THERAPEUTIC PEPTIDES

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/541,921, filed on September 30, 201 1, the entire contents of which are hereby incorporated by reference.

GOVERNMENT SUPPORT

This invention was made with Government support under Grant No. POl AI045757, awarded by the National Institutes of Health. The Government has certain rights in the invention.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 27, 2012, is named 53293W01.txt and is 90,41 1 bytes in size.

TECHNICAL FIELD

This invention relates to therapeutic compositions (e.g., peptides) related to human subjects.

BACKGROUND

Human subjects exposed to a condition or disease offer a source of antibodies with therapeutic potential and general methods for obtaining such antibodies are known in the art. However, methods for specifically obtaining antibodies with therapeutic potential are generally limited by the low frequency, slow proliferation rate, and low antibody secretion levels of B cells that express such antibodies. For example, memory B cells with defined specificity typically account for only one cell per million peripheral blood mononuclear cells or approximately one milliliter of blood (Lanzavecchia et al., Curr. Opin. Immunol, 2 1:298-304 (2009): Yoshida et al, Immunol. Rev., 237: 117-139 (2010)). The frequency of antibodies with therapeutic potential is likely to be even lower

in cancer patients, necessitating the development of novel approaches that enable isolation of such cells with high sensitivity and efficiency.

Conventional methods generally rely on conversion of memory B cells into antibody secreting cells by in vitro culture and/or use of immunized animal models (e.g., mice) (Crotty et al, J. Immunol, 171:4969-4973 (2003): Fecteau et al, Immunology, 128:e353-e365 (2009): Buisman et al, Vaccine, 28:179-186 (2009): Corti et al, PLoS One, 5:e8805 (2010)). For example, following in vitro culture for up to one week, antibodies can be measured in culture supernatants and frequencies of antibody secreting cells assessing using enzyme-linked immunosorbent spot (ELISPOT) assay. Limitations of such methods are reported (Henn et al, J. Immunol, 183:31777-3187 (2009): Cao et al, J. Immunol, Methods, 358:56-65 (2010)). For instances, in vitro culture of memory B cells alters the memory B cell phenotype to resemble plasma cells with distinct functional properties (Jiang et al, Eur. J. Immunol, 37:2205-2213 (2007): Huggins et al, Blood, 109:1611-1619 (2007): Jourdan et al, Blood, 114:5173-5181 (2009)). Limitations for fluorescent antigen-based methods are also reported (Hofer et al, Immunol. Rev, 211:295-302 (2006): Odendahl et al, Blood, 105:1614-1621 (2005); Kunkel et al, Nat. Rev. Immunol, 3:822-829 (2003): Scheid et al, Nature, 458:636-640 (2009): Wu et al, Science, 329:856-861 (2010)).

Improved methods for specifically obtaining or targeting antibodies with therapeutic potential are required.

MICA is a ligand for NKG2D, a C-type lectin-like, type II transmembrane receptor expressed on most human NK cells, γδ T cells, and CD8+ T cells. Upon ligation, NKG2D signals through the adaptor protein DAP 10 to evoke perforin dependent cytolysis and to provide co-stimulation. In humans, the NKG2D ligands include MHC class I chain-related protein A (MICA), the closely related MICB, UL-16 binding proteins (ULBP) 1-4, and RAE-IG. While NKG2D ligands are not usually found on healthy tissues, various forms of cellular stress, including DNA damage, may upregulate ligand expression, resulting in their frequent detection in multiple solid and hematologic malignancies, including melanoma. NKG2D activation through ligand positive transformed cells contributes to extrinsic tumor suppression, since NKG2D deficient and

wild type mice treated with anti-NKG2D blocking antibodies manifest enhanced tumor susceptibility. Immune escape may be achieved in patients, however, by the shedding of NKG2D ligands from tumor cells, which triggers internalization of surface NKG2D and impaired function of cytotoxic lymphocytes. Soluble NKG2D ligands may also stimulate the expansion of regulatory NKG2D+CD4+Foxp3- T cells that may antagonize anti-tumor cytotoxicity through Fas ligand, IL-10, and TGF-β. MICA is a NKG2D ligand shed from tumor cells, i.e., released from the cell surface into the surrounding medium, and sera from cancer patients typically contain elevated levels of the soluble form (sMICA). MICA shedding is accomplished in part through interactions with the protein disulfide isomerase ERp5, which forms a disulfide bond with a critical cysteine that results in unfolding of the a3 domain, rendering it susceptible to proteolysis by ADAM-10/17 and MMP14.

Angiogenesis is the process of forming new capillaries from preexisting blood vessels and has been implicated as a critical part of tumor growth and dissemination. Tumors stimulate angiogenesis to meet increasing oxygen and nutrient requirements that exceed those that can be met by diffusion alone. Consequently, tumors recruit, remodel and expand existing vascular to meet their metabolic demand. The dependence of growing tumors on new blood vessel formation has made angiogenesis an appealing target for anti-cancer therapies. Many cytokines have been are believed to play a role in the regulation of angiogenesis, including vascular endothelial growth factor (VEGF) family members and the angiopoietins. The angiopioetins were discovered as ligands for the Ties, a family of tyrosine kinases that is selectively expressed in the vascular endothelium. There are four know angiopoietins: angiopoietin-1 ("Ang-1") through angiopoietin-4 ("Ang-4"). Studies have suggested that angiopoietins (e.g., Ang-1 and Ang-2) may be involved and tumor angiogenesis. With this information, angiopoietins have been identified as potential targets of immune-based cancer therapy.

There is a need to identify new agents that specifically recognize and bind targets of immune-based cancer therapy, such as MICA and angiopoietins. Such agents would be useful for diagnostic screening and therapeutic intervention in disease states that are associated with tumor development.

SUMMARY

The present disclosure provides compositions and methods related to antibodies with therapeutic potential.

In some embodiments, the disclosure provides compositions comprising peptides that immunospecifically bind to MHC class I polypeptide-related sequence A (MICA), or an epitope thereon. In some aspects, peptides of the compositions include complementarity determining region (CDR) 3 of the $\mathbf{V_H}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions, and CDR3 of the $\mathbf{V_L}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions. In some aspects, such peptides include complementarity determining region (CDR) 3 of the $\mathbf{V_H}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1, and CDR3 of the $\mathbf{V_L}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1. In some aspects, peptides further include CDR2 of the $\mathbf{V_H}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or CDR2 of the $\mathbf{V_L}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or both. In some aspects, such peptides include complementarity determining region CDR2 of the $\mathbf{V_H}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1, or CDR2 of the $\mathbf{V_L}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1, or both. In some aspects, peptides further include CDRl of the $\mathbf{V_H}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or CDRl of the $\mathbf{V_L}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or both. In some aspects, such peptides include complementarity determining region CDRl of the $\mathbf{V_H}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1, or CDRl of the $\mathbf{V_L}$ of antibody ID 1, 6, 7, 8 or 9 shown in Table 1, or both.

In some aspects, peptides are antibody or antibody fragment that include: a $\mathbf{V_H}$ chain with identity to SEQ ID NO:2, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the $\mathbf{V_H}$ of antibody ID 1 shown in table 1 having 5 or fewer conservative amino acid substitutions, and regions within SEQ ID NO:2 corresponding to FR1, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FR1, FR2, FR3,

FR4 of the V_H of antibody ID 1 shown in table 1; and a V_L chain with identity to SEQ ID NO: 11, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 1 shown in table 1 having 5 or fewer conservative amino acid substitutions, and regions within SEQ ID NO: 11 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 1 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:2 and a V_L chain comprising SEQ ID NO: 11. In some aspects, in addition the peptides, compositions further include one or more (e.g., 1 2, 3, 4, 5, 6, 7, 8, 9, 10, or less than 20) anti-cancer therapeutics. In some aspects, compositions are formulated as pharmaceutical compositions (e.g., for administration to a subject).

In some aspects, peptides are antibody or antibody fragment that include: a V_H chain with identity to SEQ ID NO: 149, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 6 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO: 149 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 6 shown in table 1; and a V_L chain with identity to SEQ ID NO: 151, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 6 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:151 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%>, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 6 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO: 149 and a V_L chain comprising SEQ ID NO: 15 1. In some aspects, in addition the peptides, compositions further include one or more (e.g., 1 2, 3, 4, 5, 6, 7, 8, 9, 10, or less than 20) anti-cancer

therapeutics. In some aspects, compositions are formulated as pharmaceutical compositions (e.g., for administration to a subject).

In some aspects, peptides are antibody or antibody fragment that include: a V_H chain with identity to SEQ ID NO:168, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl , CDR2, and CDR3 of the V_H of antibody ID 7 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO: 168 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 7 shown in table 1; and a V_L chain with identity to SEQ ID NO:170, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 7 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:170 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%>, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 7 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO: 168 and a V_L chain comprising SEQ ID NO: 170. In some aspects, in addition the peptides, compositions further include one or more (e.g., 1 2, 3, 4, 5, 6, 7, 8, 9, 10, or less than 20) anti-cancer therapeutics. In some aspects, compositions are formulated as pharmaceutical compositions (e.g., for administration to a subject).

In some aspects, peptides are antibody or antibody fragment that include: a V_H chain with identity to SEQ ID NO: 186, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 8 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO: 186 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 8 shown in table 1; and a V_L chain with identity to SEQ ID NO: 188, wherein regions corresponding to CDRl , CDR2, and CDR3 comprise CDRl , CDR2, and CDR3 of the V_L of antibody ID 8

shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO: 188 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 8 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO: 186 and a V_L chain comprising SEQ ID NO: 188. In some aspects, in addition the peptides, compositions further include one or more (e.g., 1 2, 3, 4, 5, 6, 7, 8, 9, 10, or less than 20) anti-cancer therapeutics. In some aspects, compositions are formulated as pharmaceutical compositions (e.g., for administration to a subject).

In some aspects, peptides are antibody or antibody fragment that include: a V_H chain with identity to SEQ ID NO:204, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 9 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:204 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 9 shown in table 1; and a V_L chain with identity to SEQ ID NO:206, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 9 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:206 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%>, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 9 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:204 and a V_L chain comprising SEQ ID NO:206. In some aspects, in addition the peptides, compositions further include one or more (e.g., 1 2, 3, 4, 5, 6, 7, 8, 9, 10, or less than 20) anti-cancer therapeutics. In some aspects, compositions are formulated as pharmaceutical compositions (e.g., for administration to a subject).

In some embodiments, the disclosure provides compositions that include one or more peptides that bind to angiopoietin or an epitope thereon. In some aspects, peptides of the compositions include complementarity determining region (CDR) 3 of the V_H of antibody ID 2, 3, 4, 5 or lOshown in Table 1 having 5 or fewer conservative amino acid substitutions, and CDR3 of the V_L of antibody ID 2, 3, 4 5 or 10 shown in Table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions. In some aspects, peptides can include complementarity determining region (CDR) 3 of the V_H of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1, and CDR3 of the V_L of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1. In some aspects, peptides can further include CDR2 of the V_H of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or CDR2 of the V_L of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or both. In some aspects, such peptides can include complementarity determining region CDR2 of the V_H of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1, or CDR2 of the V_L of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1, or both. In some aspects, peptides can further include CDRl of the V_H of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or CDRl of the V_L of antibody ID 2, 3, 4, or 5 shown in Table 1 having 5 or fewer conservative amino acid substitutions, or both. In some aspects, such peptides can include complementarity determining region CDRl of the V_H of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1, or CDRl of the V_L of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1, or both.

In some aspects, peptides include an antibody or antibody fragment comprising: a V_H chain with identity to SEQ ID NO:20, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 2 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:20 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%>, 90%>, 95%>, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 2 shown in table 1; and a V_L chain with identity to SEQ ID NO:29, wherein regions

corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 2 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:29 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 2 shown in table 1. In some aspects, the peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:20 and a V_L chain comprising SEQ ID NO:29.

In some aspects, the peptides an antibody or antibody fragment comprising: a V_H chain with identity to SEQ ID NO:38, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 3 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:38 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 3 shown in table 1; and a V_L chain with identity to SEQ ID NO:47, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 3 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:47 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 3 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:38 and a V_L chain comprising SEQ ID NO:47.

In some aspects, peptides include an antibody or antibody fragment comprising: a V_H chain with identity to SEQ ID NO:56, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 4 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:56 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%,

96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 4 shown in table 1; and a V_L chain with identity to SEQ ID NO:65, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 4 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:65 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 4 shown in table 1. In some aspects, peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:56 and a V_L chain comprising SEQ ID NO:65.

In some aspects, peptides include an antibody or antibody fragment comprising: a V_H chain with identity to SEQ ID NO:74, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 5 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:74 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%>, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 5 shown in table 1; and a V_L chain with identity to SEQ ID NO:83, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 5 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:83 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 5 shown in table 1. In some aspects, the peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:74 and a V_L chain comprising SEQ ID NO:83. In some aspects, the peptides immunospecifically bind to at least angiopoietin-2. In some aspects, the compositions further include one or more anti-cancer therapeutics. In some aspects, the compositions are formulated as a pharmaceutical composition.

In some aspects, peptides include an antibody or antibody fragment comprising: a V_H chain with identity to SEQ ID NO:222, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_H of antibody ID 10 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:222 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%>, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_H of antibody ID 10 shown in table 1; and a V_L chain with identity to SEQ ID NO:224, wherein regions corresponding to CDRl, CDR2, and CDR3 comprise CDRl, CDR2, and CDR3 of the V_L of antibody ID 10 shown in table 1 having 5 or fewer conservative amino acid substitutions within the CDRl, CDR2, and CDR3 regions, and regions within SEQ ID NO:224 corresponding to FRl, FR2, FR3, FR4, comprise amino acid sequences with at least 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, or 100% identity to FRl, FR2, FR3, FR4 of the V_L of antibody ID 10 shown in table 1. In some aspects, the peptides include an antibody or antibody fragment comprising a V_H chain comprising SEQ ID NO:222 and a V_L chain comprising SEQ ID NO:224. In some aspects, the peptides immunospecifically bind to at least angiopoietin-2. In some aspects, the compositions further include one or more anti-cancer therapeutics. In some aspects, the compositions are formulated as a pharmaceutical composition.

In some embodiments, the disclosure includes methods of treating cancer in a subject. In some aspects, methods include administering to a subject a composition of any one of claims 1-27.

The present disclosure also provides provides methods of isolating human antibodies from cancer patients following immunotherapy.

In some embodiments, the disclosure includes method of obtaining immune cells directed against a self antigen from a subject, the method comprising identifying a subject exhibiting a positive immune response towards the self antigen, providing a multimeric form of the self antigen, contacting the multimeric form of the self antigen with a sample from the subject exhibiting a positive immune response towards the self antigen, and obtaining immune cells bound to the multimeric form of the self antigen.

In some embodiments, the disclosure includes method of obtaining immune cells from a cancer patient directed against a self antigen, the method comprising identifying a subject exhibiting a positive immune response towards the self antigen; providing a multimeric form of the self antigen; contacting the multimeric form of the self antigen with a sample from the subject exhibiting a positive immune response towards the self antigen; and obtaining immune cells bound to the multimeric form of the self antigen.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 INucleic acid sequence of the variable heavy (V_H) chain of antibody ID 1 (anti-MHC class I polypeptide-related sequence A (MICA) antibody) (SEQ ID NO:l).

FIG. 2 IAmino acid sequence of V_H chain of antibody ID 1 (anti-MICA antibody) (SEQ ID NO:2).

FIG. 3 INucleic acid sequence of the variable light (V_L) chain of antibody ID 1 (anti-MICA antibody) (SEQ ID NO: 10).

FIG. 4 IAmino acid sequence of V_L chain of antibody ID 1 (anti-MICA antibody) (SEQ ID NO: 11).

FIG. 5 INucleic acid sequence of the V_H chain of antibody ID 2 (anti-angiopoietin-2 antibody) (SEQ ID NO: 19).

FIG. 6 IAmino acid sequence of V_H chain of antibody ID 2 (anti- angiopoietin-2 antibody) (SEQ ID NO:20).

FIG. 7 |Nucleic acid sequence of the V_L chain of antibody ID 2 (anti-angiopoietin-2 antibody) (SEQ ID NO:28).

FIG. 8 |Amino acid sequence of V_L chain of antibody ID 2 (anti- angiopoietin-2 antibody) (SEQ ID NO:29).

FIG. 9 |Nucleic acid sequence of the V_H chain of antibody ID 3 (anti-angiopoietin-2 antibody) (SEQ ID NO: 37).

FIG. 10 |Amino acid sequence of V_H chain of antibody ID 3 (anti- angiopoietin-2 antibody) (SEQ ID NO:38).

FIG. 11 |Nucleic acid sequence of the V_L chain of antibody ID 3 (anti-angiopoietin-2 antibody) (SEQ ID NO:46).

FIG. 12 |Amino acid sequence of V_L chain of antibody ID 3 (anti- angiopoietin-2 antibody) (SEQ ID NO:47).

FIG. 13 |Nucleic acid sequence of the V_H chain of antibody ID 4 (anti-angiopoietin-2 antibody) (SEQ ID NO:55).

FIG. 14 |Amino acid sequence of V_H chain of antibody ID 4 (anti- angiopoietin-2 antibody) (SEQ ID NO:56).

FIG. 15 |Nucleic acid sequence of the V_L chain of antibody ID 4 (anti-angiopoietin-2 antibody) (SEQ ID NO: 64).

FIG. 16 |Amino acid sequence of V_L chain of antibody ID 4 (anti- angiopoietin-2 antibody) (SEQ ID NO:65).

FIG. 17 |Nucleic acid sequence of the V_H chain of antibody ID 5 (anti-angiopoietin-2 antibody) (SEQ ID NO:73).

FIG. 18 |Amino acid sequence of V_H chain of antibody ID 5 (anti- angiopoietin-2 antibody) (SEQ ID NO:74).

FIG. 19 |Nucleic acid sequence of the V_L chain of antibody ID 5 (anti-angiopoietin-2 antibody) (SEQ ID NO: 82).

FIG. 20 |Amino acid sequence of V_L chain of antibody ID 5 (anti- angiopoietin-2 antibody) (SEQ ID NO:83).

FIG. 21A-21F | Illustrates exemplary methods for making antibodies from B-cells. (A) Antigen is expressed with a BirA tag for site-specific biotinylation and

tetramerization with fluorescently-labeled streptavidin. (B) B cells are stained with tetramer and a panel of monoclonal antibodies. Tetramer+, class-switched memory B cells are single-cell sorted into PCR strips. (C) mRNA amplification is performed with T7 RNA polymerase. (D) Sequencing of PCR products is carried out using 300-400bp PCR products. (E) Overlap PCR is used for construction of full-length IgGl heavy chain and kappa/lambda light sequences which are cloned into separate vectors. Vectors are transiently transfected into CHO-S cells for expression of fully human recombinant antibodies. (F) Antibodies are tested for antigen binding and assessed for potential therapeutic properties.

FIGs. 22A-22B | Graphs showing comparison of monomeric and tetrameric antigen for identification of memory B cells. (A) Mono-biotinylated TTCF or CD80 antigens were directly labeled with Alexa-488 fluorophore; tetramers were generated with unlabeled streptavidin. Enriched B cells from each donor were split into three fractions and stained with control CD80 tetramer, TTCF monomer, or TTCF tetramer at the same total antigen concentration of 0.125 μg/mL. FACS plots depict $CD19^+$ $CD27^+$ IgM^- class-switched memory B cells; numbers adjacent to the gate represent the percentage of the parental gate. (B) Frequencies of $tetramer^+$ memory B cells detected in three different donors. Numbers are calculated as $tetramer^+$ cells per $lxlO^6$ $CD19^+$ memory B cells.

FIGs. 23A-23B | Line graphs showing high affinity binding of TTCF by antibodies generated from plasmablasts and memory B cells. Saturation binding experiments were carried out to determine the affinities of recombinant antibodies. TTCF antigen was labeled with europium, which emits a strong fluorescent signal at 615nm upon incubation with a chelating reagent. Antibodies were immobilized in a 96-well plate and incubated with TTCF-europium (lOOnM to 4pM) for two hours at 37°C. Fluorescent counts at 615nm were recorded and K_O calculated using non-linear regression analysis. Control antibody (clone 8.18.C5) that was also produced in CHO-S cells was included in all experiments. (A) Recombinant TTCF Abs 1 and 2 were generated from $TTCF\ tetramer^+$ plasmablasts (donor 1). (B) TTCF antibodies 3, 4, and 5 originated from $TTCF\ tetramer^+$ memory B cells of three different donors.

FIG. 24 IBar chart showing binding of anti-MICA antibodies to MICA-coated luminex beads.

FIGs. 25A-250 | Line graphs showing binding of anti-MICA antibodies to MICA-coated beads.

FIGs 26A-26D | Bar graphs showing binding of four human angiopoietin 2 specific antibodies as well as a control antibody to three human angiopoietins (angiopoietin- 1, 2 and 4) and ang-like-3. Recombinant angiopoietins were immobilized in an ELISAplate and binding of human recombinant antibodies was detected with europium-labeled streptavidin.

FIGs. 27A-27C | Show graphs and a gel relating to isolation of angiopoietin-specific antibodies from a lung cancer patient. (A) Angiopoietin-2 reactivity of lung cancer patient (LI 9) serum (diluted 1:1000) determined by ELISA. (B) FACS plot showing PBMC sample (timepoint- 10/98) gated on $CD19^+$, $CD27^+$ IgM-B cells with CD 19 on the X-axis and fluorescently-tagged angiopoietin-2 on the Y-axis. (C) Heavy, light chain, and hinge region PCR products from 10 angiopoietin-2 reactive memory B-cells isolated from patient L19. The 500 base pair marker is indicated on the left.

FIG. 28 INucleic acid sequence of the variable heavy (V_H) chain of antibody ID 6 (anti-MHC class I polypeptide-related sequence A (MICA) antibody) (SEQ ID NO: 148).

FIG. 29 IAmino acid sequence of V_H chain of antibody 6 (anti-MICA antibody) (SEQ ID NO: 149).

FIG. 30 INucleic acid sequence of the variable light (V_L) chain of antibody ID 6 (anti-MICA antibody) (SEQ ID NO: 150).

FIG. 31 IAmino acid sequence of V_L chain of antibody ID 6 (anti-MICA antibody) (SEQ ID NO: 151).

FIG. 32 INucleic acid sequence of the variable heavy (V_H) chain of antibody ID 7 (anti-MHC class I polypeptide-related sequence A (MICA) antibody) (SEQ ID NO: 167).

FIG. 33 IAmino acid sequence of V_H chain of antibody ID 7 (anti-MICA antibody) (SEQ ID NO: 168).

FIG. 34 INucleic acid sequence of the variable light (V_L) chain of antibody ID 7 (anti-MICA antibody) (SEQ ID NO: 169).

FIG. 35 IAmino acid sequence of V_L chain of antibody ID 7 (anti-MICA antibody) (SEQ ID NO: 170).

FIG. 36 INucleic acid sequence of the variable heavy (VH) chain of antibody ID 8 (anti-MHC class I polypeptide-related sequence A (MICA) antibody) (SEQ ID NO: 185).

FIG. 37 IAmino acid sequence of VH chain of antibody ID 8 (anti-MICA antibody) (SEQ ID NO: 186).

FIG. 38 INucleic acid sequence of the variable light (VL) chain of antibody ID 8 (anti-MICA antibody) (SEQ ID NO: 187).

FIG. 39 IAmino acid sequence of VL chain of antibody ID 8 (anti-MICA antibody) (SEQ ID NO: 188).

FIG. 40 INucleic acid sequence of the variable heavy (V_H) chain of antibody ID 9 (anti-MHC class I polypeptide-related sequence A (MICA) antibody) (SEQ ID NO:203).

FIG. 41 IAmino acid sequence of V_H chain of antibody ID 9 (anti-MICA antibody) (SEQ ID NO:204).

FIG. 42 INucleic acid sequence of the variable light (V_L) chain of antibody ID 9 (anti-MICA antibody) (SEQ ID NO:205).

FIG. 43 IAmino acid sequence of V_L chain of antibody ID 9 (anti-MICA antibody) (SEQ ID NO: 206).

FIG. 44 INucleic acid sequence of the V_H chain of antibody ID 10 (anti-angiopoietin-2 antibody) (SEQ ID NO:221).

FIG. 45 IAmino acid sequence of V_H chain of antibody ID 10 (anti- angiopoietin-2 antibody) (SEQ ID NO:222).

FIG. 46 INucleic acid sequence of the V_L chain of antibody ID 10 (anti-angiopoietin-2 antibody) (SEQ ID NO:223).

FIG. 47 IAmino acid sequence of V_L chain of antibody ID 10 (anti- angiopoietin-2 antibody) (SEQ ID NO:224).

FIGs. 48A-G | Line graphs showing assessment of MICA allele-specific binding by recombinant anti-MICA antibodies.

FIG. 49 ILine graph showing labeling of autologous tumor cells by anti-MICA antibody CM24002 Ab2.

FIG. 50 IA series of FACS plot showing regulation of NKG2D by serum MICA. Human NK cells were incubated with control serum from patient CM24002 and a 1:10 dilution for 48 hours. Indicated antibodies were added at the start of the incubation at a concentration of 10 μg/ml. NKG2D expression was assessed on CD56+ NK cells by flow cytometry.

FIG. 51 IA series of FACS plot showing regulation of NKG2D by recombinant MICA. Human NK cells were incubated with recombinant MICA at a concentration of 2 ng/ml for 48 hours. Indicated antibodies were added at the start of the incubation at a concentration of 10 μg/ml. After 48 hours, NKG2D expression was assessed on CD56+ NK cells by flow cytometry.

FIG. 52 ILine graph demonstrating enhancement of cell-mediated toxicity by anti-MiCA antibody CM24002 Ab2. Human NK cells were incubated with recombinant MICA (2ng/ml) for 48 hours in the presence of indicated antibodies at 10 μg/ml. The ability of NK cells (effectors) to kill K562 target cells was assessed by measuring LDH release following 4 hour incubation at the indicated ratios.

FIG. 53 IBar graph demonstration cell-mediated toxicity by anti-MICA antibodies CM24002 Ab2 and CM33322 Ab29. Human NK cells were incubated with recombinant MICA (2ng/ml) for 48 hours in the presence of indicated antibodies at 10 μg/ml. The ability of NK cells (effectors) to kill K562 target cells was assessed by measuring LDH release following 4 hour incubation. NKG2D blocking antibody or Fc blocking antibody was added during the 4 hr incubation of effector and target cells to assess the contribution of Fc receptor and NKG2D to cell-mediated toxicity.

FIG. 54 IA series of line graphs showing binding of MICA alpha 3 domain by recombinant anti-MICA antibodies. Recombinant MICA alpha 3 domains were biotinylated and captured on the surface of streptavidin-coated beads. Indicated antibodies were incubated at 10μg/ml with the beads coated with the individual recombinant protein for lhr. Beads were subsequently washed and incubated with FITC-conjugated anti-human IgG secondary antibody. FITC fluorescence was quantified by flow cytometry.

FIG. 55 ILine graphs demonstrating labeling of tumor cells by anti-MICA antibodies CM24002 Ab2 and CM33322 Ab29. Fluorescence was determined by flow cytometry.

FIG. 56 IBar graph demonstrating MICA allelic specificity of anti-MICA antibodies CM33322 Ab29 as determined by Luminex assay.

FIG. 57 IBar graphs showing binding of anti-angiopoietin 2 specific antibody anti-Ang6 Ab2 as well as a control antibody to three human angiopoietins (angiopoietin-1, 2 and 4) and ang-like-3. Recombinant angiopoietins were immobilized in an ELISA plate and binding of human recombinant antibodies was detected with europium-labeled streptavidin.

DETAILED DESCRIPTION

The present disclosure is based, in part, on the observation that antibodies directed against therapeutic targets important in a disease can be obtained from human subjects exposed to the disease by labeling of B cells with a tetrameric form of the antigen of interest. As described in the background section above, prior methods are limited at least in that they are inefficient at identifying appropriate B cells in human subjects and/or because they induce any captured B cells to undergo phenotypic changes, thus reducing their value. In contrast, methods are described herein that allow capture of rare memory B cells directed against specific disease-related antigens. As described below, the methods require tetramerization of the disease-related antigen, which process, as demonstrated in the Examples below, enhances the identification of appropriate memory B cells. Specifically, methods herein permit more efficient capture of appropriate memory B cells for increased periods of time following initial exposure of a subject to the antigen. Methods herein also include antibodies (and peptides generated from the sequences of such antibodies) generated using genetic material obtained from memory B cells captured using the methods disclosed herein.

Described herein are human antibodies against MHC class I polypeptide-related sequence A (MICA) and human antibodies targeted against angiopoietin-2. Both types of human antibodies were identified from patients who had received a cell-based cancer

vaccine (GM-CSF transduced autologous tumor cells) by methods that entail the use of tetrameric antigens.

In some instances, the disclosure provides methods for specifically obtaining or targeting antibodies with therapeutic potential from select human subjects and therapeutic compositions resulting therefrom. These methods can include: obtaining or targeting immune cells in a human subject, wherein immune cells include but are not limited to, for example, B cells and/or memory B cells, isolating or purifying genetic material (e.g., DNA and/or mRNA) from the obtained or targeted immune cells, and using the isolated or purified genetic material to produce therapeutic compositions, e.g., therapeutic compositions disclosed herein. Further description of the methods is provided under the section entitled "Methods," below.

In some instances, the disclosure provides therapeutic compositions (e.g., including therapeutic peptides, including antibodies, antibody fragments, antibody derivatives, and/or antibody conjugates) related to antibodies present in subjects that have or had a condition or disease and that exhibited a positive immune response towards the condition or disease.

Therapeutic Compositions

In some instances, therapeutic compositions herein can interact with (e.g., bind, bind specifically and/or bind immunospecifically) binding partners (e.g., an immunogen(s), antigen(s), and/or epitope(s)) related to a disease or condition, wherein interaction between the therapeutic composition and the binding partners results in a positive immune response towards the condition or disease (e.g., a decrease in the level of disease or symptoms thereof in a subject).

In some instances, therapeutic compositions can include peptides that include (e.g., comprise, consist essentially of, or consist of) at least one (e.g., one, two, three, four, five, and/or six) complementarity determining region (CDR) of the variable heavy chain (V_H) and/or variable light chain (V_L) of antibody ID 1, 2, 3, 4, or 5, 6, 7, 8, 9 or 10, shown in Table 1.

In some instances, therapeutic compositions can include peptides that include (e.g., comprise, consist essentially of, or consist of) at least one (e.g., one, two, three, four, five, and/or six) complementarity determining region (CDR) of the variable heavy chain (VH) and/or variable light chain (VL) of antibody ID 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, shown in Table 1, and that interact with (e.g., bind, bind specifically and/or bind immunospecifically) to MHC class I polypeptide-related sequence A (MICA (e.g., UniGene Hs.130838)) (e.g., soluble MICA (sMICA)) and/or angiopoietin-2 (e.g., UniGene Hs.583870), including epitopes thereof.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 1, 6, 7, 8 and/or 9 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, peptides can include at least two CDRs, wherein the at least two CDRs are CDRs shown in Table 1 for different antibodies. . In other words, CDRs (and FRs and/or AA sequences) shown in Table 1 for antibodies IDs 1, 6, 7, 8 and 9 are interchangeable and can be combined to generate peptides, so long as the peptides bind (e.g., bind specifically and/or bind immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 1, 6, 7, 8 and/or 9 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 1, 6, 7, 8 and/or 9 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 1, 6, 7, 8 and/or 9 shown in Table 1. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 1, 6, 7, 8 and/or 9. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 1, 6, 7, 8 and/or 9 and at least one of FRl FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 1, 6, 7, 8 and/or 9, shown in Table 1. In some instances, such peptides include one of SEQ ID NO:2, 149, 168, 186 or 204 and/or one of SEQ ID NO:l 1, 151, 170, 188, or 206. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, the affinity of binding between the peptides and MICA can be between about O.lnM to IμM , for example, about IOηM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 6 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 6 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 6 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 6 shown in Table 1. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 6. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 6 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 6, shown in Table 1. In some instances, such peptides include SEQ ID NO: 149 and/or SEQ ID NO: 151. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, the affinity of binding between the peptides and MICA can be between about O.lnM to IμM, for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 7 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 7 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 7 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 7 shown in Table 1. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 7. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 7 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 7, shown in Table 1. In some instances, such peptides include SEQ ID NO: 168 and/or SEQ ID NO: 170. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, the affinity of binding between the peptides and MICA can be between about O.lnM to IμM, for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 8 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 8 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 8 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 8 shown in Table 1. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 8. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 8 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 8, shown in Table 1. In some instances, such peptides include SEQ ID NO: 186 and/or SEQ ID NO: 188. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, the affinity of binding between the peptides and MICA can be between about O.lnM to IμM , for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 9 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 9 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 9 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 9 shown in Table 1. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 9. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 9 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 9, shown in Table 1. In some instances, such peptides include SEQ ID NO:204 and/or SEQ ID NO:206. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to MICA (e.g., human MICA (e.g., soluble MICA (sMICA))). In some instances, the affinity of binding between the peptides and MICA can be between about O.lnM to IμM , for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 2, 3, 4, 5, and/or 10 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, peptides can include at least two CDRs, wherein the at least two CDRs are CDRs shown in Table 1 for different antibodies. In other words, CDRs (and FRs and/or AA sequences) shown in Table 1 for antibodies IDs 2, 3 4, 5, and 10 are interchangeable and can be combined to generate peptides, so long as the peptides bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 2, 3, 4, 5, and/or 10 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 2, 3, 4, 5, and/or 10 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 2, 3, 4, 5, and/or 10 shown in Table 1. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 2, 3, 4, 5, and/or 10. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 2, 3, 4, 5, and/or 10 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 2, 3, 4, 5, and/or 10 5, shown in Table 1. In some instances, such peptides include one of SEQ ID NO:20, 38, 56, 74, or 222 and/or one of SEQ ID NO:29, 47, 65, 83 or 224. In some instances, peptides include one of SEQ ID NO:20, 38, 56, 74, or 222 and one of SEQ ID NO:29, 47, 65, 83 or 224. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2 (e.g, UniGene Hs.583870)).

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 2 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 2 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 2 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 2 shown in Table 1. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 2. In some instances, such peptides

include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 2 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 2, shown in Table 1. In some instances, such peptides include SEQ ID NO:20 and/or SEQ ID NO:29. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, the affinity of binding between the peptides and angiopoietin-2 can be between about 0.1nM to IμM, for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 3 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 3 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 3 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 3 shown in Table 1. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 3. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 3 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 3, shown in Table 1. In some instances, such peptides include SEQ ID NO:38 and/or SEQ ID NO:47. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, the affinity of binding between the peptides and angiopoietin-2 can be between about 0.1nM to IμM, for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 4 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 4 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 4 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 4 shown in Table 1. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 4. In some instances, such peptides

include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 4 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 4, shown in Table 1. In some instances, such peptides include SEQ ID NO:56 and/or SEQ ID NO:65. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, the affinity of binding between the peptide and angiopoietin-2 can be between X-Y, for example, X-Y, X-Y. In some instances, the affinity of binding between the peptides and angiopoietin-2 can be between about O.lnM to IμM, for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 5 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 5 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 5 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 5 shown in Table 1. In some instances, such peptides include CDRl, CDR2, and CDR3 of the V_H and/or V_L of antibody ID 5. In some instances, such peptides include CDRl CDR2, and CDR3 of the V_H and/or V_L of antibody ID 5 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 5, shown in Table 1. In some instances, such peptides include SEQ ID NO:74 and/or SEQ ID NO:83. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, the affinity of binding between the peptides and angiopoietin-2 can be between about O.lnM to IμM, for example, about lOnM.

In some instances, therapeutic compositions can include peptides that include at least one CDR of the V_H and/or V_L of antibody ID 10 shown in Table 1, wherein the peptide binds (e.g., binds specifically and/or binds immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, such peptides include CDR3 of the V_H and/or V_L of antibody ID 10 shown in Table 1. In some instances, such peptides include CDR3 of the V_H and V_L of antibody ID 10 and CDRl and/or CDR2 of the V_H and/or V_L of antibody ID 10 shown in Table 1. In some instances, such peptides include CDRl,

CDR2, and CDR3 of the V_H and/or V_L of antibody ID 10. In some instances, such peptides include CDR1 CDR2, and CDR3 of the V_H and/or V_L of antibody ID 10 and at least one of FR1 FR2 FR3, and/or FR4 of the V_H and/or V_L of antibody ID 10, shown in Table 1. In some instances, such peptides include SEQ ID NO:222 and/or SEQ ID NO:224. In each instance, the peptide can bind (e.g., bind specifically and/or bind immunospecifically) to angiopoietin-2 (e.g., human angiopoietin-2). In some instances, the affinity of binding between the peptides and angiopoietin-2 can be between about O.lnM to IμM , for example, about IOηM.

In some instances, peptides that bind to angiopoietin-2 can also bind to angiopoietin-1 (e.g., Unigene Hs.369675) and/or angiopoietin-4 (e.g., Unigene Hs.278973). For example, in some instances, peptides that bind to angiopoietin-2 can also bind specifically and/or immunospecifically relative to other antigens (other than angiopoietin-1) to angiopoietin-1. In some instances, peptides that bind to angiopoietin-2 can also bind specifically and/or immunospecifically relative to other antigens (other than angiopoietin-4) to angiopoietin-4.

In some instances, therapeutic compositions can include peptides that include: SEQ ID NO: 2 and/or SEQ ID NO:11; SEQ ID NO: 149 and/or SEQ ID NO:151; SEQ ID NO: 168 and/or SEQ ID NO:170; SEQ ID NO: 186 and/or SEQ ID NO:188; SEQ ID NO: 204 and/or SEQ ID NO:206; SEQ ID NO:20 and/or SEQ ID NO:29; SEQ ID NO:38 and/or SEQ ID NO:47; SEQ ID NO:56 and/or SEQ ID NO:65; SEQ ID NO:74 and/or SEQ ID NO:83; and SEQ ID NO: 222 and/or SEQ ID NO:224.

TABLE 1

ID	Target	V_H | V_L	FR1*	CDR1**	FR2*	CDR2**	FR3*	CDR3**	FR4*	A.A.#	Nuc. Acid ##
1	Human MICA	V_H	QVQLQQW GAGLLKP SETLALT CAVS (SEQ ID NO: 3)	GGSFTDH Y (SEQ ID NO: 4)	WSWIR QAPGK GLEWIGE (SEQ ID NO: 5)	INHSGVT (SEQ ID NO: 6)	NYNPS LKSRLT ISVDTS KSQFSL RLTSVT AADTA LYYC (SEQ ID NO: 7)	AKTG LYYD DVW GTFR PRGG FDS (SEQ ID NO: 8)	WGQGT LVTVSS (SEQ ID NO: 9)	SEQ ID NO: 2 (see FIG. 2)	SEQ ID NO: 1 (see FIG. 1)
		V_L	DIVMTQS PD SLAVSLG ERATINC KSS (SEQ ID NO: 12)	QSILYSSD NKNY (SEQ ID NO: 13)	LAWYQ HKPGQPP KLLFY (SEQ ID NO: 14)	WAS (SEQ ID NO: 15)	IRESG VPDRF SGGGSGT DFTLT ISSLQA EDVAV YYC (SEQ ID NO: 16)	QQYYSP PCS (SEQ ID NO: 17)	FGQGTK LEIQ (SEQ ID NO: 18)	SEQ ID NO: 11 (see FIG. 4)	SEQ ID NO: 10 (see FIG. 3)
		V_H	QVQLQES GPGLVEP SGTLSLT CTVS (SEQ ID NO: 152)	GGSISRS NW (SEQ ID NO: 153)	WSWVRQ PPGEGLE WIGE (SEQ ID NO: 154)	IHHIGRS (SEQ ID NO: 156)	SYNPSLK SRVTMS VDKSQN QFSLRLT SVTAAD TAVYY	CAKNGYY AMDVW (SEQ ID NO: 158)	GQGTTVT VSS (SEQ ID NO: 155)	SEQ ID NO: 149 (see	SEQ ID NO: 148 (see

6	Human MICA								
		V_L	EIVLTQS PGTLSLS PGERATL SCRAS (SEQ ID NO: 159)	QSVSSDF (SEQ ID NO: 160)	LAWYQQ KPGQAPR LLIY (SEQ ID NO: 161)	ATS (SEQ ID NO: 162)	FRATGIS DRFSGSG SGTDFSL TINRLEP EDFAVYY (SEQ ID NO: 163)	CQHYRSS PPWYTF (SEQ ID NO: 164)	AQGTKL DMRRTV AAPSV (SEQ ID NO: 165)	SEQ ID NO: 151 (see FIG. 31)	SEQ ID NO: 150 (see FIG. 30)
		V_H	QVQLQES GPGLVKP SGTLSLT CAVS (SEQ ID NO: 171)	GASITNG AW (SEQ ID NO: 172)	WSWVRQ PPGKGLE WIGE (SEQ ID NO: 173)	IYLNGNT (SEQ ID NO: 174)	NSNPSLK SRVIISVD KSKNHFS LTLNSVT AADTAV YY (SEQ ID NO: 166)	CAKNAAY NLEFW (SEQ ID NO: 176)	GQGALVT VSS (SEQ ID NO: 177)	SEQ ID NO: 168 (see FIG. 33)	SEQ ID NO: 167 (see FIG. 32)
7	Human MICA										
		V_L	EIVLTQS PGTLSLS PGERATL SCRAS (SEQ ID NO: 178)	QTVSSPY (SEQ ID NO: 179)	VAWYQQ KRGQAP RLLIY (SEQ ID NO: 180)	GAS (SEQ ID NO: 181)	TRATGIP DRFSGSG SGTDFTL TISRLEP EDFAVYY (SEQ ID NO: 182)	CQQYDRS YYYTF (SEQ ID NO: 183)	GQGTKLE IK (SEQ ID NO: 184)	SEQ ID NO: 170 (see FIG. 35)	SEQ ID NO: 169 (see FIG. 34)
		V_H	QVQLQES GPGLVKP SENLSLT CTVS	DASMSD YH (SEQ ID NO: 190)	WSWIRQ AAGKGLE WIGR (SEQ ID	MYSTGSP (SEQ ID NO: 192)	YYKPSLK GRVTMSI DTSKNQ FSLKLAS	CASGQHI GGWVPP DFW (SEQ ID	GQGTLVT VSS (SEQ ID NO: 195)	SEQ ID NO:	SEQ ID NO:

8	Human MICA		(SEQ ID NO: 189)		NO: 191)		V TAADTAI YY (SEQ ID NO: 193)	NO: 194)	185 (see FIG. 36)
		V_L	DIVMTQT PLSSPVT LGQPASI SCRSS (SEQ ID NO: 196)	EGLVYSD GDTY (SEQ ID NO: 197)	LSWFHQ RPGQPPR LLIY (SEQ ID NO: 198)	KIS (SEQ ID NO: 199)	NRFSGVP DRFSGSG AGTDFTL KISRVEA EDVGVY Y (SEQ ID NO: 200)	CMQATH FPWTF (SEQ ID NO: 201)	GQGTKVE VKR (SEQ ID NO: 202)	SEQ ID NO: 186 (see FIG. 37)	SEQ ID NO: 187 (see FIG. 38)
9	Human MICA	V_H	EVQLLES GGGLVQP GGSLRLS CAAS (SEQ ID NO: 207)	GFTFSSY G (SEQ ID NO: 208)	LTWIRQA PGKGLE WVSS (SEQ ID NO: 209)	ISGSGNN T (SEQ ID NO: 210)	YYADSVK GRFTISR DKVKKT LYLQMD SLTVGDT AVYY (SEQ ID NO: 211)	CLGVGQ (SEQ ID NO: 212)	GHGIPVI VSS (SEQ ID NO: 213)	SEQ ID NO: 204 (see FIG. 41)	SEQ ID NO: 203 (see FIG. 40)
		V_L	DIVMTQT PLSSPVT LGQPASI SCRSS (SEQ ID NO: 214)	QSLVHRD GNTY (SEQ ID NO: 215)	LSWFLQ RPGQAPR LLIY (SEQ ID NO: 216)	RIS (SEQ ID NO: 217)	NRFSGVP DRFSGSG AGTDFTL KISRVEA EDVGVY Y (SEQ ID NO: 218)	CMQATQI PNTF (SEQ ID NO: 219)	GQGTKLE IK (SEQ ID NO: 220)	SEQ ID NO: 206 (see FIG. 43)	SEQ ID NO: 205 (see FIG. 42)

2	Angiopoietin-2	V_H	EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO: 21)	GFTFSSYA (SEQ ID NO: 22)	MSWVRQAPGKGLEWVSG (SEQ ID NO: 23)	IYWSGGST (SEQ ID NO: 24)	YYADSVKGRFTISRDISKNTLYLQMNSLRADTAVYYC (SEQ ID NO: 25)	ARGDYYGSGAHFDY (SEQ ID NO: 26)	WGQGTLVTVSS (SEQ ID NO: 27)	SEQ ID NO: 20 (see FIG. 6)	SEQ ID NO: 19 (see FIG. 5)
		V_L	DIVMTQTPLSSPVTLGQPASISCRSS (SEQ ID NO: 30)	QSLVHSDGNTY (SEQ ID NO: 31)	LSWLQQRPGQPPRLLIY (SEQ ID NO: 32)	QIS (SEQ ID NO: 33)	NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 34)	MQGTQFPRT (SEQ ID NO: 35)	FGQGTKVEIK (SEQ ID NO: 36)	SEQ ID NO: 29 (see FIG. 8)	SEQ ID NO: 28 (see FIG. 7)
3	Angiopoietin-2	V_H	EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO: 39)	GFTFSNNW (SEQ ID NO: 40)	MHWVRQAPGKGLEWISE (SEQ ID NO: 41)	IRSDGNFT (SEQ ID NO: 42)	RYADSMKGRFTISRDNAKSTLYLQMNSLRVEDTGLYYC (SEQ ID NO: 43)	ARDYPYSIDY (SEQ ID NO: 44)	WGQGTLVTVSS (SEQ ID NO: 45)	SEQ ID NO: 38 (see FIG. 10)	SEQ ID NO: 37 (see FIG. 9)
		V_L	DIVMTQTPLSSPVTLGQPASISCTSS	QSLVHSNGNTY (SEQ ID NO: 49)	LSWLQQRPGQPPRLLIY (SEQ ID	EIS (SEQ ID NO: 51)	KRVSGVPDRFSGSGAGTDFTLKISRVEA	MQGKQLRT (SEQ ID NO: 53)	FGQGTKLEIK (SEQ ID NO: 54)	SEQ ID NO:	SEQ ID NO:

4	Angiopoietin-2	V_H	EVQLVESGGGLVQPGGSVRLSCAAS (SEQ ID NO: 57)	GFILSNFA (SEQ ID NO: 58)	MSWVRQAPGKGLDWVSG (SEQ ID NO: 59)	NFGGRENT (SEQ ID NO: 60)	EDVGVYYC (SEQ ID NO: 52)	ARGDYHGSGAHFDY (SEQ ID NO: 62)	WGQGILVTVSS (SEQ ID NO: 63)	SEQ ID NO: 56 (see FIG. 14)	SEQ ID NO: 55 (see FIG. 13)
		V_L	DIVMTQSPLSSPVILGQPASISCRSS (SEQ ID NO: 66)	QSLLHSDGNTY (SEQ ID NO: 67)	LSWLHQRPGQPPRLLIY (SEQ ID NO: 68)	QIS (SEQ ID NO: 69)	NRFSGVPDRFSGSGTGTDFTLKISRVEAEDAGIYYC (SEQ ID NO: 70)	MQGTEFPRT (SEQ ID NO: 71)	FGQGTKVEIK (SEQ ID NO: 72)	SEQ ID NO: 65 (see FIG. 16)	SEQ ID NO: 64 (see FIG. 15)
	Angiopoietin-2	V_H	EVQLVESGGGLIQPGGSLRLSCATS (SEQ ID NO: 75)	GFTFRTSS (SEQ ID NO: 76)	MSWVRRAPGKGLEWVSA (SEQ ID NO: 77)	IGAESHDT (SEQ ID NO: 78)	HYTDSAEGRFTISKDYSKNTVYLQMNGLRV	AHHYYYGSRQKPKDWGDAFDM (SEQ ID NO: 80)	WGQGTMVSVSS (SEQ ID NO: 81)	SEQ ID NO: 74 (see	SEQ ID NO: 73 (see

5	2		DIQMTQSPSSVSASVGDRVTITCRAS (SEQ ID NO: 84)	QDIS TW (SEQ ID NO: 85)	LTWYQQRAGKAPNLLIY (SEQ ID NO: 86)	GAS (SEQ ID NO: 87)	TLEDGVPSRFSGSGSGTDFTLTIDSLQPDDFATYYC (SEQ ID NO: 88)	QQSHSFPYT (SEQ ID NO: 89)	FGQGTQLGIS (SEQ ID NO: 90)	SEQ ID NO: 83 (see FIG. 20)	SEQ ID NO: 82 (see FIG. 19)
10	Angiopoietin-2	V_H	EVQLVESGGGLIQPGGSLRLSCAAS (SEQ ID NO: 225)	GFLISSYF (SEQ ID NO: 226)	MSWVRQAPGKGPEWVSV (SEQ ID NO: 227)	IYSDGST (SEQ ID NO: 228)	YYVDSVKGRFTISTDNSKNTLYLQMNSLRAEDTARYY (SEQ ID NO: 229)	CATRHLNYDGDHW (SEQ ID NO: 230)	GQGTLVTVSSASTK (SEQ ID NO: 175)	SEQ ID NO: 222 (see FIG. 45)	SEQ ID NO: 221 (see FIG. 44)
		V_L	DVVMTQSPLSLPVTLGQPASISCRSS (SEQ ID NO: 231)	QSLVHSDGNTY (SEQ ID NO: 232)	LNWFHQRPGQSPRRLIY (SEQ ID NO: 233)	KVS (SEQ ID NO: 234)	KRDSGVPDRFSGSGSGDFTLKISRVEAEDVGIYY (SEQ ID NO: 235)	CMQGTHWPTF (SEQ ID NO: 236)	GQGTKVEIKRTVAA (SEQ ID NO: 237)	SEQ ID NO: 224 (see FIG. 47)	SEQ ID NO: 223 (see FIG. 46)

[*] Sequences include sequences or variants with (e.g., with at least) 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, and/or 100% sequence identity to the sequences shown.

[**] Sequences can include one, two, three, four, five, less than five, or less than ten conservative amino acid modifications.

*Sequences include sequences or variants with (e.g., with at least) 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, and/or 100% sequence identity to the sequences shown, e.g., within regions corresponding to FRl, FR2, FR3, and/or FR4, and/or one, two, three, four, five, less than 5, or less than ten conservative amino acid modifications within regions corresponding to CDRs 1, 2, and/or 3.

[##] Sequences include sequences or variants with (e.g., with at least) 80%, 85%, 90%, 95%, 96%, 97%, 98, 99%, and/or 100% sequence identity to the sequences shown, wherein the sequences encode the corresponding AA.

A.A.# shows the VH or VL amino acid sequence.

Nuc. Acid ## shows the VH or VL nucleic acid sequence.

While CDR and FR regions are shown above, such regions can also be defined according to Kabat (Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md., 1987 and 1991)). Amino acid numbering of antibodies or antigen binding fragments is also according to that of Kabat.

In some instances, therapeutic compositions can include peptides, including for example, antibodies, including full length and/or intact antibodies, or antibody fragments. An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term "antibody" encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (i.e., "antigen-binding portion") or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Exemplary antibodies and antibody fragments include, but are not limited to, monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, multispecific antibodies formed from at least two different epitope binding fragments (e.g., bispecific antibodies), camelised antibodies, chimeric antibodies, single-chain Fvs (scFv), single-chain antibodies, single domain antibodies, domain antibodies, Fab fragments, F(ab')2 fragments, antibody fragments that exhibit the desired biological activity (e.g. the antigen binding portion), disulfide-linked Fvs (dsFv), and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intrabodies, and epitope-binding fragments of any of the above. Antibodies or antibody fragments can be human or humanized.

Fragments of antibodies are suitable for use in the methods provided so long as they retain the desired affinity and specificity of the full-length antibody. Thus, a

fragment of an anti- MICA antibody or the anti-Angiopoietin antibody will retain an ability to bind to MICA or angiopoietin, respectively, in the Fv portion and the ability to bind the Fc receptor on dendritic cells in the FC portion. Such fragments are characterized by properties similar to the corresponding full-length anti-MICA antibody or the anti-Angiopoietin antibody, that is, the fragments will specifically bind a human MICA antigen or the angiopoietin antigen, respectively, expressed on the surface of a human cell or the corresponding sMICA antigen that has been shed into the media.

An Fv fragment is an antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight association, which can be covalent in nature, for example in scFv. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH-VL dimer. Collectively, the six CDRs or a subset thereof confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) can have the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site.

Single-chain Fv or (scFv) antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the scFv to form the desired structure for antigen binding.

The Fab fragment contains a variable and constant domain of the light chain and a variable domain and the first constant domain (CHI) of the heavy chain. F(ab')2 antibody fragments comprise a pair of Fab fragments which are generally covalently linked near their carboxy termini by hinge cysteines between them. Other chemical couplings of antibody fragments are also known in the art.

Diabodies are small antibody fragments with two antigen-binding sites, which fragments comprise a V_H connected to a V_L in the same polypeptide chain (V_H and V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

Linear antibodies comprise a pair of tandem Fd segments (V_H-CH 1-V_H-CH 1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

Antibodies and antibody fragments of the present disclosure can be modified in the Fc region to provide desired effector functions or serum half-life. In some instances, the Fc region can be conjugated to PEG or albumin to increase the serum half-life, or some other conjugation that results in the desired effect. Alternatively, where it is desirable to eliminate or reduce effector function, so as to minimize side effects or therapeutic complications, certain other Fc regions may be used.

Human and humanized antibodies include antibodies having variable and constant regions derived from (or having the same amino acid sequence as those derived from) human germline immunoglobulin sequences. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3.

A "CDR" of a variable domain are amino acid residues within the hypervariable region that are identified in accordance with the definitions of the Kabat, Chothia, the cumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al, 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al, 1989, Nature 342:877-883. Other approaches to CDR identification include the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts, set forth in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al,

2008, Journal of Biological Chemistry, 283:1 156-1 166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

In some instances, amino acid sequences of the peptides disclosed herein can be modified and varied to create peptide variants (e.g., peptides with a defined sequence homology to the peptides disclosed herein), for example, so long as the antigen binding property of the peptide variant is maintained or improved relative to the unmodified peptide (antigen binding properties of any modified peptide can be assessed using the in vitro and/or in vivo assays described herein and/or techniques known in the art).

While peptide variants are generally observed and discussed at the amino acid level, the actual modifications are typically introduced or performed at the nucleic acid level. For example, variants with 80%, 85%, 90%, 95%, 96%, 97%, 98, or 99% amino acid sequence identity to the peptides shown in Table 1 can be generated by modifying the nucleic acids encoding SEQ ID NOs:l, 10, 19, 28, 37, 46, 55, 64, 73, and/or 82 or portions/fragments thereof, using techniques (e.g., cloning techniques) known in the art and/or that are disclosed herein.

Amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional, or deletional modifications. Insertions include amino and/or terminal fusions as well as intra-sequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site

within the protein molecule. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions can be made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional modifications are those in which at least one residue has been removed and a different residue inserted in its place. In some instances, substitutions can be conservative amino acid substitutions. In some instances, peptides herein can include one or more conservative amino acid substitutions relative to a peptide shown in Table 1. For example, variants can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, or 40-50 conservative amino acid substitutions relative to a peptide shown in Table 1. Alternatively, variants can include 50 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer conservative amino acid substitutions relative to a peptide shown in Table 1. Such substitutions generally are made in accordance with the following Table 2 and are referred to as conservative substitutions. Methods for predicting tolerance to protein modification are known in the art (see, e.g., Guo et al, Proc. Natl. Acad. Sci., USA, 101(25):9205-9210 (2004)).

Table 2: Conservative Amino Acid Substitutions

Amino Acid	Substitutions (others are known in the art)
Ala	Ser, Gly, Cys
Arg	Lys, Gin, His
Asn	Gin, His, Glu, Asp
Asp	Glu, Asn, Gin
Cys	Ser, Met, Thr
Gin	Asn, Lys, Glu, Asp, Arg

Glu	Asp, Asn, Gin
Gly	Pro, Ala, Ser
His	Asn, Gin, Lys
Ile	Leu, Val, Met, Ala
Leu	Ile, Val, Met, Ala
Lys	Arg, Gin, His
Met	Leu, He, Val, Ala, Phe
Phe	Met, Leu, Tyr, Trp, His
Ser	Thr, Cys, Ala
Thr	Ser, Val, Ala
Trp	Tyr, Phe
Tyr	Trp, Phe, His
Val	He, Leu, Met, Ala, Thr

In some instances, substitutions are not conservative. For example, an amino acid in a peptide shown in Table 1 can be replaced with an amino acid that can alter some property or aspect of the peptide. In some instances, non-conservative amino acid substitutions can be made, e.g., to change the structure of a peptide, to change the binding properties of a peptide (e.g., to increase or decrease the affinity of binding of the peptide to an antigen and/or to alter increase or decrease the binding specificity of the peptide to the antigen).

In some instances, peptides and/or peptide variants can include or can be fragments of the peptides shown in Table 1. Such fragments can include, for example, 1, 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, 30, 31, 32, 33, 34, 35, 36 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 50-100, 101-150, fewer amino acids than the CDRs, FRs, and/or AAs shown in Table 1, e.g., so long as the fragments retain at least at portion of the binding properties of the full-length peptide (e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the binding properties of the full-length peptide). Truncations can be made at the amino-terminus, the carboxy-terminus, and/or within the peptides herein.

In some instances, the interacting face of a peptide variant can be the same (e.g., substantially the same) as an unmodified peptide, e.g., to alter (e.g., increase or decrease), preserve, or maintain the binding properties of the peptide variant relative to the unmodified peptide. Methods for identifying the interacting face of a peptide are known in the art (Gong et al, BMC: Bioinformatics, 6:1471-2105 (2007); Andrade and Wei et al, Pure and Appl. Chem., 64(1 1): 1777-1781 (1992); Choi et al, Proteins: Structure, Function, and Bioinformatics, 77(1): 14-25 (2009); Park et al., BMC: and Bioinformatics, 10:1471-2105 (2009).

Those of skill in the art readily understand how to determine the identity of two polypeptides (e.g., an unmodified peptide and a peptide variant). For example, identity can be calculated after aligning the two sequences so that the identity is at its highest level. Another way of calculating identity can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, Adv. Appl. Math, 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of identity can be obtained for nucleic acids by, for example, the algorithms disclosed in Zuker, Science 244:48-52 (1989); Jaeger et al, Proc. Natl. Acad. Sci. USA 86:7706-10 (1989); Jaeger et al, Methods Enzymol. 183:281-306 (1989), which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity and to be disclosed herein.

In some instances, as described in more detail under the methods section below, therapeutic compositions disclosed herein can be produced using genetic material (e.g., DNA and/or mRNA) isolated and/or purified from immune cells (e.g., B cells, including

memory B cells) obtained using the methods disclosed herein. Once such genetic material has been obtained, methods for using it to produce the therapeutic compositions disclosed herein are known in the art and/or are summarized below.

In some instances, peptides can include a detectable label. As used herein, a "label" refers to a moiety that has at least one element, isotope, or functional group incorporated into the moiety which enables detection of the peptide to which the label is attached. Labels can be directly attached (ie, via a bond) or can be attached by a linker (e.g., such as, for example, a cyclic or acyclic, branched or unbranched, substituted or unsubstituted alkylene; cyclic or acyclic, branched or unbranched, substituted or unsubstituted alkenylene; cyclic or acyclic, branched or unbranched, substituted or unsubstituted alkynylene; cyclic or acyclic, branched or unbranched, substituted or unsubstituted heteroalkylene; cyclic or acyclic, branched or unbranched, substituted or unsubstituted heteroalkenylene; cyclic or acyclic, branched or unbranched, substituted or unsubstituted heteroalkynylene; substituted or unsubstituted arylene; substituted or unsubstituted heteroarylene; or substituted or unsubstituted acylene, or any combination thereof, which can make up a linker). Labels can be attached to a peptide at any position that does not interfere with the biological activity or characteristic of the inventive polypeptide that is being detected.

Labels can include: labels that contain isotopic moieties, which may be radioactive or heavy isotopes, including, but not limited to, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{31}P, ^{32}P, ^{35}S, ^{67}Ga, ^{99m}Tc (Tc-99m), ^{111}In, ^{123}I, ^{125}I, ^{169}Yb, and ^{186}Re; labels that include immune or immunoreactive moieties, which may be antibodies or antigens, which may be bound to enzymes {e.g., such as horseradish peroxidase); labels that are colored, luminescent, phosphorescent, or include fluorescent moieties (e.g., such as the fluorescent label FITC); labels that have one or more photoaffinity moieties; labels that have ligand moieties with one or more known binding partners (such as biotin-streptavidin, FK506-FKBP, etc.).

In some instances, labels can include one or more photoaffinity moieties for the direct elucidation of intermolecular interactions in biological systems. A variety of known photophores can be employed, most relying on photoconversion of diazo compounds, azides, or diazirines to nitrenes or carbenes (see, e.g., Bayley, FL, Photogenerated

Reagents in Biochemistry and Molecular Biology (1983), Elsevier, Amsterdam, the entire contents of which are incorporated herein by reference). In certain embodiments of the invention, the photoaffinity labels employed are o-, m- and p-azidobenzoyls, substituted with one or more halogen moieties, including, but not limited to 4-azido-2,3,5,6-tetrafluorobenzoic acid.

Labels can also be or can serve as imaging agents. Exemplary imaging agents include, but are not limited to, those used in positron emissions tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI); anti-emetics; and contrast agents. Exemplary diagnostic agents include but are not limited to, fluorescent moieties, luminescent moieties, magnetic moieties; gadolinium chelates (e.g., gadolinium chelates with DTPA, DTPA-BMA, DOTA and HP-D03A), iron chelates, magnesium chelates, manganese chelates, copper chelates, chromium chelates, iodine -based materials useful for CAT and x-ray imaging, and radionuclides. Suitable radionuclides include, but are not limited to, $^{123}1$, ^{125}I, ^{130}I, ^{131}I, ^{133}I, ^{135}I, ^{47}Sc, ^{72}As, ^{72}Se, ^{90}Y, ^{88}Y, ^{97}Ru, ^{100}Pd, ^{101}mRh, ^{119}Sb, ^{128}Ba, ^{197}Hg, ^{211}At, ^{212}Bi, ^{212}Pb, ^{109}Pd, ^{111}In, ^{67}Ga, ^{68}Ga, ^{67}Cu, ^{75}Br, ^{77}Br, ^{99}mTc, ^{14}C, ^{13}N, ^{15}0, ^{32}P, ^{33}P, and ^{18}F.

Fluorescent and luminescent moieties include, but are not limited to, a variety of different organic or inorganic small molecules commonly referred to as "dyes," "labels," or "indicators." Examples include, but are not limited to, fluorescein, rhodamine, acridine dyes, Alexa dyes, cyanine dyes, etc. Fluorescent and luminescent moieties may include a variety of naturally occurring proteins and derivatives thereof, e.g., genetically engineered variants. For example, fluorescent proteins include green fluorescent protein (GFP), enhanced GFP, red, blue, yellow, cyan, and sapphire fluorescent proteins, reef coral fluorescent protein, etc. Luminescent proteins include luciferase, aequorin and derivatives thereof. Numerous fluorescent and luminescent dyes and proteins are known in the art (see, e.g., U.S. Patent Publication 2004/0067503; Valeur, B., "Molecular Fluorescence: Principles and Applications," John Wiley and Sons, 2002; and Handbook of Fluorescent Probes and Research Products, Molecular Probes, 9th edition, 2002).

The term "purified" as used herein, refers to other molecules, e.g. polypeptide, nucleic acid molecule that have been identified and separated and/or recovered from a component of its natural environment. Thus, in one embodiment the antibodies of the invention are purified antibodies wherein they have been separated from one or more components of their natural environment.

The term "epitope" as used herein refers to a protein determinant capable of binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

In some instances, the disclosure provides nucleotide sequences corresponding to (e.g., encoding) the disclosed peptides (e.g., disclosed in Table 1). These sequences include all degenerate sequences related to the disclosed peptides, i.e., all nucleic acids having a sequence that encodes one particular peptide and variants and derivatives thereof. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed polypeptide sequences.

In some instances, nucleic acids of the disclosed can include expression vectors. Examples of suitable vectors include, but are not limited to, plasmids, artificial chromosomes, such as BACs, YACs, or PACs, and viral vectors.

The provided vectors also can include, for example, origins of replication and/or markers. A marker gene can confer a selectable phenotype, e.g., antibiotic resistance, on a cell. The marker product is used to determine if the vector has been delivered to the cell and once delivered is being expressed. Examples of selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hygromycin, puromycin, and blasticidin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. Examples of other markers include, for example, the E. coli lacZ gene, green fluorescent protein (GFP), and

luciferase. In addition, an expression vector can include a tag sequence designed to facilitate manipulation or detection (e.g., purification or localization) of the expressed polypeptide. Tag sequences, such as GFP, glutathione S-transferase (GST), polyhistidine, c-myc, hemagglutinin, or FLAG™ tag (Kodak; New Haven, CT) sequences typically are expressed as a fusion with the encoded polypeptide. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino terminus.

In some instances, the disclosure includes cells comprising the nucleic acids (e.g., vectors) and/or peptides disclosed herein. Cells can include, for example, eukaryotic and/or prokaryotic cells. In general, cells that can be used herein are commercially available from, for example, the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108. See also F. Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, (1998). Transformation and transfection methods useful in the generation of the cells disclosed herein are described, e.g., in F. Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, (1998).

Pharmaceutical Formulations

In some instances, therapeutic compositions disclosed herein can include other compounds, drugs, and/or agents used for the treatment of cancer. Such compounds, drugs, and/or agents can include, for example, chemotherapy drugs, small molecule drugs or antibodies that stimulate the immune response to a given cancer. In some instances, therapeutic compositions can include, for example, one or more peptides disclosed herein and one or more of an anti-CTLA-4 antibody or peptide, an anti-PD-1 antibody or peptide, and/or an anti-PDL-1 antibody or peptide. For example, in some instances, therapeutic compositions disclosed herein can be combined with one or more (e.g., one, two, three, four, five, or less than ten) compounds.

In some instances, therapeutic compositions disclosed herein can include other compounds including histone deacetylase inhibitors ("HDAC") inhibitors. Examples of HDAC inhibitors include, for example, hydroxamic acid, Vorinostat (Zolinza);

suberoylanilide hydroxamic acid (SAHA)(Merck), Trichostatin A (TSA), LAQ824 (Novartis), Panobinostat (LBH589) (Novartis), Belinostat (PXD101)(CuraGen), ITF2357 Italfarmaco SpA (Cinisello), Cyclic tetrapeptide; Depsipeptide (romidepsin, FK228) (Gloucester Pharmaceuticals), Benzamide; Entinostat (SNDX-275/MS-275)(Syndax Pharmaceuticals), MGCD0103 (Celgene), Short-chain aliphatic acids, Valproic acid, Phenyl butyrate, AN-9, pivanex (Titan Pharmaceutical), CHR-3996 (Chroma Therapeutics), and CHR-2845 (Chroma Therapeutics).

In some instances, therapeutic compositions disclosed herein can include other compounds including proteasome inhibitors, including, for example, Bortezomib, (Millennium Pharmaceuticals), NPI-0052 (Nereus Pharmaceuticals), Carfilzomib (PR-171)(Onyx Pharmaceuticals), CEP 18770, and MLN9708

In some instances, the therapeutic compositions disclosed herein can include alkylating agents such as mephalan and topoisomerase inhibitors such as Adriamycin (doxorubicin) have been shown to increase MICA expression, which could enhance efficacy of an anti-MICA monoclonal antibody.

In some instances, therapeutic compositions disclosed herein can be formulated for use as or in pharmaceutical compositions. Such compositions can be formulated or adapted for administration to a subject via any route, e.g., any route approved by the Food and Drug Administration (FDA). Exemplary methods are described in the FDA's CDER Data Standards Manual, version number 004 (which is available at fda.give/cder/dsm/DRG/drg0030 1.htm).

In some instances, pharmaceutical compositions can include an effective amount of one or more peptides. The terms "effective amount" and "effective to treat," as used herein, refer to an amount or a concentration of one or more peptides for a period of time (including acute or chronic administration and periodic or continuous administration) that is effective within the context of its administration for causing an intended effect or physiological outcome.

In some instances, pharmaceutical compositions can include one or more peptides and any pharmaceutically acceptable carrier, adjuvant and/or vehicle. In some instances,

pharmaceuticals can further include one or more additional therapeutic agents in amounts effective for achieving a modulation of disease or disease symptoms.

The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant that may be administered to a patient, together with a peptide of this invention, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-a-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α-, β-, and γ-cyclodextrin, may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-'articular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

Pharmaceutical compositions can be in the form of a solution or powder for inhalation and/or nasal administration. Such compositions may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for

example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

Pharmaceutical compositions can be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Alternatively or in addition, pharmaceutical compositions can be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to

enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

In some embodiments, the present disclosure provides methods for using any one or more of the peptides or pharmaceutical compositions (indicated below as 'X') disclosed herein in the following methods:

Substance X for use as a medicament in the treatment of one or more diseases or conditions disclosed herein (e.g., cancer, referred to in the following examples as 'Y'). Use of substance X for the manufacture of a medicament for the treatment of Y; and substance X for use in the treatment of Y.

In some instances, therapeutic compositions disclosed herein can be formulated for sale in the US, import into the US, and/or export from the US.

Methods

In some instances, methods can include selection of a human subject who has or had a condition or disease and who exhibits or exhibited a positive immune response towards the condition or disease. In some instances, suitable subjects include, for example, subjects who have or had a condition or disease but that resolved the disease or an aspect thereof, present reduced symptoms of disease (e.g., relative to other subjects (e.g., the majority of subjects) with the same condition or disease), and/or that survive for extended periods of time with the condition or disease (e.g., relative to other subjects (e.g., the majority of subjects) with the same condition or disease), e.g., in an asymptomatic state (e.g., relative to other subjects (e.g., the majority of subjects) with the same condition or disease). In some instances, subjects can be selected if they have been vaccinated (e.g., previously vaccinated and/or vaccinated and re-vaccinated (e.g., received a booster vaccine)) against a condition or disease.

The term "subject," as used herein, refers to any animal. In some instances, the subject is a mammal. In some instances, the term "subject", as used herein, refers to a human (e.g., a man, a woman, or a child). Samples for use in the methods can include serum samples, e.g., obtained from the selected subject.

In some instances, subject selection can include obtaining a sample from a subject (e.g., a candidate subject) and testing the sample for an indication that the subject is suitable for selection. In some instances, the subject can be confirmed or identified, e.g. by a health care professional, as having had or having a condition or disease. In some instances, exhibition of a positive immune response towards a condition or disease can be made from patient records, family history, and/or detecting an indication of a positive immune response. In some instances multiple parties can be included in subject selection. For example, a first party can obtain a sample from a candidate subject and a second party can test the sample. In some instances, subjects can be selected and/or referred by a medical practitioner (e.g., a general practitioner). In some instances, subject selection can include obtaining a sample from a selected subject and storing the sample and/or using the in the methods disclosed herein. Samples can include, for example, cells or populations of cells.

In some instances, obtaining or targeting immune cells can include one or more and/or combinations of, for example: obtaining or providing a tetrameric immunogen that can bind (e.g., bind specifically) to a target immune cell; contacting the tetrameric immunogen with a sample; detecting the tetrameric immunogen; determining whether the tetrameric immunogen is bound to a target immune cell; and, if the tetrameric immunogen is bound to a target immune cell, then obtaining the target immune cell.

Tetrameric immunogens can include immunogens related to a condition or disease and/or that bind (e.g., bind specifically) to a target immune cell, e.g., wherein the target immune cell is related to a selected condition or disease. Immunogens and target immune cells related to a condition or disease include, for example, immunogens or immune cells present in subjects with a certain condition or disease, but not subjects without the condition or disease; and/or immunogens or immune cells present at altered levels (e.g., increased) in subjects with a certain condition or disease relative to subjects without the condition or disease. In some instances, immunogens or immune cells can be cancer specific. Immunogens can be soluble. Tetrameric immunogen can include tetrameric (including, e.g., tetramerized monomeric, dimeric, and/or trimeric antigen immunogen (e.g., antigen and/or epitope). In some instances, a tetrameric immunogen has increased

binding to a cell relative to the level of binding between a non-tetrameric form of the immunogen to the cell under similar conditions. In some instances, a tetrameric antigen includes a detectable moiety, e.g., a streptavidin moiety. Tetramerization methods are known in the art and are disclosed herein.

Detecting tetrameric immunogen and/or determining whether tetrameric immunogen is bound to a target cell can be performed using methods known in the art and/or disclosed herein. For example, methods can include flow cytometry. Optimization methods for flow cytometry, including sorting and gating methods, are known in the art and/or are disclosed herein. In some instances, methods can include analysis of the level of binding, binding affinity, and/or binding specificity between a tetrameric immunogen bound to a target immune cell. For example, a target immune cell can be obtained if (e.g., only if) a pre-determined level of binding between a tetrameric immunogen and a target immune cell is determined. Pre-determined levels of binding can be specific levels and/or can be relative levels. Obtaining target immune cells can include obtaining, providing, identifying, selecting, purifying, and/or isolating the target immune cells. Such methods can include, for example, cell sorting methods, cell enrichment, and/or background reduction.

In some instances, obtaining immune cells directed against a self antigen can include one or more and/or combinations of, for example, identifying a subject exhibiting a positive immune response towards the self antigen; obtaining or providing a multimeric form of the self antigen; contacting the multimeric form of the self antigen with a sample from the subject exhibiting a positive immune response towards the self antigen; obtaining immune cells bound to the multimeric form of the self antigen.

In some instances, methods can include obtaining immune cells directed against a self antigen from a cancer patient, can include one or more and/or combinations of, for example, identifying a subject exhibiting a positive immune response towards the self antigen; providing a multimeric form of the self antigen; contacting the multimeric form of the self antigen with a sample from the subject exhibiting a positive immune response towards the self antigen; and obtaining immune cells bound to the multimeric form of the self antigen.

Multimeric forms of a self antigen can include self antigens related to a condition or disease and/or that bind (e.g., bind specifically) to a target immune cell, e.g., wherein the target immune cell is related to a selected condition or disease. Self antigens and target immune cells related to a condition or disease include, for example, antigens or immune cells present in subjects with a certain condition or disease, but not subjects without the condition or disease; and/or immunogens or immune cells present at altered levels (e.g., increased) in subjects with a certain condition or disease relative to subjects without the condition or disease. In some instances, the condition or disease can be a cancer. In some embodiments, the cancer is melanoma, lung, breast, kidney, ovarian, prostate, pancreatic, gastric, and colon carcinoma, lymphoma or leukemia. In some instances, the self antigens or immune cells can be cancer specific. The self antigens can be soluble. Multimeric form of the self antigen can include a tetrameric form (including, e.g., tetramerized monomeric, dimeric, and/or trimeric antigen) of the self-antigen (e.g., antigen and/or epitope). In some instances, a multimeric form of the self antigen includes a detectable moiety, e.g., a streptavidin moiety. Multimerization methods are known in the art and are disclosed herein.

Methods for isolating or purifying genetic material (e.g., DNA and/or mRNA) from the obtained target immune cell are known in the art and are exemplified herein. Once such genetic material has been obtained, methods for using it to produce the therapeutic compositions disclosed herein are known in the art and/or are summarized below. As discussed above, genetic material can be varied, using techniques known in the art to create peptide variants disclosed herein.

Generating peptides from nucleic acids (e.g., cDNA) contained within or obtained from the target cell can include, for example, analysis, e.g., sequencing of heavy and light chain variable domains from target immune cells (e.g., single or isolated identified target immune cells). In some instances, methods can include generating fully human antibodies, or fragments thereof (e.g., as disclosed above), and humanization of non-human antibodies. DNA can be readily isolated and/or sequenced from the obtained immune cells using conventional procedures (e.g., by using oligonucleotide probes that

are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies).

Once isolated, DNA can be placed into expression vectors, which are then transfected into host cells such as *Escherichia coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., Curr. Opinion in Immunol., 5:256-262 (1993) and Pluckthun, Immunol. Revs., 130:151-188 (1992).

Recombinant expression of an antibody or variant thereof generally requires construction of an expression vector containing a polynucleotide that encodes the antibody. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody or a portion thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., US. Patent Nos. 5,981,216; 5,591,639; 5,658,759 and 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

Once the expression vector is transferred to a host cell by conventional techniques, the transfected cells are then cultured by conventional techniques to produce an antibody. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention or fragments thereof, or a heavy or light chain thereof, or portion thereof, or a single-chain antibody of the invention, operably linked to a heterologous promoter. In certain embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

Mammalian cell lines available as hosts for expression of recombinant antibodies are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster

ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), human epithelial kidney 293 cells, and a number of other cell lines. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the antibody or portion thereof expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, NSO (a murine myeloma cell line that does not endogenously produce any functional immunoglobulin chains), SP20, CRL7030 and HsS78Bst cells. In one embodiment, human cell lines developed by immortalizing human lymphocytes can be used to recombinantly produce monoclonal antibodies. In one embodiment, the human cell line PER.C6. (Crucell, Netherlands) can be used to recombinantly produce monoclonal antibodies.

In some instances, peptides disclosed herein can be generated synthetically. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing peptides described herein are known in the art and include, for example, those such as described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 3d. Ed., John Wiley and Sons (1999); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof.

Peptides can also be made by chemical synthesis methods, which are well known to the ordinarily skilled artisan. See, for example, Fields et al., Chapter 3 in Synthetic Peptides: A User's Guide, ed. Grant, W. H. Freeman & Co., New York, N.Y., 1992, p. 77. Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the a-NH_2 protected by either t-Boc or Fmoc chemistry using

side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431.

One manner of making of the peptides described herein is using solid phase peptide synthesis (SPPS). The C-terminal amino acid is attached to a cross-linked polystyrene resin via an acid labile bond with a linker molecule. This resin is insoluble in the solvents used for synthesis, making it relatively simple and fast to wash away excess reagents and by-products. The N-terminus is protected with the Fmoc group, which is stable in acid, but removable by base. Any side chain functional groups are protected with base stable, acid labile groups.

Longer peptides could be made by conjoining individual synthetic peptides using native chemical ligation. Alternatively, the longer synthetic peptides can be synthesized by well-known recombinant DNA techniques. Such techniques are provided in well-known standard manuals with detailed protocols. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated to obtain a nucleic acid sequence encoding the amino acid sequence, preferably with codons that are optimum for the organism in which the gene is to be expressed. Next, a synthetic gene is made, typically by synthesizing oligonucleotides which encode the peptide and any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and transfected into a host cell. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

The peptides can be made in a high-throughput, combinatorial fashion, e.g., using a high-throughput multiple channel combinatorial synthesizer available from Advanced Chemtech.

Peptide bonds can be replaced, e.g., to increase physiological stability of the peptide, by: a retro-inverso bonds (C(O)-NH); a reduced amide bond (NH-CH_2); a thiomethylene bond (S-CH_2 or CH_2-S); an oxomethylene bond (0-CH_2 or CH_2-0); an ethylene bond (CH_2-CH_2); a thioamide bond (C(S)-NH); a trans-olefm bond (CH=CH); a fluoro substituted trans-olefm bond (CF=CH); a ketomethylene bond (C(O)-CHR) or

CHR-C(O) wherein R is H or CH_3; and a fluoro-ketomethylene bond (C(O)-CFR or CFR-C(O) wherein R is H or F or CH_3.

Peptides can be further modified by: acetylation, amidation, biotinylation, cinnamoylation, farnesylation, fluoresceination, formylation, myristoylation, palmitoylation, phosphorylation (Ser, Tyr or Thr), stearoylation, succinylation and sulfurylation. As indicated above, peptides can be conjugated to, for example, polyethylene glycol (PEG); alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; and combinations thereof.

In some instances, peptides can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigens Protein A or Protein G, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies of the present invention or fragments thereof may be fused to heterologous polypeptide sequences (refered to herein as "tags") described above or otherwise known in the art to facilitate purification.

An exemplary, non-limiting, overview of the methods is shown in FIG. 21. Ordering is not implied.

Methods of Use

In some instances, the disclosure provides methods of treatment that include administering to a subject a composition disclosed herein.

Provided herein are methods for treating and/or preventing cancer or symptoms of cancer in a subject comprising administering to the subject a therapeutically effective amount of a composition comprising a peptide that immunospecifically binds to MHC class I polypeptide-related sequence A (MICA), wherein the peptide comprises complementarity determining region (CDR) 3 of the V_H of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions, and CDR3 of the V_L of antibody ID 1, 6, 7, 8 or 9 shown in Table 1 having 5 or fewer conservative amino acid substitutions. In some embodiments the cancer is a cancer associated with

overexpression of MICA. In some embodiments, the cancer is melanoma, lung, breast, kidney, ovarian, prostate, pancreatic, gastric, and colon carcinoma, lymphoma or leukemia. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is a plasma cell malignancy, for example, multiple myeloma (MM) or pre-malignant condition of plasma cells. In some embodiments the subject has been diagnosed as having a cancer or as being predisposed to cancer.

In some instances, the disclosure provides methods for treating and/or preventing cancer or symptoms of cancer in a subject comprising administering to the subject a therapeutically effective amount of a composition comprising an isolated antibody which specifically binds to MHC class I polypeptide -related sequence A (MICA), wherein the antibody comprises a heavy chain variable region (VH) comprising the VH CDR1, VH CDR2, and VH CDR3 as shown in the VH sequence of SEQ ID NO: 11, 149, 168, 186, or 204 and a light chain variable region (VL) sequence of SEQ ID No: 4, 151, 170, 189, or 206.

Also provided herein are methods for treating and/or preventing cancer or symptoms of cancer in a subject comprising administering to the subject a therapeutically effective amount of a peptide that immunospecifically binds to angiopoietin, wherein the peptide comprises complementarity determining region (CDR) 3 of the VH of antibody ID 2, 3, 4 or 5 or 10 shown in Table 1 having 5 or fewer conservative amino acid substitutions, and CDR3 of the VL of antibody ID 2, 3, 4 or 5 shown in Table 1 having 5 or fewer conservative amino acid substitutions. In some embodiments the cancer is a cancer associated with overexpression of MICA. In some embodiments, the cancer is melanoma, lung, breast, kidney, ovarian, prostate, pancreatic, gastric, and colon carcinoma, lymphoma or leukemia. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is a plasma cell malignancy, for example, multiple myeloma (MM) or pre-malignant condition of plasma cells. In some embodiments the subject has been diagnosed as having a cancer or as being predisposed to cancer.

In some instances, the disclosure provides methods for treating and/or preventing cancer or symptoms of cancer in a subject comprising administering to the subject a therapeutically effective amount of a composition comprising an isolated antibody which

specifically binds to angiopoietin (e.g., angiopoietin-2), wherein the antibody comprises a heavy chain variable region (VH) comprising the VH CDR1, VH CDR2, and VH CDR3 as shown in the VH sequence of SEQ ID NO: 20, 38, 56, 74, 222 and a light chain variable region (VL) sequence of SEQ ID No: 29, 47, 65, 83, or 224.

Symptoms of cancer are well-known to those of skill in the art and include, without limitation, unusual mole features, a change in the appearance of a mole, including asymmetry, border, color and/or diameter, a newly pigmented skin area, an abnormal mole, darkened area under nail, breast lumps, nipple changes, breast cysts, breast pain, death, weight loss, weakness, excessive fatigue, difficulty eating, loss of appetite, chronic cough, worsening breathlessness, coughing up blood, blood in the urine, blood in stool, nausea, vomiting, liver metastases, lung metastases, bone metastases, abdominal fullness, bloating, fluid in peritoneal cavity, vaginal bleeding, constipation, abdominal distension, perforation of colon, acute peritonitis (infection, fever, pain), pain, vomiting blood, heavy sweating, fever, high blood pressure, anemia, diarrhea, jaundice, dizziness, chills, muscle spasms, colon metastases, lung metastases, bladder metastases, liver metastases, bone metastases, kidney metastases, and pancreatic metastases, difficulty swallowing, and the like.

The methods disclosed herein can be applied to a wide range of species, e.g., humans, non-human primates (e.g., monkeys), horses, cattle, pigs, sheep, deer, elk, goats, dogs, cats, mustelids, rabbits, guinea pigs, hamsters, rats, and mice.

The terms "treat" or "treating," as used herein, refers to partially or completely alleviating, inhibiting, ameliorating, and/or relieving the disease or condition from which the subject is suffering. In some instances, treatment can result in the continued absence of the disease or condition from which the subject is suffering.

In general, methods include selecting a subject at risk for or with a condition or disease. In some instances, the subject's condition or disease can be treated with a pharmaceutical composition disclosed herein. For example, in some instances, methods include selecting a subject with cancer, e.g., wherein the subject's cancer can be treated by targeting one or both of MICA and/or angiopoetin-2.

In some instances, treatments methods can include a single administration, multiple administrations, and repeating administration as required for the prophylaxis or treatment of the disease or condition from which the subject is suffering. In some instances treatment methods can include assessing a level of disease in the subject prior to treatment, during treatment, and/or after treatment. In some instances, treatment can continue until a decrease in the level of disease in the subject is detected.

The terms "administer," "administering," or "administration," as used herein refers to implanting, absorbing, ingesting, injecting, or inhaling, the inventive peptide, regardless of form. In some instances, one or more of the peptides disclosed herein can be administered to a subject topically (e.g., nasally) and/or orally. For example, the methods herein include administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Following administration, the subject can be evaluated to detect, assess, or determine their level of disease. In some instances, treatment can continue until a change (e.g., reduction) in the level of disease in the subject is detected.

Upon improvement of a patient's condition (e.g., a change (e.g., decrease) in the level of disease in the subject), a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

In some instances, the disclosure provides methods for detecting immune cells e.g., B cells and/or memory B cells, from a human subject. Such methods can be used, for example, to monitor the levels of immune cells e.g., B cells and/or memory B cells, in

a human subject, e.g., following an event. Exemplary events can include, but are not limited to, detection of diseases, infection; administration of a therapeutic composition disclosed herein, administration of a therapeutic agent or treatment regimen, administration of a vaccine, induction of an immune response. Such methods can be used clinically and/or for research.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Methods are described herein that allow sensitive, specific, and reliable detection of rare memory B cells, with defined antigen specificity, from limited quantities of peripheral blood. Methods allowed visualization and isolation of memory B cells months to years after antigen had been cleared.

Proof of principle for the methods disclosed herein was established using tetramers of tetanus toxin C-fragment (TTCF), as reported in detail in Franz et al. (Blood, 118(2):348-357 (201 1)), which reference is hereby incorporated by reference in its entirety.

TTCF (i.e., the 52 kDa, non-toxic, C-terminal fragment of TTCF) was selected as a model antigen because the majority of individuals have been vaccinated with tetanus toxoid and persistent IgG antibody titers are induced by the vaccine (Amanna et al., N. Engl. J. Med., 357:1903-1915, 2007). Accordingly, use of TTCF afforded a large pool of subjects in which the methods disclosed herein could be verified. One of skill in the art will appreciate, however, that the present methods can be adapted to include any disease-related antigen using routine skill. As demonstrated in the examples below, such adaption has been shown through the acquisition of antibodies directed against MICA and angiopoietin-2, which are cancer-related antigens.

Example 1: Antigen Expression and Tetramer Formation

As described in further detail below, TTCF was expressed in *Eschericia coli* and a BirA site was attached to the N-terminus for site-specific mono-biotinylation by BirA

enzyme. A flexible linker was placed between the protein and the biotinylation site to prevent steric hindrance of antibody binding. TTCF was purified by anion-exchange chromatography, biotinylated with BirA, and separated from free biotin and BirA by gel filtration chromatography. TTCF tetramers were generated by incubating fluorescently tagged streptavidin with biotinylated TTCF antigen at a molar ratio of 1:4. These tetramers were then used along with a panel of mAbs for the identification of tetanus toxoid specific memory B cells.

TTCF was cloned in pET-15b (Novagen). Protein expression was induced in BL21(DE3) *Eschericia coli* with ImM isopropyl β-D-l-thiogalactopyranoside (IPTG) for 4 hours at 28°C. Cells were washed, lysed, and resulting supernatant was collected. TTCF was purified using a HIS-Select affinity column (Sigma). The His-tag was removed proteolytically. Murine CD80 membrane proximal domain was produced using similar methods. Proteins were mono-biotinylated. For certain experiments, Alexa-488 dye molecules (Molecular probes) were linked to primary amines on biotinylated TTCF or CD80.

Antigen tetramers were prepared by incubating biotinyated antigen with premium grade PE labeled streptavidin (Molecular Probes) for at least 20 minutes on ice at a molar ratio of 4:1. Prior to use, tetramer preparations were centrifuged to remove aggregates. In some experiments, tetramers were formed with Alexa-fluor-488 tagged antigens and non-fluorescent streptavidin at a 4:1 ratio.

Example 2: Identification Methods

Methods were performed as described in Franz et al, Blood, 118(2):348-357 (201 1).

Cells were sorted on a BD FACS Aria II cell sorter. Cells were single-cell sorted. Samples were first gated on $CD19^+$ cells that were negative for a panel of exclusion markers (CD3, CD 14, CD 16, 7AAD) then gated on plasmablasts, identified by high levels of CD27 and an immediate level of CD 19 expression, and finally on $tetramer^+$ $CD19^+$ cells.

Due to the low frequency of memory B cells, it was necessary to carefully reduce background as much as possible. B cells were first enriched by negative selection (cocktail of antibodies to CD2, CD3, CD14, CD16, CD56 and glycophorin A) to remove most cells that could non-specifically bind the tetramer. Enriched cells were split evenly and stained with TTCF or a control tetramer followed by labeling with CD19, CD27 and IgM to specifically select class-switched memory B cells. The gating strategy considered expression of CD19, lack of labeling with a panel of exclusion markers (CD3, CD14, CD16, 7AAD), expression of the memory marker CD27 and lack of IgM expression as evidence of class switching. Tetramer staining was plotted versus CD27 staining for visualization of memory B cells with the antigen specificity of interest. Tetramer-positive B cells were directly sorted into PCR strips containing 3 µï mRNA extraction buffer.

Tubes were kept cold during sorting and sorted cells were frozen and stored at -80°C. CD19+ CD27+ IgM- B cells were used as positive controls.

A previously reported nest PCR protocol was used to amplify heavy and light chain variable segments (Wang et al., J. Immunol. Methods., 244:217-225, 2000). mRNA amplification was carried out under conditions suitable to minimize contamination. Primers used included:

TAATACGACTCACTATAGGTTCGGGGAAGTAGTCCTTGACCAGG (SEQ ID NO: 91);

TAATACGACTCACTATAGGGATAGAAGTTATTCAGCAGGCACAC (SEQ ID NO:92);

TAATACGACTCACTATAGGCGTCAGGCTCAGRTAGCTGCTGGCCGC (SEQ ID NO:93).

Nested RT-PCR was performed as described in Franz et al, Blood, 118(2):348-357 (2011).

Negative controls were included to monitor and guard against contamination. From a total of 35 single cells labeled with the TTCF tetramer, 32 heavy and 30 light chain segments were amplified and directly sequence from gel-purified PCR products, corresponding to an overall PCR efficiency of 89%. Sequence analysis revealed that

TTCF tetramer^{+} cells employed a variety of different VHD-J_h gene segments, without dominance of one particular gene segment. Sequences observed supported that clones represented cells diversified by somatic hypermutation.

Antibody production and purification included cloning heavy and light variable domain DNA into separate pcDNA3.3 expression vectors containing the bovine prolactin signal peptide sequence as well as full length IgGl heavy or kappa light chain constant domains. Antibodies were expressed in CHO-S media (Invitrogen) supplemented with 8mM Glutamax (Gibco) in 100ml sinner flasks at 37 °C with 8% $C0_2$. One day prior to transfection, cells were split to $6xl0^5$ cells/ml. On the day of transfection, cells were adjusted, were necessary, to $lxl\ 0^6$ cells/ml. 25 μg of heavy and light chain plasmid DNA were co-transfected using MAX transfection reagent (Invitrogen) and transfected cells were cultured for 6-8 days. Protein was obtained using Protein G sepharose beads and antibody was eluted using lOOmM glycine pH2.5 and separated from beads using Spin-X centrifuge tubes. Purified antibody was exchanged into phosphate buffered saline (PBS) using Micro Bio-Spin columns (BioRad). Protein concentration was assessed by absorbance at 280nm.

For saturation binding assay, non-biotinylated, MonoQ purified TTCF was labeled with europium and free europium was removed. 96-well flat bottom plates were coated overnight with 20ng of antibody per well in lOOmM NaHCOs buffer at pH 9.6. Blocking was performed with assay buffer supplemented with bovine serum albumin (BSA) and bovine gamma globulins. TTCF-europium was diluted in assay buffer (IOOηM to 4pM) and 200μ1was added per well in triplicate. Plates were incubated for 2 hours at 37 °C and washed three times with 200 μï wash buffer (50mM Tris pH 8, 150mM NaCl, 20 μM EDTA, 0.05% Tween). 100 μï enhancement solution was added to each well and fluorescence counts measured using a Victor^3 plate reader at 615nm.

Heavy and light chain variable domain sequences were analyzed using IMGT/V-Quest and JIONSOLVER software. Flow cytometry data were evaluated using FlowJo analysis software. Statistical analyses were carried out using GraphPad Prism 5 software using unpaired t-test. To determine antibody K_D values, saturation binding data were fitted using GraphPad Prism 5 software using non-linear regression analysis.

Example 3: Multimerization Enhances Identification of Memory B Cells

Tetrameric and monomeric TTCF were compared. TTCF was fluorescently labeled with Alexa-488 and then used in monomeric form or was converted to a tetramer using unlabeled streptavidin (see above). Enriched B cells were then incubated with tetrameric or monomeric TTCF-Alexa-488 at the same concentration. Control protein (CD80 membrane proximal domain) was labeled in the same way and also used as a tetramer.

As shown in FIGs. 22A and 22B, TTCF labeled some memory B cells, but frequencies identified with tetramer were substantially larger (1.6-7.3 fold) using cells from three donors. In one of the three donors TTCF specific memory B cells could be detected with the tetramer but not with the monomer.

These results demonstrate that antigen tetramers enable sensitive detection of memory B cells based on the antigen specificity of their BCR, despite such cells being very rare in peripheral blood. Class-switched memory B cells specific for TTCF were brightly labeled by the appropriate tetrameric TTCF antigen, while background labeling with control tetramer was consistently low.

Example 4: Method/Antibody Validation

Fully human antibodies were generated by joining constant regions of IgG heavy and kappa chains to isolated variable segments via overlap PCR. Antibodies were expressed in a transient, serum free mammalian expression system using CHO-S cells for a period of 6-8 days. Antibodies were purified using protein G and gel filtration chromatography.

As shown in FIG. 23, antibodies isolated from TTCF-specific plasmablasts showed high binding affinities to TTCF antigen, with a K_D of 2.2 nM (TTCF Ab 1) and 323 pM (TTCF Ab 2)(FIG. 23B. Antibodies isolated from memory B cells also exhibited high binding affinities, with K_D of 382 pM, 228 pM, and 1.4 nM, for other antibodies (TTCF Abs 3, 4, and 5)(FIG. 23B).

These data support the specificity of the methods disclosed herein. Moreover, the specificity of the methods herein was demonstrated by the construction of five anti-TTCF antibodies from three different donors, all of which bound to TTCF with high affinities.

Data herein also demonstrate that antigen tetramers enable sensitive detection of memory B cells long after clearance of the antigen from the host.

Example 5: Obtaining Anti-MICA Antibodies

Antibodies that immunospecifically bind to MICA were developed using the methods herein.

Briefly, MICA antigen (UniGene Hs. 130838) was expressed with a C-terminal BirA tag (GLNDIFEAQKIEWHE (SEQ ID NO: 238)), which enables mono-biotinylation of the antigen. Antigen was tetramerized with streptavidin (SA) labeled with R-Phycoerythrin (PE) at a molar ration of 4 MICA: 1 SA. Peripheral blood mononuclear cells were obtained from advanced stage melanoma patients who had been vaccinated with autologous tumor cells transduced with a GM-CSF expression vector (GVAX) (PNAS 103: 9190, 2006), and subsequently treated with the anti-CTLA-4 monoclonal antibody ipilimumab (YERVOY™ (available from Bristol Myers Squib)) Peripheral blood mononuclear cells were quickly thawed, washed and resuspended at $5xl0^{6}$ in phosphate buffered saline (pH 7.2) supplemented with 2% fetal calf serum and stained with approximately 0.lug/ml tetramer for 30 minutes on ice. Antibodies were added to identify class-switched, memory B-cells ($CD19^{+}$, $CD27^{+}$, and IgM^{-}). A panel of exclusion antibodies labeling T-cells, natural killer-cells, marcrophages, and dead cells were included to reduce background tetramer staining (CD3, CD14, CD16, 7-AAD). Single B-cells that bound to the MICA tetramer were sorted into 8-tube-PCR strips using the BD FACS Aria II. The B-cell receptor (BCR) mRNA was amplified using a commercial kit from Epicentre Biotechnologies (catalog number: MBCL90310) using gene specific primers shown below:

mRNA Amplification

IgG-T7 : AATACGACTC ACTATAGGTTCGGGGAAGT AGTCCTTGACC AGG (SEQ ID NO:94)

Kappa-T7:
TAATACGACTCACTATAGGGATAGAAGTTATTCAGCAGGCACAC (SEQ ID NO:95)

Lambda-T7:
TAATACGACTCACTATAGGCGTCAGGCTCAGRTAGCTGCTGGCCGC (SEQ ID NO:96)

PCR One

VHL-1: TCACCATGGACTG(C/G)ACCTGGA (SEQ ID NO:97)

VHL-2: CCATGGACACACTTTG(C/T)TCCAC (SEQ ID NO:98)

VHL-3: TCACCATGGAGTTTGGGCTGAGC (SEQ ID NO:99)

VHL-4: AGAACATGAAACA(C/T)CTGTGGTTCTT (SEQ ID NO: 100)

VHL-5: ATGGGGTCAACCGCCATCCT (SEQ ID NO: 101)

VHL-6: ACAATGTCTGTCTCCTTCCTCAT (SEQ ID NO: 102)

VkL-1: GCTCAGCTCCTGGGGCTCCTG (SEQ ID NO: 103)

VkL-2 : CTGGGGCTGCT AATGCTCTGG (SEQ ID NO:104)

VkL-3: TTCCTCCTGCTACTCTGGCTC (SEQ ID NO: 105)

VkL-4: CAGACCCAGGTCTTCATTTCT (SEQ ID NO: 106)

VlL-1: CCTCTCCTCCTCACCCTCCT (SEQ ID NO: 107)

V1L-2: CTCCTCACTCAGGGCACA (SEQ ID NO: 108)

V1L-3 : ATGGCCTGGA(T/C)C(C/G)CTCTCC (SEQ ID NO:109)

Cgll: GCCAGGGGGAAGAC(C/G)GATG (SEQ ID NO:l 10)

Ckll: TTTCAACTGCTCATCAGATGGCGG (SEQ ID NO: 111)

CHI: AGCTCCTCAGAGGAGGG(C/T)GG (SEQ ID NO: 112)

PCR Two

VH-1 : CAGGT(G/ C)CAGCTGGT(G/A)C AGTC (SEQ ID NO: 113)

VH-2: CAG(A/G)TCACCTTGAAGGAGTC (SEQ ID NO: 114)

VH-3: (G/C)AGGTGCAGCTGGTGGAGTC (SEQ ID NO: 115)

VH-4: CAGGTGCAGCTGCAGGAGTC (SEQ ID NO: 116)

VH-5: GA(G/A)GTGCAGCTGGTGCAGTC (SEQ ID NO: 117)

VH-6 : CAGGTAC AGCTGC AGC AGTC (SEQ ID NO:118)

Vk-1: CG(A/C)CATCC(A/G)G(A/T)TGACCCAGT (SEQ ID NO: 119)

Vk-2: CGAT(A/G)TTGTGATGAC(C/T)CAG (SEQ ID NO: 120)

Vk-3: CGAAAT(T/A)GTG(T/A)TGAC(G/A)CAGTCT (SEQ ID NO: 121)

Vk-4: CGACATCGTGATGACCCAGT (SEQ ID NO: 122)

VI-1: CCAGTCTGTGCTGACTCAGC (SEQ ID NO:123)

Vl-2: CCAGTCTGCCCTGACTCAGC (SEQ ID NO: 124)

Vl-3: CTCCTATGAGCTGAC(T/A)CAGC (SEQ ID NO: 125)

Cglll: GAC(C/G)GATGGGCCCTTGGTGGA (SEQ ID NO: 126)

Cklll: AAGATGAAGACAGATGGTGC (SEQ ID NO: 127)

C1III: GGGAACAGAGTGACCG (SEQ ID NO: 128)

The primers and PCR cycling conditions used in PCR one and PCR two are adapted from Wang and Stollar et al. (journal of immunological methods2000).

An alternate heavy chain variable region forward primer set was developed to cover heavy chain variable region sequences potentially not adequately covered by the above primer set. The following alternate primers were generated:

PCR One

VHL1-58: TCACTATGGACTGGATTTGGA (SEQ ID NO: 129)

VHL2-5: CCATGGACA(C/T)ACTTTG(C/T)TCCAC (SEQ ID NO:130)

VHL3-7: GTAGGAGACATGCAAATAGGGCC (SEQ ID NO: 131)

VHL3-11: AACAAAGCTATGACATATAGATC (SEQ ID NO: 132)

VHL3-13.1: ATGGAGTTGGGGCTGAGCTGGGTT (SEQ ID NO: 133)

VHL3-13.2: AGTTGTTAAATGTTTATCGCAGA (SEQ ID NO:134)

VHL3-23: AGGTAATTCATGGAGAAATAGAA (SEQ ID NO:135)

VHL4-39: AGAACATGAAGCA(C/T)CTGTGGTTCTT (SEQ ID NO: 136)

VHL4-61 : ATGGACTGGACCTGGAGCATC (SEQ ID NO: 137)

VHL-9: CCTCTGCTGATGAAAACCAGCCC (SEQ ID NO: 138)

PCR Two

VH1-3/18: CAGGT(C/T)CAGCT(T/G)GTGCAGTC (SEQ ID NO:139)

VH1-45/58: CA(A/G)ATGCAGCTGGTGCAGTC (SEQ ID NO: 140)

VH2-5 : CAG(A/G)TC ACCTTGA(A/G)GGAGTCTGGT (SEQ ID NO:141)

VH3-9/23/43: GA(A/G)GTGCAGCTG(T/G)TGGAGTC (SEQ ID NO: 142)
VH3-16: GAGGTACAACTGGTGGAGTC (SEQ ID NO: 143)
VH3-47: GAGGATCAGCTGGTGGAGTC (SEQ ID NO: 144)
V4-34: CAGGTGCAGCTACAGCAGTG (SEQ ID NO: 145)
V4-30-2/ 39: CAGCTGCAGCTGCAGGAGTC (SEQ ID NO: 146)
VH7-4-1: CAGGTGCAGCTGGTGCAATC (SEQ ID NO: 147)

Briefly, 2ul cDNA generated via mRNA amplification was used as a template for first-round PCR, with the following cycling conditions: 3 cycles of preamplification (94°C/45 seconds, 45°C/45 seconds, 72°C/105 seconds); 30 cycles of amplification (94°C/45 seconds, 50°C/45 seconds, 72°C/105 seconds); 10 minutes of final extension at 72°C.

3ul of first-round PCR product served as a template for the second round of nested PCR. The same cycling conditions were used for the first round of PCR, but the 3 cycles of preamplification were omitted. Both PCR steps were performed by the use of cloned Pfu polymerase AD (Agilent Technologies). PCR products were separated on 1% agarose gels and products of 300-400 nucleotides in size isolated with the use of Zymoclean DNA gel recovery kit (Zymo Research). Sequencing was performed by the use of forward and reverse primers used for the second-round nested PCR. A two-step nested PCR amplifies the BCR variable domains of heavy and light chains (see above). Peripheral blood mononuclear cells were obtained from advanced stage melanoma patients who had been vaccinated with autologous tumor cells transduced with a GM-CSF expression vector (GVAX) (PNAS 103: 9190, 2006). The antibodies were expressed as full-length IgGl antibodies in a transient CHO-S expression system.

Validation of anti-MICA antibody binding to MICA was performed using two independent bead-based assays. The first assay used a commercially available solution-based bead assay kit designed for detection of anti-MICA antibodies reactive to a variety of MICA alleles (One Lambda, catalog number LSMICAOOl). Varying concentrations of the MICA antibody were incubated with beads, then washed, and incubated with an anti-human IgG antibody conjugated with phycoerythrin. Following a

second wash step, beads were analyzed on a Luminex machine. A negative control consisted of incubation of beads with anti-human IgG antibody conjugated with phycoerythrin alone (no anti-MICA antibody). A positive control consisted of incubation of beads with a commercially available anti-MICA/MICB monoclonal antibody (clone 6D4) directly conjugated to phycoerythrin (BioLegend catalog #320906). The second assay was developed internally using polystyrene beads conjugated with streptavidin. Beads were coated with monobiotinylated MICA protein, and incubated with varying concentrations of anti-MICA antibody, anti-TTCF antibody (isotype negative control), or BioLegend anti-MICA/MICB antibody directly conjugated to phycoerythrin (positive control). Beads incubated with anti-MICA antibody or anti-TTCF antibody were washed and then incubated with anti-human IgG antibody conjugated with Alexa488. To determine background binding to the beads, the same incubation was performed using streptavidin-conjugated beads not coated with MICA protein for comparison. Beads were analyzed for binding to antibodies on a FACS Caliber flow cytometer.

As shown in FIGs. 24 and 25, anti-MICA antibodies (MICA-AM2 and MICA-Ab20) bind with high affinity to MICA. MICA-Ab20 corresponds to the anti-MICA antibody ID-1 described in Table 1.

Example 6: Anti-MICA Antibodies

Additional anti- MICA antibodies with clinically relevant biological properties were developed using the methods herein. MICA-specific antibodies reactive to common alleles were identified in patients who had received a cellular cancer vaccine (GM-CSF transduced cancer cells, referred to as GVAX) and an antibody that blocks the inhibitory CTLA-4 receptor on T cells ipilimumab (YERVOY™ (available from Bristol Myers Squib)). MICA tetramers were then used to isolate B cells from peripheral blood mononuclear cells of patients with the highest serum MICA reactivity. Heavy and light chain sequences were determined from these B cells by single cell PCR, as outlined in the in Example 5. This effort led to the identification of antibodies that recognize alleles common in the North American population.

CM24002 Ab2 (anti-MICA antibody ID-6 described in Table 1) is an antibody isolated from a patient with acute myeloid leukemia (AML) who demonstrated a significant clinical response to the GVAX + Ipilimumab combination therapy and whose plasma reacted strongly with MICA. The CM24002 Ab2 light chain (FIGs. 30 and 31) and heavy chain (FIGs. 28 and 29) nucleotide and amino acid sequences are shown, with CDR1, CDR2 and CDR3 sequences underlined. An additional antibody with strong binding was obtained from the same patient and is labeled as CM24002 Ab4 (anti-MICA antibody ID-7 described in Table 1) The CM24002 Ab4 light chain (FIGs. 34 and 35) and heavy chain (FIGs. 23 and 32) nucleotide and amino acid sequences are shown, with CDR1, CDR2 and CDR3 sequences underlined.

CM33322 Abll (anti-MICA antibody ID-8 described in Table 1) and CM33322 Ab29 (anti-MICA antibody ID-9 described in Table 1) are antibodies isolated from a patient with metastatic melanoma who is a long-term responder (>15 years) to the GVAX + Ipilimumab combination therapy. The CM33322 Abll light chain ((FIGs. 38 and 39) and heavy chain (FIGs. 36 and 37) nucleotide and amino acid sequences are shown, with CDR1, CDR2 and CDR3 sequences underlined. The CM33322 Ab29 light chain ((FIGs. 42 and 43) and heavy chain (FIGs. 40 and 41) nucleotide and amino acid sequences are shown, with CDR1, CDR2 and CDR3 sequences underlined. Due to the long-term clinical response of this patient, these antibodies are of particular interest.

After initial identification, cloning, and expression of the antibodies of interest, the specificity of these antibodies for different MICA alleles was determined with a cytometric bead assay. Briefly, soluble, recombinant MICA alleles 002, 008, 009 and MICB with a single BirA biotinylation site were expressed, purified, and captured on streptavidin beads. Indicated anti-MICA antibodies were then incubated with the beads coated with recombinant MICA at different concentrations for one hour, then washed, and incubated with a FITC-labeled anti-human IgG secondary antibody. Following a second wash step, quantification of bead-bound FITC fluorescence was completed by flow cytometry. MICA alleles 002, 008, 009 as well as the related MICB protein were chosen based on their prevalence in the North American population (FIG. 48). MICA alleles 002, 008, 009 as well as the related MICB protein were also chosen based on their generally

high prevalence worldwide. Importantly, CM24002 Ab2 and CM33322 Ab29 bound strongly to all MICA alleles as well as to MICB. The other two antibodies bound to a subset of alleles: CM24002 Ab4 bound highly to MICA*009 and MICB, and CM33322 Abll bound highly to MICA*002, MICA*008, and MICB. (FIGs. 48A-F) Specificity was documented by use of a negative human control antibody generated with the same technology (specific for tetanus toxoid C-terminal fragment, TTCF) and a positive control antibody to MICA (a commercial murine antibody from BioLegend directed against MICA). These studies identified CM24002 Ab2 and CM33322 Ab29 as potential candidates for clinical application.

Example 7: Binding of Anti-MICA Antibody to Autologous Tumor Cells

The ability of isolated anti-MICA antibody CM24002 Ab2 to bind to autologous tumor cells was examined by flow cytometry (FIG 49). Bone marrow obtained from patient CM24002 and tested binding to tumor cells by CM24002 Ab2. Tumor cells were then identified from the bone marrow sample as CD33+ CD34+ cells. The tumor cells were then stained with 10 μg/ml with anti-MICA antibody CM24002 Ab2, positive control commercial MICA antibody (BioLegend) or a negative control antibody (TTCF specific). As shown in FIG. 49, CM24002 Ab2 strongly bound to these cells. CM24002 Ab2 did not display binding to non-tumor cells (CD 16+ and CD3+ cells) and only background binding to CD 14+ cells, demonstrating anti-tumor specificity (data not shown).

Example 8. Anti-MICA Antibody Inhibition of NKG2D Receptor on NK Cells.

The ability of isolated anti-MICA antibody CM24002 Ab2 to prevent soluble MICA-mediated down-regulation of is cognate receptor, NKG2D was examined. Serum from patient CM24002 was used at a 1:10 dilution and incubated with human NK cells for a period of 48 hours. CM24002 Ab2 (concentration of 10μg/ml), positive control commercial MICA antibody (BioLegend) or a negative control antibody (TTCF specific) were added to these cultures. NKG2D expression was assessed by flow cytometry at 48hr (FIG. 50). Serum from patient CM24002 strongly down-regulated expression of

NKG2D (thus disabling the function of this receptor). CM24002 Ab2 and the positive control MICA antibody partially restored NKG2D surface expression by NK cells. To demonstrate specificity, we repeated the above experiment by incubating cells with recombinant MICA at 2ng/ml instead of patient serum (FIG. 51). CM24002 Ab2 completely prevented MICA-mediated down-regulation of NKG2D expression, while the negative control antibody (specific for TTCF) had no effect (FIG. 51). These data demonstrate that human MICA antibodies can prevent inhibition of the critical NKG2D receptor on human NK cells.

Example 9: Anti-MICA Antibody Cell-Mediated Cytotoxicity

To determine if CM24002 Ab2 enables cell-mediated cytotoxicity, human NK cells (effector cells) were incubated for 48 hours with recombinant MICA (2ng/ml) in the presence of CM24002 Ab2, a negative control antibody (TTCF specific) or a positive control antibody (BioLegend), all at l(^g/ml. After 48 hours, cells were washed and incubated with K562 tumor cells at 20:1, 10:1, and 5:1 effector:target ratios for 4 hours. Specific lysis of target cells by NK cells was determined by release of a cytosolic protein (LDH) from K562 tumor cells. In the absence of MICA antibodies, there was no killing of K562 tumor cells by NK cells. However, CM24002 Ab2 greatly enhanced NK cell mediated lysis of K562 tumor cells and was more effective than the positive control murine MICA antibody at all effector:target ratios (FIG. 52). It was further demonstrated that killing of K562 tumor cells was indeed mediated by the NKG2D pathway (rather than Fc receptors). The above experiment was repeated, with the addition two experimental groups: a blocking antibody for NKG2D and human Fc block. In addition, CM33322 Ab29 was also tested. The data show that addition of CM24002 Ab2 and CM33322 Ab29 enabled NK cell mediated cytotoxicity. Killing of K562 cells did not occur when a blocking NKG2D antibody was added, while the Fc blocking reagent had little effect (FIG. 53). These data show that CM24002 Ab2 and CM33322 Ab29 restore the anti-tumor function of the NKG2D pathway.

Example 10: Binding of Anti-MICA Antibody to Alpha 3 MICA domain

The NKG2D receptor binds to the top alpha 1 and alpha 2 domains of MICA, and antibodies that bind to the same site may compete with the NKG2D receptor and thereby block killing of tumor cells by NK cells. Antibodies that bind to the alpha 3 domain are of particular interest because they cannot block NKG2D receptor binding. At the same time, such antibodies can interfere with proteolytic cleavage of MICA from the tumor cell surface. The ability of anti-MICA antibodies to the MICA alpha 3 domain was assessed using the previously described cytometric bead assay. The biotinylated recombinant protein was captured on streptavidin beads. Beads were then incubated with antibodies CM24002 Ab2, CM24002 Ab4, CM33322 Abll, CM33322 AB29, a negative control antibody (TTCF specific) or a positive control antibody (BioLegend), at l(^g/ml followed by a FITC-labeled anti-human IgG secondary antibody and quantification of bead-bound FITC fluorescence by flow cytometry (FIG. 54). As shown in FIG. 54, CM33322 Ab29 bound to the MICA alpha 3 domain and is therefore of great interest for therapeutic applications.

Example 11: Binding of Anti-MICA Antibody to Tumor Cells

The potential of CM24002 Ab2 and CM33322 Ab29 to be used to target a broad range of cancers was assessed. A panel of multiple myeloma (RPMI 8226 and Xg-1), ovarian cancer (OVCAR3), acute myeloid leukemia (U937), melanoma (K028), lung cancer (1792 and 827), and breast cancer (MCF7) cells were tested for labeling by CM24002 Ab2 and CM33322 Ab29. The tumor cells were resuspended at a concentration of lxl 0^6 cells/ml in PBS with 1% BSA and stained with the CM24002 Ab2 and CM33322 Ab29, as well as positive and negative controls (murine MICA antibody and TTCF-specific antibody, respective ly)(directly conjugated) at a concentration of 10 µg/ml for 1 hour at 4°C. Labeling was assessed by flow cytometry (FIG. 55). CM24002 Ab2 and CM33322 Ab29 both bound every tumor cell type tested, with labeling being greater than the commercial positive control for the majority of tested cell lines.

Example 11: MICA Allele Specificity of Anti-MICA antibody

The allelic specificity of CM33322 Ab29 was assessed using a commercially available Luminex assay. The commercial test kit contains recombinant MICA alleles (MICA*001, *002, *007, *012, *017, *018, *027, *004, *009, and*015) directly conjugated to Luminex beads, each with intrinsic fluorescent properties enabling binding to be assessed in a single sample. Luminex beads coated with the indicated MICA alleles were incubated with CM33322 Ab29, BioLegend positive control, and the negative control (TTCF), at 10 μg/ml for 1 hr, with subsequent incubation with PE-conjugated anti-human IgG secondary antibody. Fluorescence was determined following incubation for 60 minutes with the indicated antibodies and subsequent incubation with anti-human PE-conjugated secondary antibody using a Luminex 200 instrument (FIG 56). CM33322 Ab29 was able to bind to all alleles present in the commercial assay, indicating that it may be used in patients regardless of MICA genotype.

These data demonstrate the high biological activity of CM24002 Ab2 and CM33322 Ab29 and their ability to restore NK cell mediated lysis of tumor cells. These data demonstrate that cancer patients who responded to immunotherapies produced MICA antibodies that restored the anti-tumor activity of NK cells. Together, these results highlight the therapeutic potential of anti-MICA antibodies to overcome immune suppression and promote tumor destruction in cancer patients.

Example 12: Obtaining Anti-Angiopoietin-2 Antibodies

Antibodies that bind to angiopoietin-2 were developed using the methods herein. Briefly, biotinylated angiopoietin-2 (UniGene Hs.583870) was purchased from R&D Systems. Peripheral blood mononuclear cells were quickly thawed, washed and resuspended at $5xl0^{6}$ in phosphate buffered saline (pH 7.2) supplemented with 2% fetal calf serum and stained with approximately 0.5ug/ml angiopoietin-2 for 30 minutes on ice. Cells were washed twice with 4ml PBS/2% FCS. Then antibodies were added to identify class-switched, memory B-cells (CD19+, CD27+, and IgM-) as well as SA-PE to label B-cells with biotinylated angiopoietin on the surface. A panel of exclusion antibodies labeling T-cells, natural killer-cells, marcrophages, and dead cells were included to

reduce background tetramer staining (CD3, CD14, CD16, 7-AAD). Single B-cells that bound to angiopoietin-2 were sorted into 8-tube-PCR strips using the BD FACS Aria II. The B-cell receptor (BCR) mRNA was amplified using a commercial kit from Epicentre Biotechnologies (catalog number: MBCL90310) using gene specific primers (see above). A two-step nested PCR amplifies the BCR variable domains of heavy and light chains (see above). Peripheral blood mononuclear cells were obtained from a patient with malignant non-small cell lung carcimonnoma who had been vaccinated with autologous tumor cells transduced with a GM-CSF expression vector (GVAX) (Cancer Res. 70: 10150, 2010). The antibodies were expressed as full-length IgGl antibodies in a transient CHO-S expression system.

Validation of anti-angiopoietin-2 antibodies binding to angiopoietin-2 was performed using ELISA assays. Briefly, angiopoietin-2 was coated overnight at 4μg/ml in lOOmM sodium bicarbonate buffer pH 9.6 in 96-well flat bottom plates (PerkinElmer) at 4°C. Plates were blocked with assay buffer containing bovine serum albumin and bovine gamma globulins (PerkinElmer) at room temperature for three hours. Antibodies were diluted in assay buffer at 20ug/ml-0.16ug/ml and incubated for 1 hour at 4°C. Plates were washed three times with 200μ1wash buffer (50mM Tris pH8, 150mM NaCl, 20mM EDTA, 0.05% Tween). IOOμI enhancement solution (PerkinElmer) was added to each well and fluorescence counts measured using a Victor3 plate reader (PerkinElmer) at a wavelength of 615nm. Human angiopoietin-1 and -4 was also tested for binding and showed similar reactivity.

Relevant data is shown in FIGs. 27A-27C, that provide graphs and a gel relating to isolation of angiopoietin-specific antibodies from a lung cancer patient. (A) Angiopoietin-2 reactivity of lung cancer patient (LI 9) serum (diluted 1:1000) determined by ELISA. Dates of serum collection are shown on the X-axis. The control protein bovine serum albumin (BSA) was included as a negative control. (B) FACS plot showing PBMC sample (timepoint- 10/98) gated on CD19+, CD27+ IgM-B cells with CD19 on the X-axis and fluorescently-tagged angiopoietin-2 on the Y-axis. The gate indicates approximately where the sorting cut-off was made. Ten B-cells were sorted from this sample. (C) Heavy, light chain, and hinge region PCR products from 10

angiopoietin-2 reactive memory B-cells isolated from pateitn LI9. Heavy (top) and light (bottom) chain PCR products after two rounds of nested PCR of approximately 350 base pairs.

Example 13: Binding of Anti-Angiopoietin-2 Antibodies Against Human Recombinant Angiopoietin Family Members

96 well plates were coated overnight with 4 μg/mL recombinant angiopoietin- 1, -2, and -4 (R&D Systems) in sodium bicarbonate buffer at pH9.6. Plates were subsequently blocked for 3 hours at room temperature with assay buffer (Perkin Elmer) containing bovine serum albumin (BSA) and bovine gamma-globulins. Antibodies ID 2, 3, 4, and 5 (see Table 1), diluted between 20 μg/mL-0.16 μg/mL, were incubated on plates for 1 hour at 4°C with rotation. Plates were subsequently washed before being incubated with anti-human IgG-Europium antibody (Perkin Elmer). Fluorescent counts at 615 nm were obtained via plate reader. A negative control antibody (clone 8.18.C5) was used to determine specificity. Data was determined in duplicate.

As shown in FIGs. 26A-26C, antibodies ID 2, 3, 4, and 5 (see Table l)bind with high specificity to angiopoietin- 1 -2, and -4. Antibodies do not bind to Ang-like-3, a structurally-related protein *(see* FIG. 26D).

An additional anti- angiopoietin antibody, designated anti-Ang2 Ab6 (anti-MICA antibody ID- 10 described in Table 1) with clinically relevant biological properties were developed using the methods herein. Binding of anti-Ang2 Ab6 to human recombinant angiopoietin family members was analyzed as described above. Briefly, ELISA plates were coated with 4 μg/ml of angiopoietins Ang- 1, Ang-2, Ang4, and Ang-like-3 binding, and detection by anti-Ang2 Ab6 was tested at 20 μg/ml. 4 μg/ml, 0.8 μg/ml, and 0.16 μg/ml. Europium conjugated anti-human IgG secondary was used, with europium counts measured after 45 minutes. As shown in FIG. 57, anti-Ang2 Ab6 binds to all angiopoietins in a dose dependent manner.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate

and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A composition having anti-tumor activity comprising an antibody or antibody fragment that immunospecifically binds to MHC class I polypeptide-related sequence A (MICA), wherein the antibody or antibody fragment comprises a heavy chain variable region (V_H) and a light chain variable region (V_L) and wherein,

(a) the V_H CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 208, the V_H CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 210, and the V_H CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 212, and the V_L CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 215, the V_L CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 217, and the V_L CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 219; or

(b) the V_H CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 153, the V_H CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 156, and the V_H CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 158, and the V_L CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 160, the V_L CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 162, and the V_L CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 164.

2. The composition of claim 1, wherein the antibody or antibody fragment comprises:

(a) a V_H chain with at least 95%, 96%, 97%, 98 or 99% identity to the amino acid sequence set forth in SEQ ID NO: 204, and a V_L chain with at least 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 206; or

(b) a V_H chain with at least 95%, 96%, 97%, 98 or 99% identity to the amino acid sequence set forth in SEQ ID NO: 149, and a V_L with at least 95%, 96%, 97%, 98% or 99% identity to the amino acid sequence set forth in SEQ ID NO: 151.

3. The composition of claim 1, wherein the antibody or antibody fragment comprises

(a) a V_H chain comprising the amino acid sequence set forth in SEQ ID NO: 204 and a V_L chain comprising the amino acid sequence set forth in SEQ ID NO: 206; or

(b) V_H chain comprising the amino acid sequence set forth in SEQ ID NO: 149 and a V_L chain comprising the amino acid sequence set forth in SEQ ID NO: 151.

4. The composition of any one of claims 1-3, further comprising an anti-cancer therapeutic.

5. The composition of any one of claims 1-4, formulated as a pharmaceutical composition.

6. The composition of any one of claims 1-5, further comprising an histone deacetylase inhibitor (HDAC) selected from the group consisting of hydroxamic acid, vorinostat, suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), LAQ824, panobinostat (LBH589), belinostat (PXD101), givinostat (ITF2357), cyclic tetrapeptide, depsipeptide (romidepsin, FK228), benzamide; entinostat (SNDX-275/MS-275), MGCD0103, short-chain aliphatic acids, valproic acid, phenyl butyrate, pivaloyloxymethyl butyrate (AN-9), CHR-3996, and CHR-2845.

7. The composition of any one of claims 1-6, further comprising a proteasome inhibitor selected from the group consisting of bortezomib, NPI-0052, carfilzomib (PR-171), CEP 18770, and MLN9708.

8. The composition of any one of claims 1-7, further comprising an antibody selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, an anti-PDL-1 antibody and a combination of one or more thereof.

9. Use of the composition of any one of claims 1-8, for preparation of a medicament for treating cancer in a subject.

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGGCCCTCACCTGCGCTGTCTCT
GGTGGGTCCTTCACTGATCATTACTGGAGTTGGATCCGTCAGGCCCCAGGGAAGGGGCTGGAGTGGATTGGAGAA
ATCAATCATAGTGGAGTCACCAACTACAACCCGTCCCTCAAGAGTCGACTCACCATATCAGTAGACACGTCCAAG
AGCCAGTTCTCCCTGAGGCTGACCTCTGTGACCGCCGCGGACACGGCTCTGTACTACTGTGCGAAAACTGGCCTG
TATTATGATGACGTTTGGGGGACTTTTCGTCCACGGGGCGGGTTCGACTCCTGGGGCCAGGGAACCCTGGTCACC
GTCTCCTCA (SEQ ID NO: 1)

FIG. 1

Q	V	Q	L	Q	Q	W	G	A	G	L	L	K	P	S	E	T	L	A	L	T	C	A	V	S
G	G	S	F	T	D	H	Y	W	S	W	I	R	Q	A	P	G	K	G	L	E	W	I	G	E
I	N	H	S	G	V	T	N	Y	N	P	S	L	K	S	R	L	T	I	S	V	D	T	S	K
S	Q	F	S	L	R	L	T	S	V	T	A	A	D	T	A	L	Y	Y	C	A	K	T	G	L
Y	Y	D	D	V	W	G	T	F	R	P	R	G	G	F	D	S	W	G	Q	G	T	L	V	T
V	S	S	(SEQ ID NO: 2)																			

FIG. 2

GACATCGTGATGACCCAGTCTCCGGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCC
AGCCAGAGTATTTTATATAGCTCCGACAATAAGAATTACTTAGCTTGGTACCAGCACAAGCCAGGACAGCCTCCT
AAGCTCCTCTTTTACTGGGCATCTATCCGGGAATCCGGGTCCCTGACCGATTCAGTGGCGGCGGGTCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATATTATAGTCCT
CCTTGCAGTTTTGGCCAGGGGACCAAGCTGGAGATCCAA (SEQ ID NO: 10)

FIG. 3

D I V M T Q S P D S L A V S L G E R A T I N C K S
S Q S L L Y S S D N K N Y L A W Y Q H K P G Q P P
K L L F Y W A S I R E S G V P D R F S G G G S G T
D F T L T I S S L Q A E D V A V Y Y C Q Q Y Y S P
P C S F G Q G T K L E I Q (SEQ ID NO: 11)

FIG. 4

```
1    GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGTC CCTGAGACTC
61   TCCTGTGCAG CCTCTGGATT CACCTTTAGT AGTTATGCCA TGAGCTGGGT CCGCCAGGCT
121  CCAGGGAAGG GGCTGGAGTG GGTCTCAGGT ATTTATTGGA GTGGTGGTAG CACATACTAC
181  GCAGACTCCG TGAAGGGCCG GTTCACCATC TCCAGAGACA TATCCAAGAA CACGCTGTAT
241  CTGCAAATGA ACAGTCTGAG AGCCGACGAC ACGGCCGTGT ATTACTGTGC GAGAGGCGAT
301  TACTATGGTT CGGGGGCTCA CTTTGACTAC TGGGGCCAGG GAACCCTGGT CACCGTCTCC
361  TCA    (SEQ ID NO: 19)
```

FIG. 5

```
1    EVQLVESGGG LVQPGGSLRL
21   SCAASGFTFS SYAMSWVRQA CDR1
41   PGKGLEWVSG IYWSGGSTYY CDR2
61   ADSVKGRFTI SRDISKNTLY
81   LQMNSLRADD TAVYYCARGD CDR3
101  YYGSGAHFDY WGQGTLVTVS
121  S    (SEQ ID NO: 20)
```

FIG. 6

```
1    GATATTGTGA TGACCCAGAC TCCACTCTCC TCACCTGTCA CCCTTGGACA GCCGGCCTCC
61   ATCTCCTGCA GGTCTAGCCA AAGCCTCGTA CACAGTGATG GAAACACCTA CTTGAGTTGG
121  CTTCAGCAGA GGCCAGGCCA GCCTCCAAGA CTCCTAATTT ATCAGATTTC TAACCGGTTC
181  TCTGGGGTCC CAGACAGATT CAGTGGCAGT GGGGCAGGGA CAGATTTCAC ACTGAAAATC
241  AGCAGGGTGG AAGCTGAGGA TGTCGGGGTT TACTACTGCA TGCAAGGTAC ACAATTTCCT
301  CGGACGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAA
```

(SEQ ID NO: 28)

FIG. 7

```
1    DIVMTQTPLS SPVTLGQPAS
21   ISCRSSQSLV HSDGNTYLSW CDR1
41   LQQRPGQPPR LLIYQISNRF CDR2
61   SGVPDRFSGS GAGTDFTLKI
81   SRVEAEDVGV YYCMQGTQFP CDR3
101  RTFGQGTKVE IK
```

(SEQ ID NO: 29)

FIG. 8

```
1    GAGGTGCAGC TGGTGGAGTC CGGGGGAGGC TTAGTTCAGC CTGGGGGATC CCTGAGACTC
61   TCCTGTGCAG CCTCAGGGTT CACCTTTAGT AATAACTGGA TGCACTGGGT CCGCCAGGCT
121  CCAGGGAAGG GGCTGGAGTG GATCTCAGAG ATTAGAAGTG ATGGGAATTT CACAAGGTAC
181  GCGGACTCCA TGAAGGGCCG ATTCACCATC TCCAGAGACA ACGCCAAGAG CACACTGTAT
241  TTGCAAATGA ACAGTCTGAG AGTCGAGGAC ACGGGTCTGT ATTACTGTGC AAGAGACTAC
301  CCCTATAGCA TTGACTACTG GGGCCAGGGA ACCCTGGTCA CCGTCTCCTC A    (SEQ ID NO: 37)
```

FIG. 9

```
1    EVQLVESGGG LVQPGGSLRL
21   SCAASGFTFS NNWMHWVRQA CDR1
41   PGKGLEWISE IRSDGNFTRY CDR2
61   ADSMKGRFTI SRDNAKSTLY
81   LQMNSLRVED TGLYYCARDY CDR3
101  PYSIDYWGQG TLVTVSS    (SEQ ID NO: 38)
```

FIG. 10

```
1    GATATTGTGA TGACCCAGAC TCCACTCTCC TCACCTGTCA CCCTTGGACA GCCGGCCTCC
61   ATCTCCTGCA CATCTAGTCA AAGCCTCGTA CACAGTAATG GAAACACCTA CTTGAGTTGG
121  CTTCAGCAGA GGCCAGGCCA GCCCCCAAGA CTCCTAATTT ATGAGATTTC TAAGCGGGTC
181  TCTGGGGTCC CAGACAGATT CAGTGGCAGT GGGGCAGGGA CAGATTTCAC ACTGAAAATC
241  AGCAGGGTGG AAGCTGAGGA TGTCGGGGTT TATTACTGCA TGCAAGGTAA ACAACTTCGG
301  ACTTTTGGCC AGGGGACCAA GCTGGAGATC AAA      (SEQ ID NO: 46)
```

FIG. 11

```
1    DIVMTQTPLS SPVTLGQPAS
21   ISCTSSQSLV HSNGNTYLSW CDR1
41   LQQRPGQPPR LLIYEISKRV CDR2
61   SGVPDRFSGS GAGTDFTLKI
81   SRVEAEDVGV YYCMQGKQLR CDR3
101  TFGQGTKLEI K      (SEQ ID NO: 47)
```

FIG. 12

```
1    GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTACAGC CTGGGGGGCTC CGTGAGACTG
61   TCTTGTGCGG CCTCAGGCTT CATTCTTAGC AACTTTGCCA TGAGTTGGGT CCGCCAGGCT
121  CCAGGGAAGG GGCTGGACTG GGTCTCAGGT AATTTTGGTG GTCGTGAAAA TACATATTAC
181  GCAGACTCCG TGAAGGGCCG GTTCACCATC TCCAGAGACA GTTCCAAGAG CACACTGTAT
241  CTGCAAATGA ACAATTTGAG AGCCGAGGAC ACGGCCGTAT ATTACTGTGC GCGAGGCCGAT
301  TACCATGGTT CGGGGGCTCA CTTTGACTAC TGGGGCCAGG GAATACTGGT CACCGTCTCC
361  TCA    (SEQ ID NO: 55)
```

FIG. 13

```
1     EVQLVESGGG LVQPGGSVRL
21    SCAASGFILS NFAMSWVRQA CDR1
41    PGKGLDWVSG NFGGRENTYY CDR2
61    ADSVKGRFTI SRDSSKSTLY
81    LQMNNLRAED TAVYYCARGD CDR3
101   YHGSGAHFDY WGQGILVTVS
121   S      (SEQ ID NO: 56)
```

FIG. 14

```
1    GATATTGTGA TGACCCAGAG TCCACTCTCC TCACCTGTCA TCCTTGGACA GCCGGCCTCC
61   ATCTCCTGCA GGTCTAGTCA AAGCCTCCTA CACAGTGATG GAAACACCTA CTTGAGTTGG
121  CTTCACCAGA GGCCAGGCCA GCCTCCTAGA CTCCTAATTT ATCAGATTTC TAACCGGTTC
181  TCTGGGGTCC CAGACAGATT CAGTGGCAGT GGGACAGGGA CAGATTTCAC ACTGAAAATC
241  AGCAGGGTGG AAGCTGAGGA TGCCGGGATT TATTACTGCA TGCAAGGTAC AGAATTTCCT
301  CGGACGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAA          (SEQ ID NO: 64)
```

FIG. 15

```
1    DIVMTQSPLS SPVILGQPAS
21   ISCRSSQSLL HSDGNTYLSW CDR1
41   LHQRPGQPPR LLIYQISNRF CDR2
61   SGVPDRFSGS GTGTDFTLKI
81   SRVEAEDAGI YYCMQGTEFP CDR3
101  RTFGQGTKVE IK          (SEQ ID NO: 65)
```

FIG. 16

```
1    GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGATACAGC CTGGGGGGTC CCTGAGACTC
61   TCCTGTGCAA CCTCTGGATT CACCTTTAGA ACTTCTTCCA TGAGTTGGGT CCGTCGGGCT
121  CCAGGGAAGG GGCTGGAATG GGTCTCAGCT ATTGGTGCTG AAAGTCATGA CACGCACTAC
181  ACAGACTCCG CGGAGGGCCG GTTCACCATC TCCAAAGACT ATTCAAAGAA CACAGTATAT
241  CTGCAGATGA ACGGCCTGAG AGTCGACGAC ACGGCCATAT ATTATTGTGC CCATCACTAT
301  TACTATGGCT CGCGGCAGAA ACCCAAAGAT TGGGGAGATG CTTTTGATAT GTGGGGCCAG
361  GGGACAATGG TCTCCGTCTC TTCA          (SEQ ID NO: 73)
```

FIG. 17

```
1    EVQLVESGGG LIQPGGSLRL
21   SCATSGFTFR TSSMSWVRRA CDR1
41   PGKGLEWVSA IGAESHDTHY CDR2
61   TDSAEGRFTI SKDYSKNTVY
81   LQMNGLRVDD TAIYYCAHHY CDR3
101  YYGSRQKPKD WGDAFDMWGQ
121  GTMVSVSS          (SEQ ID NO: 74)
```

FIG. 18

```
1    GACATCCAGA TGACCCAGTC TCCATCTTCT GTGTCTGCAT CTGTAGGAGA CAGAGTCACC
61   ATCACTTGTC GGGCGAGTCA GGATATTAGC ACCTGGTTAA CCTGGTATCA GCAGAGAGCA
121  GGGAAGGCCC CTAACCTCCT GATCTATGGT GCATCCACTT TGGAAGATGG GGTCCCATCC
181  AGGTTCAGCG GCAGTGGATC CGGGACAGAT TTCACTCTCA CTATCGACAG CCTGCAGCCT
241  GACGATTTTG CAACTTACTA TTGTCAACAG TCTCACAGTT TCCCCTACAC TTTTGGCCAG
301  GGGACCCAGC TGGGGATCTC A          (SEQ ID NO: 82)
```

FIG. 19

```
1    DIQMTQSPSS VSASVGDRVT
21   ITCRASQDIS TWLTWYQQRA CDR1
41   GKAPNLLIYG ASTLEDGVPS CDR2
61   RFSGSGSGTD FTLTIDSLQP
81   DDFATYYCQQ SHSFPYTFGQ CDR3
101  GTQLGIS          (SEQ ID NO: 83)
```

FIG. 20

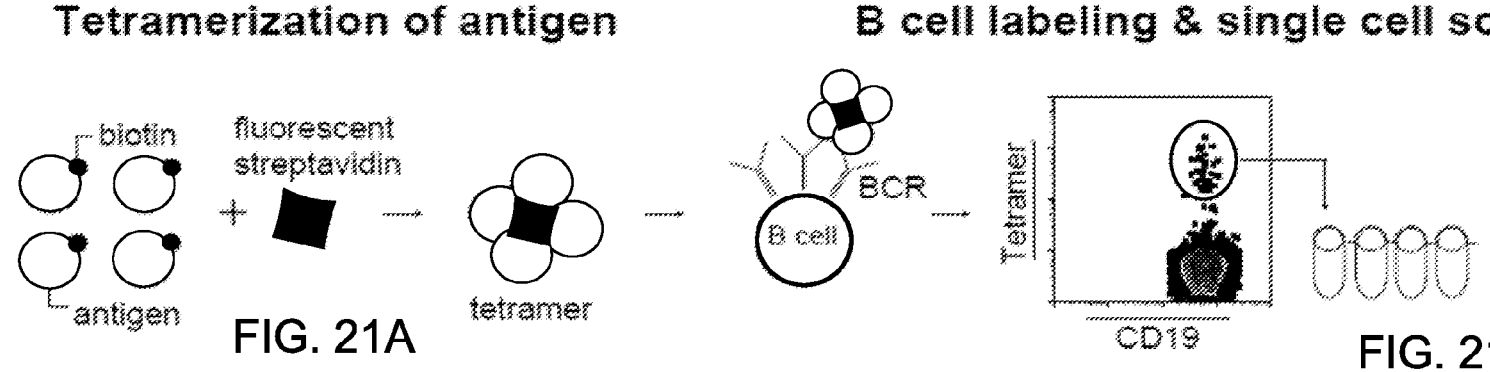


FIG. 21A

FIG. 21B

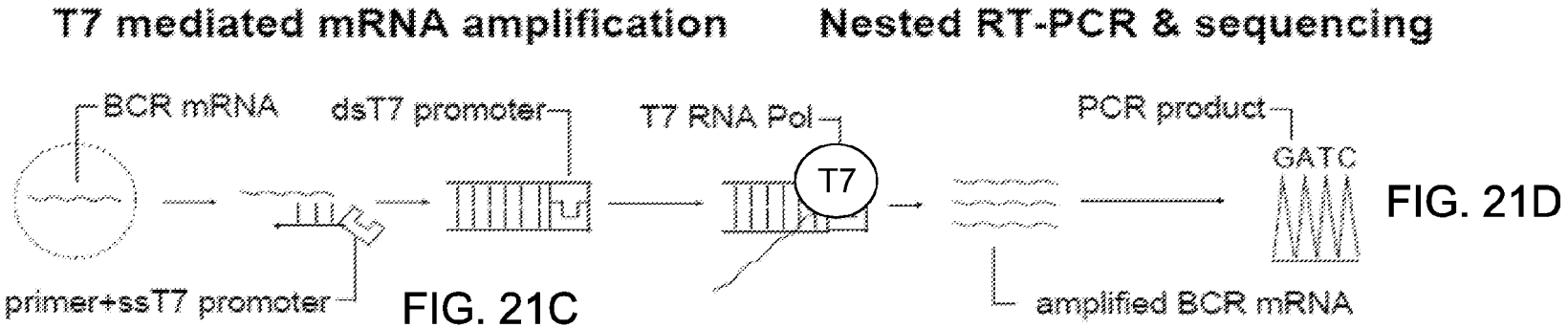


FIG. 21C

FIG. 21D

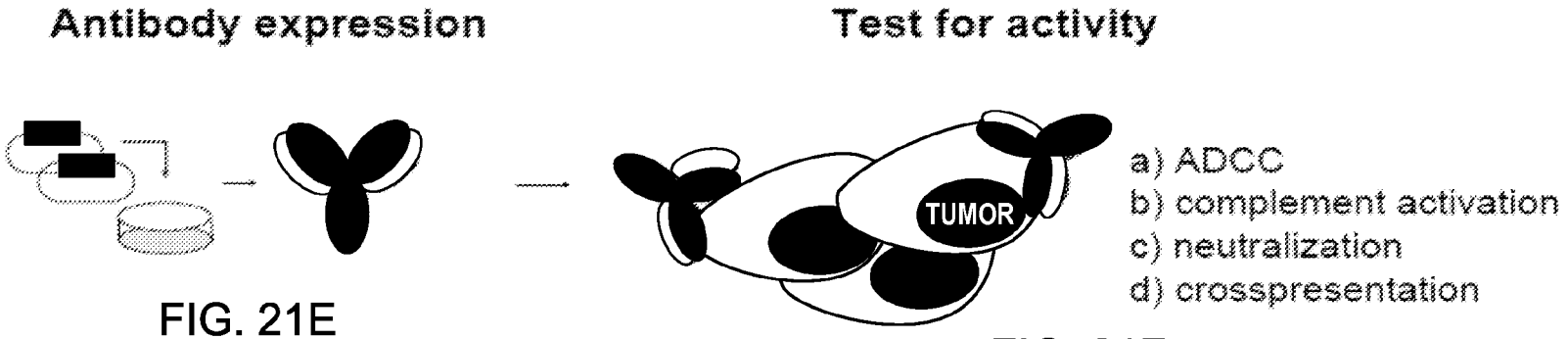


FIG. 21E

FIG. 21F

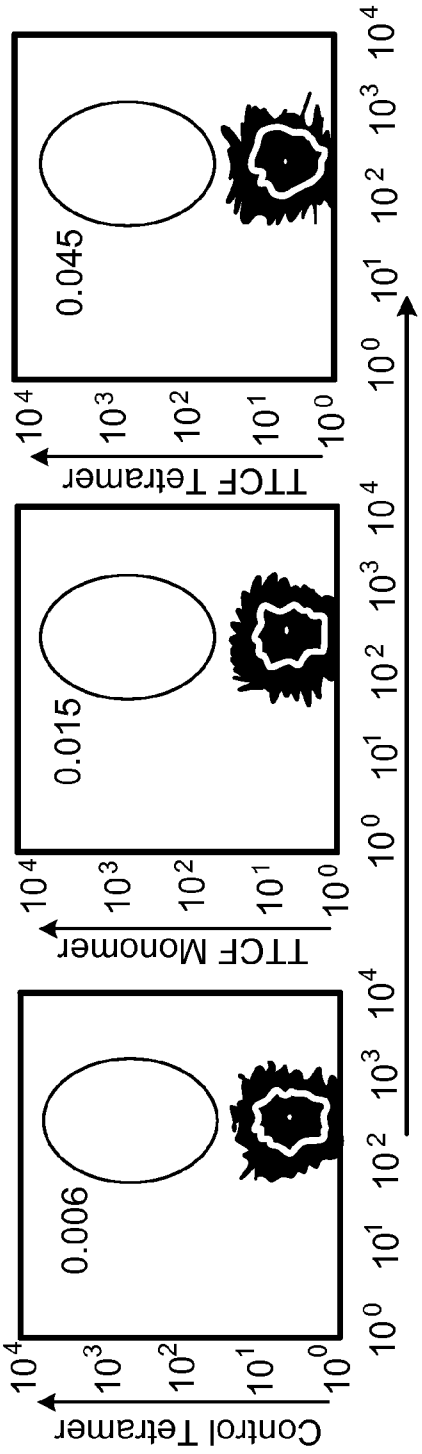


FIG. 22A

	Control Tet/ 10^5 B cells	TTCF Moncmen/ 10^4 B cells	TTCF Tet/# 10^4 B cells	#Tet/#Mono Foldchange
Donor 1	3.98	2.53	18.41	7.3
Donor 2	15.03	38.64	117.18	3.1
Donor 3	4.14	48.92	77.55	1.6

FIG. 22B

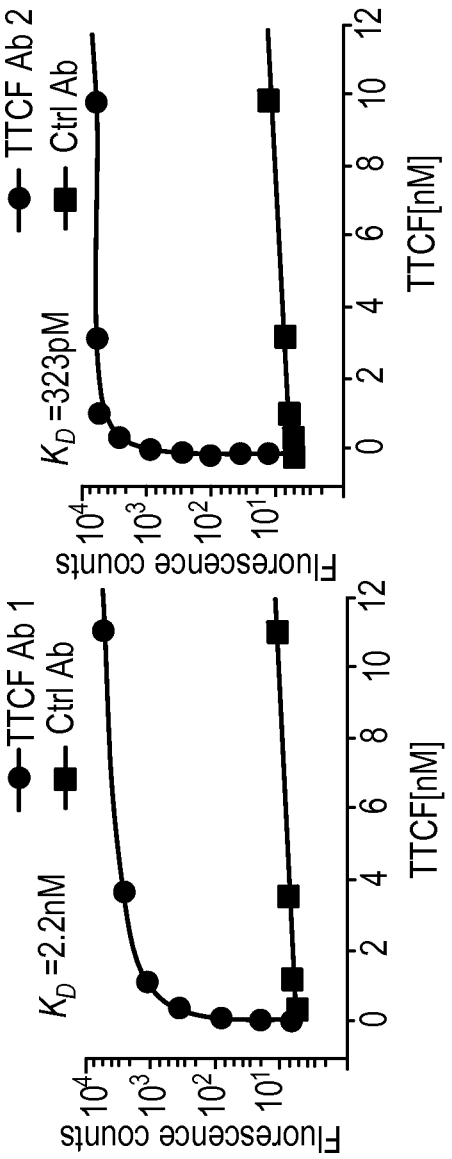


FIG. 23A

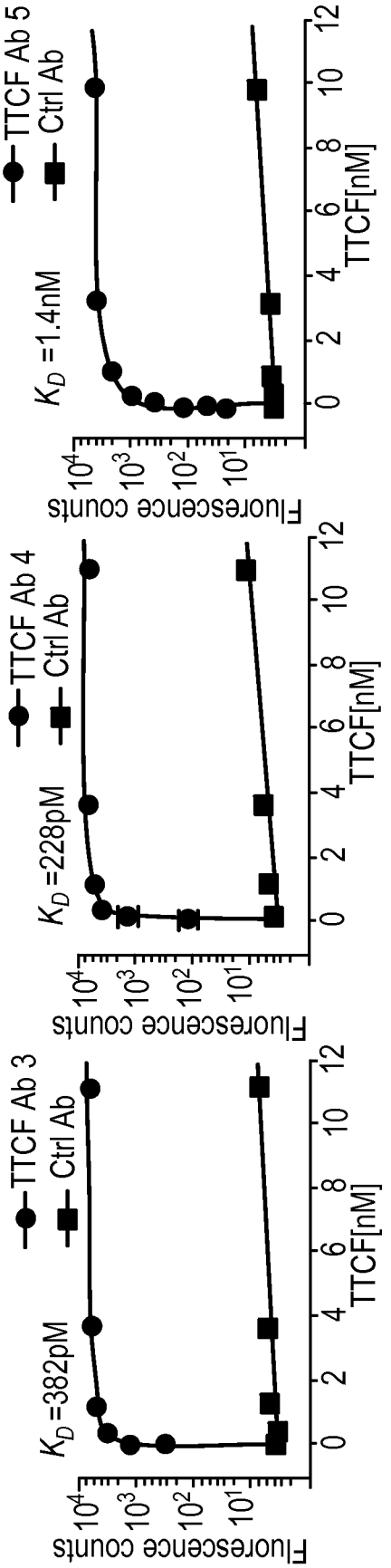


FIG. 23B

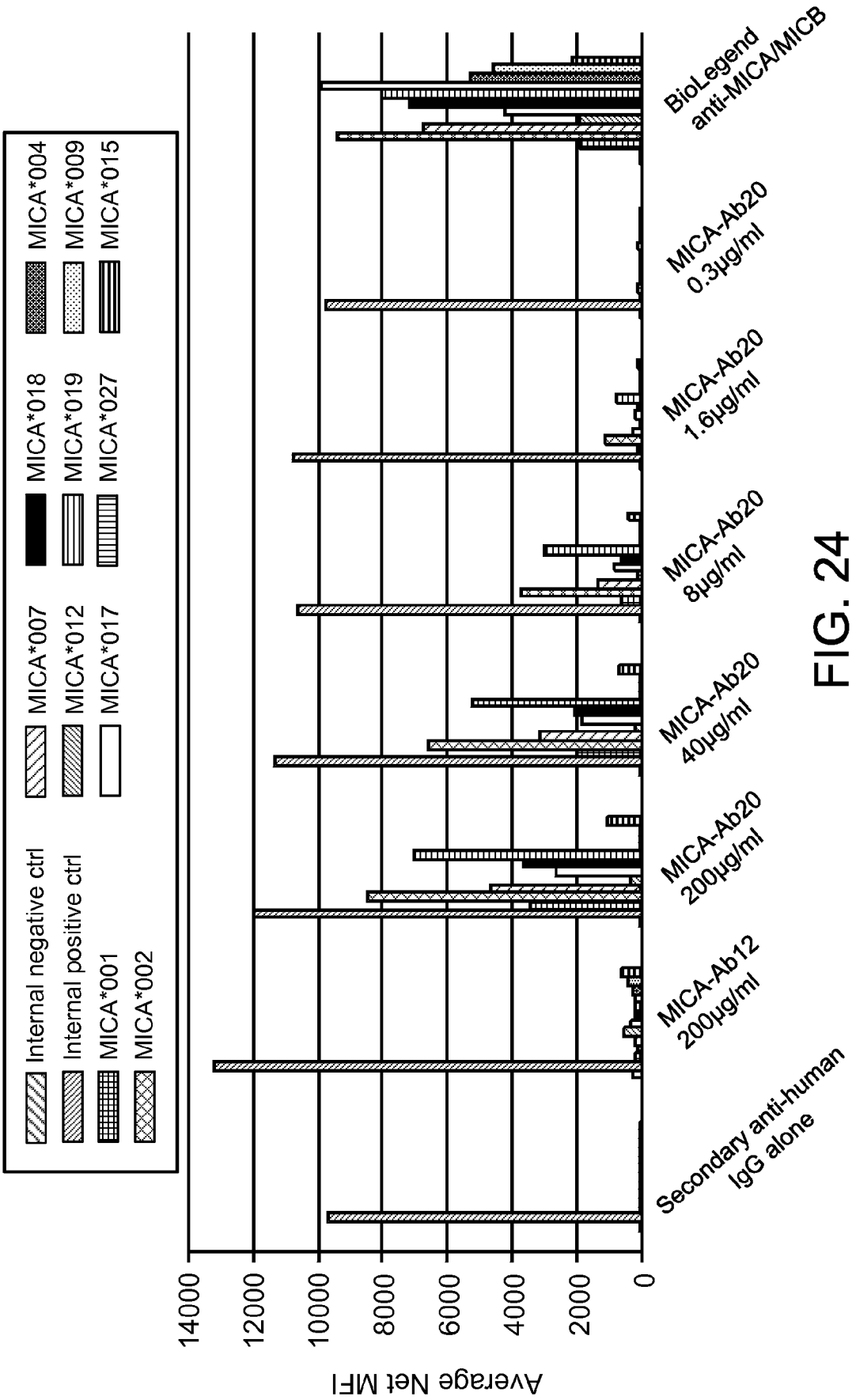

Internal negative ctrl
Internal positive ctrl
MICA*001
MICA*002
MICA*007
MICA*012
MICA*017
MICA*018
MICA*019
MICA*027
MICA*004
MICA*009
MICA*015
Average Net MFI
14000
12000
10000
8000
6000
4000
2000
0
Secondary anti-human IgG alone
MICA-Ab12 200µg/ml
MICA-Ab20 200µg/ml
MICA-Ab20 40µg/ml
MICA-Ab20 8µg/ml
MICA-Ab20 1.6µg/ml
MICA-Ab20 0.3µg/ml
BioLegend anti-MICA/MICB

FIG. 24

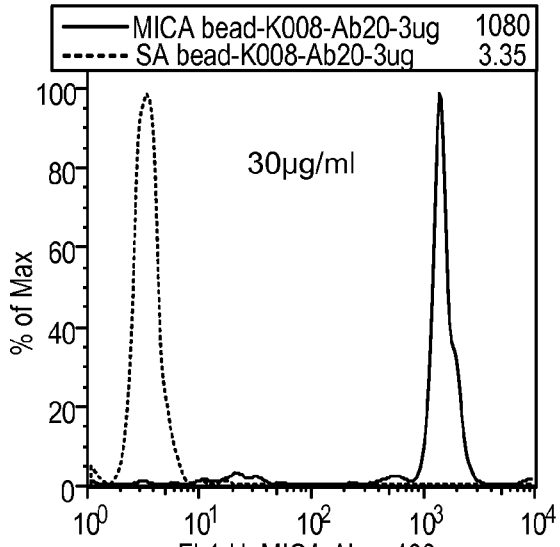


FIG. 25A

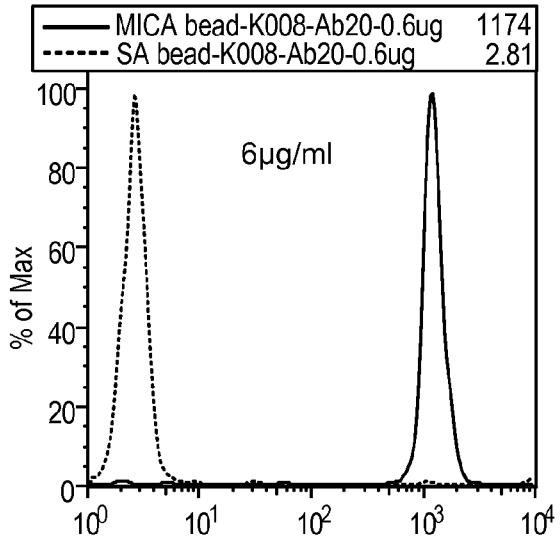
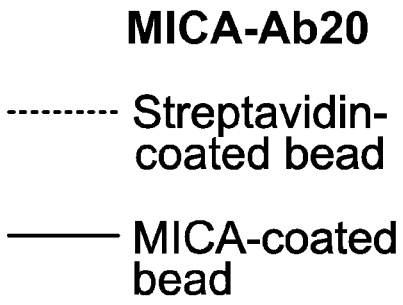


FIG. 25B



MICA

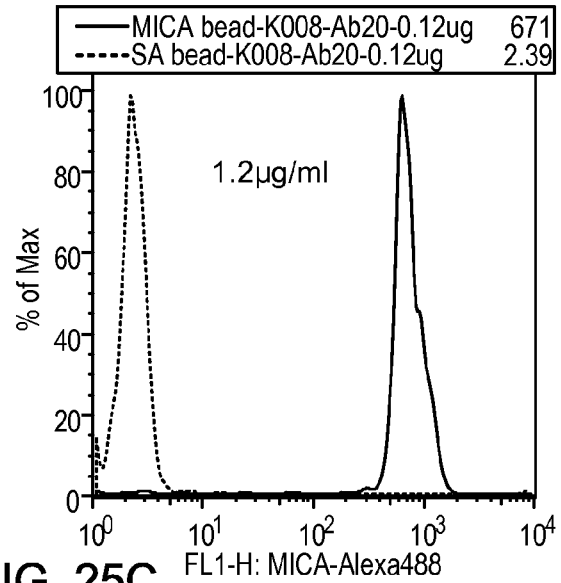


FIG. 25C

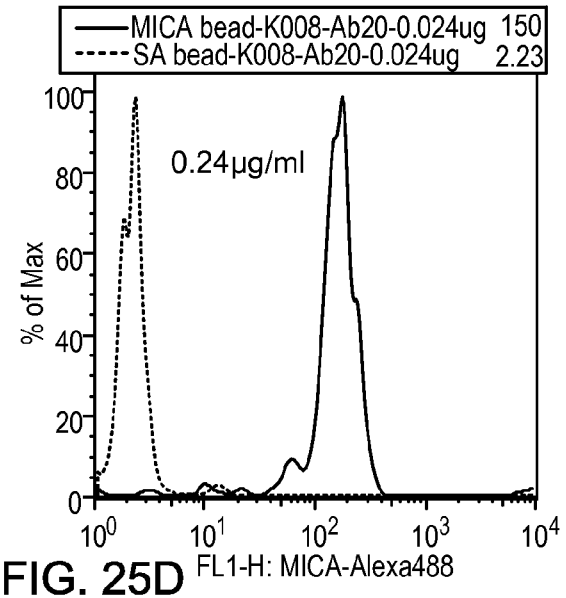


FIG. 25D

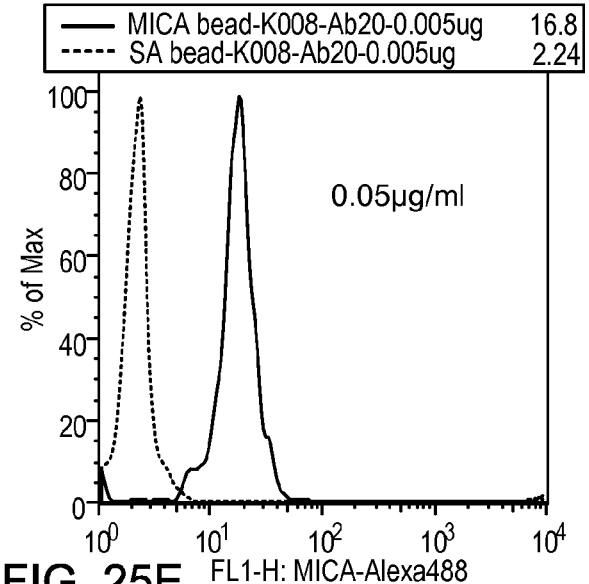


FIG. 25E

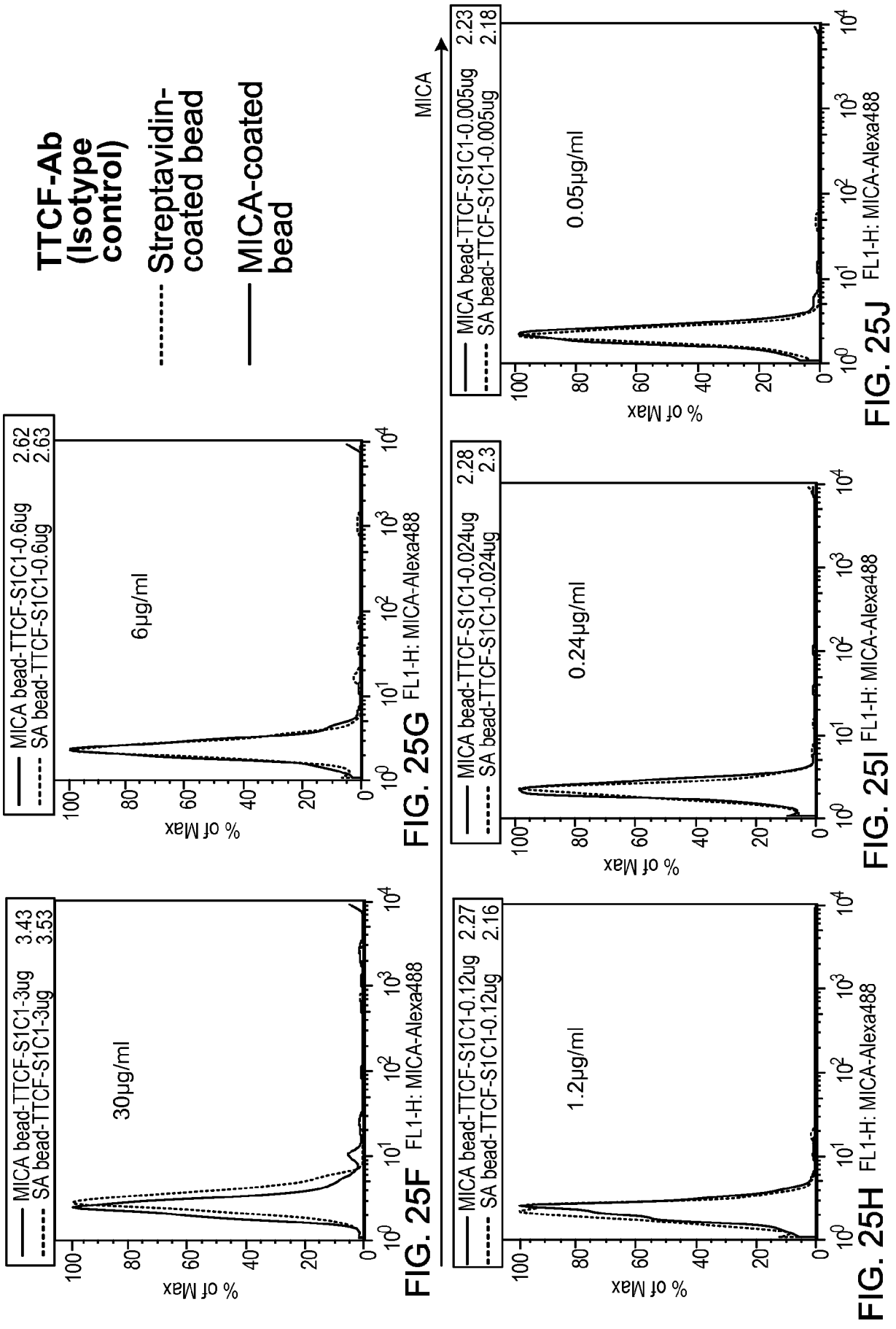


FIG. 25F

FIG. 25G

FIG. 25H

FIG. 25I

FIG. 25J

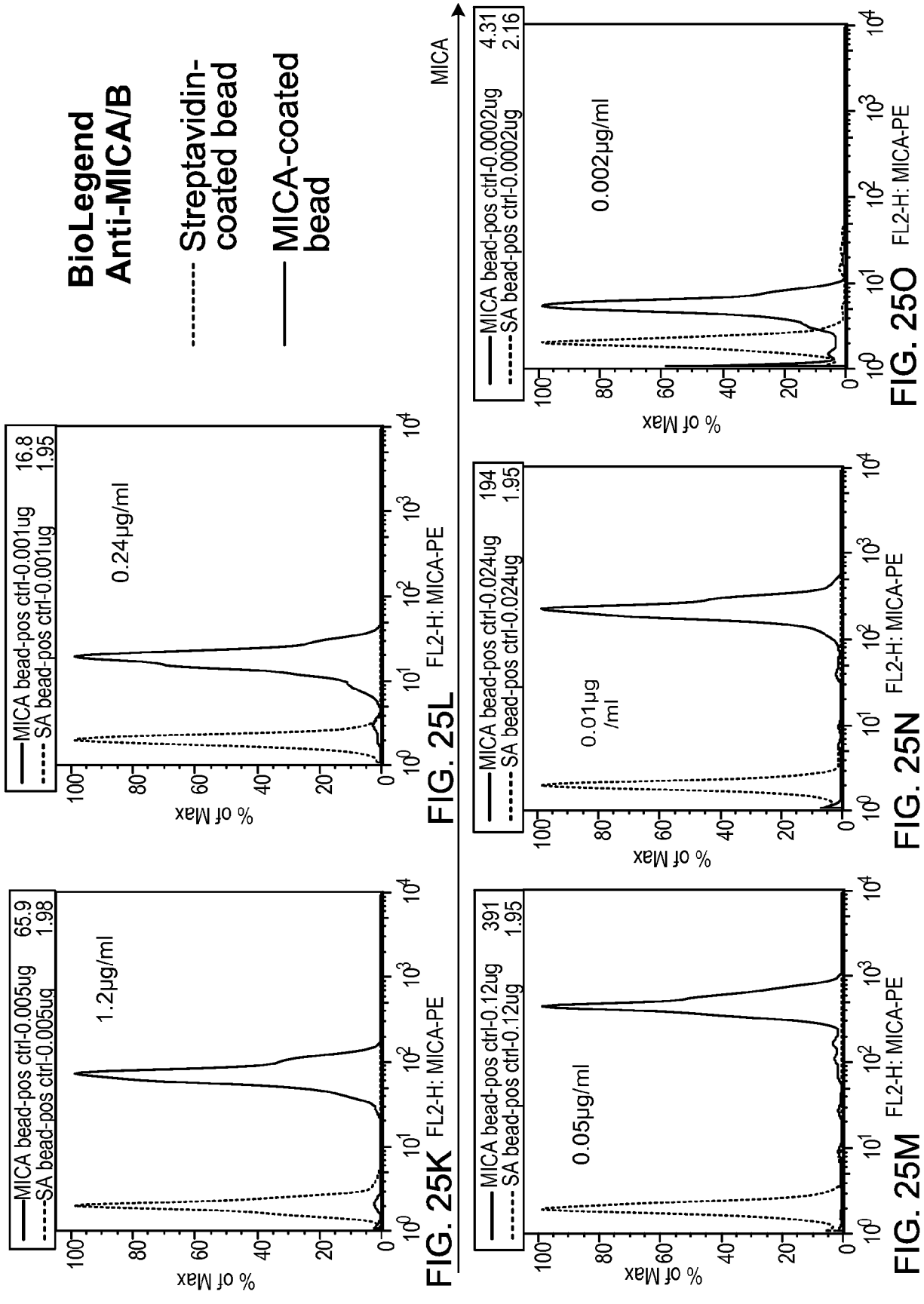


FIG. 25K FIG. 25L FIG. 25M FIG. 25N FIG. 25O

FIG. 26A

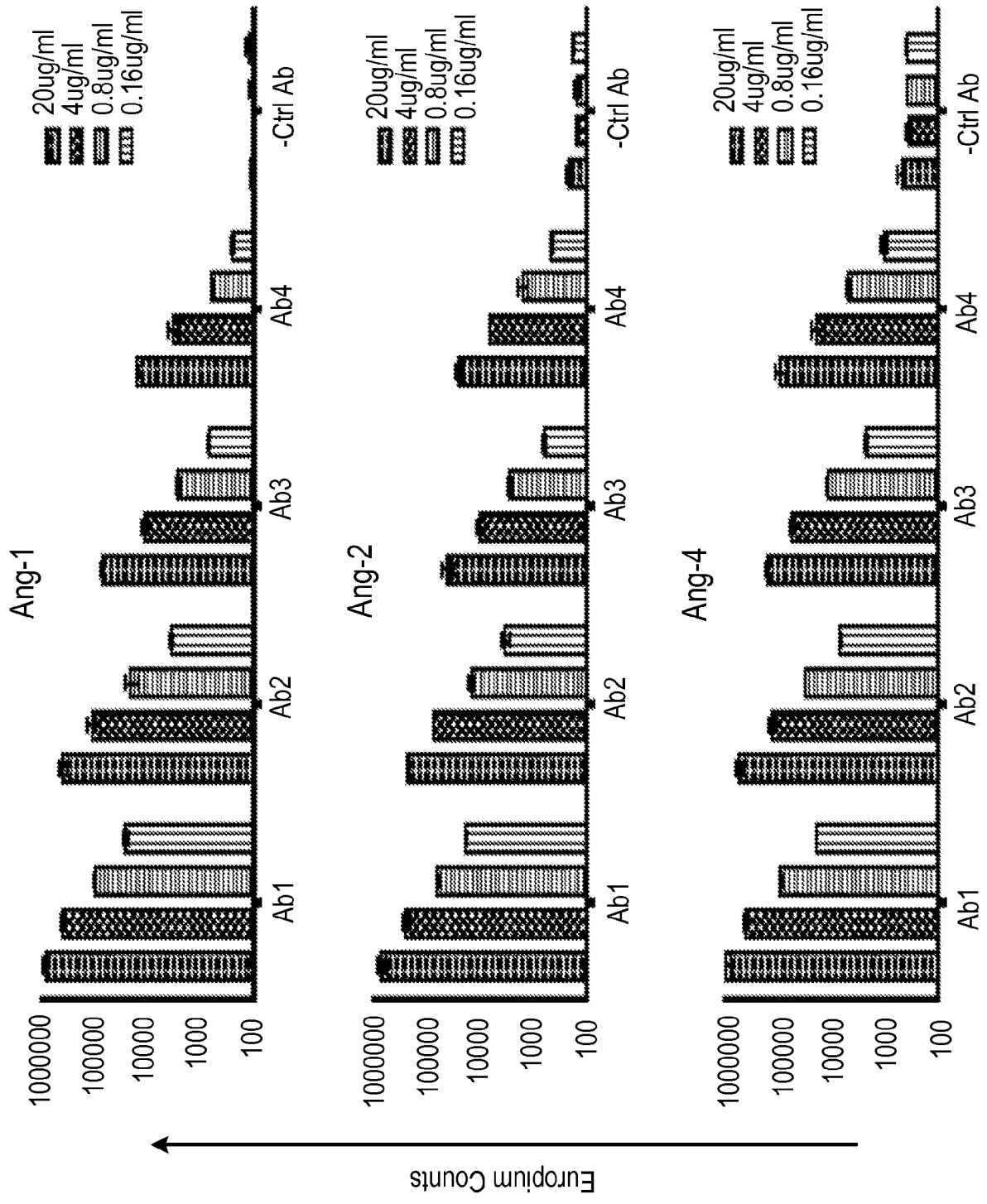


FIG. 26B

FIG. 26C


Ang-like-3
20ug/ml
4ug/ml
0.8ug/ml
0.16ug/ml
1000000
100000
10000
1000
100
Ab1
Ab2
Ab3
Ab4
-Ctrl Ab

FIG. 26D

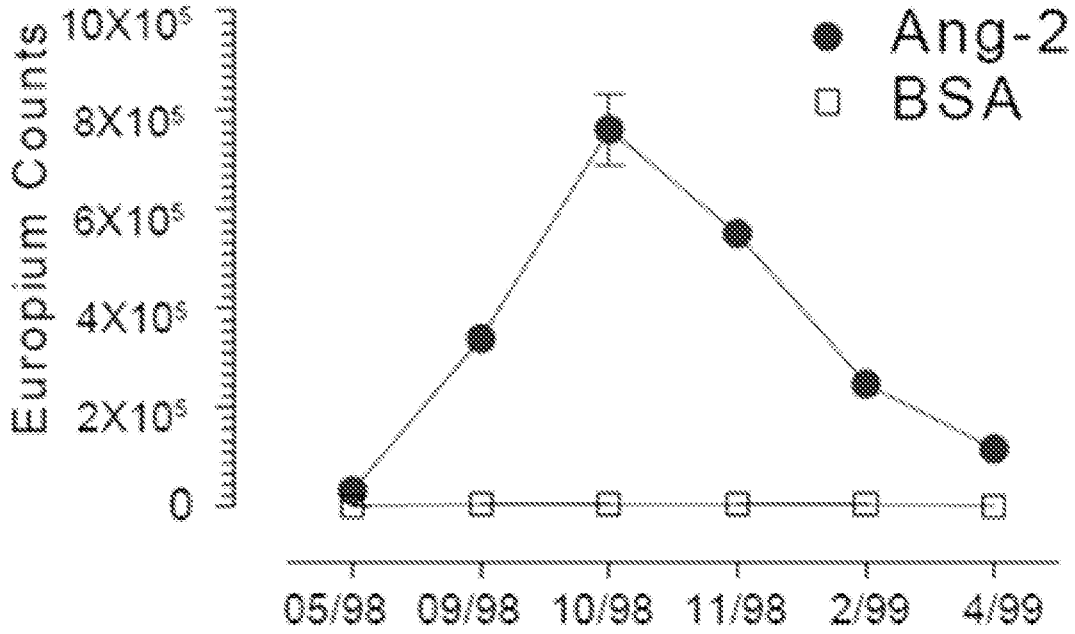


FIG. 27A

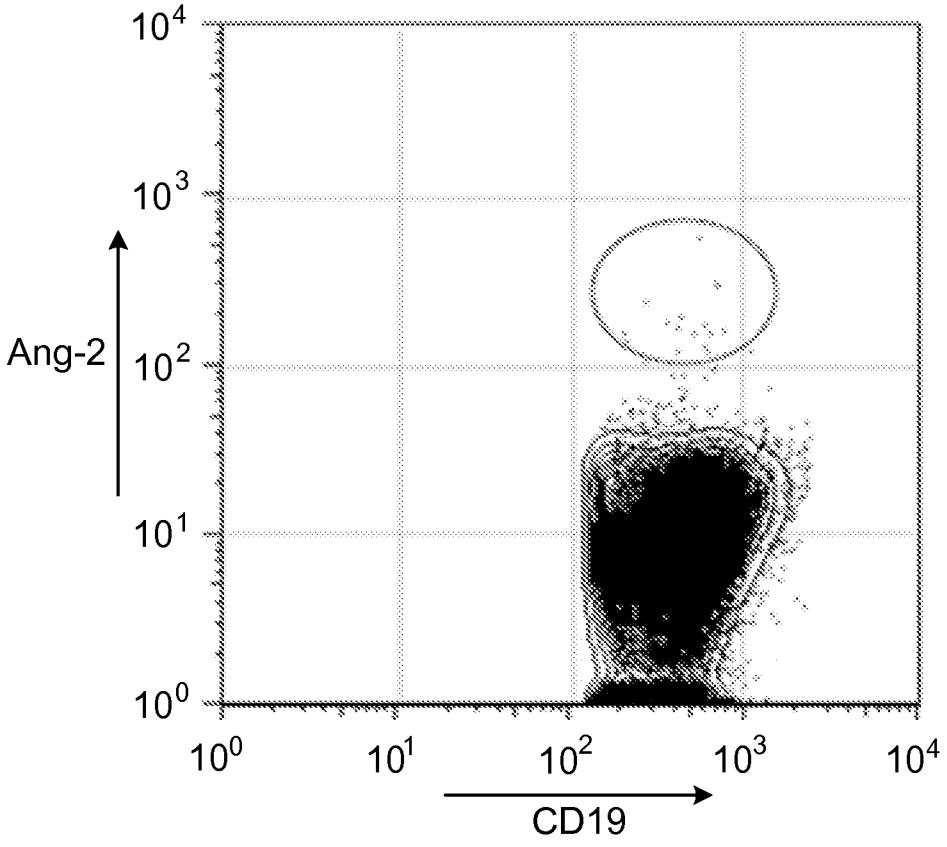


FIG. 27B

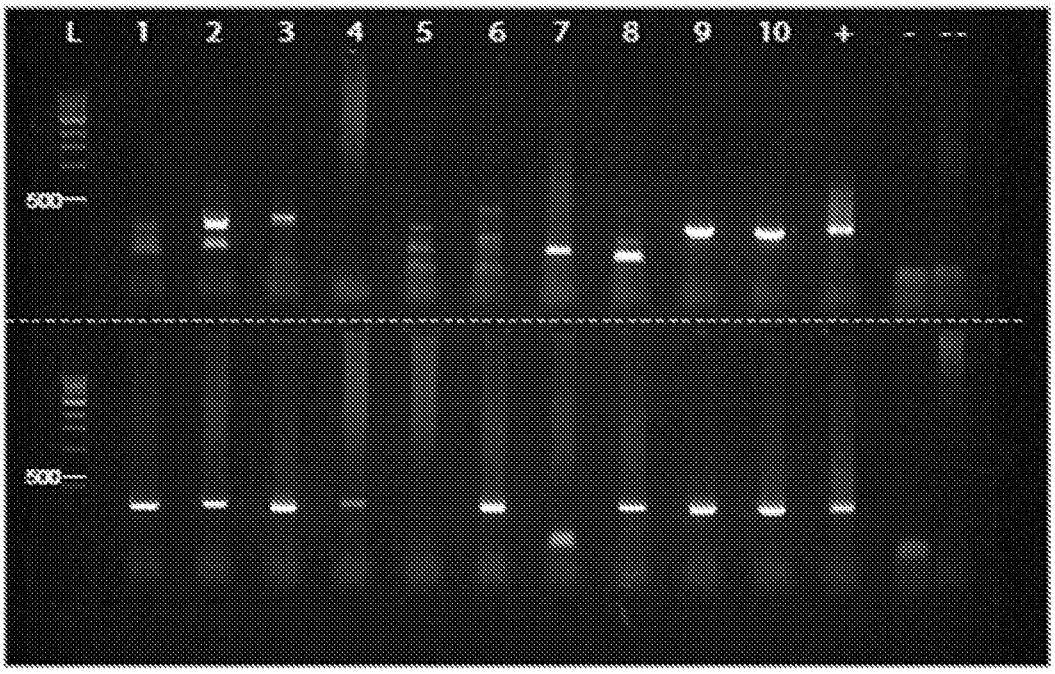


FIG. 27C

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGGAGCCTTCGGGGACCCTGTCCCT
CACCTGCACTGTGTCTGGTGGCTCCATCAGCAGGAGTAACTGGTGGAGTTGGGTCCGCC
AGCCCCCAGGGGAGGGGCTGGAATGGATTGGAGAAATCCATCACATTGGGAGGTCCAGC
TACAATCCGTCCCTCAAGAGTCGAGTCACCATGTCTGTAGACAAGTCCCAGAACCAGTT
CTCCCTGAGGCTGACCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCGAAAA
ATGGCTACTACGCTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCG
(SEQ ID NO. 148)

FIG. 28

QVQLQESGPGLVEPSGTLSLTCTVSGGSISRSNWWSWVRQPPGEGLEWIGEIHHIGRSS
YNPSLKSRVTMSVDKSQNQFSLRLTSVTAADTAVYYCAKNGYYAMDVWGQGTTVTVSS
(SEQ ID NO. 149)

FIG. 29

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCAC
CCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCGACTTCCTAGCCTGGTACCAGCAGA
AACCTGGCCAGGCTCCCAGGCTCCTCATCTACGCTACATCCTTCAGGGCCACTGGCATC
TCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCTCTCTCACCATCAACAGACT
GGAACCTGAAGATTTTGCAGTGTATTACTGTCAGCACTATCGTAGTTCACCTCCGTGGT
ACACTTTTGCCCAGGGGACCAAGCTGGACATGAGACGTACGGTGGCTGCACCATCTGTC
(SEQ ID NO. 150)

FIG. 30

EIVLTQSPGTLSLSPGERATLSCRASQSVSSDFLAWYQQKPGQAPRLLIYATSFRATGI
SDRFSGSGSGTDFSLTINRLEPEDFAVYYCQHYRSSPPWYTFAQGTKLDMRRTVAAPSV
(SEQ ID NO. 151)

FIG. 31

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGGGACCCTGTCCCTC
ACCTGCGCTGTCTCTGGTGCCTCCATTACCAATGGTGCCTGGTGGAGTTGGGTCCGCCAG
CCCCCAGGGAAGGGGCTGGAGTGGATTGGAGAAATCTATCTTAATGGGAACACCAACTCC
AACCCGTCCCTGAAGAGTCGAGTCATCATATCAGTGGACAAGTCCAAGAACCACTTCTCG
CTGACCCTGAACTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCGAAGAACGCT
GCCTACAACCTTGAGTTCTGGGGCCAGGGAGCCCTGGTCACCGTCTCCTCA (SEQ ID NO:
167)

FIG. 32

QVQLQESGPGLVKPSGTLSLTCAVSGASITNGAWWSWVRQPPGKGLEWIGEIYLNGNTNS
NPSLKSRVIISVDKSKNHFSLTLNSVTAADTAVYYCAKNAAYNLEFWGQGALVTVSS (SEQ
ID NO: 168)

FIG. 33

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC

CTCTCCTGCAGGGCCAGTCAGACTGTTAGCAGCCCCTACGTAGCCTGGTACCAGCAGAAA

CGTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGCCACCGGCATCCCAG

ACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC

CTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGATAGATCATACTATTACACTTTT

GGCCAGGGGACCAAGCTGGAGATCAAA (SEQ ID NO: 169)

FIG. 34

EIVLTQSPGTLSLSPGERATLSCRASQTVSSPYVAWYQQKRGQAPRLLIYGASTRATGIPDR

FSGSGSGTDFTLTISRLEPEDFAVYYCQQYDRSYYYTFGQGTKLEIK (SEQ ID NO: 170)

FIG. 35

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGAACCTGTCGCTC
ACCTGCACTGTCTCTGATGCCTCCATGAGTGATTATCACTGGAGCTGGATCCGGCAGGCC
GCCGGGAAGGGACTGGAGTGGATTGGGCGTATGTACAGCACTGGGAGTCCCTACTACAA
ACCCTCCCTCAAAGGTCGGGTCACCATGTCAATAGACACGTCCAAGAACCAGTTCTCCCT
GAAGCTGGCCTCTGTGACCGCCGCAGACACGGCCATCTATTATTGTGCGAGCGGACAACA
TATTGGTGGCTGGGTCCCCCCTGACTTCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC
A (SEQ ID NO: 185)

FIG. 36

QVQLQESGPGLVKPSENLSLTCTVSDASMSDYHWSWIRQAAGKGLEWIGRMYSTGSPYY
KPSLKGRVTMSIDTSKNQFSLKLASVTAADTAIYYCASGQHIGGWVPPDFWGQGTLVTVS
S (SEQ ID NO: 186)

FIG. 37

GATATTGTGATGACCCAGACTCCACTCTCCTCACCTGTCACCCTTGGACAGCCGGCCTCCA
TCTCCTGCAGGTCTAGTGAAGGCCTCGTATATAGTGATGGAGACACCTACTTGAGTTGGT
TTCACCAGAGGCCAGGCCAGCCTCCAAGACTCCTGATTTATAAAATTTCTAACCGGTTCT
CTGGGGTCCCCGACAGATTCAGTGGCAGTGGGGCAGGCACAGATTTCACACTGAAAATCA
GCAGGGTGGAGGCTGAGGATGTCGGGGTTTATTACTGCATGCAAGCTACACATTTTCCGT
GGACGTTCGGCCAGGGGACCAAAGTGGAAGTCAAACGT (SEQ ID NO: 187)

FIG. 38

DIVMTQTPLSSPVTLGQPASISCRSSEGLVYSDGDTYLSWFHQRPGQPPRLLIYKISNRFSG
VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATHFPWTFGQGTKVEVKR (SEQ ID NO:
188)

FIG. 39

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTTAGTTCATATGGCTTGACCTGGATACGCCAGGCT
CCGGGGAAGGGCCTGGAGTGGGTCTCAAGTATCAGTGGCAGTGGCAATAACACATACTA
CGCAGACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAAAGTCAAGAAGACACTATA
TCTACAAATGGACAGCCTGACAGTCGGAGACACGGCCGTCTATTACTGCTTAGGAGTCGG
TCAGGGCCACGGAATTCCGGTCATCGTCTCCTCA (SEQ ID NO. 203)

FIG. 40

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYGLTWIRQAPGKGLEWVSSISGSGNNTYYA
DSVKGRFTISRDKVKKTLYLQMDSLTVGDTAVYYCLGVGQGHGIPVIVSS (SEQ ID NO.
204)

FIG. 41

GATATTGTGATGACCCAGACTCCACTCTCCTCACCTGTCACCCTTGGACAGCCGGCCTCCA
TCTCCTGCAGGTCTAGTCAGAGCCTCGTACACCGTGATGGAAACACCTACTTGAGTTGGT
TTCTGCAGAGGCCAGGCCAGGCTCCAAGACTCCTAATTTATCGGATTTCTAACCGGTTCT
CTGGGGTCCCAGACAGATTCAGTGGCAGTGGGGCAGGGACGGATTTCACACTGAAAATC
AGCAGGGTGGAAGCTGAGGATGTCGGCGTTTACTACTGCATGCAAGCTACACAAATCCCC
AACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAG (SEQ ID NO. 205)

FIG. 42

DIVMTQTPLSSPVTLGQPASISCRSSQSLVHRDGNTYLSWFLQRPGQAPRLLIYRISNRFSG
VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATQIPNTFGQGTKLEIK (SEQ ID NO.
206)

FIG. 43

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTAATCCAGCCGGGGGGGTCCCTAAGACT
CTCCTGTGCAGCCTCGGGCTTCCTCATCAGTAGTTATTTCATGAGCTGGGTCCGCCAGG
CTCCAGGGAAGGGGCCGGAGTGGGTCTCAGTTATTTATAGCGATGGTAGTACATATTAC
GTAGACTCCGTGAAGGGCCGATTCACCATCTCCACAGACAATTCCAAGAACACACTATA
TCTTCAGATGAACAGCCTGAGAGCCGAGGACACGGCCCGATATTACTGTGCGACACGGC
ATTTGAATTATGACGGTGACCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCC
TCCACCAAG (SEQ ID NO: 221)

FIG. 44

EVQLVESGGGLIQPGGSLRLSCAASGFLISSYFMSWVRQAPGKGPEWVSVIYSDGSTYY
VDSVKGRFTISTDNSKNTLYLQMNSLRAEDTARYYCATRHLNYDGDHWGQGTLVTVSSA
STK (SEQ ID NO: 222)

FIG. 45

GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGGCCTC
CATCTCCTGCAGGTCTAGTCAAAGCCTCGTACACAGTGACGGAAACACCTACTTGAATT
GGTTTCACCAGAGGCCAGGCCAATCTCCAAGGCGCCTAATTTATAAGGTTTCTAAGCGG
GACTCTGGGGTCCCAGACAGATTCAGCGGCAGTGGGTCAGGTAGTGATTTCACACTGAA
AATCAGCAGGGTGGAGGCTGAGGATGTTGGAATTTATTACTGCATGCAAGGTACACATT
GGCCGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGAACTGTGGCTGCA (SEQ
ID NO: 223)

FIG. 46

DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSDGNTYLNWFHQRPGQSPRRLIYKVSKR
DSGVPDRFSGSGSGSDFTLKISRVEAEDVGIYYCMQGTHWPTFGQGTKVEIKRTVAA
(SEQ ID NO: 224)

FIG. 47

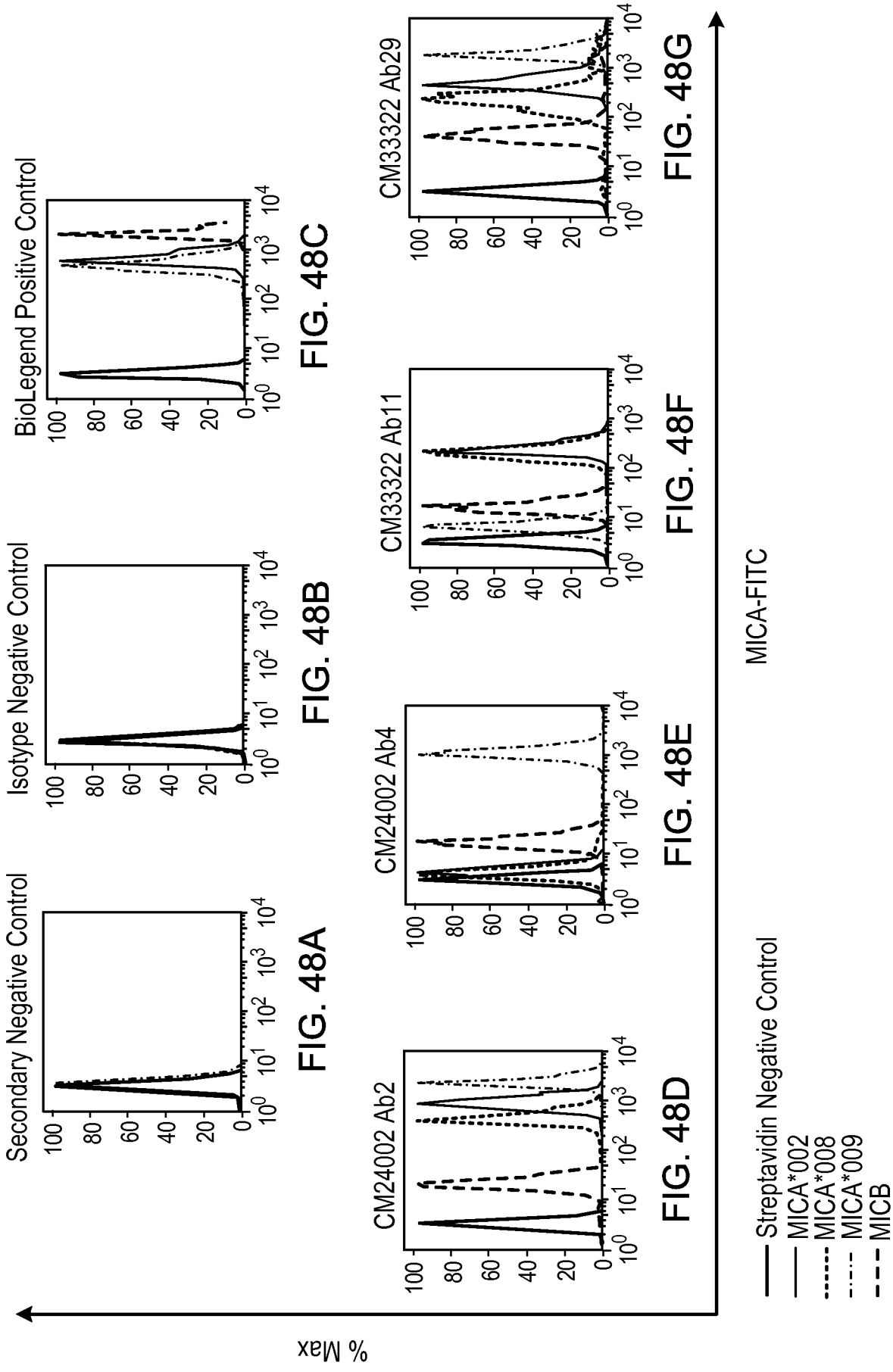


FIG. 48A FIG. 48B FIG. 48C

FIG. 48D FIG. 48E FIG. 48F FIG. 48G

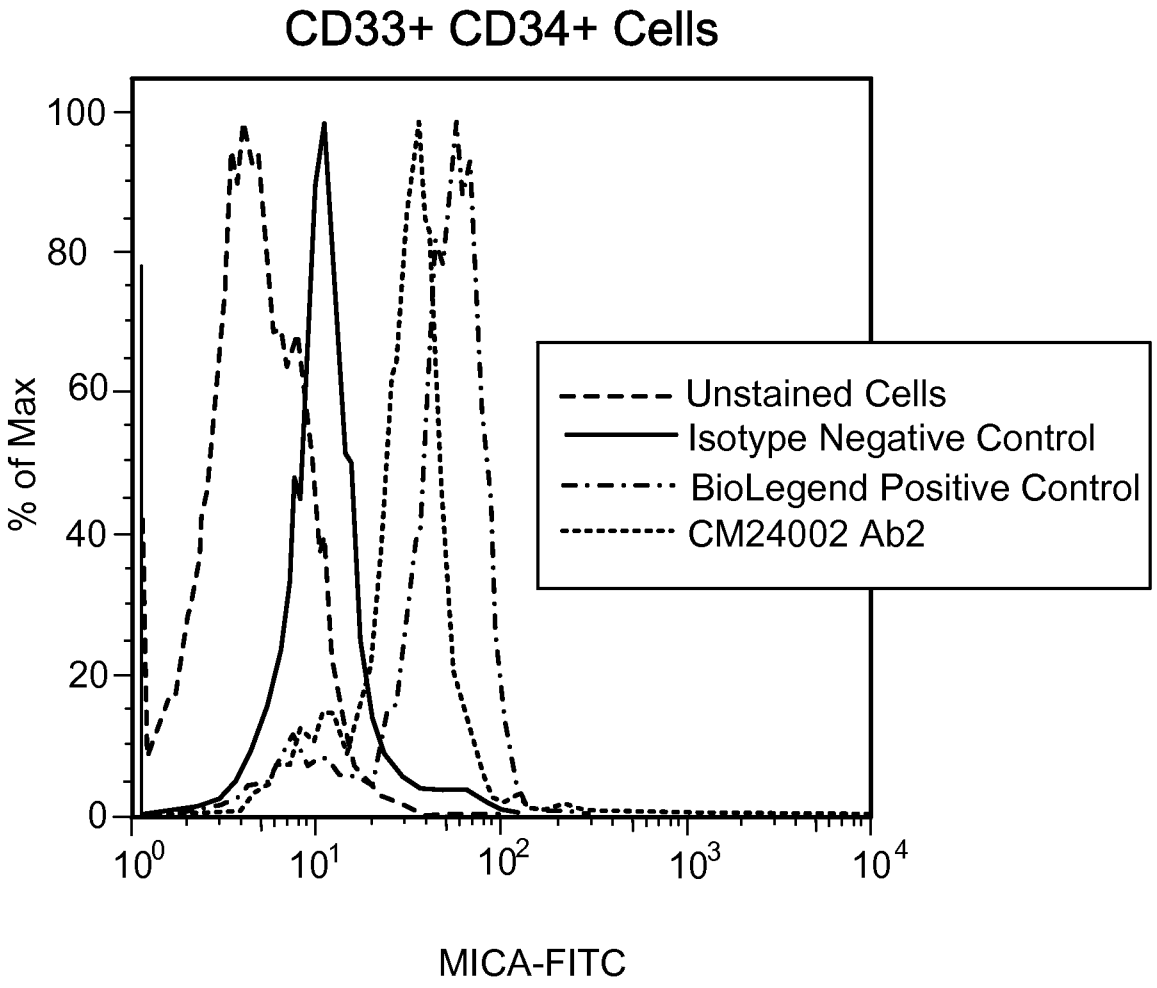

CD33+ CD34+ Cells
100
80
60
40
20
0
% of Max
10^0
10^1
10^2
10^3
10^4
MICA-FITC
Unstained Cells
Isotype Negative Control
BioLegend Positive Control
CM24002 Ab2

FIG. 49

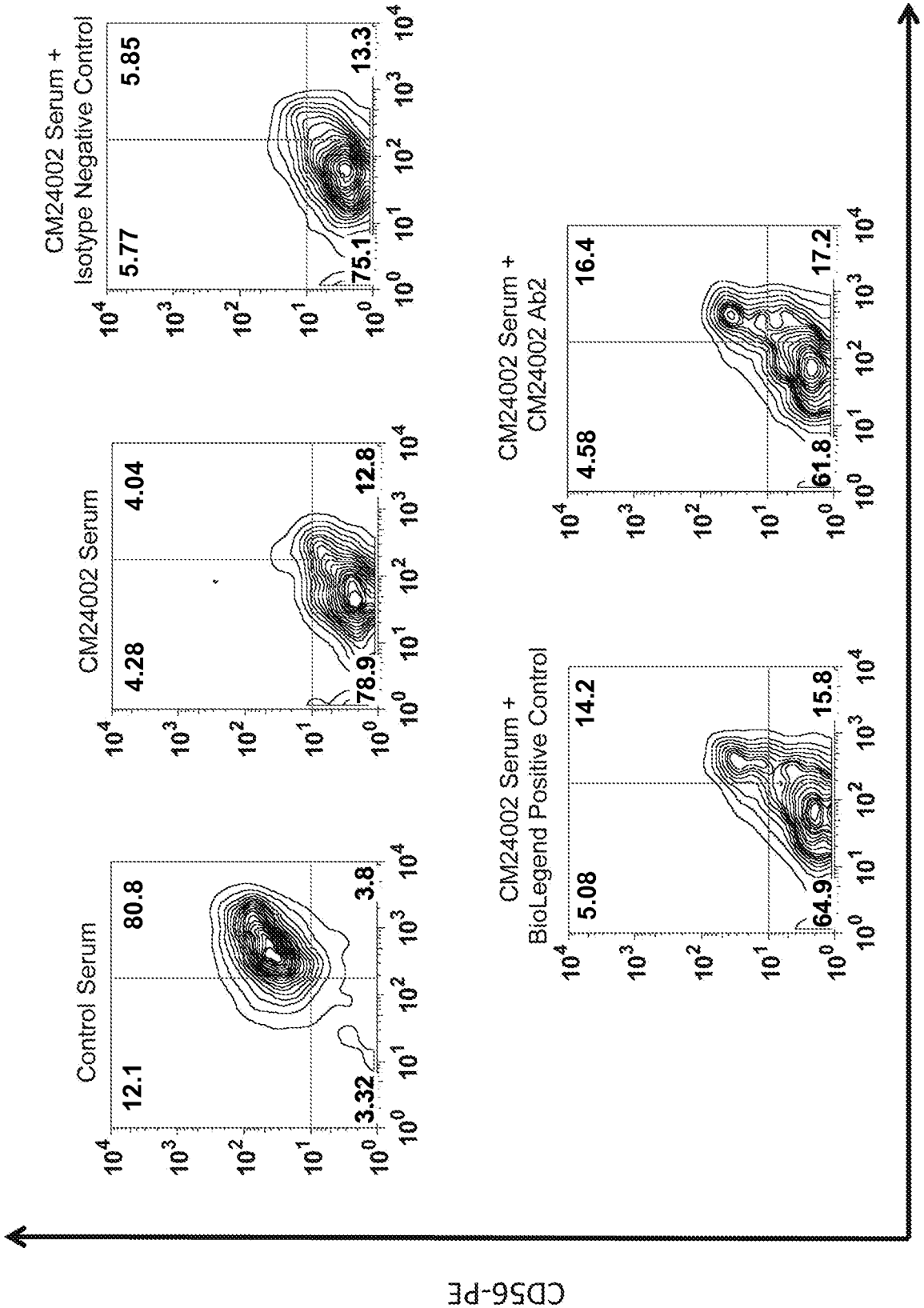

Control Serum
12.1
80.8
3.32
3.8
CM24002 Serum
4.28
4.04
78.9
12.8
CM24002 Serum +
Isotype Negative Control
5.77
5.85
75.1
13.3
CM24002 Serum +
BioLegend Positive Control
5.08
14.2
64.9
15.8
CM24002 Serum +
CM24002 Ab2
4.58
16.4
61.8
17.2
CD56-PE
NKG2D-APC

FIG. 50

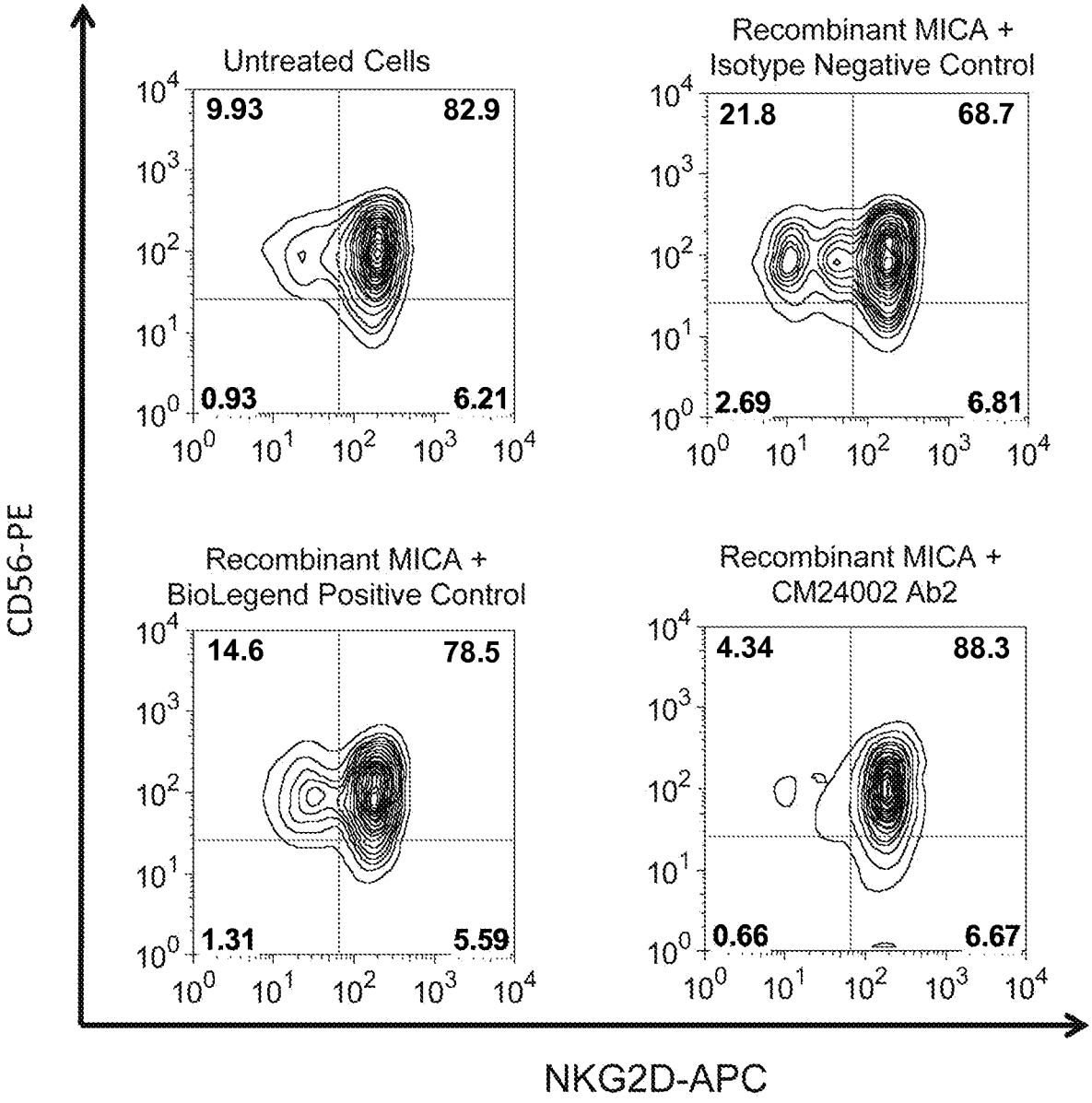

Untreated Cells
9.93
82.9
0.93
6.21
Recombinant MICA +
Isotype Negative Control
21.8
68.7
2.69
6.81
Recombinant MICA +
BioLegend Positive Control
14.6
78.5
1.31
5.59
Recombinant MICA +
CM24002 Ab2
4.34
88.3
0.66
6.67
CD56-PE
NKG2D-APC

FIG. 51

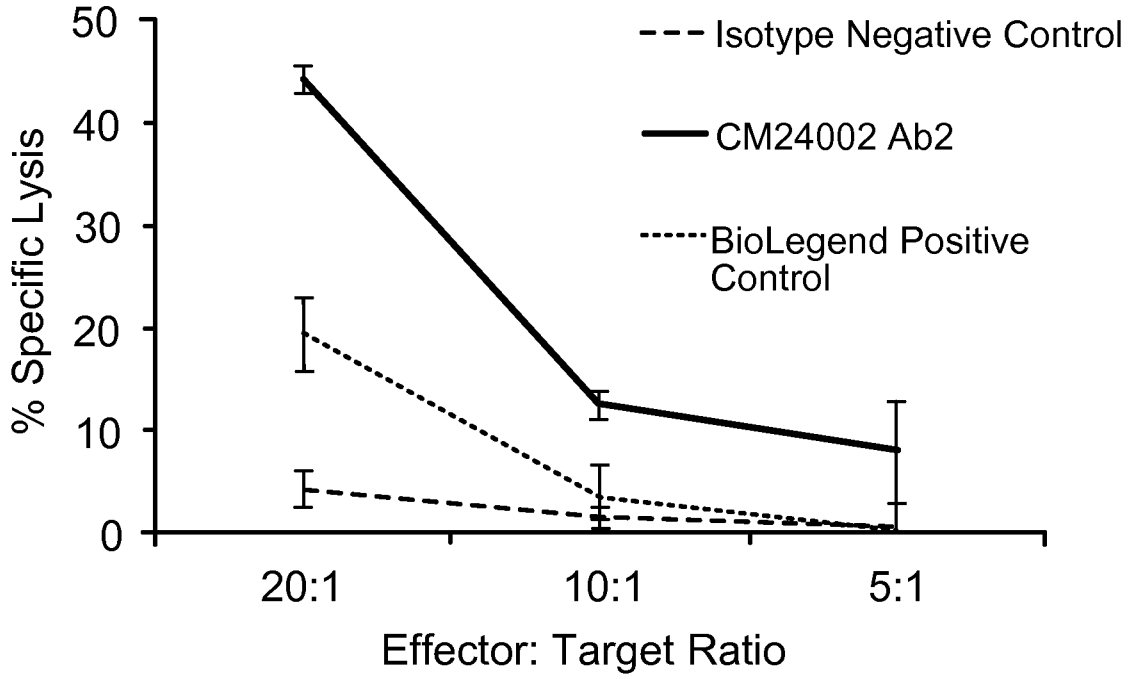

50
40
30
20
10
0
% Specific Lysis
Isotype Negative Control
CM24002 Ab2
BioLegend Positive Control
20:1
10:1
5:1
Effector: Target Ratio

FIG. 52

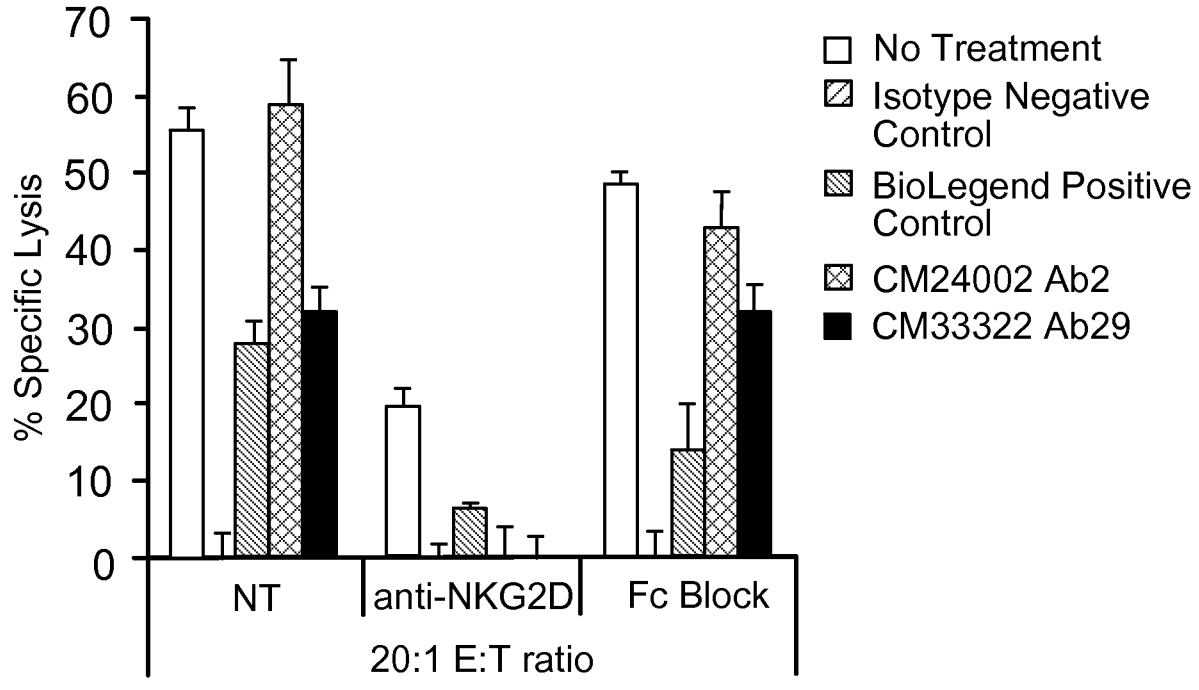

70
60
50
40
30
20
10
0
% Specific Lysis
No Treatment
Isotype Negative Control
BioLegend Positive Control
CM24002 Ab2
CM33322 Ab29
NT
anti-NKG2D
Fc Block
20:1 E:T ratio

FIG. 53

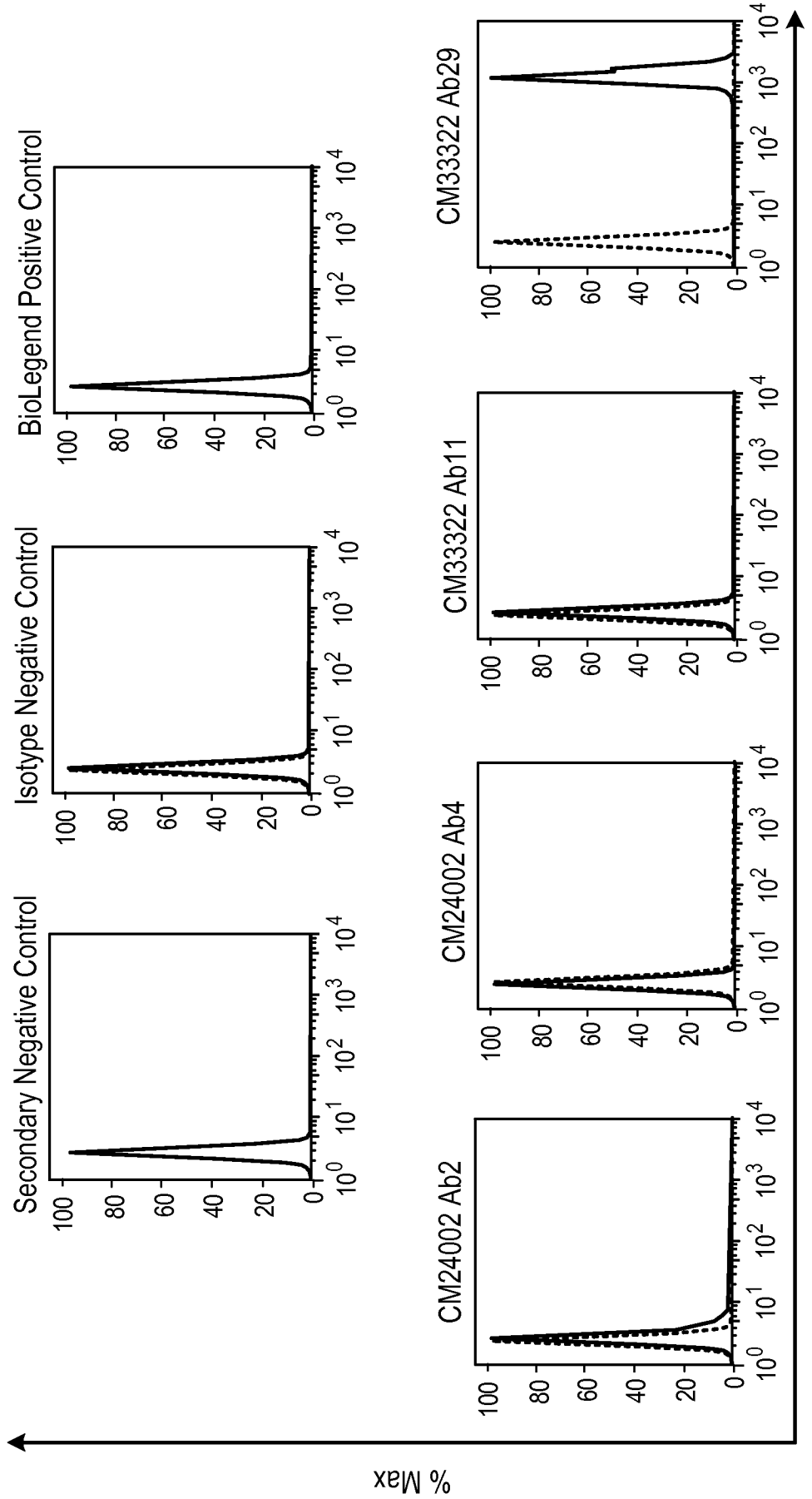

Secondary Negative Control
Isotype Negative Control
BioLegend Positive Control
CM24002 Ab2
CM24002 Ab4
CM33322 Ab11
CM33322 Ab29
% Max
MICA-α3 DOMAIN-FITC
Streptavidin Negative Control
MICA α3 Domain

FIG. 54

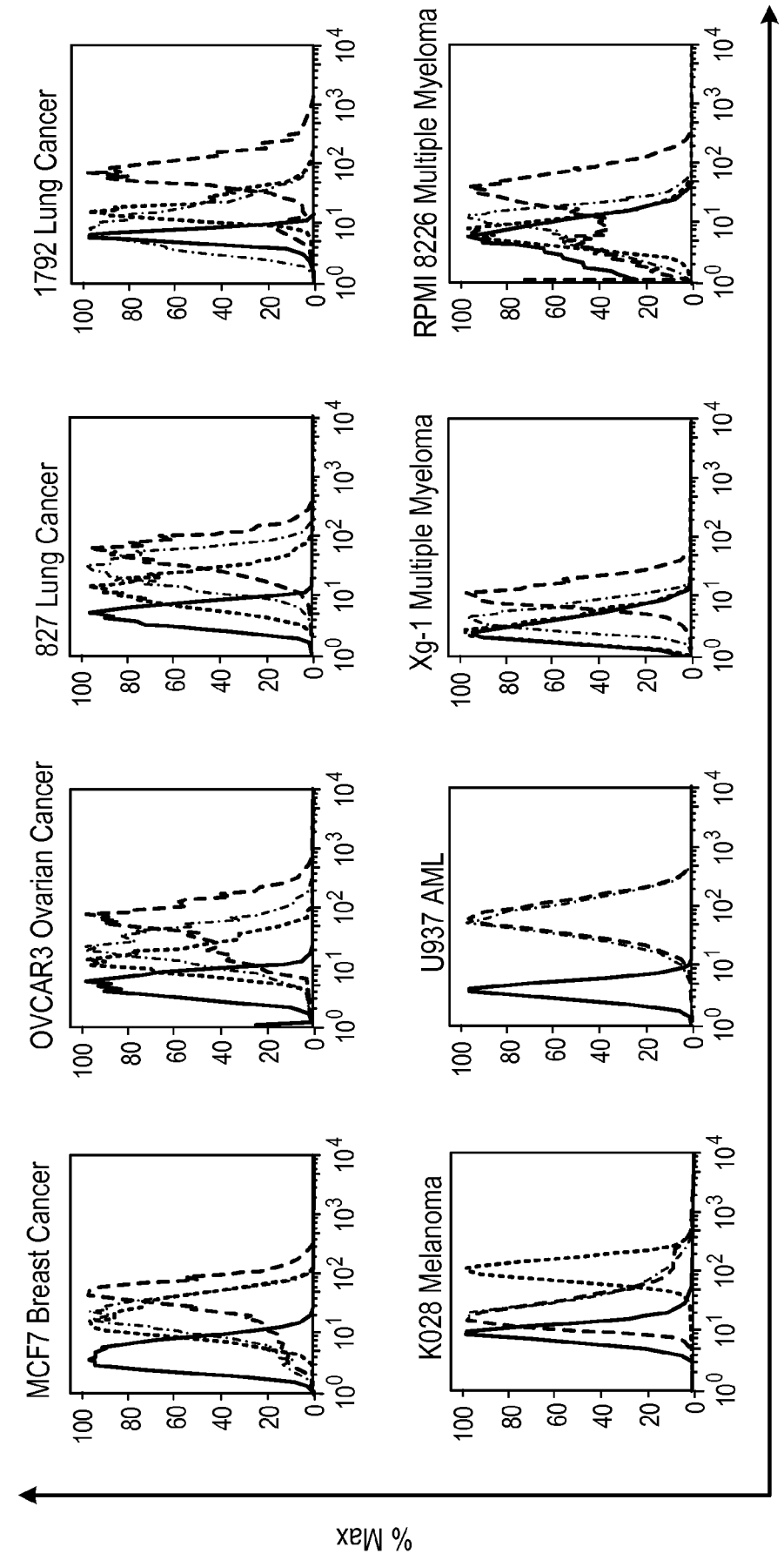

MCF7 Breast Cancer
OVCAR3 Ovarian Cancer
827 Lung Cancer
1792 Lung Cancer
K028 Melanoma
U937 AML
Xg-1 Multiple Myeloma
RPMI 8226 Multiple Myeloma
% Max
MICA-FITC
Unstained Cells
Isotype Negative Control
BioLegend Positive Control
CM24002 Ab2
CM33322 Ab29

FIG. 55

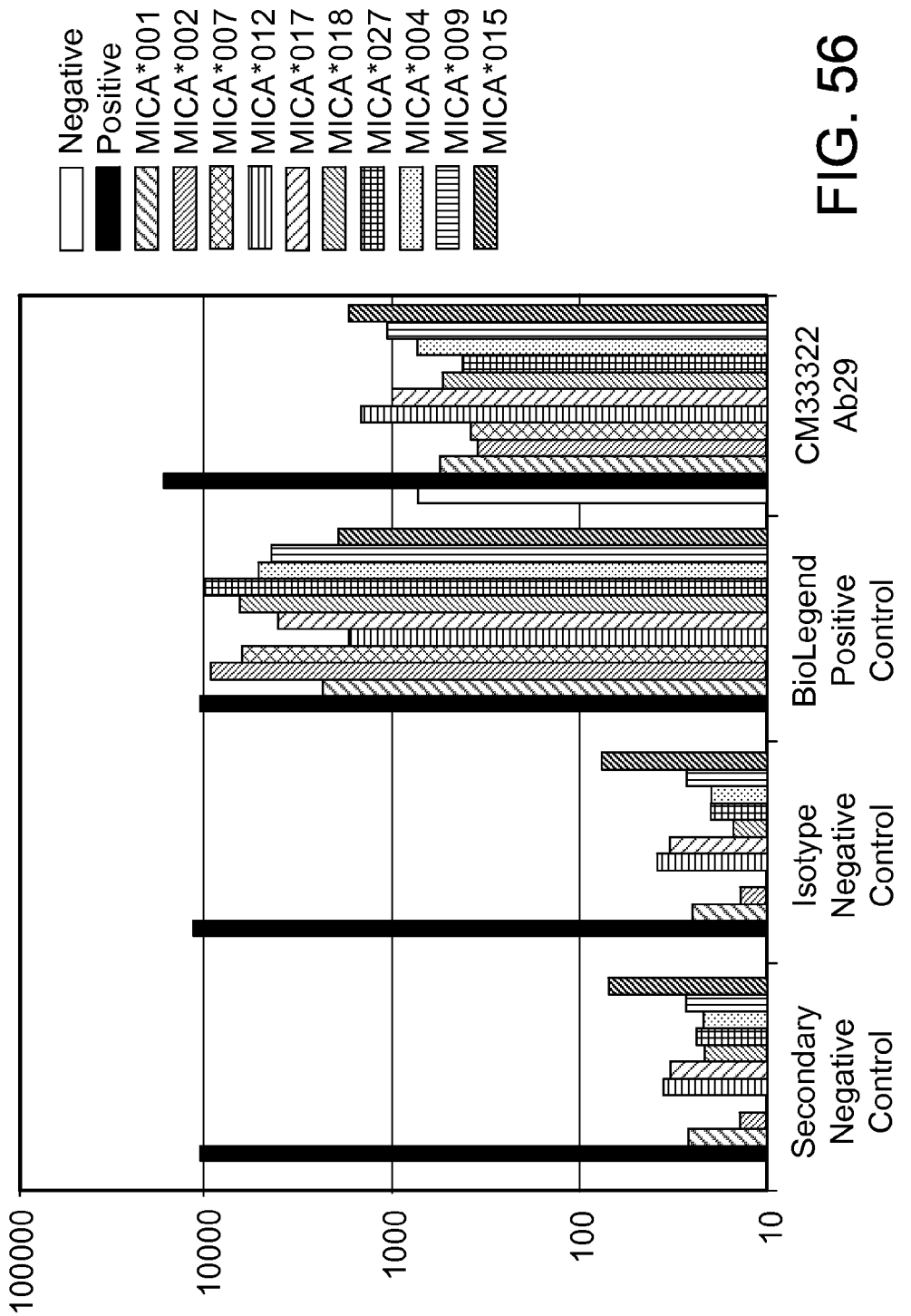

Negative
Positive
MICA*001
MICA*002
MICA*007
MICA*012
MICA*017
MICA*018
MICA*027
MICA*004
MICA*009
MICA*015
100000
10000
1000
100
10
Secondary Negative Control
Isotype Negative Control
BioLegend Positive Control
CM33322 Ab29

FIG. 56

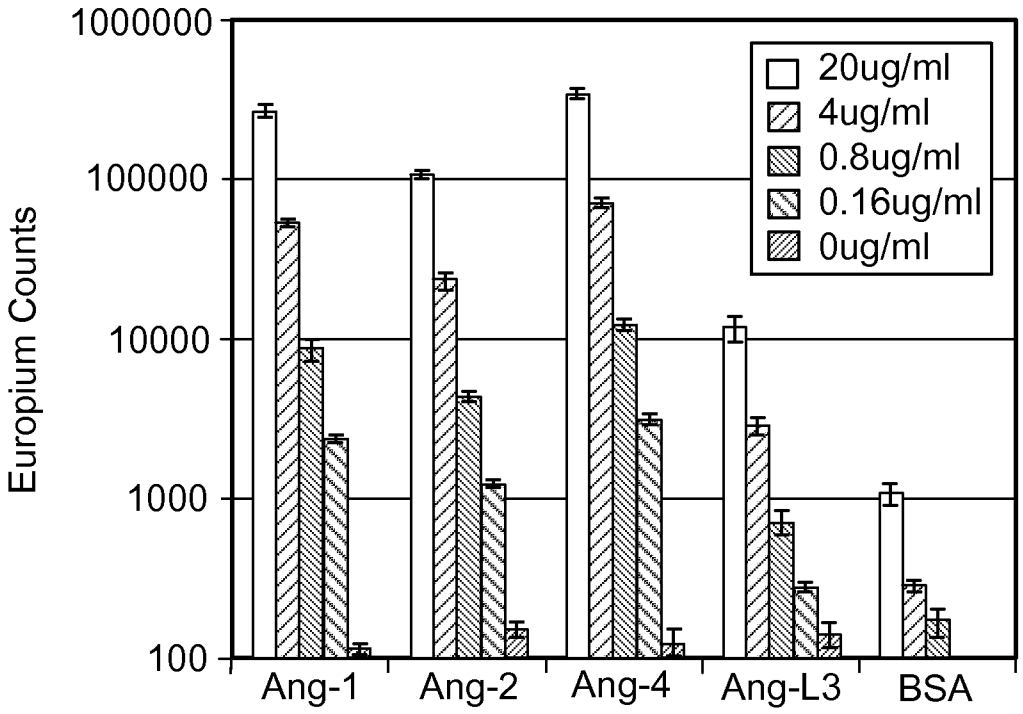

1000000
100000
10000
1000
100
Europium Counts
20ug/ml
4ug/ml
0.8ug/ml
0.16ug/ml
0ug/ml
Ang-1
Ang-2
Ang-4
Ang-L3
BSA

FIG. 57

SEQUENCE LISTING

<110> LEVICEPT LTD

<120> THERAPEUTIC USE OF P75NTR NEUROTROPHIN BINDING PROTEIN

<130> LEVI01102WO.el

<150> 60/610,682
<151> 2012-03-14

<160> 19

<170> PatentIn version 3.5

<210> 1
<211> 427
<212> PRT
<213> Homo sapiens

<400> 1

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1 5 10 15

Leu Leu Leu Leu Leu Gly Val Ser Leu Gly Gly Ala Lys Glu Ala Cys
20 25 30

Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala Cys Asn
35 40 45

Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn Gln Thr Val Cys
50 55 60

Glu Pro Cys Leu Asp Ser Val Thr Phe Ser Asp Val Val Ser Ala Thr
65 70 75 80

Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu Gln Ser Met Ser
85 90 95

Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala Tyr Gly
100 105 110

Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala Cys Arg Val Cys
115 120 125

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Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln Asn Thr
    130                 135                 140

Val Cys Glu Glu Cys Pro Asp Gly Thr Tyr Ser Asp Glu Ala Asn His
145                 150                 155                 160

Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu Arg Gln
                165                 170                 175

Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro
            180                 185                 190

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

Ala Pro Ser Thr Gln Glu Pro Glu Ala Pro Pro Glu Gln Asp Leu Ile
    210                 215                 220

Ala Ser Thr Val Ala Gly Val Val Thr Thr Val Met Gly Ser Ser Gln
225                 230                 235                 240

Pro Val Val Thr Arg Gly Thr Thr Asp Asn Leu Ile Pro Val Tyr Cys
                245                 250                 255

Ser Ile Leu Ala Ala Val Val Val Gly Leu Val Ala Tyr Ile Ala Phe
            260                 265                 270

Lys Arg Trp Asn Ser Cys Lys Gln Asn Lys Gln Gly Ala Asn Ser Arg
        275                 280                 285

Pro Val Asn Gln Thr Pro Pro Pro Glu Gly Glu Lys Leu His Ser Asp
    290                 295                 300

Ser Gly Ile Ser Val Asp Ser Gln Ser Leu His Asp Gln Gln Pro His
305                 310                 315                 320

Thr Gln Thr Ala Ser Gly Gln Ala Leu Lys Gly Asp Gly Gly Leu Tyr
                325                 330                 335
```

```
Ser Ser Leu Pro Pro Ala Lys Arg Glu Glu Val Glu Lys Leu Leu Asn
            340                 345                 350

Gly Ser Ala Gly Asp Thr Trp Arg His Leu Ala Gly Glu Leu Gly Tyr
        355                 360                 365

Gln Pro Glu His Ile Asp Ser Phe Thr His Glu Ala Cys Pro Val Arg
    370                 375                 380

Ala Leu Leu Ala Ser Trp Ala Thr Gln Asp Ser Ala Thr Leu Asp Ala
385                 390                 395                 400

Leu Leu Ala Ala Leu Arg Arg Ile Gln Arg Ala Asp Leu Val Glu Ser
                405                 410                 415

Leu Cys Ser Glu Ser Thr Ala Thr Ser Pro Val
            420                 425
```

<210> 2
<211> 250
<212> PRT
<213> Homo sapiens

<400> 2

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Met Gly Ala Gly Ala Thr Gly Arg Ala Met Asp Gly Pro Arg Leu Leu
1               5                   10                  15

Leu Leu Leu Leu Leu Gly Val Ser Leu Gly Gly Ala Lys Glu Ala Cys
            20                  25                  30

Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala Cys Asn
        35                  40                  45

Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn Gln Thr Val Cys
    50                  55                  60

Glu Pro Cys Leu Asp Ser Val Thr Phe Ser Asp Val Val Ser Ala Thr
65                  70                  75                  80

Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu Gln Ser Met Ser
                85                  90                  95
```

```
Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala Tyr Gly
            100                 105                 110

Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala Cys Arg Val Cys
        115                 120                 125

Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln Asn Thr
    130                 135                 140

Val Cys Glu Glu Cys Pro Asp Gly Thr Tyr Ser Asp Glu Ala Asn His
145                 150                 155                 160

Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu Arg Gln
                165                 170                 175

Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro
            180                 185                 190

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

Ala Pro Ser Thr Gln Glu Pro Glu Ala Pro Pro Glu Gln Asp Leu Ile
    210                 215                 220

Ala Ser Thr Val Ala Gly Val Val Thr Thr Val Met Gly Ser Ser Gln
225                 230                 235                 240

Pro Val Val Thr Arg Gly Thr Thr Asp Asn
                245                 250
```

<210> 3
<211> 221
<212> PRT
<213> Homo sapiens

<400> 3

```
Lys Glu Ala Cys Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys
1               5                   10                  15
```

```
Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn
            20                  25                  30

Gln Thr Val Cys Glu Pro Cys Leu Asp Ser Val Thr Phe Ser Asp Val
        35                  40                  45

Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu
    50                  55                  60

Gln Ser Met Ser Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg
65                  70                  75                  80

Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala
                85                  90                  95

Cys Arg Val Cys Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp
            100                 105                 110

Lys Gln Asn Thr Val Cys Glu Glu Cys Pro Gly Thr Tyr Ser Asp Glu
        115                 120                 125

Ala Asn His Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr
    130                 135                 140

Glu Arg Gln Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu
145                 150                 155                 160

Glu Ile Pro Gly Arg Trp Ile Thr Arg Ser Thr Pro Pro Glu Gly Ser
                165                 170                 175

Asp Ser Thr Ala Pro Ser Thr Gln Glu Pro Glu Ala Pro Pro Glu Gln
            180                 185                 190

Asp Leu Ile Ala Ser Thr Val Ala Gly Val Val Thr Thr Val Met Gly
        195                 200                 205

Ser Ser Gln Pro Val Val Thr Arg Gly Thr Thr Asp Asn
    210                 215                 220
```

<210> 4

<211> 13
<212> PRT
<213> Homo sapiens

<400> 4

Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Thr Thr Gly Arg
1 5 10

<210> 5
<211> 49
<212> PRT
<213> Homo sapiens

<400> 5

Val Cys Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln
1 5 10 15

Asn Thr Val Cys Glu Glu Cys Pro Gly Gly Thr Tyr Ser Asp Glu Ala
20 25 30

Asn His Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu
35 40 45

Arg

<210> 6
<211> 15
<212> PRT
<213> Homo sapiens

<400> 6

Val Cys Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys
1 5 10 15

<210> 7
<211> 12
<212> PRT
<213> Homo sapiens

<400> 7

Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro Gly Arg
1 5 10

<210> 8
<211> 17
<212> PRT
<213> Homo sapiens

<400> 8

Leu Asp Ser Val Thr Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys
1 5 10 15

Pro

<210> 9
<211> 698
<212> PRT
<213> Homo sapiens

<400> 9

Met Arg Leu Ala Val Gly Ala Leu Leu Val Cys Ala Val Leu Gly Leu
1 5 10 15

Cys Leu Ala Val Pro Asp Lys Thr Val Arg Trp Cys Ala Val Ser Glu
20 25 30

His Glu Ala Thr Lys Cys Gln Ser Phe Arg Asp His Met Lys Ser Val
35 40 45

Ile Pro Ser Asp Gly Pro Ser Val Ala Cys Val Lys Lys Ala Ser Tyr
50 55 60

Leu Asp Cys Ile Arg Ala Ile Ala Ala Asn Glu Ala Asp Ala Val Thr
65 70 75 80

Leu Asp Ala Gly Leu Val Tyr Asp Ala Tyr Leu Ala Pro Asn Asn Leu
85 90 95

Lys Pro Val Val Ala Glu Phe Tyr Gly Ser Lys Glu Asp Pro Gln Thr
100 105 110

Phe Tyr Tyr Ala Val Ala Val Val Lys Lys Asp Ser Gly Phe Gln Met

```
        115                 120                 125

Asn Gln Leu Arg Gly Lys Lys Ser Cys His Thr Gly Leu Gly Arg Ser
    130                 135                 140

Ala Gly Trp Asn Ile Pro Ile Gly Leu Leu Tyr Cys Asp Leu Pro Glu
145                 150                 155                 160

Pro Arg Lys Pro Leu Glu Lys Ala Val Ala Asn Phe Phe Ser Gly Ser
                165                 170                 175

Cys Ala Pro Cys Ala Asp Gly Thr Asp Phe Pro Gln Leu Cys Gln Leu
            180                 185                 190

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

Gly Ala Phe Lys Cys Leu Lys Asp Gly Ala Gly Asp Val Ala Phe Val
    210                 215                 220

Lys His Ser Thr Ile Phe Glu Asn Leu Ala Asn Lys Ala Asp Arg Asp
225                 230                 235                 240

Gln Tyr Glu Leu Leu Cys Leu Asp Asn Thr Arg Lys Pro Val Asp Glu
                245                 250                 255

Tyr Lys Asp Cys His Leu Ala Gln Val Pro Ser His Thr Val Val Ala
            260                 265                 270

Arg Ser Met Gly Gly Lys Glu Asp Leu Ile Trp Glu Leu Leu Asn Gln
        275                 280                 285

Ala Gln Glu His Phe Gly Lys Asp Lys Ser Lys Glu Phe Gln Leu Phe
    290                 295                 300

Ser Ser Pro His Gly Lys Asp Leu Leu Phe Lys Asp Ser Ala His Gly
305                 310                 315                 320

Phe Leu Lys Val Pro Pro Arg Met Asp Ala Lys Met Tyr Leu Gly Tyr
                325                 330                 335
```

```
Glu Tyr Val Thr Ala Ile Arg Asn Leu Arg Glu Gly Thr Cys Pro Glu
            340                 345                 350

Ala Pro Thr Asp Glu Cys Lys Pro Val Lys Trp Cys Ala Leu Ser His
        355                 360                 365

His Glu Arg Leu Lys Cys Asp Glu Trp Ser Val Asn Ser Val Gly Lys
    370                 375                 380

Ile Glu Cys Val Ser Ala Glu Thr Thr Glu Asp Cys Ile Ala Lys Ile
385                 390                 395                 400

Met Asn Gly Glu Ala Asp Ala Met Ser Leu Asp Gly Gly Phe Val Tyr
                405                 410                 415

Ile Ala Gly Lys Cys Gly Leu Val Pro Val Leu Ala Glu Asn Tyr Asn
            420                 425                 430

Lys Ser Asp Asn Cys Glu Asp Thr Pro Glu Ala Gly Tyr Phe Ala Val
        435                 440                 445

Ala Val Val Lys Lys Ser Ala Ser Asp Leu Thr Trp Asp Asn Leu Lys
    450                 455                 460

Gly Lys Lys Ser Cys His Thr Ala Val Gly Arg Thr Ala Gly Trp Asn
465                 470                 475                 480

Ile Pro Met Gly Leu Leu Tyr Asn Lys Ile Asn His Cys Arg Phe Asp
                485                 490                 495

Glu Phe Phe Ser Glu Gly Cys Ala Pro Gly Ser Lys Lys Asp Ser Ser
            500                 505                 510

Leu Cys Lys Leu Cys Met Gly Ser Gly Leu Asn Leu Cys Glu Pro Asn
        515                 520                 525

Asn Lys Glu Gly Tyr Tyr Gly Tyr Thr Gly Ala Phe Arg Cys Leu Val
    530                 535                 540
```

```
Glu Lys Gly Asp Val Ala Phe Val Lys His Gln Thr Val Pro Gln Asn
545                 550                 555                 560

Thr Gly Gly Lys Asn Pro Asp Pro Trp Ala Lys Asn Leu Asn Glu Lys
                565                 570                 575

Asp Tyr Glu Leu Leu Cys Leu Asp Gly Thr Arg Lys Pro Val Glu Glu
            580                 585                 590

Tyr Ala Asn Cys His Leu Ala Arg Ala Pro Asn His Ala Val Val Thr
        595                 600                 605

Arg Lys Asp Lys Glu Ala Cys Val His Lys Ile Leu Arg Gln Gln Gln
    610                 615                 620

His Leu Phe Gly Ser Asn Val Thr Asp Cys Ser Gly Asn Phe Cys Leu
625                 630                 635                 640

Phe Arg Ser Glu Thr Lys Asp Leu Leu Phe Arg Asp Asp Thr Val Cys
                645                 650                 655

Leu Ala Lys Leu His Asp Arg Asn Thr Tyr Glu Lys Tyr Leu Gly Glu
            660                 665                 670

Glu Tyr Val Lys Ala Val Gly Asn Leu Arg Lys Cys Ser Thr Ser Ser
        675                 680                 685

Leu Leu Glu Ala Cys Thr Phe Arg Arg Pro
    690                 695
```

<210> 10
<211> 609
<212> PRT
<213> Homo sapiens

<400> 10

```
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1               5                   10                  15

Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
```

20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

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

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

```
Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    370                 375                 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385                 390                 395                 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
                405                 410                 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
            420                 425                 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
        435                 440                 445
```

```
Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
    450                 455                 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465                 470                 475                 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
                485                 490                 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
            500                 505                 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
        515                 520                 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
    530                 535                 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545                 550                 555                 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
                565                 570                 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
            580                 585                 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
        595                 600                 605

Leu
```

<210> 11
<211> 212
<212> PRT
<213> Homo sapiens

<400> 11

```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
```

```
1               5                   10                  15

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            20                  25                  30

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        35                  40                  45

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asp Ser Thr
    50                  55                  60

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
65                  70                  75                  80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                85                  90                  95

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            100                 105                 110

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
        115                 120                 125

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    130                 135                 140

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
145                 150                 155                 160

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                165                 170                 175

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            180                 185                 190

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        195                 200                 205

Ser Pro Gly Lys
    210
```

<210> 12
<211> 326
<212> PRT
<213> Homo sapiens

<400> 12

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15


Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45


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


Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80


Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95


Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110


Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125


Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140


Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160


Met Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
                165                 170                 175
```

```
Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
            180                 185                 190


Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
        195                 200                 205


Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
    210                 215                 220


Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225                 230                 235                 240


Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255


Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            260                 265                 270


Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285


Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300


Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320


Ser Leu Ser Pro Gly Lys
                325


<210>  13
<211>  165
<212>  PRT
<213>  Homo sapiens

<400>  13

Cys Tyr Thr Leu Leu Leu Leu Thr Thr Pro Ser Trp Val Leu Ser Gln
1               5                   10                  15


Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu Thr
            20                  25                  30
```

```
Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Asn Ala Lys
        35                  40                  45

Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp
    50                  55                  60

Leu Ala His Ile Phe Ser Asn Asp Glu Lys Ser Tyr Ser Thr Ser Leu
65                  70                  75                  80

Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val Val
                85                  90                  95

Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys
            100                 105                 110

Ala Arg Ile Phe Thr Ile Thr Tyr Ser Asn Tyr Val Leu Gln Tyr Tyr
        115                 120                 125

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser
    130                 135                 140

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

Ser Thr Ser Gly Gly
                165
```

<210> 14
<211> 217
<212> PRT
<213> Homo sapiens

<400> 14

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

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30
```

```
Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
        35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60

Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                85                  90                  95

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            100                 105                 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val
            180                 185                 190

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        195                 200                 205

Lys Ser Leu Ser Leu Ser Leu Gly Lys
    210                 215
```

<210> 15
<211> 209
<212> PRT
<213> Homo sapiens

<400> 15

```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
1               5                  10                  15

Tyr Ile Thr Arg Glu Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            20                  25                  30

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        35                  40                  45

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    50                  55                  60

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
65                  70                  75                  80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                85                  90                  95

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            100                 105                 110

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
        115                 120                 125

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    130                 135                 140

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
145                 150                 155                 160

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                165                 170                 175

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            180                 185                 190

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        195                 200                 205
```

Ser

<210> 16
<211> 225
<212> PRT
<213> Homo sapiens

<400> 16

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro
1 5 10 15

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
20 25 30

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
35 40 45

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

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

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
85 90 95

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

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

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

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

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu

165 170 175

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

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
195 200 205

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
210 215 220

Lys
225

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

<220>
<223> Linker sequence

<220>
<221> MISC_FEATURE
<222> (1)..(5)
<223> (GGGGS)n (n = 3 to 4)

<400> 17

Gly Gly Gly Gly Ser
1 5

<210> 18
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Linker sequence

<220>
<221> MISC_FEATURE
<222> (1)..(5)
<223> (EAAAK)n (n = 2 to 5)

<400> 18

Glu Ala Ala Ala Lys
1 5

<210> 19
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Linker sequence

<400> 19

Gly Gly Gly Gly Ser
1 5