt cttctcatgc 2100
tccgtgatgc atgaggctct gcacaaccac tacacgcaga agagcctctc cctgtctccg 2160
ggtaaacatc atcatcatca tcattga 2187

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<210> 86  
 <211> 736  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence of anti-FOLR1/CD3-epsilon Bi-Fc  
 (PL-30058) ahuFolateR-1(5G1)-scFv-ahuCD3(I2C)scFv-FCAAKK"

<400> 86  
 Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln  
 1 5 10 15

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

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

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

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

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

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

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser  
115 120 125

Gly Gly Gly Gly Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser  
130 135 140

Glu Ala Pro Arg Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser  
145 150 155 160

Asn Ile Gly Asn Asn Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys  
165 170 175

Ala Pro Lys Leu Leu Ile Tyr Tyr Asp Asp Met Leu Ser Ser Gly Val  
180 185 190

Ser Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala  
195 200 205

Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala  
210 215 220

Trp Asp Asp Ser Leu Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu  
225 230 235 240

Thr Val Leu Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser  
245 250 255

Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala  
260 265 270

Ala Ser Gly Phe Thr Phe Asn Lys Tyr Ala Met Asn Trp Val Arg Gln  
275 280 285

Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys Tyr  
290 295 300

Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Asp Arg Phe Thr  
305 310 315 320

Ile Ser Arg Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Asn  
325 330 335

Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn  
340 345 350



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Phe Gly Asn Ser Tyr Ile Ser Tyr Trp Ala Tyr Trp Gly Gln Gly Thr  
355 360 365

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
370 375 380

Gly Gly Gly Gly Ser Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr  
385 390 395 400

Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly  
405 410 415

Ala Val Thr Ser Gly Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly  
420 425 430

Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly  
435 440 445

Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu  
450 455 460

Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val  
465 470 475 480

Leu Trp Tyr Ser Asn Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr  
485 490 495

Val Leu Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys  
500 505 510

Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro  
515 520 525

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys  
530 535 540

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp  
545 550 555 560

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu  
565 570 575

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
580 585 590

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn  
595 600 605

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
610 615 620

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Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Lys Glu  
625 630 635 640

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr  
645 650 655

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn  
660 665 670

Asn Tyr Lys Thr Thr Pro Pro Val Leu Lys Ser Asp Gly Ser Phe Phe  
675 680 685

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn  
690 695 700

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
705 710 715 720

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys His His His His His His  
725 730 735

<210> 87  
<211> 2211  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:86"

<400> 87  
caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc 60  
acctgcactg tctctggtgg ctccatcagc agtgggtgctt actactggac ctggatccgc 120  
cagcaccag ggaagggcct ggagtggatt gggtagatctt attacagtgg gagcacctac 180  
tacaaccgt ccctcaagag tcgagttagc atatcaatag acacgtctaa gaaccagttc 240  
tccctgaagc tgagctctgt gactgccgcg gacacggccg tgtattactg tgcgcgagggc 300  
agcagcagct ggttcgacta ctggggccag ggaaccctgg tcaccgtctc ctcaggaggc 360  
ggcggttcag gcggaggtgg ctctggcggg ggcggaagtc agtctgtgct gactcagcca 420  
ccctcgggtg ctgaagcccc caggcagagg gtcaccatct cctgttcttg aagcagctcc 480  
aacatcgga ataatgctgt aaactggtag cagcagctcc caggaaaggc tcccaaactc 540  
ctcatctatt atgatgatat gttgtcttca ggggtctcgg accgattttc tggctccaag 600  
tctggcacct cagcctccct ggccatcagt gggctccagt ctgaggatga ggctgattat 660  
tactgtgcag catgggatga cagcctgaat ggtgtggtat tcggcggagg gaccaagctg 720  
accgtcctat ccggaggtgg tggatccgaa gtgcagctcg tggagagtgg cggaggactg 780  
gtgcagccag gcggctccct gaagctgtct tgcgccgcca gcggcttcac cttcaataag 840  
tacgctatga attgggtccg gcaggcacct ggaaaagggc tcgaatgggt cgcaaggatt 900

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aggctctaagt acaacaacta cgccacctat tacgccgact ctgtgaagga ccggttcacc      960
atctcccgagg acgactctaa gaacaccgct tacctgcaga tgaacaacct gaaaaccgag      1020
gataccgctg  tgtactattg tgtgcggcac ggcaacttcg gcaactccta catctcctac      1080
tgggcctatt  ggggacaggg cacactgggc accgtgtcct ctggcggtgg aggatctggt      1140
ggcgggcggt  ctggcgggcg aggttcccag accgtggtca cccaggaacc ttctctgacc      1200
gtcagtcccc  gcggaaccgt gaccctgacc tgtggctcct ctaccggcgc tgtgacctcc      1260
ggcaactacc  ctaactgggt gcagcagaaa cccggccagg ctcccagagg actcatcggc      1320
ggcaccaagt  ttctggcccc tggcaccctt gccagattct ctggctcctt gctgggcggc      1380
aaggctgctc  tgaccctgag cggagtccag ccagaggacg aggccgagta ctactgtgtg      1440
ctgtggtact  ccaacagatg ggtgttcggc ggtggcacca agctgaccgt gctggagccc      1500
aaatcttctg  acaaaactca cacatgcccc ccgtgcccag cacctgaagc agctggggga      1560
ccgtcagtct  tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccctt      1620
gaggtcacat  gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg      1680
tacgtggacg  gcgtggaggt gcataatgcc aagacaaagc cgcgagagga gcagtacaac      1740
agcacgtacc  gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag      1800
gagtacaagt  gcaaggcttc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc      1860
aaagccaaag  ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccggaaggag      1920
atgaccaaga  accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc      1980
gccgtggagt  gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg      2040
ctgaagtccg  acggctcctt cttcctctat agcaagctca ccgtggacaa gagcaggtgg      2100
cagcagggga  acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg      2160
cagaagagcc  tctccctgtc tccgggtaaa catcaccacc accatcactg a      2211

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<210> 88  
 <211> 243  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <221> source  
 <223> /note="Description of Artificial Sequence: Synthetic  
 amino acid sequence encoding an Fc polypeptide chain"

<400> 88  
 His Met Ser Ser Val Ser Ala Gln Ala Ala Ala Glu Pro Lys Ser Ser  
 1 5 10 15

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly  
 20 25 30

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 35 40 45

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Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
50 55 60

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
65 70 75 80

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
85 90 95

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
100 105 110

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
115 120 125

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

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
145 150 155 160

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
165 170 175

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asp Thr Thr Pro Pro  
180 185 190

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Asp Leu Thr Val  
195 200 205

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
210 215 220

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
225 230 235 240

Pro Gly Lys

<210> 89

<211> 729

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
nucleic acid sequence encoding SEQ ID NO:88"

<400> 89

cacatgtctt cggtaagtgc acaggcggcc gcagagccca aatcttctga caaaactcac 60

acatgcccac cgtgcccagc acctgaagca gctgggggac cgtcagtctt cctcttcccc 120

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ccaaaaccca aggacaccct catgatctcc cggaccctg aggtcacatg cgtggtggtg      180
gacgtgagcc acgaagaccc tgaggtaag ttcaactggt acgtggacgg cgtggaggtg      240
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc      300
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc      360
aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga      420
gaaccacagg tgtacaccct gccccatcc cgggaggaga tgaccaagaa ccaggtcagc      480
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat      540
gggcagccgg agaacaacta cgacaccacg cctcccgtgc tggactccga cggctccttc      600
ttcctctata gcgacctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca      660
tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct      720
ccgggtaaa                                     729

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<210> 90

<211> 504

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
Amino acid sequence of a single chain anti-FOLR1/CD3-epsilon  
Bispecific PL-30055 aFOLR1(5G1)scFv-aCD3(I2C)scFv"

<400> 90

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

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

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

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

Leu Lys Ser Arg Val Ser Ile Ser Ile 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 Ser Ser Trp Phe Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
115 120 125

Gly Gly Gly Gly Ser Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser

130

135

140

Glu Ala Pro Arg Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser  
 145 150 155 160

Asn Ile Gly Asn Asn Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys  
 165 170 175

Ala Pro Lys Leu Leu Ile Tyr Tyr Asp Asp Met Leu Ser Ser Gly Val  
 180 185 190

Ser Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala  
 195 200 205

Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala  
 210 215 220

Trp Asp Asp Ser Leu Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu  
 225 230 235 240

Thr Val Leu Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser  
 245 250 255

Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala  
 260 265 270

Ala Ser Gly Phe Thr Phe Asn Lys Tyr Ala Met Asn Trp Val Arg Gln  
 275 280 285

Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys Tyr  
 290 295 300

Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Asp Arg Phe Thr  
 305 310 315 320

Ile Ser Arg Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Asn  
 325 330 335

Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn  
 340 345 350

Phe Gly Asn Ser Tyr Ile Ser Tyr Trp Ala Tyr Trp Gly Gln Gly Thr  
 355 360 365

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 370 375 380

Gly Gly Gly Gly Ser Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr  
 385 390 395 400

Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly

405

410

415

Ala Val Thr Ser Gly Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly  
                   420                                  425                                  430

Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly  
                   435                                  440                                  445

Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu  
           450                                  455                                  460

Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val  
   465                                  470                                  475                                  480

Leu Trp Tyr Ser Asn Arg Trp Val Phe Gly Gly Gly Thr Lys Leu Thr  
                   485                                  490                                  495

Val Leu His His His His His  
                   500

&lt;210&gt; 91

&lt;211&gt; 1515

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; source

<223> /note="Description of Artificial Sequence: Synthetic  
           nucleic acid sequence encoding SEQ ID NO:90"

&lt;400&gt; 91

cagggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc	60
acctgcactg tctctggtgg ctccatcagc agtgggtgctt actactggac ctggatccgc	120
cagcaccag ggaagggcct ggagtggatt gggtagatctt attacagtgg gagcacctac	180
tacaacccgt ccctcaagag tcgagttagc atatcaatag acacgtctaa gaaccagttc	240
tccctgaagc tgagctctgt gactgccgcg gacacggccg tgtattactg tgcgcgagggc	300
agcagcagct ggttcgacta ctggggccag ggaaccctgg tcaccgtctc ctcaggaggc	360
ggcggttcag gcggagggtg ctctggcggt ggcggaagtc agtctgtgct gactcagcca	420
ccctcgggtg ctgaagcccc caggcagagg gtcaccatct cctgttctgg aagcagctcc	480
aacatcgga ataagtctgt aaactggtac cagcagctcc caggaaaggc tcccaaactc	540
ctcatctatt atgatgatat gttgtcttca ggggtctcgg accgattttc tggctccaag	600
tctggcacct cagcctccct ggccatcagt gggctccagt ctgaggatga ggctgattat	660
tactgtgcag catgggatga cagcctgaat ggtgtggtat tcggcgaggg gaccaagctg	720
accgtcctat ccggagggtg tggatccgaa gtgcagctcg tggagagtgg cggaggactg	780
gtgcagccag gcggctccct gaagctgtct tgcgccgcca gcggcttcac cttcaataag	840
tacgctatga attgggtccg gcaggcacct ggaaaagggc tcgaatgggt cgcaaggatt	900

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aggtctaagt acaacaacta cgccacctat tacgccgact ctgtgaagga ccggttcacc      960
atctcccggg acgactctaa gaacaccgct tacctgcaga tgaacaacct gaaaaccgag      1020
gataccgctg tgtactattg tgtgcggcac ggcaacttcg gcaactccta catctcctac      1080
tgggcctatt ggggacaggg cacactggtc accgtgtcct ctggcggtgg aggatctggt      1140
ggcggcggat ctggcggcgg aggttcccag accgtggtca cccaggaacc ttctctgacc      1200
gtcagtcccg gcggaaccgt gaccctgacc tgtggctcct ctaccggcgc tgtgacctcc      1260
ggcaactacc ctaactgggt gcagcagaaa cccggccagg ctcccagagg actcatcggc      1320
ggcaccaagt ttctggcccc tggcacccct gccagattct ctggctccct gctgggcggc      1380
aaggctgctc tgacctgag cggagtccag ccagaggacg aggccgagta ctactgtgtg      1440
ctgtggtact ccaacagatg ggtgttcggc ggtggcacca agctgaccgt gctgcatcac      1500
caccaccatc actga                                                         1515

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<210> 92

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic  
6xHis tag"

<400> 92

His His His His His His

1

5