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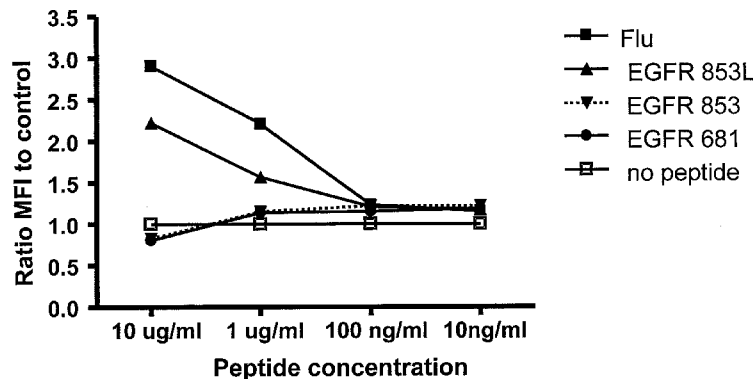
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(54) Title: CYTOTOXIC T CELL DEFINED EGFR PEPTIDE AND AN OPTIMIZED DERIVATIVE PEPTIDE

FIG. 1



(57) Abstract: The invention provides a polypeptide having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1), as well as a nucleic acid encoding the polypeptide, vector comprising the nucleic acid, cell comprising the vector, and compositions thereof. The invention also provides a method of inducing a T-cell response in a patient with epithelial cancer, and a method inhibiting epithelial cancer, wherein the methods comprise administering the composition of the invention.

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CYTOTOXIC T CELL DEFINED EGFR PEPTIDE AND AN OPTIMIZED DERIVATIVE  
PEPTIDE

## SEQUENCE LISTING

[0001] Incorporated by reference in its entirety herein is a sequence listing submitted concurrently herewith.

## BACKGROUND OF THE INVENTION

[0002] The epidermal growth factor receptor (EGFR) is an attractive target for cancer therapy because it is highly overexpressed and correlated with poor prognosis in epithelial malignancies, including head and neck squamous cell carcinoma (HNSCC). Although clinical responses have been achieved using EGFR inhibition through blocking antibodies or EGFR tyrosine-kinase inhibitors, many individuals do not respond to these treatments. Thus, the desire exists for additional EGFR inhibition therapies.

## BRIEF SUMMARY OF THE INVENTION

[0003] The invention provides a polypeptide having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1), as well as a nucleic acid encoding the polypeptide, vector comprising the nucleic acid, cell comprising the vector, and compositions thereof.

[0004] The invention also provides a method of inducing a T-cell response in a patient with epithelial cancer, and a method of inhibiting epithelial cancer. The methods comprise administering the above-described composition.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0005] Fig. 1 depicts the results of an HLA-A\*0201 stabilization assay by EGFR peptides and flu. The ratio of mean fluorescence intensity (MFI) relative to control is on the y axis and peptide concentration is on the x-axis. The graph represents results for (i) Flu<sub>58-66</sub> (Flu), (ii) EGFR<sub>853-861</sub>, corresponding to SEQ ID NO: 1, wherein the second position is a leucine (EGFR 853L), (iii) EGFR<sub>853-861</sub>, corresponding to SEQ ID NO: 1, wherein the second position is a threonine (EGFR 853), (iv) EGFR<sub>681-689</sub> (EGFR 681), and (v) no peptide.

[0006] Figs. 2A, 2B, 3, and 4 depict the results of ELISPOT assays. The targets are indicated on the x-axis and the number of interferon- $\gamma$  (IFN- $\gamma$ ) spots/ $5.0 \times 10^4$  CD8<sup>+</sup> T cells is indicated on the y-axis for SCC4 (Fig. 2A), PCI-30 (Fig. 2B and Fig. 4), and PCI-13 (Fig. 3) cancer cells.

[0007] Fig. 5 depicts the results of an ELISPOT assay of CD8<sup>+</sup> T cells from a healthy HLA:A0201 donor following three *in vitro* stimulations with the peptide EGFR<sub>853</sub> and challenge with T2 cells, T2 cells plus peptide (titrated), or T2 cells plus negative control peptide (HIV 476-484). The challenge material is indicated on the x-axis and the number of IFN- $\gamma$  spots/5.0 x 10<sup>4</sup> CD8<sup>+</sup> T cells is indicated on the y-axis.

[0008] Figs. 6A and 6B depict the results of ELISPOT assays. The targets are indicated on the x-axis and the number of IFN- $\gamma$  spots/5.0 x 10<sup>4</sup> CD8<sup>+</sup> T cells is indicated on the y-axis. The HLA-A, -B, and -C specific monoclonal antibody (mAb) W6/32, HLA-A specific mAb LGIII, and the HLA-DR specific mAb L.243 were used to determine class I restricted activity and specificity with wild-type peptide EGFR<sub>853</sub> (Fig. 6A) and the optimized peptide EGFR<sub>853L</sub> (Fig. 6B).

[0009] Fig. 7 depicts the results of an ELISPOT assay of CD8<sup>+</sup> T cells against different concentrations of wild-type peptide (EGFR<sub>853</sub>) and optimized peptide (EGFR<sub>853L</sub>) loaded with T2 cells. The concentrations of peptides is indicated on the x-axis and the number of IFN- $\gamma$  spots/5.0 x 10<sup>4</sup> CD8<sup>+</sup> T cells is indicated on the y-axis.

[0010] Figs. 8A-8D depict the HLA-A2 peptide EGFR<sub>853</sub> and EGFR<sub>853L</sub> tetramer-sorted population of healthy donor stained after *in vitro* expansion.

[0011] Fig. 9A depicts the results of ELISPOT assays. EGFR<sub>853</sub> peptide-specific CD8<sup>+</sup> T cells recognize three Head and Neck cell lines (SCC-4, PCI-30, and PCI-13), which recognition was blocked by anti-HLA Class I mAb (Fig. 9A). The targets are indicated on the x-axis and the number of IFN- $\gamma$  spots/5.0 x 10<sup>4</sup> CD8<sup>+</sup> T cells is indicated on the y-axis. Fig. 9B depicts the EGFR expression of the three Head and Neck cell lines (SCC-4, PCI-30, and PCI-13) using flow-cytometry with cetuximab.

[0012] Fig. 10 depicts the results of an ELISPOT assay. EGFR<sub>853</sub> peptide-specific CD8<sup>+</sup> T cells recognize PCI-30, which recognition was (i) blocked by anti-HLA Class I mAb but not by anti-HLA Class II mAb and (ii) increased after treatment with cetuximab. The targets are indicated on the x-axis and the number of IFN- $\gamma$  spots/5.0 x 10<sup>4</sup> CD8<sup>+</sup> T cells is indicated on the y-axis.

[0013] Fig. 11 depicts the results of an ELISPOT assay of CD8<sup>+</sup> T cells from a healthy HLA:A0201 donor following three *in vitro* stimulations with the peptide EGFR<sub>853</sub> or EGFR<sub>853L</sub>. The targets are indicated on the x-axis and the number of IFN- $\gamma$  spots/5.0 x 10<sup>4</sup> CD8<sup>+</sup> T cells is indicated on the y-axis.

## DETAILED DESCRIPTION OF THE INVENTION

**[0014]** The invention provides a polypeptide having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1), wherein the X at position 2 can be leucine or threonine. IXDFGLAKL (SEQ ID NO: 1) corresponds to positions 853-861 of the epidermal growth factor receptor (EGFR) (see, e.g., GenBank Accession No. AY588246; and SEQ ID NO: 2). In one embodiment, the polypeptide is isolated or substantially purified.

**[0015]** The polypeptide having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1) is an immunogenic peptide with an HLA-A2 binding motif. In one embodiment, the polypeptide has a leucine at position 2 of SEQ ID NO: 1, which enhances HLA-A2 binding stimulation of anti-EGFR T cells. The invention also encompasses variants of SEQ ID NO: 1, which contain one, two, or three modifications (e.g., deletions, substitutions, or additions) of SEQ ID NO: 1. The variants of SEQ ID NO: 1 maintain the activity of the polypeptide of SEQ ID NO: 1, or have an increased activity, antigenicity, stability, or solubility.

**[0016]** In some applications, the polypeptide can elicit a cellular immune response against (i) an EGFR family member protein (e.g., EGFR) or a portion thereof, (ii) cells expressing an EGFR family member protein or portion thereof, and/or (iii) cells that bind an EGFR family member protein or a portion thereof. The EGFR (ErbB) family consists of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). For example, in some applications, the polypeptide can generate cytotoxic T lymphocytes that inhibit or lyse (i) target cells that express or have bound thereto an EGFR family member protein (e.g., EGFR) or a portion thereof or (ii) target cells that express or have bound thereto one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) of the polypeptide of the invention.

**[0017]** The invention also provides a fusion polypeptide comprising one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) polypeptides having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1), which, optionally, can be joined by an amino acid linker sequence. Amino acid linker sequences are known in the art and can comprise about one to about ten (e.g., two, three, four, five, six, seven, eight, or nine) amino acids. The fusion polypeptide also can comprise other helper peptides or carrier molecules to enhance the immunogenicity of the polypeptide. Such molecules include, but are not limited to, an influenza peptide, tetanus toxoid, tetanus toxoid CD4 epitope, *Pseudomonas* exotoxin A, and poly-L-lysine.

[0018] The polypeptides of the invention can be obtained by any suitable methods, including, but not limited to, recombinant nucleic acid technology, chemical peptide synthesis, and protease cleavage of isolated EGFR.

[0019] The invention also provides an isolated nucleic acid comprising a sequence that encodes a polypeptide of the invention. The nucleic acid can be single or double stranded and can include DNA or RNA molecules (e.g., DNA, DNA/DNA, RNA, or RNA/DNA). The nucleic acid encompasses variants containing one or more (e.g., two, three, four, or five) substitutions, deletions, or additions that maintain or increase the ability of the polypeptide to elicit a cellular immune response against EGFR family member protein or portion thereof or against cells expressing or binding an EGFR family member protein or portion thereof. The nucleic acid can be derived from a variety of sources including genomic DNA, cDNA, synthetic DNA, synthetic RNA, or combinations thereof. In one embodiment, the nucleic acid is isolated or substantially purified.

[0020] Isolated refers to material (e.g., a polypeptide or nucleic acid) that is largely free from components which normally accompany or interact with it as found in its naturally occurring environment, or if in its natural environment, the material has been non-naturally altered to a composition. In the case of a nucleic acid, an isolated nucleic acid refers to a nucleic acid placed at a locus in the cell not native to a material found in that environment. The isolated material optionally comprises material not found with the material in its natural environment. For example, a naturally occurring nucleic acid becomes an isolated nucleic acid if it is altered, or if it is transcribed from DNA which is altered, by non-natural, synthetic methods performed within the cell from which it originates.

[0021] Similarly, substantially purified refers to material (e.g., a polypeptide or nucleic acid) that is considerably (e.g., about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99%) free from contaminants or materials that accompany or interact with it as found in its naturally occurring environment.

[0022] The nucleic acid can be in the form of a vector. Any suitable vector can be used, including, but not limited to, bacterial vectors, viral vectors, and nucleic acid-based vectors. Viral vectors include, but are not limited to, poxvirus, Herpes virus, lentivirus, adeno-associated virus, alphavirus, retrovirus, picornavirus, and iridovirus.

[0023] Preferably, the vector is a poxvirus. Examples of poxviruses include replicating and non-replicating vectors, such as orthopox (e.g., vaccinia, raccoon pox, and rabbit pox),

avipox (e.g., canarypox, pigeonpox, and fowlpox), suipox (e.g., swinepox), and capripox (e.g., goatpox and sheeppox). In particular, poxviruses can be selected from the group consisting of vaccinia-Copenhagen, vaccinia-Wyeth strain, vaccinia-MVA strain, NYVAC, fowlpox, TROVAC, canarypox, ALVAC, and swinepox. Parental poxviruses useful in constructing the recombinant poxvirus include, but are not limited to, orthopox virus such as replicating vaccinia virus (see, e.g., Perkus et al., *Science*, 229: 981-984 (1985); Kaufman et al., *Int. J. Cancer*, 48: 900-907 (1991); and Moss, *Science*, 252: 1662 (1991)), highly attenuated vaccinia viruses such as MVA, modified vaccinia Ankara (see, e.g., Sutter et al., *Proc. Nat'l Acad. Sci. U.S.A.*, 89: 10847-10851 (1992)), vaccinia-Copenhagen and NYVAC, avipoxviruses, such as fowlpox virus, canarypox virus, such as ALVAC (see, e.g., Baxby et al., *Vaccine*, 10: 8-9 (1992); and Paoletti, *Proc. Nat'l Acad. Sci. USA*, 93: 11349-11353 (1996)), and suipoxvirus, and capripoxvirus.

**[0024]** In one embodiment, the poxvirus is a vaccinia virus, such as a Wyeth strain or derivative thereof. A derivative of the Wyeth strain includes, but is not limited to, vTBC33. In yet another embodiment, the virus is Dry-Vax, which is available as a smallpox vaccine from the Centers for Disease Control, Atlanta, Ga. In another embodiment, the poxvirus is a strain of fowlpox, such as POXVAC-TC (Schering-Plough Corporation).

**[0025]** The vector comprises at least one expression control element operably linked to the nucleic acid sequence. The expression control elements are inserted in the vector to control and regulate the expression of the nucleic acid sequence (see, e.g., Ausubel et al, 1987, in Current Protocols in Molecular Biology, John Wiley and Sons, New York, N.Y.). Expression control elements are known in the art (e.g., promoters). Promoters useful in the vector include, but are not limited to, poxviral promoters, such as 30K, I3, sE/L, 7.5K, 40K, C1, and those described in the art (see, e.g., Davison et al., *J. Mol. Biol.*, 210: 749 769, (1989)).

**[0026]** When the vector is a nucleic acid-based vector, the constructs can be DNA or RNA or associated with/or encapsulated in a lipid carrier (e.g., a liposome). Liposomes can serve to target a nucleic acid or polypeptide to a particular tissue. Liposomes also can be used to increase the half-life of a nucleic acid or polypeptide. Many methods are available for preparing liposomes, as described in, for example, Szoka et al., *Ann. Rev. Biophys. Bioeng.*, 9, 467 (1980) and U.S. Patents 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

**[0027]** Naked DNA vectors can be prepared by methods described in the art (see, e.g., U.S. Patent 5,827,703). The transcriptional initiation region, or promoter element, can be

native to or homologous to the cell and/or to the DNA to be transcribed, or foreign or heterologous to the cell and/or the DNA sequence to be transcribed. Efficient promoter elements for transcription initiation of naked DNA include, but are not limited to, the SV40 early promoter, RSV promoter, adenovirus major late promoter, and CMV immediate early I promoter.

**[0028]** The vector is able to infect, transfect, or transduce a cell, which can be *in vitro* or within in a subject. The subject includes, but is not limited to, mammals (e.g., mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, pigs, cows, horses, and primates, such as humans), birds, and fish. The cell can be any cell amenable to infection, transfection, or transduction by the vector and capable of expressing the foreign genes from the vector. Desirably, the cell includes, but is not limited to, professional antigen presenting cells (APCs) and antigen presenting precursor cells, such as monocytes, macrophages, dendritic cells (DCs), and Langerhans; lymphocytes (e.g., T cells, B cells, natural killer(NK) cells; a heat inactivated recombinant yeast cell; and an artificial antigen presenting construct.

**[0029]** The vector also desirably can infect, transfect, or transduce tumor cells or other cell types, such as fibroblasts or muscle cells. The cells express, or are engineered to express, the appropriate MHC (HLA) Class I or II molecules for appropriate antigenic presentation to CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells. It will be understood that the invention provides a cell comprising a nucleic acid of the present invention.

**[0030]** A polypeptide, nucleic acid, vector, or cell of the invention can be administered alone or in a composition (e.g., pharmaceutically acceptable composition). The composition of the invention comprises a carrier (e.g., a pharmaceutically acceptable carrier), such as those known in the art. The pharmaceutically acceptable carrier (or excipient) is preferably one that is chemically inert to the polypeptide, nucleic acid, vector, or cell and one that has no detrimental side effects or toxicity under the conditions of use. Such pharmaceutically acceptable carriers include, but are not limited to, water, saline, Cremophor EL (Sigma Chemical Co., St. Louis, MO), propylene glycol, polyethylene glycol, alcohol, and combinations thereof. The choice of carrier will be determined in part by the polypeptide, nucleic acid, vector, or cell, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the composition.

**[0031]** Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or

orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent, or emulsifying agent. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and cornstarch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

**[0032]** A polypeptide, nucleic acid, vector, or cell of the invention, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer.

**[0033]** Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. A polypeptide, nucleic acid, vector, or cell of the invention can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as

poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0034] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0035] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylene-polypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-beta-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (3) mixtures thereof.

[0036] Suitable preservatives and buffers can be used in such formulations. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5% to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[0037] A polypeptide, nucleic acid, vector, or cell of the invention can be administered as an injectable formulation. The requirements for effective pharmaceutical carriers for injectable compositions are well known to those of ordinary skill in the art. See

*Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Co., Philadelphia, Pa., Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986).

[0038] Topical formulations, including those that are useful for transdermal drug release, are well known to those of skill in the art and are suitable in the context of the invention for application to skin.

[0039] A polypeptide, nucleic acid, vector, or cell of the invention can be administered as a suppository by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0040] The concentration of a polypeptide, nucleic acid, vector, or cell of the invention in the pharmaceutical formulations can vary, e.g., from less than about 1%, usually at or at least about 10%, to as much as 20% to 50% or more by weight, and can be selected primarily by fluid volumes, and viscosities, in accordance with the particular mode of administration selected.

[0041] Methods for preparing administrable (e.g., parenterally administrable) polypeptides, nucleic acids, vectors, cells, and compositions thereof are known or apparent to those skilled in the art and are described in more detail in, for example, *Remington's Pharmaceutical Science* (17th ed., Mack Publishing Company, Easton, PA, 1985).

[0042] The composition can comprise an adjuvant. The adjuvant can be any suitable adjuvant, such as those described in the part. For example, the adjuvant can be selected from the group consisting of, but not limited to, interleukin (IL)-2, IL-6, IL-12, IL-15, IL-21, interferon, tumor necrosis factor, granulocyte macrophage colony stimulating factor (GM-CSF), incomplete Freund's adjuvant, alum, aluminum salts, aluminum phosphate, aluminum hydroxide, aluminum silica, Montanide, calcium phosphate, cyclophosphamide, and combinations thereof.

[0043] The composition can comprise a chemotherapeutic agent. The chemotherapeutic agent can be any suitable chemotherapeutic agent, such as those described in the art. For example, the chemotherapeutic agent can be selected from the group consisting of, but not limited to, alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumor agents.

[0044] Preferably, the chemotherapeutic agent is an epidermal growth factor receptor (EGFR) family specific antibody. The EGFR family specific antibody can be any suitable antibody that recognizes one or more of the EGFR family (e.g., an EGFR-specific antibody, such as cetuximab and panitumumab). The antibody can be a monoclonal, polyclonal, human, and/or humanized antibody or an antibody fragment, such as F(ab')<sub>2</sub>, Fab', Fv, and single chain Fv (scFv).

[0045] The composition can comprise a target antigen, which refers to an antigen or immunological epitope on the antigen that is crucial in immune recognition and ultimate elimination or control of the disease-causing agent or disease state in a mammal. The immune recognition can be cellular and/or humoral. In the case of intracellular pathogens and cancer, immune recognition is preferably a T lymphocyte response.

[0046] Target antigen includes an antigen associated with a preneoplastic or hyperplastic state. Such target antigen can be tumor specific antigen, tumor associated antigen (TAA), or tissue specific antigen, epitope thereof, and epitope agonist thereof. Examples of target antigens include, but are not limited to, carcinoembryonic antigen (CEA) and epitopes thereof such as CAP-1 and CAP-1-6D (see, e.g., GenBank Accession No. M29540), MART-1 (see, e.g., Kawakami et al., *J. Exp. Med.*, 180: 347-352 (1994)), MAGE-1 (see, e.g., U.S. Patent 5,750,395), MAGE-3, GAGE (see, e.g., U.S. Patent 5,648,226), GP-100 (see, e.g., Kawakami et al., *Proc. Nat'l Acad. Sci. USA*, 91:6458-6462 (1992)), MUC-1, MUC-2, point mutated ras oncogene, normal and point mutated p53 oncogenes (see, e.g., Hollstein et al., *Nucleic Acids Res.*, 22: 3551-3555 (1994)), PSMA (see, e.g., Israeli et al., *Cancer Res.*, 53: 227-230 (1993)), tyrosinase (see, e.g., Kwon et al., *Proc. Nat'l Acad. Sci. USA*, 84: 7473-7477 (1987)), TRP-1 (gp75) (see, e.g., Cohen et al., *Nucleic Acid Res.*, 18: 2807-2808 (1990) and U.S. Patent 5,840,839), NY-ESO-1 (see, e.g., Chen et al., *Proc. Nat'l Acad. Sci. USA*, 94: 1914-1918 (1997)), TRP-2 (see, e.g., Jackson et al., *EMBO J*, 11:527-535 (1992)), TAG72, KSA, CA-125, PSA, HER-2/neu/c-erb/B2, BRC-I, BRC-II, bcr-abl, pax3-fkhr, ewe-fli-1, HAGE, modifications of TAAs and tissue specific antigens, splice variants of TAAs, and epitope agonists. Preferably, the composition comprises one or more TAAs selected from the group consisting of MAGE (e.g., MAGE-1 or MAGE-3), p53, Erb2, MUC-1, HAGE, and human papilloma virus.

[0047] The polypeptide, nucleic acid, vector, cell, and compositions thereof can be administered using any suitable method including, but not limited to, oral, aerosol, parenteral

(e.g., subcutaneous, intravenous, intraarterial, intramuscular, intradermal, interperitoneal, and intrathecal), rectal, and vaginal administration.

[0048] The amount (e.g., therapeutically effective amount) of a polypeptide, nucleic acid, vector, cell, and compositions thereof of the invention to be administered depends on the compound used and the particular route of administration. For example, the polypeptide or composition thereof can be administered in a dose of about 0.5 mg to about 100 mg (e.g., 1 mg, 2 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, or 90 mg) per kilogram body weight of the subject. Several doses can be provided over a period of weeks.

[0049] When the polypeptide, cell, vector, or cell is administered with one or more additional therapeutic agents (e.g., chemotherapeutic agents, adjuvants, or target antigens), the polypeptide, cell, vector, or cell and one or more additional therapeutic agents can be coadministered. By "coadministering" is meant administering one or more additional therapeutic agents and the polypeptide, cell, vector, or cell sufficiently close in time such that the polypeptide, cell, vector, or cell can enhance the effect of one or more additional therapeutic agents. In this regard, the polypeptide, cell, vector, or cell can be administered first and the one or more additional therapeutic agents can be administered second, or *vice versa*. Alternatively, the polypeptide, cell, vector, or cell and the one or more additional therapeutic agents can be administered simultaneously.

[0050] The vector or composition thereof (e.g., vaccine) can generate an immune response as a result of the administration of, for example, about  $10^5$  to about  $10^{10}$  (e.g.,  $10^6$ ,  $10^7$ ,  $10^8$ , or  $10^9$ ) pfu of the viral vector (e.g., poxvirus). The immune response can be boosted by administering additional antigen to the subject (e.g., in the form of a vector, polypeptide, or compositions thereof).

[0051] The ability of the polypeptide, nucleic acid, vector, and cell and compositions thereof to generate and EGFR-specific cellular immune response can be determined by *in vivo* or *in vitro* parameters known in the art, such as antigen specific cytotoxicity assays, regression of EGFR<sup>+</sup> tumors, inhibition of EGFR<sup>+</sup> cancer cells, and the production of cytokines.

[0052] The invention also provides a method of inducing a T-cell response in a patient with epithelial cancer, comprising administering the composition of the invention to the subject, such that a T-cell response against the epithelial cancer is induced. In particular, the invention provides a method of generating EGFR-specific cytotoxic T lymphocytes (both *in*

*vivo* and *in vitro*) by stimulation of lymphocytes with a polypeptide, nucleic acid, vector, or cell of the invention, alone or in combination with one or more adjuvants, such as cytokines (e.g., IL-2, IL-6, IL-12, RANTES, GM-CSF, TNF-alpha, or IFN-gamma) or costimulatory molecules (e.g., B7.1, B7.2, ICAM-1, LFA-3, or CD72). The lymphocytes can be obtained from, for example, peripheral blood lymphocytes, tumor infiltrating lymphocytes, and lymph nodes.

**[0053]** The invention also provides a method of inhibiting epithelial cancer comprising administering the composition to the subject, such that epithelial cancer is inhibited.

Inhibition of the cancer includes (i) a reduction (e.g., by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%) in the number of epithelial cancer cells, (ii) prevention of an increase in the number of epithelial cancer cells, (iii) an increase (e.g., by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%) in apoptosis of epithelial cancer cells, and/or (iv) a decrease or plateau in solid tumor size.

**[0054]** The epithelial cancer can be any suitable cancer, including, but not limited to head and neck squamous cell carcinoma (HNSCC), ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, gastric cancer, breast cancer, endometrial cancer, and colorectal (colon) cancer.

**[0055]** The polypeptide, nucleic acid, vector, and cell therapy can be used individually or in any combination. In addition, these treatments can be used as adjuncts to other treatments for epithelial cancer, such as hormone therapy, chemotherapy, surgery, cryosurgery, radiation therapy, and immunotherapy (e.g., anti-HER2 antibodies for breast cancer).

**[0056]** The invention also includes the administration of the polypeptide, cell, vector, cell, or composition thereof followed by a second administration (which may be the same or different) of the polypeptide, cell, vector, cell, or composition thereof as a "boost." The second administration can occur at any time frame, but is typically one to three months after the initial administration. The invention encompasses additional (e.g., one, two, three, or four) administrations following the first and second administrations as an additional "boost."

**[0057]** The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

## EXAMPLE 1

[0058] This example demonstrates the generation of the polypeptide of the invention.

[0059] Two polypeptides having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1) were synthesized using standard F-moc chemistry by the University of Pittsburgh Peptide Synthesis Facility. One polypeptide had a leucine at position 2 of SEQ ID NO: 1 and the other had a threonine at position 2 of SEQ ID NO: 1. The polypeptides were substantially pure, in that they were >90% pure as indicated by analytical HPLC and were validated by mass spectrometry. Lyophilized polypeptides were dissolved in 100% DMSO at a concentration of 2 mg/ml and stored at -20°C until use.

[0060] It is interesting to note that EGFR<sub>853-861</sub> (i.e., SEQ ID NO: 1, wherein the residue at position 2 is threonine) shares identity with a HER-2 peptide that binds HLA-A\*0201 and has demonstrated immunogenicity. In particular, EGFR<sub>853-861</sub> differs from the HER2<sub>861-869</sub> sequence at position 868.

## EXAMPLE 2

[0061] This example describes a polypeptide-induced stabilization assay.

[0062] The polypeptide-induced stabilization assay of the HLA-A\*0201 class I molecule expressed by the T2 cell line (a human T cell leukemia/B cell line hybrid) was performed using methods known in the art. Briefly,  $5 \times 10^5$  T2 cells were incubated in the presence of 10-fold dilutions of polypeptide (i.e., EGFR<sub>853-861</sub>, EGFR<sub>853-861L</sub>, HER2<sub>861-869</sub>, Flu<sub>58-66</sub>, or EGFR<sub>681-689</sub>) in AIM-V medium for 18 hours at 37°C. Surface HLA-A\*0201 molecule expression was detected using the anti-HLA -A, -B, -C (W6/32) or anti-HLA-A2 mAb (BB7.2). After incubation with a FITC-conjugated secondary mAb, T2 cells were analyzed by flow cytometry using a FACScan (BD Biosciences).

[0063] The results of the experiment are set forth in Figure 1.

## EXAMPLE 3

[0064] This example demonstrates the ability of the immunogenicity of the inventive polypeptide.

[0065] *In vitro* stimulation (IVS) was performed using EGFR<sub>853-861</sub> (i.e., SEQ ID NO: 1, wherein the residue at position 2 is threonine) or EGFR<sub>853-861L</sub> (i.e., SEQ ID NO: 1, wherein the residue at position 2 is leucine) to induce cytotoxic T lymphocytes (CTL) using CD8+ PBMC from five HLA-A\*0201<sup>+</sup> healthy donors and one head and neck squamous cell carcinoma (SCCHN) patient. The resulting EGFR-specific CTL were tested for EGFR<sub>853-861</sub>

specificity and HLA class I antigen restriction using polypeptide pulsed T2 cells. CTL generated against EGFR<sub>853-861</sub> polypeptide recognized T2 cells pulsed with either EGFR<sub>853-861</sub> or EGFR<sub>853-861L</sub> polypeptide, but not T2 cells alone or T2 cells incubated with the HIV Pol<sub>476-484</sub> polypeptide (10 µg/ml at 37°C) (see Figs. 6A and 6B). This CTL recognition of T2 cells, which only express HLA-A2 molecules, was blocked by incubation with the anti-HLA-A, -B, -C mAb W6/32 and the anti-HLA-A mAb LGIII.147.4 (see Figs. 6A and 6B).

[0066] To compare the ability of EGFR<sub>853-861</sub> or EGFR<sub>853-861L</sub> polypeptide to stimulate EGFR<sub>853-861</sub> specific CTL, a peptide titration experiment was performed with ten-fold dilutions of EGFR<sub>853-861</sub> or EGFR<sub>853-861L</sub> polypeptide (see Fig. 7). Recognition of T2 cells incubated with the EGFR<sub>853-861L</sub> polypeptide was detectable at approximately 20-fold lower concentrations than CTL recognition of T2 cells incubated with EGFR<sub>853-861</sub> polypeptide (see Fig. 7), indicating improved immunogenicity of the EGFR<sub>853-861L</sub> polypeptide, which is potentially due to enhanced HLA-A\*0201 binding and/or stabilization.

[0067] CTL derived from EGFR<sub>853-861</sub> or EGFR<sub>853-861L</sub> polypeptide, as well as a HER2 encoded polypeptide, demonstrated cross-reactivity, which supports the therapeutic application of EGFR-targeted vaccine approaches.

#### EXAMPLE 4

[0068] This example describes the purification of T cells comprising the polypeptide of the invention using HLA-A2/peptide tetrameric complexes (tetramer).

[0069] The PE-labeled HLA-A\*0201 EGFR<sub>853-861</sub> and HLA-A\*0201 EGFR<sub>853-861L</sub> tetramers were obtained from the Tetramer Facility of the National Institute of Allergy and Infectious Disease (Atlanta, GA). Specificity was confirmed by staining of the CTL line specific for each polypeptide after IVS and the lack of staining of irrelevant (e.g., MAGE-3<sub>271-279</sub> specific) CTL or HLA-A2<sup>-</sup> PBMCs obtained from normal donors. Three-color flow cytometry assays (FACScan; BD Biosciences) were performed with energy-coupled dye labeled anti-CD3 and FITC-anti-CD8 Abs (Beckman Coulter) and PE-tetramer. Flow cytometry was performed on a CyAn<sup>TM</sup> flow cytometer (Dako, Ft. Collins, CO) machine, and data analyzed using Summit V4.3 software. Generally, 100,000 events per sample were collected after gating on lymphocytes by forward- and side-scatter. CTL clones were sorted using PE-labeled HLA-A2EGFR<sub>853-861</sub> polypeptide loaded tetramers. Clones were plated in 96-well-U-bottom plates and 50 µl/well of cytokine stimulation mixture was added. IVS was performed using 10<sup>6</sup> allogeneic PBLs/ml with a concentration of 5 x 10<sup>6</sup> per plate, and

irradiated with 50 Gy, IL-2 at 200 IU/ml and PHA-L at 1 µg/ml. The clones were restimulated every 7 to 10 days with 50 µl of stimulation mixture.

#### EXAMPLE 5

[0070] This example describes the induction of CD8<sup>+</sup> T cells with the polypeptide of the invention.

[0071] PBMCs were isolated by density centrifugation on Ficoll-Paque™ PLUS (GE Healthcare Bio-Science AB; SE-751 84 Uppsala) and used to prepare mature DCs using methods described in the art. PBMCs were resuspended at  $5 \times 10^7$ /ml in AIM-V medium (Invitrogen) and were incubated for 90 min in 75-cm<sup>2</sup> tissue culture flasks or six-well plates (37°C; 5% CO<sub>2</sub>). Non-adherent (T cell-enriched) cells were gently washed out with HBSS and subsequently frozen. The plastic adherent cells were cultured in AIM-V medium supplemented with 1000 units/ml rhGM-CSF and 1000 units/ml rhIL-4. Six days later, the culture medium was removed, and the immature DCs were cultured in AIM-V supplemented with 1000 units/ml rhGM-CSF and 1000 units/ml rhIL-4, 10 ng/ml rhIL-6, 10 ng/ml recombinant human tumor necrosis factor (TNF)-α, and 10 ng/ml IL-1β and 1 µg/ml PGE<sub>2</sub>. Mature DCs were harvested on day 8, centrifuged and frozen or used to stimulate autologous T cells. The stimulator cells were resuspended in AIM-V at 10<sup>6</sup>/ml supplemented with 10 µg/ml of each polypeptide (EGFR<sub>853-861</sub> and EGFR<sub>853-861L</sub>) and incubated for 4 hours at 37°C. The polypeptide-pulsed DCs were then irradiated (50 Gy) and washed and resuspended in culture medium (Iscove's medium supplemented with 10% human serum, L-arginine, L-asparagine, and L-glutamine). Autologous CD8<sup>+</sup> T cells were negatively isolated from PBMCs with immunomagnetic beads (Miltenyi Biotech, Germany) and added to the polypeptide-pulsed DCs at a ratio of  $1 \times 10^6$  cell/ml to  $1 \times 10^5$  polypeptide-pulsed DC in a final volume of 2 ml of culture medium (24-well tissue culture plate). The cells were cultured for 48 hours at 37°C with IL-2 (20 unites/ml) and IL-7 (5 ng/ml). On day 7 and weekly thereafter, lymphocytes were restimulated with autologous irradiated DCs pulsed with polypeptide in culture medium supplemented with 20 unites/ml IL-2 and 5 ng/ml IL-7. The stimulated CD8<sup>+</sup> T cells were analyzed for specificity in IFN-γ ELISPOT and <sup>51</sup>Cr release assays at day 21 and then every 7 days thereafter.

#### EXAMPLE 6

[0072] This example describes the assessment of T cell responses to the polypeptide of the invention and tumor cells.

**[0073]** The recognition of APCs pulsed with polypeptide (EGFR<sub>853-861</sub> or EGFR<sub>853-861L</sub>) and head and neck tumor cells was assessed by ELISPOT assays specific for hu-IFN- $\gamma$ . For the ELISPOT assays, multiscreen HTS plates (Millipore, Bedford, MA) were coated with 10  $\mu$ g/ml of either mAb antihuman IFN- $\gamma$  (1-D1K; Mabtech, Stockholm, Sweden) in PBS (Life Technologies, Inc.) overnight at 4°C. Unbound mAb was removed by four successive washings with PBS. After the plates were blocked with Iscove's modified Dulbecco's Medium with 10% human serum (1 hour at 37°C), CD8<sup>+</sup> T cells were seeded in triplicate (5 x 10<sup>4</sup> for bulk CD4<sup>+</sup> T cells and 10<sup>3</sup> for CD8<sup>+</sup> T cell clones) in multiscreen HTS plates. Nonirradiated T2 cells (5 x 10<sup>4</sup>) or SCC4, PCI-30, or PCI-13 head and neck tumor cell lines (5 x 10<sup>4</sup>) were added. Synthetic polypeptides were then added into ELISPOT assays after APCs were seeded. Control wells contained unstimulated T cells, T cells in the presence of unloaded T2 cells, or tumor cells alone. Culture medium was AIM-V at a final volume of 200  $\mu$ l/well.

**[0074]** After incubation at 37°C in 5% CO<sub>2</sub> for 40 hours (IFN- $\gamma$  ELISPOT assays), cells were removed by washings with PBS-0.05% Tween 20 (PBS-T). Captured cytokine was detected at sites of its secretion by incubation for 4 hours with biotinylated mAb anti-hIFN- $\gamma$  (7-B6-1; Mabtech) at 2  $\mu$ g/ml in PBS-0.5% BSA. Plates were washed six times with PBS-T, and avidin-peroxidase complex, diluted 1:100 (Vectastain Elite Kit: Vector, Burlingame, CA), was added for 1 hour at room temperature. Unbound complex was removed by three successive washings with PBS-T and three with PBS alone. Peroxidase staining was performed with 3,3',5'-tetramethylbenzidine (Vector Laboratories) for 4 minutes and stopped by rinsing the plates under running tap water. Spot numbers and spot sizes were automatically determined with the use of computer-assisted video image analysis.

**[0075]** The stimulated CD8<sup>+</sup> T cells recognized the HLA-A2<sup>+</sup> head and neck tumor cells, SCC4, PCI-30, and PCI-13, as indicated by the amount of IFN- $\gamma$  spots in the ELISPOT assay. For SCC4 cells, there were about 300 IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells and about 600 IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells when the SCC4 cells were treated with IFN- $\gamma$  (see Figs. 2A and 9A). For PCI-30 cells, there were about 450 IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells and about 550 IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells when the PCI-30 were treated with IFN- $\gamma$  (see Figs. 2B and 9A). For PCI-13 cells, there were about 8 IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells and about 27 IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells when the PCI-13 cells were treated with IFN- $\gamma$  (see Figs. 3 and 9A). Little or no spots were detected following the additions of HLA-A, -B, and -C

mAb, demonstrating T cell reactivity in an HLA dependent manner (see Figs. 2A, 2B, 3, and 9A).

[0076] To provide further evidence that the polypeptide of the invention is a class I restricted epitope, class I and class II mAb were separately added to compositions comprising CTLs (stimulated with EGFR<sub>853-861</sub>) and PCI-30 cells. Addition of the class II mAb resulted in no change in the amount of IFN- $\gamma$  spots/5 x 10<sup>4</sup> CD8<sup>+</sup> T cells; however, no spots were detected following the addition of the class I mAb (see Figs. 4 and 10). The addition of anti-EGFR antibody (cetuximab or panitumumab) increased the number of IFN- $\gamma$  spots (Figs. 4 and 10). Similarly, addition of the polypeptide of the invention (EGFR<sub>853-861</sub> or EGFR<sub>853-861L</sub>) resulted in a significant increase in IFN- $\gamma$  spots (see Fig. 4).

#### EXAMPLE 7

[0077] This example describes the generation of a vector comprising a nucleic acid encoding the polypeptide of the invention.

[0078] A nucleic acid encoding the polypeptide of the invention is inserted under the control of the vaccinia 40K promoter (see, e.g., Gritz et al., *J. Virol.*, 64: 5948-5957 (1990)) into the HindIII M genomic region of the Wyeth strain of vaccinia virus. A selection marker, such as the lacZ gene, can be used to identify recombinant viruses. For example, the *E. coli* lacZ gene, under the control of the fowlpox C1 promoter (see, e.g., Jenkins et al., *AIDS Res. Hum. Retroviruses*, 7: 991-998 (1991)) can be included as a calorimetric marker for recombinant viruses. Then, recombinant viruses can be identified using a chromogenic assay for the lacZ gene product.

[0079] 1 x 10<sup>6</sup> immune cells (e.g., DCs, APCs, lymphocytes, etc.) are incubated with the recombinant virus encoding the polypeptide of the invention. Titration experiments are performed to determine the amount of virus necessary to consistently induce production of the polypeptide of the invention. The infected cells are suspended in 10 ml RPMI-1640 complete medium containing 100 ng/ml of rhGM-CSF, 20 ng/ml rhIL-4, and 20 ng/ml of TNF-alpha cultured for 24 hours.

[0080] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

**[0081]** The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

**[0082]** Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

## CLAIM(S):

1. A polypeptide having a sequence of amino acids consisting of IXDFGLAKL (SEQ ID NO: 1).
2. The polypeptide of claim 1, wherein the polypeptide is isolated or substantially purified.
3. An isolated or substantially purified nucleic acid encoding the polypeptide of claim 1.
4. A vector comprising the nucleic acid of claim 3.
5. A cell comprising the vector of claim 4.
6. The cell of claim 5, wherein the cell is selected from the group consisting of an antigen presenting cell, heat inactivated recombinant yeast cell, and artificial antigen presenting construct.
7. A composition comprising the polypeptide of claim 1 and a carrier.
8. A composition comprising the nucleic acid of claim 3 and a carrier.
9. A composition comprising the vector of claim 4 and a carrier.
10. A composition comprising the cell of claim 5 and a carrier.
11. The composition of any of claims 7-10, further comprising an adjuvant.
12. The composition of claim 11, wherein the adjuvant is selected from the group consisting of IL-2, IL-15, IL-21, GM-CSF, incomplete Freund's adjuvant, alum, aluminum salts, aluminum phosphate, aluminum hydroxide, aluminum silica, Montanide, calcium phosphate, and combinations thereof.
13. The composition of any of claims 7-11, further comprising a chemotherapeutic agent.
14. The composition of claim 13, wherein the chemotherapeutic agent is an EGFR family-specific antibody.

15. The composition of any of claims 7-14, further comprising a tumor associated antigen selected from the group consisting of MAGE, p53, Erb2, MUC-1, HAGE, and human papilloma virus.
16. The composition of any of claims 7-15, wherein the composition is a pharmaceutical composition and the carrier is a pharmaceutically acceptable carrier.
17. A method of inducing a T-cell response in a patient with epithelial cancer, comprising administering to the patient the composition of any of claims 7-16, such that a T-cell response against the epithelial cancer is induced.
18. A method of inhibiting epithelial cancer comprising administering the composition of any of claims 7-16, such that epithelial cancer is inhibited.
19. The method of claim 17 or 18, wherein the epithelial cancer is selected from the group consisting of head and neck squamous cell carcinoma (HNSCC), ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, gastric cancer, breast cancer, endometrial cancer, and colorectal cancer.

FIG. 1

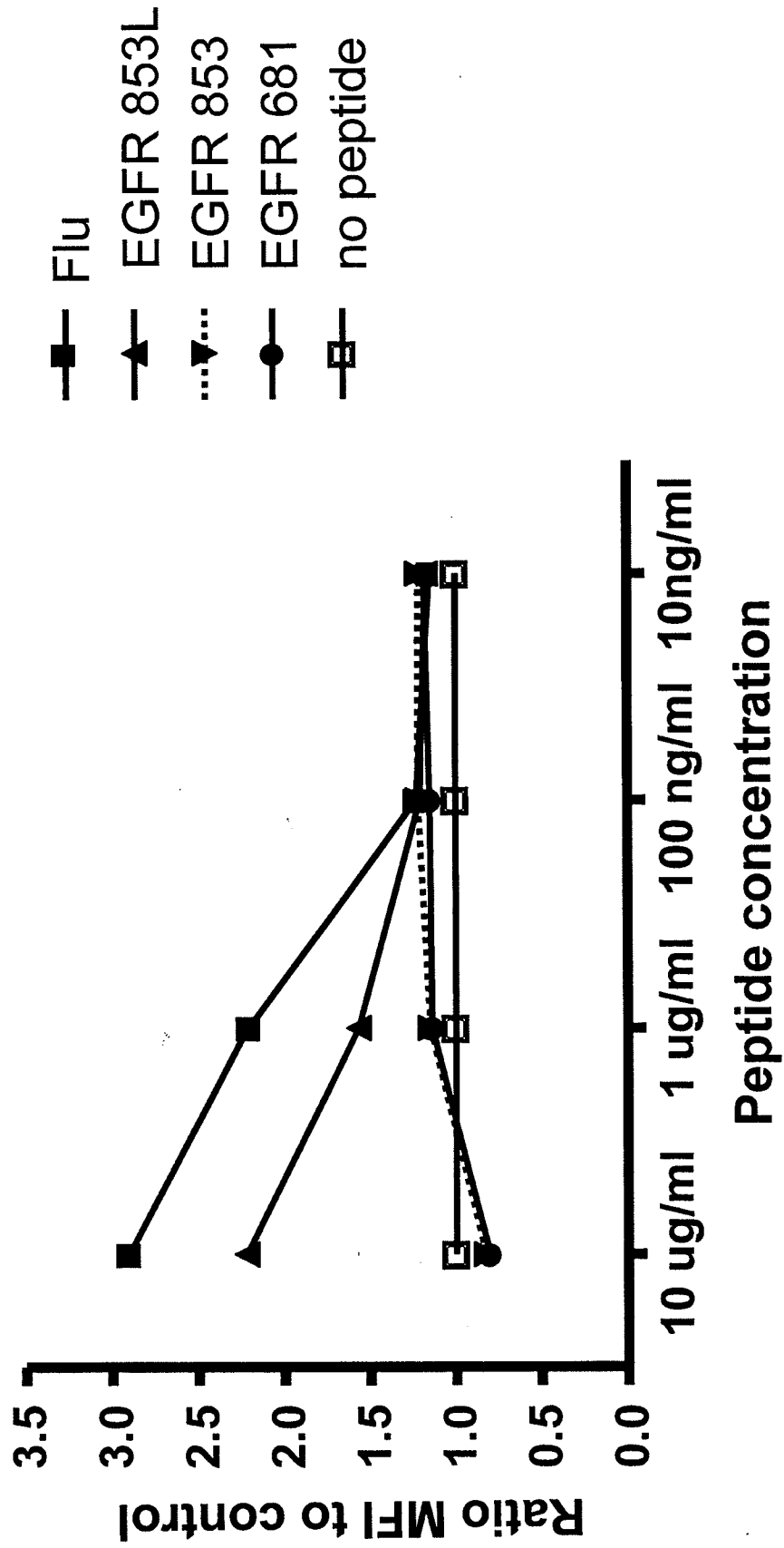


FIG. 2A

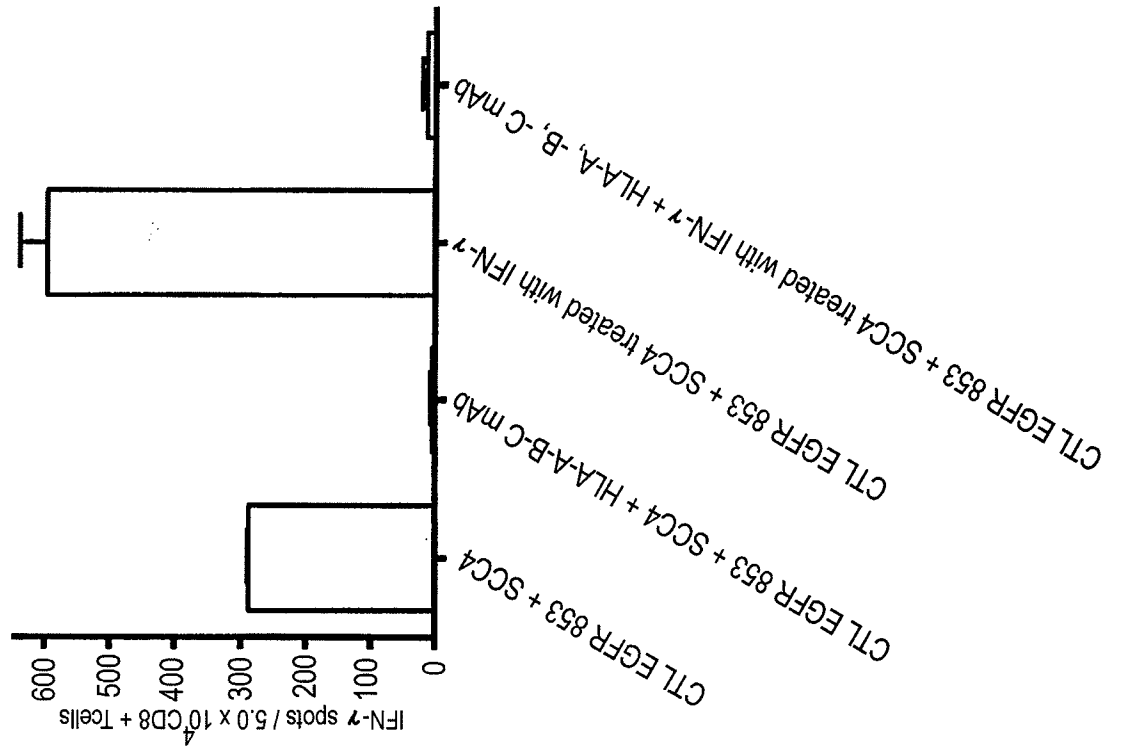


FIG. 2B

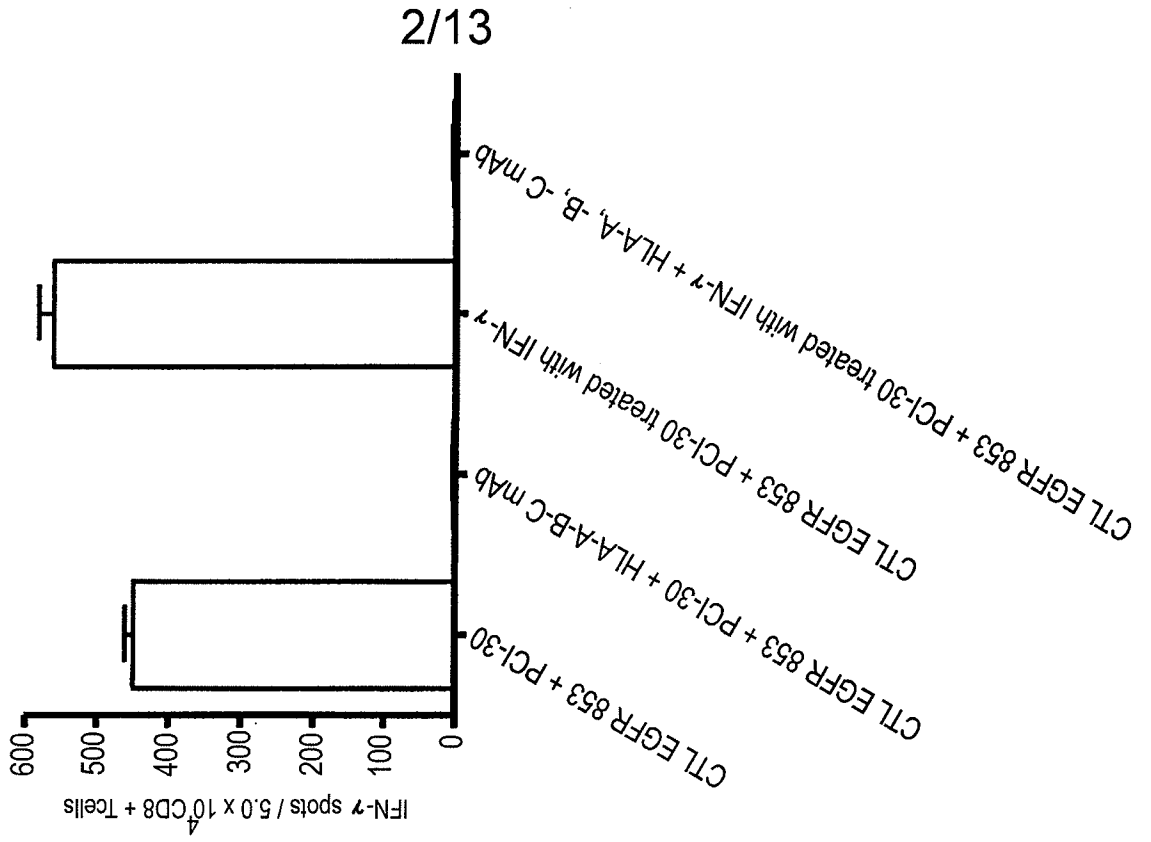


FIG. 3

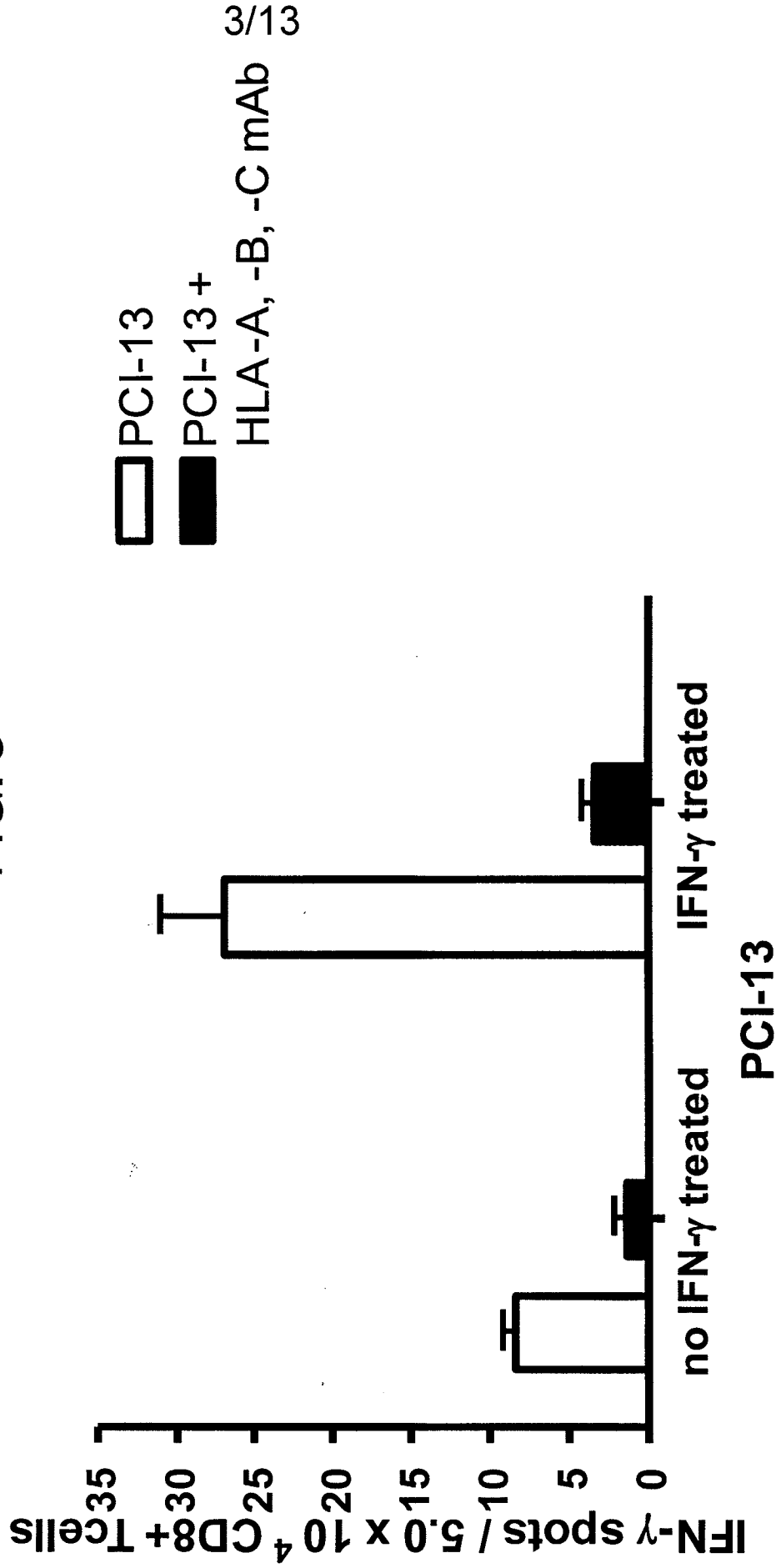


FIG. 4

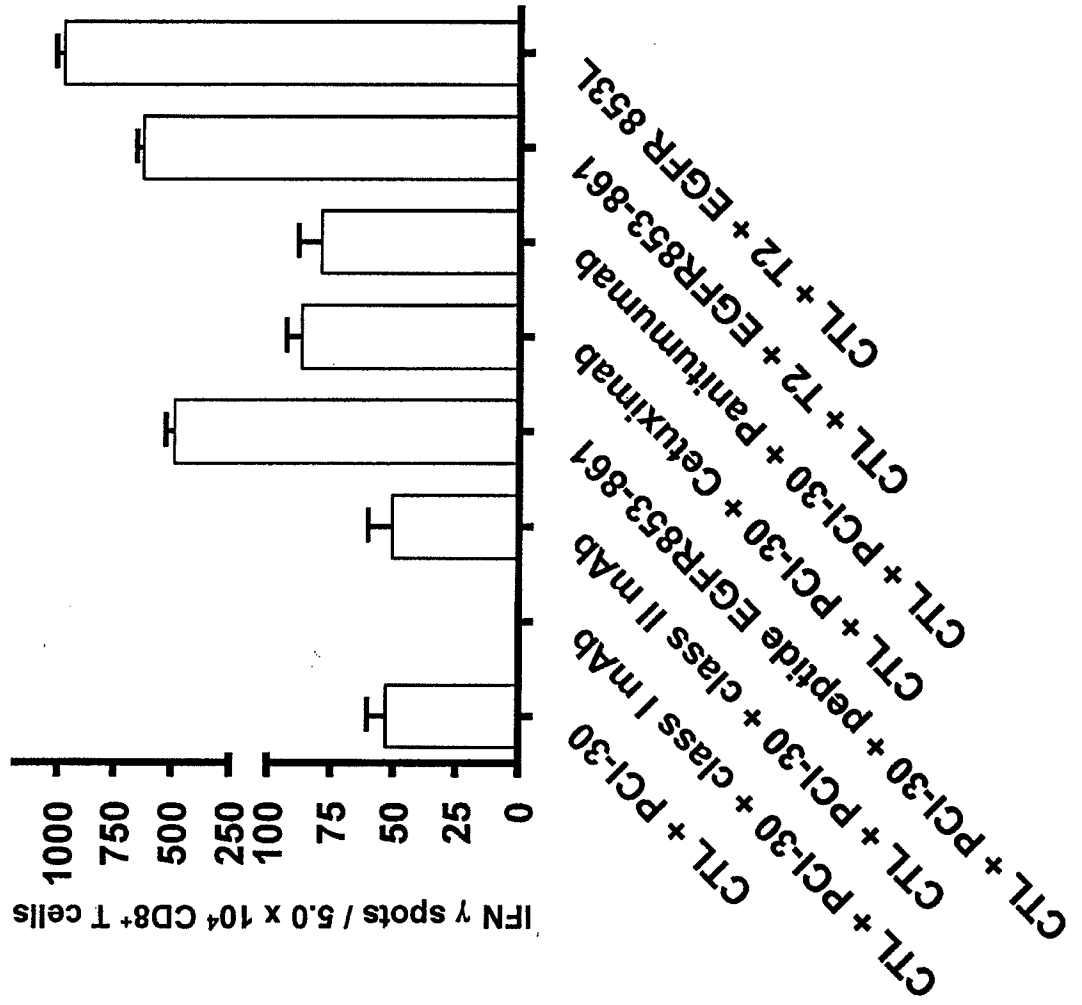


FIG. 5

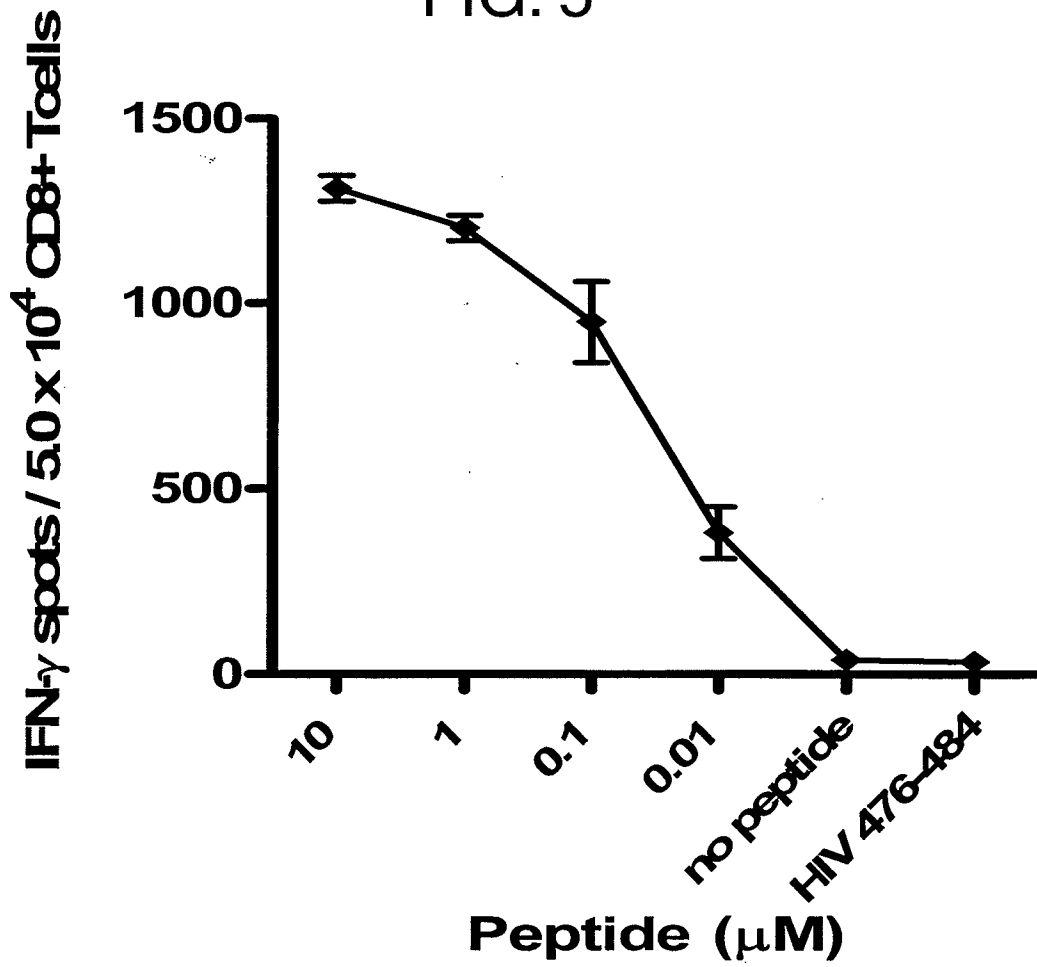


FIG. 6A

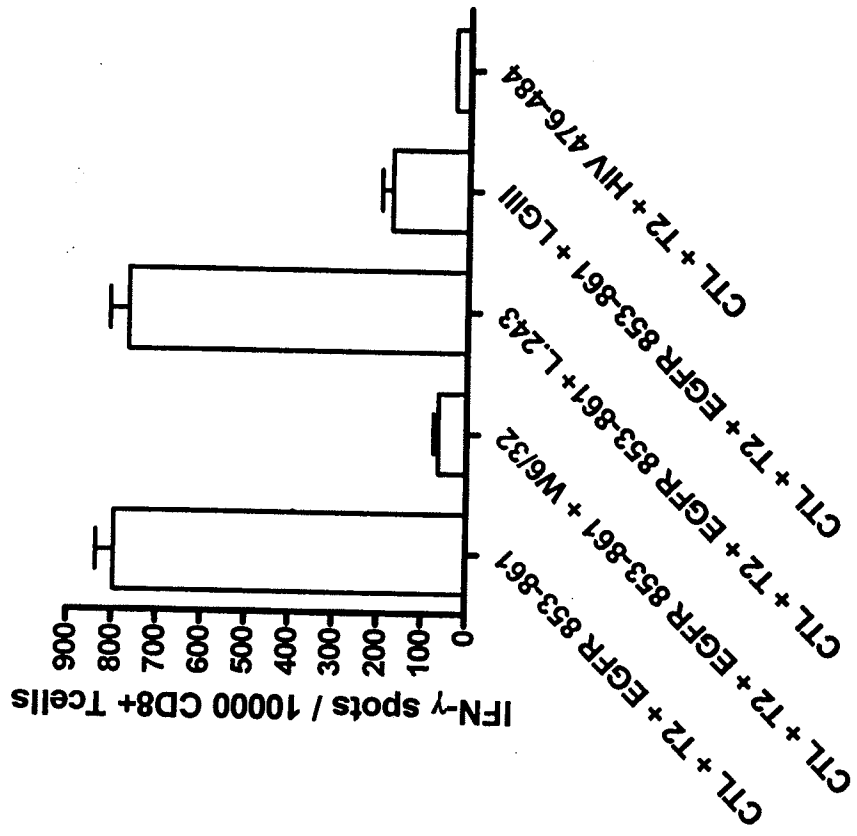
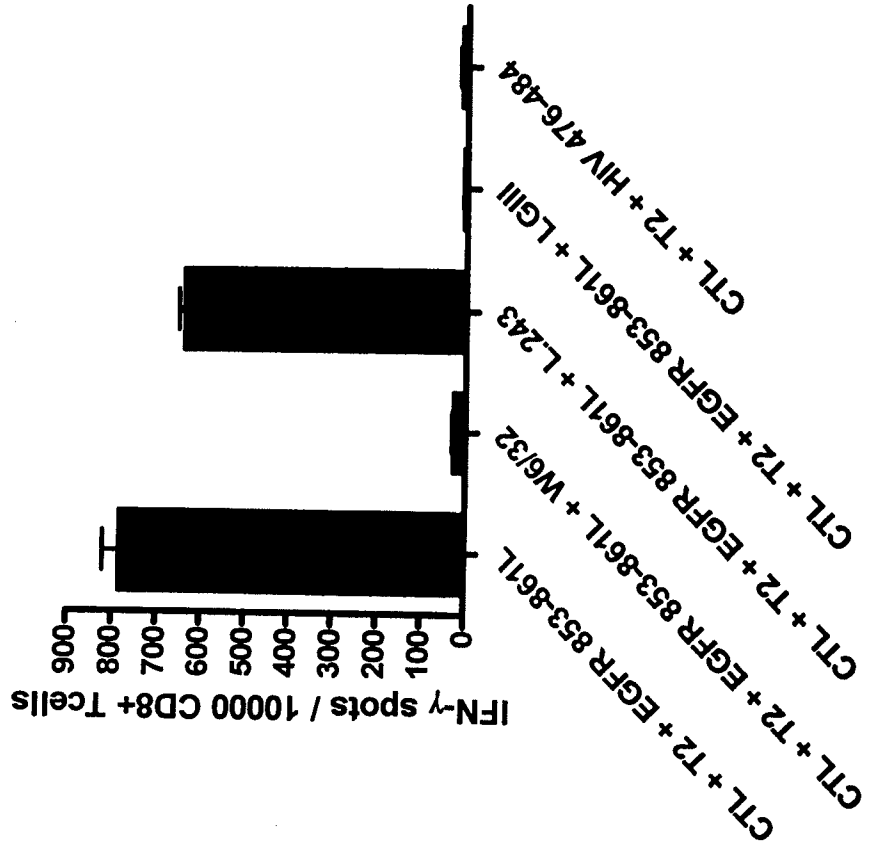


FIG. 6B



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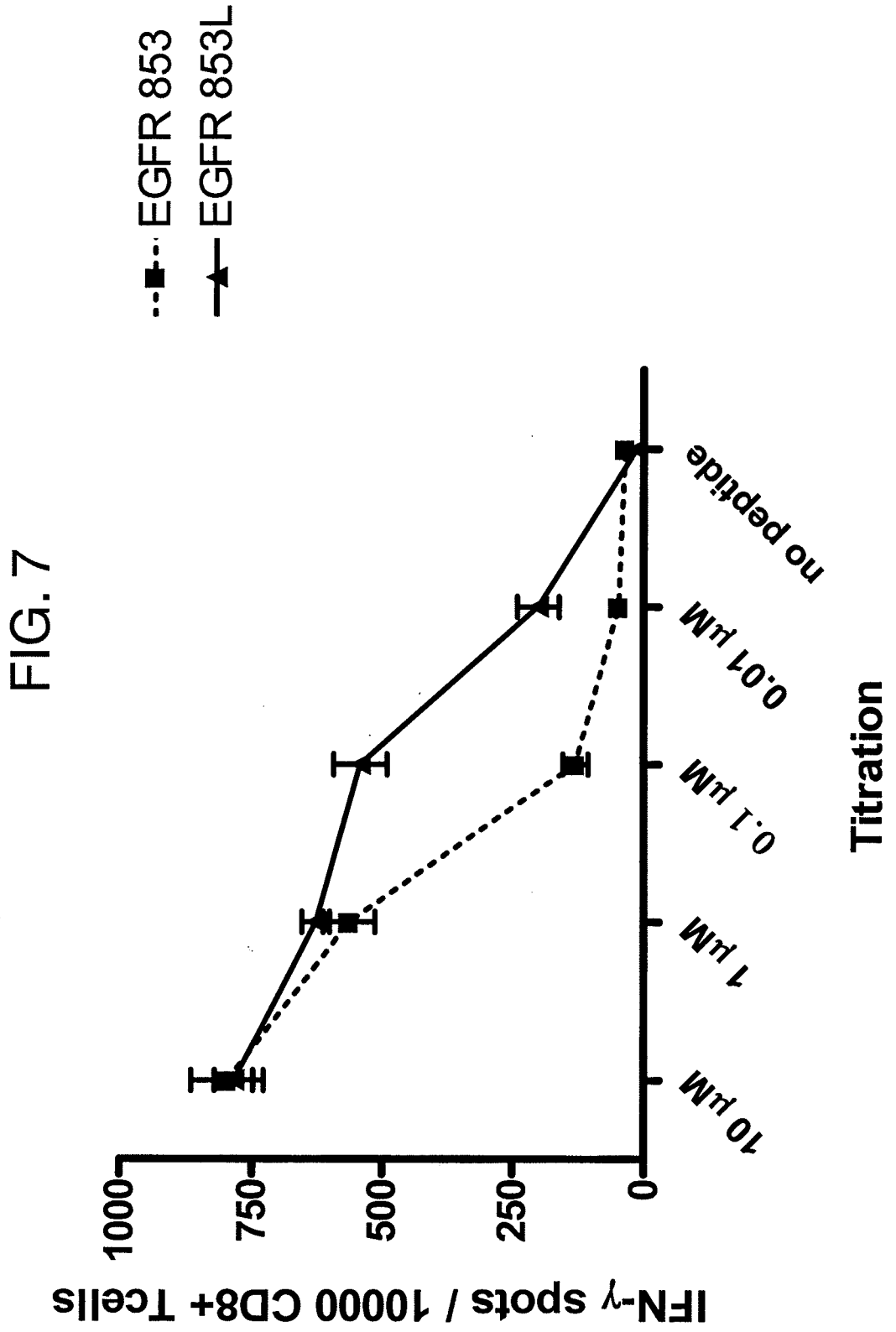


FIG. 8A

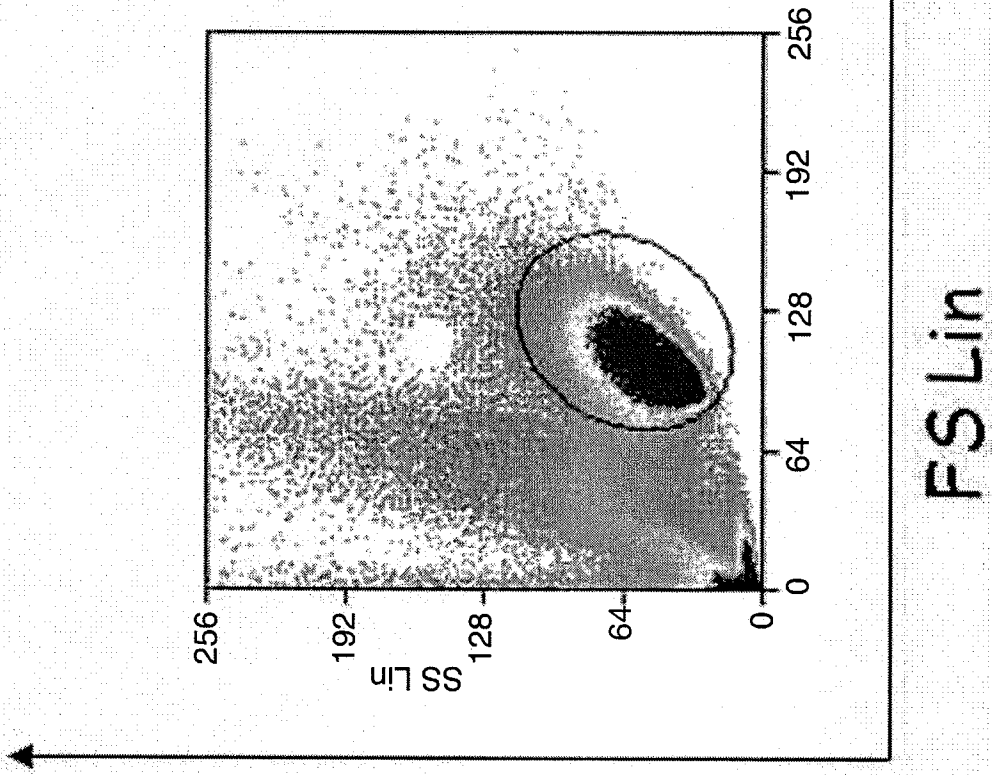


FIG. 8B

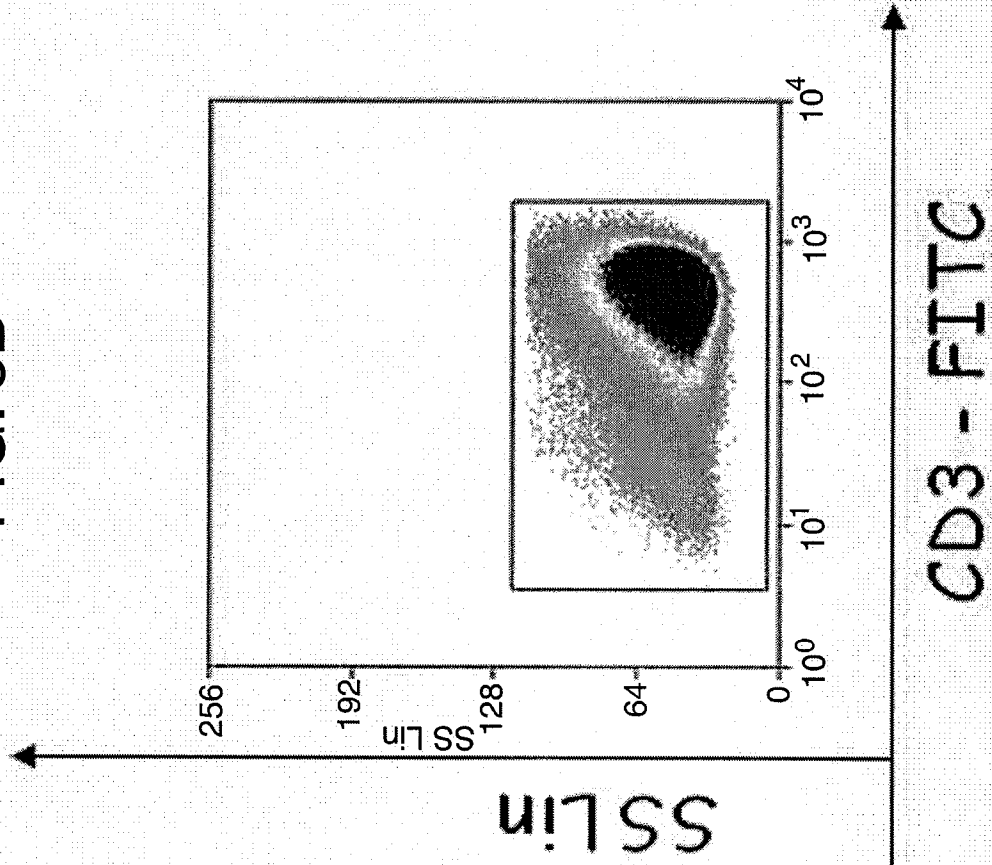


FIG. 8C

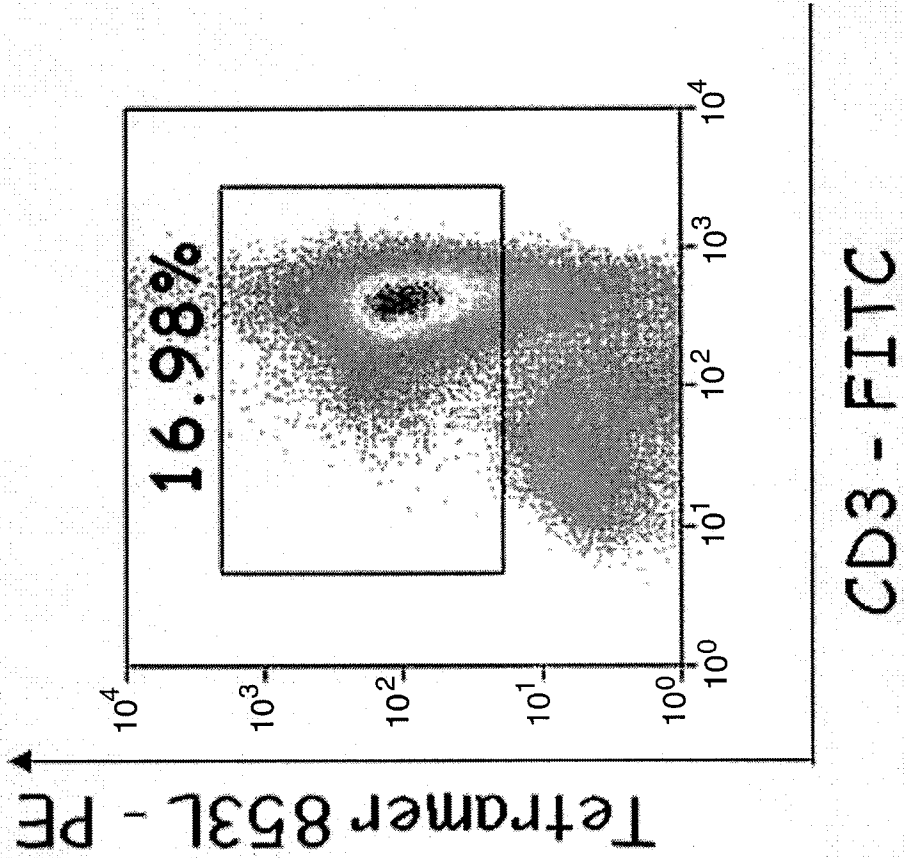


FIG. 8D

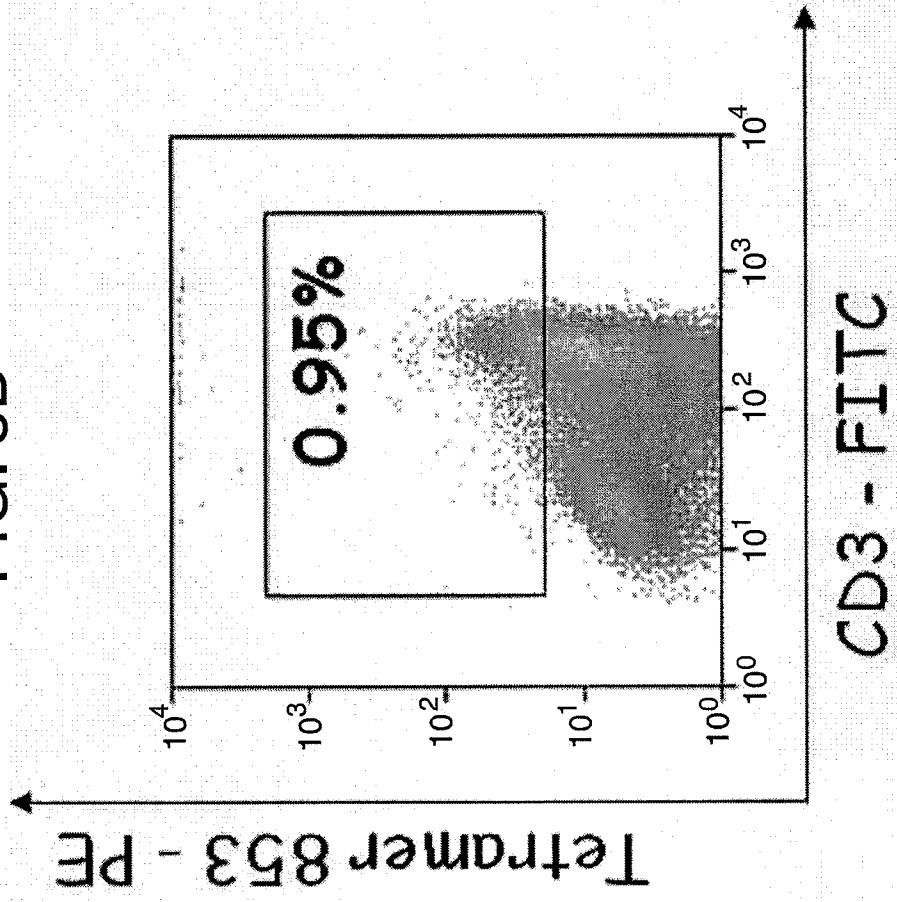


FIG. 9A

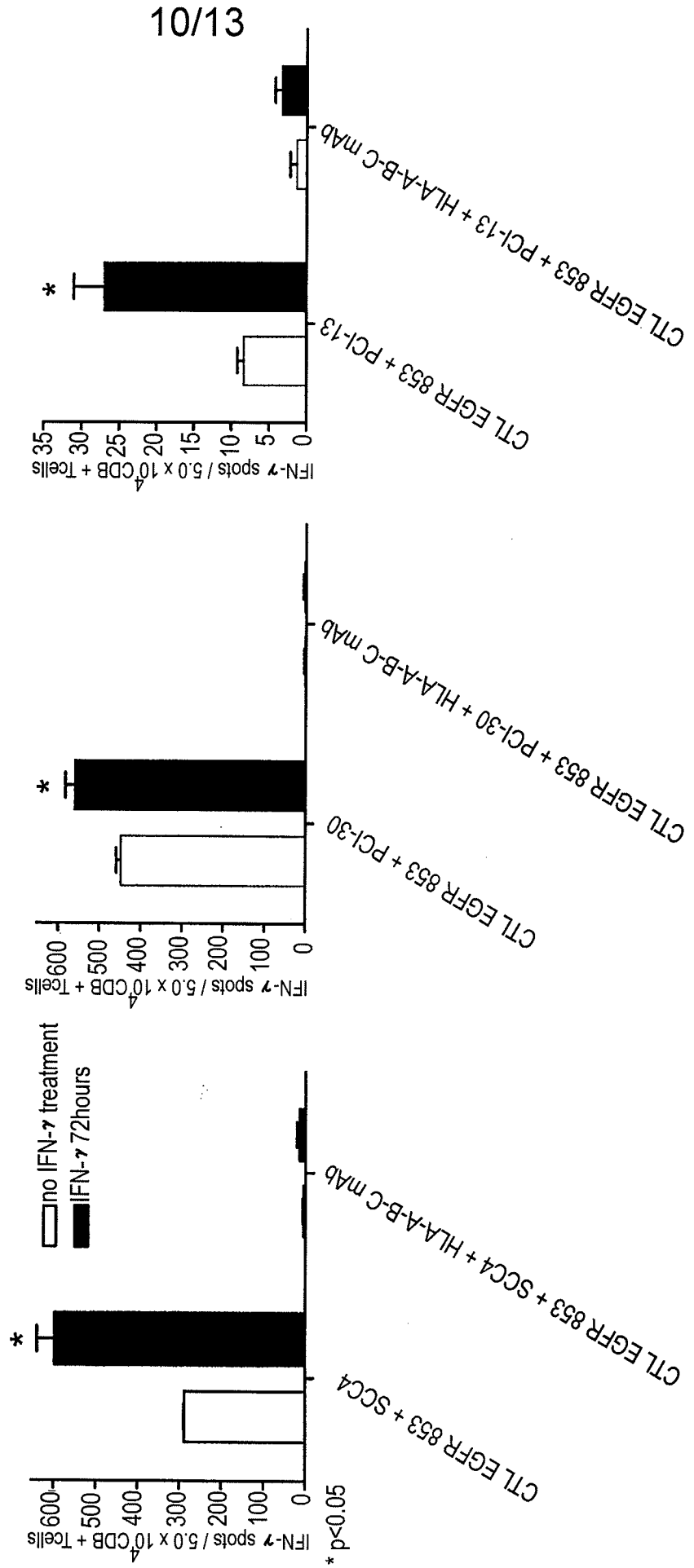
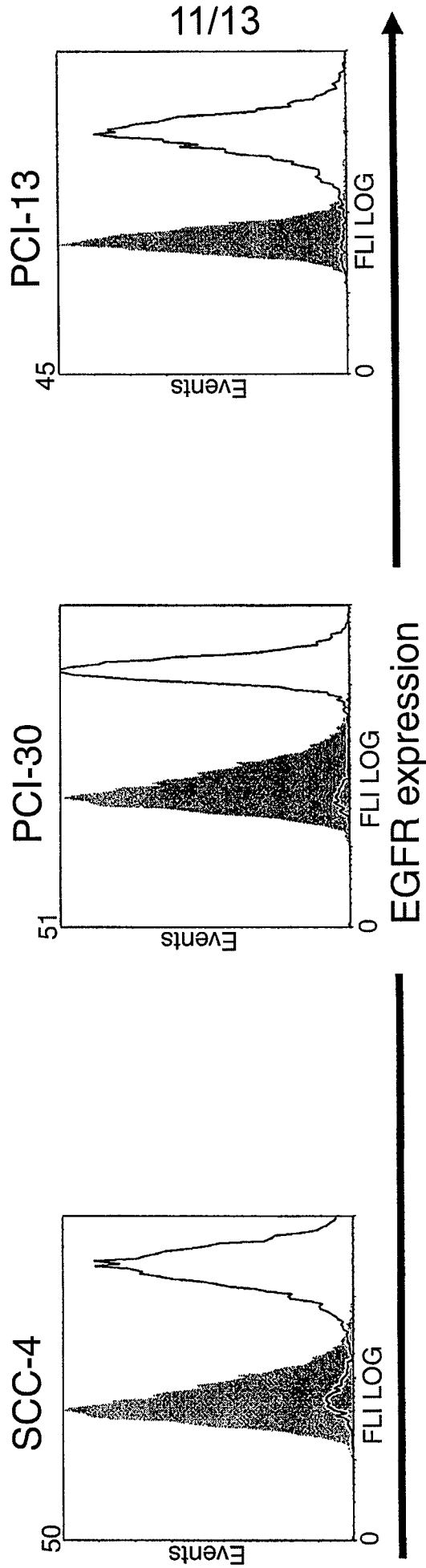
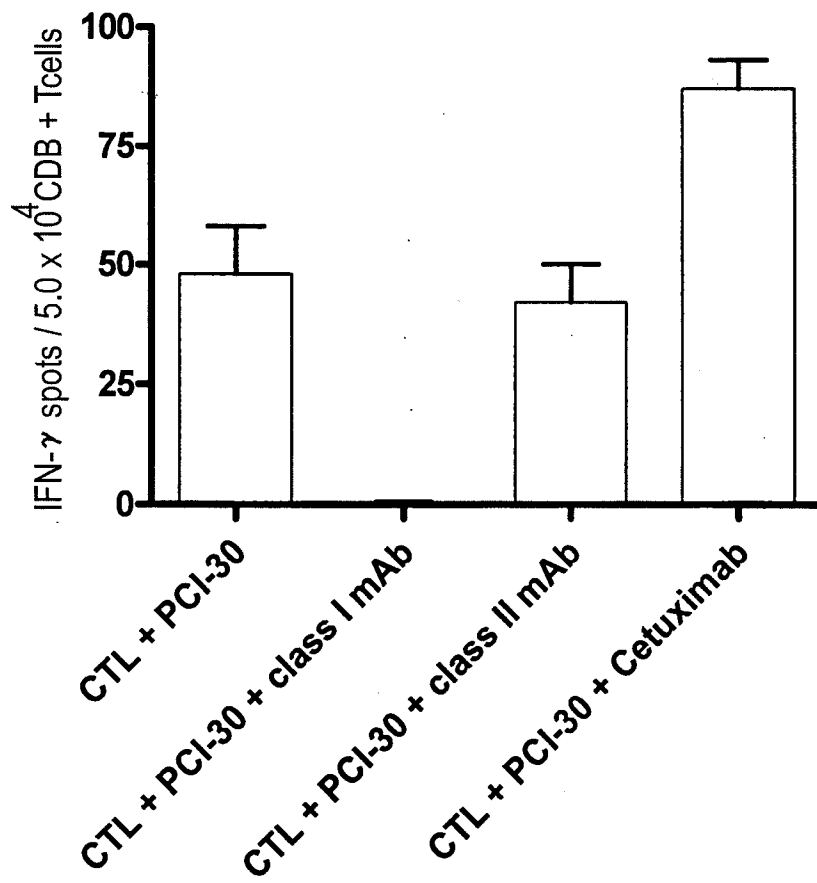


FIG. 9B



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FIG. 10



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FIG. 11

