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(57) Abstract: The present invention relates to chimeric binding agents and compositions comprising the same. The invention further relates to polynucleotides encoding the chimeric binding agent and vectors and host cells comprising the same. The invention further relates to methods of using the chimeric binding agents to mediate antibody-dependent cellular cytotoxicity of epithelial cancer cells and methods of treating epithelial cell cancers.

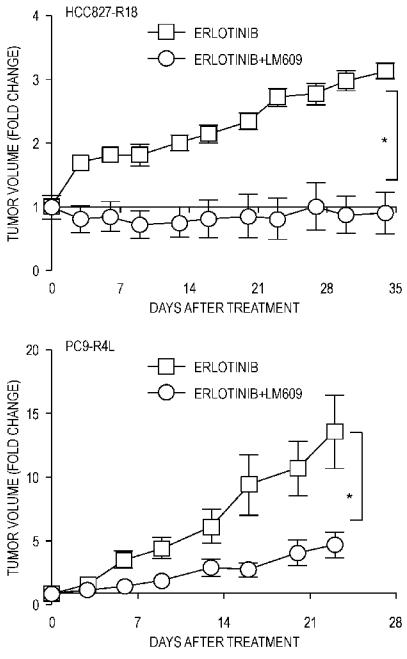

FIG. 1

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COMPOSITIONS AND METHODS FOR TREATING CANCER

STATEMENT OF PRIORITY

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/014,550, filed April 23, 2020, the entire contents of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to chimeric binding agents and compositions comprising the same. The invention further relates to polynucleotides encoding the chimeric binding agent and vectors and host cells comprising the same. The invention further relates to methods of using the chimeric binding agents to mediate antibody-dependent cellular cytotoxicity of epithelial cancer cells and methods of treating epithelial cell cancers.

BACKGROUND

[0003] Antibodies are proteins that bind to a specific antigen. Monoclonal antibodies (mAbs) and mAb-based reagents approved for cancer therapy include several that are directed against antigens expressed on malignant B cells and plasma cells (CD19, CD20, CD22, CD30, CD38, CD52, CD79B, SLAMF7), epithelial cancer cells (EpCAM, EGFR, HER2, VEGFR2, nectin-4), acute myeloid leukemia (CD33), cutaneous T-cell lymphoma (CCR4), neuroblastoma (GD2), and sarcoma (PDGFRA), as well as immune checkpoint targets (PD-1, PD-L1, CTLA-4) (Gasser, 2016; Carter, 2018). A total of 42 antibody-based cancer therapies are currently FDA-approved and marketed. The efficacy of a therapeutic antibody for cancer can be influenced by a combination of mechanisms (Chiavenna, 2017). Antibody binding to an antigen selectively expressed on a cancer cell may produce anti-tumor effects by directly blocking the function of the antigen that promotes tumor cell growth or survival pathways. An antibody can also act as a bridge to bring together a tumor cell with an immune effector cell that can indirectly induce tumor cell destruction.

[0004] The properties of therapeutic antibodies can be modified to either enhance or suppress engagement with certain types of immune effector cells using a growing arsenal of glycoengineering and Fc engineering approaches or through the creation of bispecific or trispecific antibodies (Saxena, 2016; Rader, 2020). These tools can be utilized for the rational design of "antigen-effector matching" to create a personalized medicine approach for cancer therapy.

[0005] Antibody engineering strategies focused on improving the engagement of monocytes or natural killer (NK) cells include a vast collection of glycoengineered and Fc engineered variants that promote binding of the Fc portion of a therapeutic antibody to FcγRIIIA (CD16A), the only Fc receptor expressed on NK cells (Lazar, 2006). Although less common, several strategies have generated antibody variants with enhanced binding to macrophages, including a G236A Fc mutant that promotes binding to FcγRIIA (CD32A) (Richards, 2008) or a bispecific antibody that recruits macrophages via FcαRI (CD89) (Li, 2017).

[0006] Selecting an antigen for antibody therapy needs to address a cancer phenotype that evolves over time. A class of antibody therapeutics has been developed for epithelial cancers that express high levels of markers such as EpCAM, EGFR, HER2, or VEGFR2. While antibodies targeting such antigens may be effective for early stage tumors, epithelial cancers are known to undergo an epithelial-to-mesenchymal transition (EMT) that involves not only a loss of epithelial markers and a gain of mesenchymal markers (Karacosta, 2019), but also a change in the tumor microenvironment and immune cell infiltration (Dongre, 2019). As such, targeting epithelial tumors that convert toward a mesenchymal state may require different antigen-effector cell combinations.

[0007] EMT is a dynamic process that describes a tumor cell phenotype in crosstalk with stromal constituents of the tumor (Dongre, 2019). Cancer-associated fibroblasts, macrophages, and other immune cells secrete a variety of cytokines and factors that engage tumor cells to activate the expression of transcription factors that induce EMT. Mesenchymal-like carcinoma cells also shift the immune component of the tumor toward an immunocompromised state that excludes anti-tumor immune cell types and recruits pro-tumor macrophages.

[0008] As such, targeting mesenchymal tumors that are often "immune-cold" with antibody therapeutics may require different antigen-effector cell combinations than those developed for epithelial tumors which are often "immune-hot". Antibodies that recognize epithelial markers such as EpCAM, EGFR, HER2, or VEGFR2 have been developed and optimized to engage receptors on peripheral blood mononuclear cells (PBMC) or NK cells. For an epithelial-like tumor, a number of approved therapeutic antibodies provide a good match for such antigen-effector combinations. In contrast, an antibody that recognizes an antigen expressed on the surface of mesenchymal-like tumor cells that is capable of engaging macrophages as effector cells represents an unmet need in the field of therapeutic antibody development for solid tumors. Cancers that have undergone EMT tend to be more aggressive, metastatic and drug resistance. Therefore, having a drug that attacks tumor cells that have undergone EMT is likely to decrease tumor progression and drug resistance.

[0009] Thus, there is a need for new compositions, and methods of using such compositions, to treat cancers, including in particular late-stage epithelial cancers that have undergone EMT.

SUMMARY OF THE DISCLOSURE

[0010] The present invention is based in part on an understanding of epithelial cancer cells and the EMT process, including changes in the immune cell populations in the tumor microenvironment. Central to this transformation process, the epithelial tumor cells gain expression of avβ3 integrin on their cell surface, becoming drug resistant and more stem-like in phenotype as well as insensitive to hypoxia or other environmental stress. Expression of avβ3 on epithelial cancer cells is triggered by the various forms of cellular stress as well within the microenvironment or the application of a wide range of anti-cancer drugs. Patients that have progressed on standard of care therapeutics and thereby express avβ3 are therefore candidates for therapies targeting the avβ3 antigen. Given that avβ3 is necessary and sufficient for drug resistance it is likely that by selectively targeting avβ3 positive tumor cells it would be possible to prevent or reverse cancer acquired drug resistance.

[0011] The present invention provides compositions and methods for engaging the appropriate immune effector cells to effectively mediate antibody-dependent cellular cytotoxicity (ADCC) against epithelial cancer cells that have undergone EMT and have gained the cell surface marker avβ3.

[0012] The inventors have determined that ADCC is mediated by macrophages, and not NK cells, that leads to the death of cancer cells targeted by the antibody. Further, the cell death does not involve antibody-dependent cellular phagocytosis (ADCP) or direct killing via the antibody alone. It was previously understood that antibody engagement of macrophages as an effector cell typically promoted ADCP. The present inventors have surprisingly determined that the chimeric binding agents of the invention do not induce ADCP, but instead exclusively promote macrophage-dependent ADCC of human cell targets. This unexpected finding, among other benefits, advantageously permits treatment of CD47-positive tumor cells that would normally be resistant to phagocytosis or ADCP. A binding agent that promotes ADCC exclusively will kill every cell it encounters while a binding agent that promotes ADCP will not be able to kill CD47-positive cells. Thus, the chimeric binding agents of the present invention are expected to be more efficient. Without being bound by theory, it is thought that the advantages of the present invention are based on the structure of the chimeric binding agent (e.g., the IgG4 domain) and/or the antigen being recognized (e.g., integrin αvβ3) that make cells expressing the antigen particularly sensitive to ADCC rather than ADCP.

[0013] Mesenchymal tumors are identified by expression of transcription factors (ZEB, SNAIL, SLUG, and TWIST1) that repress epithelial markers (including E-cadherin, EpCAM, occludins, claudins, and cytokeratins) and promote the expression of mesenchymal markers (including cell adhesion-related proteins N-cadherin, vimentin, fibronectin, β1 and β3 integrins, and MMPs) (Dongre, 2019). An ideal tumor cell antigen for a mesenchymal-like tumor would be a cell surface marker with high expression on tumor cells but low expression on all other normal cell types. Since EMT has been closely linked with a cancer stem phenotype (Marie-Egyptienne, 2013; Singh, 2010; Ye, 2015), and drug resistance, cancer stem cell markers may represent another type of antigen for targeting mesenchymal tumors, although these often vary between tumor types.

[0014] Among potential cell surface mesenchymal markers, N-cadherin and β1 integrin are expressed on many normal cell types and could thus contribute to issues with toxicity or compete with tumor cells for antibody binding. In contrast, integrin avβ3 is a more selective candidate for a mesenchymal tumor cell antigen based on its low expression in normal adult tissues and its enrichment on epithelial tumors as they become more aggressive, late-stage, and drug resistant.

[0015] The present invention is based on the development of agents that can mediate ADCC by engaging myeloid-derived cells found in mesenchymal tumors and targeting them to antigens that are expressed on epithelial cancer cells that have undergone EMT.

[0016] Thus, one aspect of the invention relates to a chimeric binding agent comprising a first domain that specifically binds to an antigen on an epithelial cancer cell expressing at least one mesenchymal cell marker and a second domain that mediates ADCC by engaging a myeloid-derived cell that accumulates in mesenchymal tumors, and compositions or pharmaceutical compositions comprising the chimeric binding agents.

[0017] Another aspect of the invention relates to a polynucleotide encoding the chimeric binding agent of the invention and vectors and host cells comprising the polynucleotide.

[0018] An additional aspect of the invention relates to a method of targeting a myeloid-derived cell that accumulates in mesenchymal tumors to an epithelial cancer cell expressing at least one mesenchymal cell marker, comprising contacting the cancer cell and the myeloid-derived cell with an effective amount of the chimeric binding agent of the invention.

[0019] A further aspect of the invention relates to a method of treating an epithelial cell cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the invention to the subject,

thereby treating the epithelial cell cancer. In particular, avβ3 is expressed in increased amounts on drug resistant cancers making it possible to prevent or reverse drug resistance.

[0020] Another aspect of the invention relates to a method of treating an epithelial cell cancer in a subject in need thereof, comprising the steps of:

a) selecting a subject having epithelial cancer cells that are enriched for an antigen specifically bound by the chimeric binding agent of the invention and enriched for myeloid-derived cells that accumulate in mesenchymal tumors; and

b) administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the invention to the subject, thereby treating the epithelial cell cancer. Cancer patients that become drug resistant gain the expression of avβ3 and thereby become candidates for such therapies targeting this marker.

[0021] Another aspect of this invention relates to antigen-effector cell matching of tumors such that the antigen is specifically present on the tumor cell (e.g., a tumor cell antigen) and a therapeutic antibody contains effector cell binding regions that are specific to those effector cells found in the tumor (e.g., neutrophils, dendritic cells, NK cells etc.).

[0022] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **FIG. 1** shows anti-αvβ3 mouse monoclonal antibody LM609 sensitizes tumor xenografts to erlotinib. LM609 re-sensitizes resistant tumors to erlotinib. HCC827-R18 and PC9-R4L erlotinib-resistant tumor cells generated as reported in Wettersten *et al., Cancer Res.* *79*:5048 (2019), incorporated by reference herein in its entirety, were injected to form subcutaneous flank tumors in nu/nu recipient mice. Once tumors reached a volume of 100 mm^3, mice were randomized to receive erlotinib alone (6.25 mg/kg) or the combination of erlotinib and LM609 (10 mg/kg). Tumor dimensions were measured biweekly and volume calculated as $V = ½ (\text{length} \times \text{width}^2)$. Graph shows mean ± SE. *, $P < 0.05$ for erlotinib vs. erlotinib/LM609 using ANOVA.

[0024] **FIG. 2** shows the amino acid sequence for mAb LM609-mIgG1-kappa heavy chain (**SEQ ID NO:11**) and light chain (**SEQ ID NO:12**).

[0025] **FIG. 3** shows the amino acid sequence for hLM609-hIgG1-WT (humanized LM609) heavy chain (**SEQ ID NO:9**) and light chain (**SEQ ID NO:10**).

[0026] **FIG. 4** shows the amino acid sequence for two distinct forms of shLM609-hIgG1-WT (super-humanized LM609): LM609_7 Fab domain of heavy chain (**SEQ ID NO:5**) and light

chain (**SEQ ID NO:6**) and JC7U Fab domain of heavy chain (**SEQ ID NO:7**) and light chain (**SEQ ID NO:8**).

[0027] **FIG. 5** shows the amino acid sequence for hLM609-hIgG4-S228P (humanized LM609) heavy chain (**SEQ ID NO:1**) and light chain (**SEQ ID NO:2**).

[0028] **FIG. 6** shows an amino acid sequence alignment for hLM609-hIgG1-WT heavy chain (**SEQ ID NO:9**) vs. hLM609-hIgG4-S228P heavy chain (**SEQ ID NO:1**). Sequence alignment was performed using the Align Sequences Protein BLAST tool from ncbi.nih.gov. The "Query" sequence is hLM609-hIgG1-WT and the "Sbjct" sequence is hLM609-hIgG4-S228P. Sequence differences are shown in boldface.

[0029] **FIG. 7** shows that hLM609-IgG4-S228P engages and activates FcγRI in a cell-based ADCC reporter bioassay. Integrin αvβ3-expressing human pancreatic cancer cells were utilized as "target cells" to assess the ability of anti-αvβ3 antibodies to elicit effector cell activation using a Promega ADCC Reporter Bioassay in which "effector cell" activation is evaluated using a Raji cell line stably expressing the human FcγR I or III and NFAT-induced luciferase. Six antibody dilutions were tested per antibody, with FcγR activation shown as fold change relative to treatment with assay buffer containing no antibody.

[0030] **FIG. 8** shows equivalent blocking of αvβ3-mediated adhesion by hLM609 IgG1 and IgG4-S228P variants. Antibody affinity is evaluated using an in vitro cell adhesion assay. 48 well tissue culture plates were coated with the integrin αvβ3 ligand fibrinogen or the integrin β1 ligand type I collagen, and 2000-10000 cells were added in the presence of each antibody for a range of 2-fold dilutions starting at 5 μg/mL in duplicate. Plates were washed at the endpoint and cells attached to the substrate detected using crystal violet.

[0031] **FIGS. 9A-9C** show in vitro ADCC by NK cells (NK-ADCC) and macrophages (Mac-ADCC). (A) In vitro NK-ADCC to compare hIgG4-S228P vs. hIgG1-WT isotypes of hLM609. Luminescence-based cell killing assay in which CD16-V176.NK92 cells were engaged to kill HCC827+β3 target cells. Graphs show effect of increasing effector-to-target ratio (E:T). Target cells: HCC827+β3 human lung cancer; Effector cells: CD16-V176.NK92. (B) In vitro Macrophage-ADCC to compare hIgG4-S228P vs. hIgG1-WT isotypes of hLM609. Primary human macrophages were isolated from blood from two different healthy donors and used as effector cells in killing assays for H1975 target cells with endogenous β3 expression. Target cells: H1975 human lung cancer (endogenous β3); Effector cells: Primary human macrophages isolated from normal donor blood; Donor 980-A has CD32 high affinity variant (H131) and CD16 low affinity variant (F158); Variant genotype not determined for Donor 980-B. (C) In vitro Macrophage-ADCC induced by hLM609-hIgG4-S228P using macrophages

isolated from multiple donors. Primary human macrophages were isolated from blood from three different healthy donors and used as effector cells in killing assays for HCC827+β3 target cells. Target cells: HCC827+β3 human lung cancer; Effector cells: Primary human macrophages isolated from normal donor blood.

[0032] FIGS. 10A-10B show that LM609 and hLM609-hIgG4-S228P induce ADCC mediated by macrophages, but not NK cells, isolated from healthy blood donors. (A) In vitro ADCC for primary human monocyte-derived macrophages as effector cells. (B). In vitro ADCC for human NK cells as effector cells. Graphs show effect of increasing effector-to-target ratio (E:T) on death of αvβ3-expressing human lung cancer cells.

[0033] FIG. 11 shows in vitro ADCC by mouse bone marrow derived macrophages. In vitro ADCC for mouse primary macrophage effector cells. Primary mouse macrophages were isolated from mouse bone marrow and used as effector cells to kill HCC827+β3 target cells.

[0034] Fig. 12 shows that hLM609-hIgG4-S228P inhibits growth of αvβ3-expressing tumors in mice with no body weight loss over two weeks of treatment. Human pancreatic cancer cells expressing αvβ3 were subcutaneously injected to the flank region of nu/nu mice. Tumor dimensions were measured twice weekly using calipers. Once the tumors were palpable (approximately 150 mm^3), the mice were randomly assigned to groups. The mice were treated with either PBS (vehicle, n = 8), LM609 (10 mg/kg, n = 8), or hLM609-IgG4-S228P (10 mg/kg, n = 9) on day 0, 4, 7, and 11. Body weight was measured on day 0, 7, and 14. Error bars show standard error, *P<0.05, **P<0.01 compared to PBS using one-way ANOVA.

[0035] Fig. 13 shows that the anti-tumor activity of hLM609-hIgG4-S228P is superior to hLM609-hIgG1 for xenografts in mice. Human αvβ3+ pancreatic cancer cells were subcutaneously injected to nu/nu mice. Tumor dimensions were measured twice weekly using calipers. Once tumors were palpable (approximately 100 mm^3), mice were dosed twice weekly with: PBS (vehicle, n=13), hLM609-hIgG1 (10 mg/kg, n=8), or hLM609-hIgG4-S228P (10 mg/kg, n=9). *P < 0.05 compared to PBS using One way-ANOVA.

[0036] Fig. 14 shows that the tumor accumulation of hLM609-hIgG1 to hLM609-hIgG4-S228P is superior to hLM609-hIgG1 for xenografts in mice. Nude mice injected with FG-β3 cells (human pancreatic cancer cells that express αvβ3) were randomly divided into 3 groups. The mice were treated with PBS, hLM609-hIgG4-S228P (10 mg/kg, i.p.), or hLM609-hIgG1 (10 mg/kg, i.p.) at 10 mg/kg twice per week for 14 days. 30 min after the last dosing, animals were sacrificed, and tumor tissues were collected and stored at -80°C until further analyses. The tumor tissues were lysed in RPMI at 6.4 μL/mg. The concentration of hLM609-hIgG4-

S228P and hLM609-hIgG1 in the lysates were measured using a human IgG ELISA kit (Thermo). *P < 0.001 compared to PBS using Bonferroni and Tukey tests.

DETAILED DESCRIPTION

[0037] The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

[0038] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0039] 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. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

[0040] Except as otherwise indicated, standard methods known to those skilled in the art may be used for production of recombinant and synthetic polypeptides, antibodies or antigen-binding fragments thereof, manipulation of nucleic acid sequences, and production of transformed cells. Such techniques are known to those skilled in the art. See, e.g., SAMBROOK et al., MOLECULAR CLONING: A LABORATORY MANUAL 4th Ed. (Cold Spring Harbor, N.Y., 2012); F. M. AUSUBEL et al. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

[0041] All publications, patent applications, patents, nucleotide sequences, amino acid sequences and other references mentioned herein are incorporated by reference in their entirety.

Definitions

[0042] As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0043] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0044] Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted.

[0045] Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound or agent of this invention, dose, time, temperature, and the like, is meant to encompass variations of ±10%, ±5%, ±1%, ±0.5%, or even ±0.1% of the specified amount.

[0046] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently-disclosed subject matter.

[0047] As used herein, ranges can be expressed as from "about" one particular value, and/or to "about" another particular value. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units is also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0048] The transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim, and those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

[0049] The term "consists essentially of" (and grammatical variants), as applied to a polynucleotide or polypeptide sequence of this invention, means a polynucleotide or polypeptide that consists of both the recited sequence (*e.g.,* SEQ ID NO) and a total of ten or

less (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional nucleotides or amino acids on the 5' and/or 3' or N-terminal and/or C-terminal ends of the recited sequence or between the two ends (*e.g.*, between domains) such that the function of the polynucleotide or polypeptide is not materially altered. The total of ten or less additional nucleotides or amino acids includes the total number of additional nucleotides or amino acids added together.

[0050] As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

[0051] The term "chimeric" refers to a molecule having two or more portions that are not naturally found together in the same molecule.

[0052] A "nucleic acid" or "nucleotide sequence" is a sequence of nucleotide bases, and may be RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotide) but is preferably either single or double stranded DNA sequences.

[0053] As used herein, the term "isolated" means a molecule, e.g., a protein, polynucleotide, or cell, separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell structural components or other polypeptides or nucleic acids commonly found associated with the molecule. The term also encompasses molecules that have been prepared synthetically.

[0054] By the terms "treat," "treating," or "treatment of" (or grammatically equivalent terms) it is meant that the severity of the subject's condition is reduced or at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one clinical symptom is achieved and/or there is a delay in the progression of the condition.

[0055] As used herein, the terms "prevent," "prevents," or "prevention" and "inhibit," "inhibits," or "inhibition" (and grammatical equivalents thereof) are not meant to imply complete abolition of disease and encompasses any type of prophylactic treatment that reduces the incidence of the condition, delays the onset of the condition, and/or reduces the symptoms associated with the condition after onset.

[0056] An "effective," "prophylactically effective," or "therapeutically effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, an "effective," "prophylactically effective," or "therapeutically effective" amount is an amount that will provide some delay, alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the effects need not be complete or curative, as long as some benefit is provided to the subject.

[0057] As used herein, the term "bind specifically" or "specifically binds" in reference to a chimeric binding agent of the invention means that the agent will bind with an epitope (including one or more epitopes) of a target, but does not substantially bind to other unrelated epitopes or molecules. In certain embodiments, the term refers to an agent that exhibits at least about 60% binding, e.g., at least about 70%, 80%, 90%, or 95% binding, to the target epitope relative to binding to other unrelated epitopes or molecules.

Chimeric binding agents

[0058] A first aspect of the invention relates to a chimeric binding agent comprising a first domain that specifically binds to an antigen on an epithelial cancer cell expressing at least one mesenchymal cell marker and a second domain that mediates antibody-dependent cellular cytotoxicity (ADCC) by engaging a myeloid-derived cell that accumulates in mesenchymal tumors.

[0059] A myeloid-derived cell that accumulates in mesenchymal tumors is a cell type that is enriched in epithelial cell tumors as they undergo an epithelial-to-mesenchymal transition. In some embodiments, the level of the myeloid-derived cell in the tumor increases by 2-fold, 5-fold, 10-fold or more relative to the level before the transition. In some embodiments, the myeloid-derived cell is a macrophage, dendritic cell, or a granulocyte, such as a neutrophil, basophil, eosinophil, or mast cell. In some embodiments, the myeloid-derived cell is a macrophage.

[0060] The epithelial cancer may be any known type of carcinoma. Examples of epithelial cancers include, without limitation, cancers of the gastrointestinal tract, breast, lungs (e.g., non-small cell lung cancer), colon, prostate, or bladder. In some embodiments, the epithelial cancer cell is a late-stage epithelial cancer cell. Late stage or advanced stage, as used herein, refers to stage III or stage IV cancers based on the TNM staging system. In some embodiments, the epithelial cancer cell has at least partially transitioned to a mesenchymal cell, *e.g.*, expresses one or more mesenchymal antigens. In certain embodiments, the epithelial cancer cell is chemotherapy resistant or refractory, which may be due to the epithelial-to-mesenchymal transition.

[0061] The chimeric binding agent may be any structure that is capable of binding to an antigen on the epithelial cancer cell and engaging a myeloid-derived cell to mediate ADCC. In some embodiments, the chimeric binding agent is an antibody or an antigen-binding fragment thereof. In some embodiments, one or more portions of the chimeric binding agent are composed of antibody fragments. In some embodiments, one or both domains of the chimeric

binding agent is a non-immunoglobulin scaffold, an aptamer, a small molecule (*e.g.*, a receptor ligand), or other binding moiety.

[0062] In certain embodiments, the first domain of the chimeric binding agent is an antibody domain. In certain embodiments, the second domain of the chimeric binding agent is an antibody domain. In some embodiments, both domains are antibody domains. In some embodiments, the first domain is a humanized or human antibody domain. In some embodiments, the second domain is a humanized or human antibody domain. In some embodiments, the first domain and the second domain are humanized or human antibody domains.

[0063] In some embodiments, the first domain specifically binds an antigen on the surface of the epithelial cancer cell. In some embodiments, the antigen is a receptor found on the surface of epithelial-like tumor cells, such as, without limitation, EGFR, HER2, EpCAM, E-cadherin, ZO-1, or integrin α6β4. In some embodiments, the antigen is a receptor found on the surface of mesenchymal-like tumor cells, such as, without limitation, integrin αvβ3, integrin β1, integrin αvβ6, N-cadherin, OB-cadherin, or syndecan-1.

[0064] In some embodiments, the antigen may be one that is not present or present at low levels on the surface of normal epithelial cells. In some embodiments, the antigen may be one that is not present or present at low levels on the surface of epithelial cancer cells. In some embodiments, the antigen may be one that that is present or present at increased levels only after the epithelial cancer cell begins to transition to a mesenchymal cell. In some embodiments, the antigen is a mesenchymal cell antigen that is not present or only present at low levels on the epithelial cancer cell until it begins to transition to a mesenchymal cell. In some embodiments, the antigen is a neoantigen that has not been previously recognized by the immune system.

[0065] In certain embodiments, the first domain specifically binds an integrin. The integrin may be, without limitation, integrin αv, integrin β3, or integrin αvβ3.

[0066] In certain embodiments, the first domain comprises, consists essentially of, or consists of a Fab domain of an antibody. The Fab domain may be from any antibody isotype. In some embodiments, the first domain comprises a Fab domain of an IgG antibody, *e.g.*, an IgG1 or IgG4 antibody. In some embodiments, the first domain comprises the amino acid sequence of the light chain of hLM609-hIgG4-S228P (**SEQ ID NO:2**) and the Fab portion (also known as the Fd fragment) of the heavy chain of hLM609-hIgG4-S228P (**SEQ ID NO:3**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto. In some embodiments, the first domain comprises the amino acid

sequence of a superhumanized variant of shLM609-hIgG1-WT, *e.g.*, the LM609_7 Fab domain of heavy chain (**SEQ ID NO:5**) and light chain (**SEQ ID NO:6**) or the JC7U Fab domain of heavy chain (**SEQ ID NO:7**) and light chain (**SEQ ID NO:8**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto. In some embodiments, the first domain comprises the amino acid sequence of the light chain of hLM609-hIgG1-WT (**SEQ ID NO:9**) and the Fab portion of the heavy chain of hLM609-hIgG1-WT (**SEQ ID NO:10**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto.

[0067] In certain embodiments, the first domain may specifically bind a second antigen in addition to an antigen on the surface of the epithelial cancer cell. In some embodiments, the first domain may be a bispecific antibody domain, trispecific antibody domain, or other structure that can specifically bind more than one antigen. The second antigen may be, for example, the binding target of an antibody used to treat cancer, e.g., an immune checkpoint molecule such as PD-1, PD-L1, or CTLA-4. In some embodiments, the second antigen is a cancer stem cell marker (e.g., CD133, CD44, CD90, CD117, CD166, CD105). In some embodiments, the second antigen is an antigen on an effector cell that is different from the effector cell targeted by the second domain. In some embodiments, the different effector cell is a myeloid-derived cell, e.g., a macrophage, dendritic cell, or a granulocyte, such as a neutrophil, basophil, eosinophil, or mast cell. In this aspect, the chimeric binding agent is capable of localizing two or more classes of effector cells to the tumor cells, e.g., macrophages and dendritic cells or macrophages and neutrophils.

[0068] The second domain of the chimeric binding agent preferably engages one or more types of myeloid-derived cells. In some embodiments, the second domain predominately engages one type of myeloid-derived cells, e.g., macrophages or dendritic cells or granulocytes, such as a neutrophils, basophils, eosinophils, or mast cells. In some embodiments, the second domain predominately engages macrophages. "Predominantly engage," as used herein, refers to engaging at least 80% of the target cell type, *e.g.*, macrophages, relative to other cell types, *e.g.*, at least 85%, 90%, or 95%.

[0069] In certain embodiments, the second domain does not significantly engage natural killer (NK) cells. In certain embodiments, the second domain does not significantly engage one or more types of lymphocytes, e.g., NK cells, B cells, or T cells. "Does not significantly engage," as used herein, refers to less than 30% of the total engaged cells being the indicated cell type, *e.g.*, less than 25%, 20%, 15%, 10%, or 5%.

[0070] In some embodiments, the second domain specifically binds a protein on the surface of the myeloid-derived cell. The protein is one that can mediate ADCC when engaged. In some embodiments, the protein is not present or only present at low levels on other cell types, *e.g.*, natural killer cells. In some embodiments, the second domain specifically binds to an Fc-gamma receptor. In some embodiments, the second domain specifically binds Fc-gamma receptor I (FcγRI, CD64).

[0071] In certain embodiments, the second domain comprises, consists essentially of, or consists of a Fc domain of an antibody. The Fc domain may be from any antibody isotype. In some embodiments, the second domain comprises a Fc domain of an IgG antibody, *e.g.*, an IgG4 antibody. In some embodiments, the second domain comprises a Fc domain of an IgA or IgE antibody. In certain embodiments, the second domain further comprises a hinge domain of an antibody. In some embodiments, the second domain comprises the amino acid sequence of the heavy chain Fc domain and hinge domain of hLM609-hIgG4-S228P (**SEQ ID NO:4**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto. In some embodiments, the second domain comprises the amino acid sequence of the heavy chain Fc domain and hinge domain of hLM609-hIgG1-WT (**SEQ ID NO:9**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto.

[0072] In certain embodiments, the chimeric binding agent comprises the amino acid sequence of the hLM609-hIgG4-S228P heavy chain (**SEQ ID NO:1**) and light chain (**SEQ ID NO:2**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto. In certain embodiments, the chimeric binding agent comprises the amino acid sequence of the hLM609-hIgG1-WT heavy chain (**SEQ ID NO:9**) and light chain (**SEQ ID NO:10**) or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical thereto.

[0073] The chimeric binding agent may include sequence modifications that are known to enhance the characteristics of an antibody, *e.g.*, stability, or alter the binding of the antibody to Fc-gamma receptors. In some embodiments, the amino acid sequence of the chimeric binding agent comprises a S228P (Eu numbering system) mutation in the hinge region. In some embodiments, the amino acid sequence comprises a mutation selected from:

a) S239D/A330L/I332E;

b) I332E;

c) G236A/S239D/I332E;

d) G236A;

e) N297A/E382V/M428I;

f) M252Y/S254T/T256E;

g) Q295R/L328W/A330V/P331A/I332Y/E382V/M428I;

h) L234A/L235A/P329G;

i) M428L/N434S;

j) L234A/L235A/P331S;

k) L234A/L235A/P329G/M252Y/S254T/T256E;

l) S298A/E333A/K334/A;

m) S239D/I332E;

n) G236A/S239D/A330L/I332E;

o) S239D/I332E/G236A;

p) L234Y/G236W/S298A;

q) F243L/R292P/Y300L/V305I/P396L;

r) K326W/E333S;

s) K326A/E333A;

t) K326M/E333S;

u) C221D/D222C;

v) S267E/H268F/S324W;

w) H268F/S324W;

x) E345R

y) R435H;

z) N434A;

aa) M252Y/S254T/T256E;

ab) M428L/N434S;

ac) T252L/T/253S/T254F;

ad) E294delta/T307P/N434Y;

ae) T256N/A378V/S383N/N434Y;

af) E294delta

ag) L235E;

ah) L234A/L235A;

ai) S228P/L235E;

aj) P331S/L234E/L225F;

ak) D265A;

al) G237A;

am) E318A;

an) E233P;

ao) G236R/L328R;

ap) H268Q/V309L/A330S/P331S;

aq) L234A/L235A/G237A/P238S/H268A/A330S/P331S;

ar) A330L;

as) D270A;

at) K322A;

au) P329A;

av) P331A;

aw V264A;

ax) F241A;

ay) N297A or G or N

az) S228P/F234A/L235A; or

ba) any combination of a) to az);

(Eu numbering system) with or without the S228P mutation.

[0074] The following discussion is presented as a general overview of the techniques available for the production of antibodies; however, one of skill in the art will recognize that many variations upon the following methods are known.

[0075] The term "antibody" or "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibody can be monoclonal, oligoclonal, or polyclonal and can be of any species of origin, including (for example) mouse, rat, hamster, rabbit, horse, cow, goat, sheep, pig, camel, monkey, or human, or can be a chimeric or humanized antibody. See, e.g., Walker et al., *Molec. Immunol.* 26:403 (1989). The antibodies can be recombinant monoclonal antibodies produced according to the methods disclosed in U.S. Pat. No. 4,474,893 or U.S. Pat. No. 4,816,567. The antibodies can also be chemically constructed according to the method disclosed in U.S. Pat. No. 4,676,980.

[0076] Antibody fragments included within the scope of the present invention include, for example, Fab, Fab', $F(ab)_2$, and Fv fragments; domain antibodies, diabodies; vaccibodies, linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Such fragments can be produced by known techniques. For example, $F(ab')_2$ fragments can be produced by pepsin digestion of the antibody molecule, and Fab fragments can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification

of monoclonal Fab fragments with the desired specificity (Huse et al., *Science* 254:1275 (1989)). In some embodiments, the term "antibody fragment" as used herein may also include any protein construct that is capable of binding a target antigen.

[0077] Antibodies of the invention may be altered or mutated for compatibility with species other than the species in which the antibody was produced. For example, antibodies may be humanized or camelized. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab′, $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework (FR) regions (i.e., the sequences between the CDR regions) are those of a human immunoglobulin consensus sequence. The humanized antibody can be a superhumanized antibody where only two CDRs are non-human (US Patent No. 7,087,409). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., *Nature* 321:522 (1986); Riechmann et al., *Nature*, 332:323 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593 (1992)).

[0078] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can essentially be performed following the method of Winter and co-workers (Jones et al., *Nature* 321:522 (1986); Riechmann et al., *Nature* 332:323 (1988); Verhoeyen et al., *Science* 239:1534 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized

antibodies are typically human antibodies in which some CDR residues (e.g., all of the CDRs or a portion thereof) and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0079] Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, *J. Mol. Biol.* 227:381 (1991); Marks et al., *J. Mol. Biol.* 222:581 (1991)). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.* 147:86 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10:779 (1992); Lonberg et al., *Nature* 368:856 (1994); Morrison, *Nature* 368:812 (1994); Fishwild et al., *Nature Biotechnol.* 14:845 (1996); Neuberger, *Nature Biotechnol.* 14:826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65 (1995).

[0080] Immunogens (antigens) are used to produce antibodies specifically reactive with target polypeptides. Recombinant or synthetic polypeptides and peptides, e.g., of at least 5 (e.g., at least 7 or 10) amino acids in length, or greater, are the preferred immunogens for the production of monoclonal or polyclonal antibodies. In one embodiment, an immunogenic polypeptide conjugate is also included as an immunogen. The peptides are used either in pure, partially pure or impure form. Suitable polypeptides and epitopes for target pathogens and sperm are well known in the art. Polynucleotide and polypeptide sequences are available in public sequence databases such as GENBANK®/GENPEPT®. Large numbers of antibodies that specifically bind to target cancer cell antigens have been described in the art and can be used as starting material to prepare the antibodies of the present invention. Alternatively, new antibodies can be raised against target antigens using the techniques described herein and well known in the art.

[0081] Recombinant polypeptides are expressed in eukaryotic or prokaryotic cells and purified using standard techniques. The polypeptide, or a synthetic version thereof, is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal

antibodies can be generated for subsequent use in immunoassays to measure the presence and quantity of the polypeptide.

[0082] Methods of producing polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, e.g., a purified or synthetic peptide, a peptide coupled to an appropriate carrier (e.g., glutathione-S-transferase, keyhole limpet hemocyanin, etc.), or a peptide incorporated into an immunization vector such as a recombinant vaccinia virus is optionally mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the peptide of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the peptide is performed where desired. Antibodies, including binding fragments and single chain recombinant versions thereof, against the polypeptides are raised by immunizing animals, e.g., using immunogenic conjugates comprising a polypeptide covalently attached (conjugated) to a carrier protein as described above. Typically, the immunogen of interest is a polypeptide of at least about 10 amino acids, in another embodiment the polypeptide is at least about 20 amino acids in length, and in another embodiment, the fragment is at least about 30 amino acids in length. The immunogenic conjugates are typically prepared by coupling the polypeptide to a carrier protein (e.g., as a fusion protein) or, alternatively, they are recombinantly expressed in an immunization vector.

[0083] Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies are screened for binding to normal or modified peptides, or screened for agonistic or antagonistic activity. Specific monoclonal and polyclonal antibodies will usually bind with a K_D of at least about 50 mM, e.g., at least about 1 mM, e.g., at least about 0.1 mM or better. In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as rodents, lagomorphs, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies are found in Kohler and Milstein 1975 *Nature* 256:495-497. Summarized briefly, this method proceeds by injecting an animal with an immunogen, e.g., an immunogenic peptide either alone or optionally linked to a carrier protein. The animal is then sacrificed and cells taken from its spleen, which are fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained

are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

[0084] Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells is enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate (preferably mammalian) host. The polypeptides and antibodies of the present invention are used with or without modification, and include chimeric antibodies such as humanized murine antibodies. Other suitable techniques involve selection of libraries of recombinant antibodies in phage or similar vectors. See, Huse et al. 1989 *Science* 246:1275-1281; and Ward et al. 1989 *Nature* 341:544-546.

[0085] Antibodies specific to the target polypeptide can also be obtained by phage display techniques known in the art.

[0086] The present invention additionally provides polynucleotides encoding the chimeric binding agent of this invention. In some embodiments, the polynucleotides comprise a heavy chain encoding nucleotide sequence of **SEQ ID NO:13** and a light chain encoding sequence of **SEQ ID NO:14** or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical. In some embodiments, the polynucleotides comprise a heavy chain encoding nucleotide sequence of **SEQ ID NO:15** and a light chain encoding sequence of **SEQ ID NO:14** or a sequence at least 90% identical thereto, e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical.

[0087] Further provided herein is a vector comprising the polynucleotide of the invention. Vectors include, but are not limited to, plasmid vectors, phage vectors, virus vectors, or cosmid vectors.

[0088] In some embodiments, the present invention provides a host cell comprising the polynucleotide and/or vector of this invention. The host cell can be a eukaryotic or prokaryotic cell and may be used for expressing the chimeric binding agent or other purposes.

[0089] A further aspect of the invention relates to a composition comprising the chimeric binding agent of the invention and a carrier. In some embodiments, the composition is a pharmaceutical composition and the carrier is a pharmaceutically acceptable carrier.

[0090] In some embodiments, the pharmaceutical composition may further comprise an additional therapeutic agent, e.g., a chemotherapeutic agent. Agents useful for treating cancer include, without limitation: 1) vinca alkaloids (*e.g.*, vinblastine, vincristine); 2)

epipodophyllotoxins (*e.g.*, etoposide and teniposide); 3) antibiotics (*e.g.*, dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin), and mitomycin (mitomycin C)); 4) enzymes (*e.g.*, L-asparaginase); 5) biological response modifiers (*e.g.*, interferon-alfa); 6) platinum coordinating complexes (*e.g.*, cisplatin and carboplatin); 7) anthracenediones (*e.g.*, mitoxantrone); 8) substituted ureas (*e.g.*, hydroxyurea); 9) methylhydrazine derivatives (*e.g.*, procarbazine (N-methylhydrazine; MIH)); 10) adrenocortical suppressants (*e.g.*, mitotane (o,p'-DDD) and aminoglutethimide); 11) adrenocorticosteroids (*e.g.*, prednisone); 12) progestins (*e.g.*, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate); 13) estrogens (*e.g.*, diethylstilbestrol and ethinyl estradiol); 14) antiestrogens (*e.g.*, tamoxifen); 15) androgens (*e.g.*, testosterone propionate and fluoxymesterone); 16) antiandrogens (*e.g.*, flutamide): and 17) gonadotropin-releasing hormone analogs (*e.g.*, leuprolide). In another embodiment, the agents of the invention are administered in conjunction with anti-angiogenesis agents, such as antibodies to VEGF (*e.g.*, bevacizumab (AVASTIN), ranibizumab (LUCENTIS)) and other promoters of angiogenesis (*e.g.*, bFGF, angiopoietin-1), antibodies to alpha-v/beta-3 vascular integrin (*e.g.*, VITAXIN), angiostatin, endostatin, dalteparin, ABT-510, CNGRC peptide TNF alpha conjugate, cyclophosphamide, combretastatin A4 phosphate, dimethylxanthenone acetic acid, docetaxel, lenalidomide, enzastaurin, paclitaxel, paclitaxel albumin-stabilized nanoparticle formulation (Abraxane), soy isoflavone (Genistein), tamoxifen citrate, thalidomide, ADH-1 (EXHERIN), AG-013736, AMG-706, AZD2171, sorafenib tosylate, BMS-582664, CHIR-265, pazopanib, PI-88, vatalanib, everolimus, suramin, sunitinib malate, XL184, ZD6474, ATN-161, cilenigtide, and celecoxib, or any combination thereof. In other embodiments, the agents of the invention are administered in conjunction with one or more therapeutic antibodies, e.g., anti-cancer antibodies or antibodies to immune checkpoints. In other embodiments, the agents of the invention are administered in conjunction with one or more immune checkpoint inhibitors. The immune checkpoint inhibitor may be any molecule that inhibits an immune checkpoint. Immune checkpoints are well known in the art and include, without limitation, PD-1, PD-L1, PD-L2, CTLA4, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, A2AR, TIM-3, and VISTA. In some embodiments, the inhibitor is an antibody against the immune checkpoint protein. In certain embodiments, the immune checkpoint inhibitor is an inhibitor of PD-1 or PD-L1, e.g., an antibody that specifically binds PD-1 or PD-L1. In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, ipilimumab, durvalumab, or atezolizumab. In some embodiments, the chimeric binding agent

may be directly or indirectly linked with an additional therapeutic agent to form an antibody drug conjugate.

[0091] An additional aspect of the invention relates to a kit comprising the chimeric binding agent of the invention or cells for producing the chimeric binding agent of the invention. In some embodiments, the kit can include multiple chimeric binding agents and/or compositions containing such agents. In some embodiments, each of multiple chimeric binding agents provided in such a kit can specifically bind to a different antigen and/or engage a different myeloid-derived cell. In some embodiments, the kit can further include an additional active agent, e.g., a chemotherapeutic agent as would be known to one of skill in the art. In some embodiments, the kit can further include additional reagents, buffers, containers, etc.

Methods using chimeric binding agents

[0092] One aspect of the invention relates to a method of targeting a myeloid-derived cell (e.g., a macrophage) to a cancer cell expressing an antigen recognized by the chimeric binding agent of the invention (e.g., integrin αvβ3), comprising contacting the cancer cell and the myeloid-derived cell with an effective amount of the chimeric binding agent of the invention.

[0093] Another aspect of the invention relates to a method of targeting a myeloid-derived cell that accumulates in mesenchymal tumors to an epithelial cancer cell expressing at least one mesenchymal cell marker, comprising contacting the cancer cell and the myeloid-derived cell with an effective amount of the chimeric binding agent of the invention.

[0094] A further aspect of the invention relates to a method of treating a cancer expressing an antigen recognized by the chimeric binding agent of the invention (e.g., integrin αvβ3) in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the invention to the subject, thereby treating the cancer.

[0095] An additional aspect of the invention relates to a method of treating an epithelial cell cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the invention to the subject, thereby treating the epithelial cell cancer.

[0096] Another aspect of the invention relates to a method of treating a cancer in a subject in need thereof, comprising the steps of:

a) selecting a subject having cancer cells that are enriched for an antigen specifically bound by the chimeric binding agent of the invention (e.g., integrin αvβ3) and enriched for myeloid-derived cells; and

b) administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the invention to the subject, thereby treating the cancer.

[0097] A further aspect of the invention relates to a method of treating an epithelial cell cancer in a subject in need thereof, comprising the steps of:

a) selecting a subject having epithelial cancer cells that are enriched for an antigen specifically bound by the chimeric binding agent of the invention and enriched for myeloid-derived cells that accumulate in mesenchymal tumors; and

b) administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the invention to the subject, thereby treating the epithelial cell cancer.

[0098] The term "enriched", as used herein, refers to a level of antigen on a cancer cell or level of myeloid-derived cells in a tumor that is greater than the level found in the cancer cell or tumor at an earlier point in time (e.g., prior to the start of the EMT) or greater than the average level found in similar cancer cells or tumors at a similar stage in the general population.

[0099] The selection step may be carried out by any method known to measure antigens and cells. In some embodiments, step a) comprises obtaining a sample of the cancer from the subject and measuring the level of antigen and myeloid-derived cells in the sample. The level of antigen may be measured by, e.g., an immunoassay, protein analysis, RNA analysis, or immunohistochemistry. The level of myeloid-derived cells may be measured by, e.g., an immunoassay, protein analysis, RNA analysis, or flow cytometry.

[0100] Another aspect of this invention relates to antigen-effector cell matching of tumors such that the antigen is specifically present on the tumor cell (e.g., a tumor cell antigen) and a therapeutic antibody contains effector cell binding regions that are specific to those effector cells found in the tumor (e.g., neutrophils, dendritic cells, NK cells etc.).

[0101] As one embodiment of the methods of the invention, the inventors have determined that the αvβ3 integrin appears on the surface of cancer cells that have gained drug resistance. This helps identify those patients most likely to be effectively treated with therapeutic monoclonal antibody approaches that are directed against αvβ3 integrin. This provides a precision medicine approach to the correct patient populations that allows one to include other therapeutic monoclonal antibodies that target αvβ3 integrin. As cancer patients become resistant to standard of care therapeutics their tumors gain αvβ3 expression and thereby become candidates for treatment with an αvβ3 targeted antibody that is capable of promoting immune cell mediated ADCC of the αvβ3 expressing tumor cells.

[0102] In the methods of the invention, the myeloid-derived cell is a macrophage, dendritic cell, or granulocyte, such as a neutrophil, basophil, eosinophil, or mast cell. In some embodiments, the myeloid-derived cell is a macrophage.

[0103] The epithelial cancer may be any known type of carcinoma. Examples of epithelial cancers include, without limitation, cancers of the gastrointestinal tract, breast, lungs (e.g., non-small cell lung cancer), colon, prostate, or bladder. In some embodiments, the epithelial cancer cell is a late stage epithelial cancer cell. In some embodiments, the epithelial cancer cell has at least partially transitioned to a mesenchymal cell, *e.g.*, expresses one or more mesenchymal antigens. In certain embodiments, the epithelial cancer cell is chemotherapy resistant or refractory, which may be due to the epithelial-to-mesenchymal transition.

[0104] In some embodiments, the methods may further comprise the step of isolating myeloid-derived cells from the subject, contacting the myeloid-derived cells with the chimeric binding agent or pharmaceutical composition, and administering the contacted myeloid-derived cells to the subject.

[0105] In some embodiments, more than one chimeric binding agent may be delivered to a subject. For example, if a cancer sample shows expression of more than one targetable antigen or more than one type of myeloid-derived cell is enriched in the cancer, agents targeting each of the antigens and/or myeloid-derived cells may be administered. In some embodiments, the chimeric binding agent may be multispecific (e.g., bispecific or trispecific) in order to engage multiple targetable antigens and/or more than one type of myeloid-derived cell.

[0106] The methods of the invention may further comprise administering to the subject an additional cancer therapeutic agent or treatment (e.g., surgery, radiation). Cancer therapeutic agents include, without limitation, 1) vinca alkaloids (e.g., vinblastine, vincristine); 2) epipodophyllotoxins (e.g., etoposide and teniposide); 3) antibiotics (e.g., dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin), and mitomycin (mitomycin C)); 4) enzymes (e.g., L-asparaginase); 5) biological response modifiers (e.g., interferon-alfa); 6) platinum coordinating complexes (e.g., cisplatin and carboplatin); 7) anthracenediones (e.g., mitoxantrone); 8) substituted ureas (e.g., hydroxyurea); 9) methylhydrazine derivatives (e.g., procarbazine (N-methylhydrazine; MIH)); 10) adrenocortical suppressants (e.g., mitotane (o,p'-DDD) and aminoglutethimide); 11) adrenocorticosteroids (e.g., prednisone); 12) progestins (e.g., hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate); 13) estrogens (e.g., diethylstilbestrol and ethinyl estradiol); 14) antiestrogens (e.g., tamoxifen); 15) androgens (e.g., testosterone propionate and fluoxymesterone); 16) antiandrogens (e.g., flutamide): and

17) gonadotropin-releasing hormone analogs (e.g., leuprolide). Other cancer therapeutic agents include, without limitation, anti-angiogenesis agents, such as antibodies to VEGF (e.g., bevacizumab (AVASTIN), ranibizumab (LUCENTIS)) and other promoters of angiogenesis (e.g., bFGF, angiopoietin-1), angiostatin, endostatin, dalteparin, ABT-510, CNGRC peptide TNF alpha conjugate, cyclophosphamide, combretastatin A4 phosphate, dimethylxanthenone acetic acid, docetaxel, lenalidomide, enzastaurin, paclitaxel, paclitaxel albumin-stabilized nanoparticle formulation (Abraxane), soy isoflavone (Genistein), tamoxifen citrate, thalidomide, ADH-1 (EXHERIN), AG-013736, AMG-706, AZD2171, sorafenib tosylate, BMS-582664, CHIR-265, pazopanib, PI-88, vatalanib, everolimus, suramin, sunitinib malate, XL184, ZD6474, ATN-161, cilenigtide, and celecoxib.

[0107] In some embodiments, the methods further comprise administering to the subject a CD47 blocking agent to enhance phagocytosis of the cancer cells. Such agents include CD47-blocking monoclonal antibodies (Hu5F9-G4, CC-90002, Ti-061, or SRF231) or SIRPα-Fc fusion proteins (TTI-621, TTI-622, ALX148). However, one of the advantages of the present invention is that the method is effective against cancers whether or not the cancer cells express CD47. Thus, in some embodiments, the methods of the invention are used to treat cancers that express CD47. in some embodiments, the methods of the invention are used to treat cancers that express CD47. In some embodiments, the methods of the invention do not comprise administering to the subject a CD47 blocking agent.

[0108] In some embodiments, the methods further comprise administering to the subject an immune checkpoint inhibitor. Immune checkpoints are well known in the art and include, without limitation, PD-1, PD-L1, PD-L2, CTLA4, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, A2AR, TIM-3, and VISTA. In some embodiments, the inhibitor is an antibody against the immune checkpoint protein. In certain embodiments, the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, or CTLA-4 that are enriched in mesenchymal tumors, e.g., an antibody that specifically binds PD-1, PD-L1, or CTLA-4. In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, ipilimumab, durvalumab, or atezolizumab.

[0109] In some embodiments, the methods further comprise administering to the subject an EGFR inhibitor. Such agents include tyrosine kinase inhibitors (e.g., erlotinib, gefitinib, lapatinib, Osimertinib, neratinib) and monoclonal antibodies (e.g., cetuximab, necitumumab, panitumumab).

[0110] In certain embodiments, the chimeric binding agents used in the methods of the present invention are administered directly to a subject. In some embodiments, the chimeric binding agents will be suspended in a pharmaceutically-acceptable carrier (*e.g.*, physiological

saline) and administered orally or by intravenous infusion, or administered subcutaneously, intramuscularly, intrathecally, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. In another embodiment, the intratracheal or intrapulmonary delivery can be accomplished using a standard nebulizer, jet nebulizer, wire mesh nebulizer, dry powder inhaler, or metered dose inhaler. The agents can be delivered directly to the site of the disease or disorder, such as lungs, kidney, or intestines, e.g., injected in situ into or near a tumor. The dosage required depends on the choice of the route of administration; the nature of the formulation; the nature of the patient's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages for each agent are in the range of 0.01-100 μg/kg. Wide variations in the needed dosage are to be expected in view of the variety of agents available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by i.v. injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (*e.g.*, 2-, 3-, 4-, 6-, 8-, 10-; 20-, 50-, 100-, 150-, or more fold). Encapsulation of the compound in a suitable delivery vehicle (*e.g.*, polymeric microparticles or nanoparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

[0111] By "pharmaceutically acceptable" it is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material can be administered to a subject without causing any undesirable biological effects such as toxicity.

[0112] The formulations of the invention can optionally comprise medicinal agents, pharmaceutical agents, carriers, adjuvants, dispersing agents, diluents, and the like.

[0113] The chimeric binding agents of the invention can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.,* Remington, *The Science and Practice of Pharmacy* (21st Ed. 2006). In the manufacture of a pharmaceutical formulation according to the invention, the agent is typically admixed with, *inter alia*, an acceptable carrier. The carrier can be a solid or a liquid, or both, and may be formulated with the agent as a unit-dose formulation, for example, a capsule or vial, which can contain from 0.01 or 0.5% to 95% or 99% by weight of the agent. One or more agents can be incorporated in the formulations of the invention, which can be prepared by any of the well-known techniques of pharmacy.

[0114] The formulations of the invention include those suitable for oral, rectal, topical, buccal (*e.g.*, sub-lingual), vaginal, parenteral (*e.g.*, subcutaneous, intramuscular including skeletal

muscle, cardiac muscle, diaphragm muscle and smooth muscle, intradermal, intravenous, intraperitoneal), topical (*i.e.*, both skin and mucosal surfaces, including airway surfaces), intranasal, transdermal, intraarticular, intrathecal, and inhalation administration, administration to the liver by intraportal delivery, as well as direct organ injection (*e.g.*, into the liver, into the brain for delivery to the central nervous system, or into the pancreas) or injection into a body cavity. The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular agent which is being used.

[0115] For injection, the carrier will typically be a liquid, such as sterile pyrogen-free water, pyrogen-free phosphate-buffered saline solution, bacteriostatic water, or Cremophor EL[R] (BASF, Parsippany, N.J.). For other methods of administration, the carrier can be either solid or liquid.

[0116] For oral administration, the agent can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Agents can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that can be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric- coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0117] Formulations suitable for buccal (sub-lingual) administration include lozenges comprising the agent in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the agent in an inert base such as gelatin and glycerin or sucrose and acacia.

[0118] Formulations of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the agent, which preparations are preferably isotonic with the blood of the intended recipient. These preparations can contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions can include suspending agents and thickening agents. The formulations can be presented in unit/dose or

multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

[0119] Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, in one aspect of the present invention, there is provided an injectable, stable, sterile composition comprising an agent of the invention, in a unit dosage form in a sealed container. The agent is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 1 mg to about 10 grams of the agent. When the agent is substantially water-insoluble, a sufficient amount of emulsifying agent which is pharmaceutically acceptable can be employed in sufficient quantity to emulsify the agent in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

[0120] Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These can be prepared by admixing the agent with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

[0121] Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

[0122] Formulations suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration can also be delivered by iontophoresis (*see*, for example, Tyle, *Pharm. Res. 3*:318 (1986)) and typically take the form of an optionally buffered aqueous solution of the compounds. Suitable formulations comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M of the compound.

[0123] The agent can alternatively be formulated for nasal administration or otherwise administered to the lungs of a subject by any suitable means, *e.g.*, administered by an aerosol suspension of respirable particles comprising the agent, which the subject inhales. The respirable particles can be liquid or solid. The term "aerosol" includes any gas-borne suspended phase, which is capable of being inhaled into the bronchioles or nasal passages. Specifically, aerosol includes a gas-borne suspension of droplets, as can be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. Aerosol also includes a dry powder

composition suspended in air or other carrier gas, which can be delivered by insufflation from an inhaler device, for example. *See* Ganderton & Jones, *Drug Delivery to the Respiratory Tract*, Ellis Horwood (1987); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn *et al., J. Pharmacol. Toxicol. Meth.* 27:143 (1992). Aerosols of liquid particles comprising the agent can be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the agent can likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0124] Alternatively, one can administer the compound in a local rather than systemic manner, for example, in a depot or sustained-release formulation.

[0125] Further, the present invention provides liposomal formulations of the agents disclosed herein and salts thereof. The technology for forming liposomal suspensions is well known in the art. When the compound or salt thereof is an aqueous-soluble salt, using conventional liposome technology, the same can be incorporated into lipid vesicles. In such an instance, due to the water solubility of the agent, the agent will be substantially entrained within the hydrophilic center or core of the liposomes. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. When the compound or salt of interest is water-insoluble, again employing conventional liposome formation technology, the salt can be substantially entrained within the hydrophobic lipid bilayer which forms the structure of the liposome. In either instance, the liposomes which are produced can be reduced in size, as through the use of standard sonication and homogenization techniques.

[0126] The liposomal formulations containing the agent can be lyophilized to produce a lyophilizate which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

[0127] In the case of water-insoluble agents, a pharmaceutical composition can be prepared containing the water-insoluble agent, such as for example, in an aqueous base emulsion. In such an instance, the composition will contain a sufficient amount of pharmaceutically acceptable emulsifying agent to emulsify the desired amount of the agent. Particularly useful emulsifying agents include phosphatidyl cholines and lecithin.

[0128] In particular embodiments, the compound is administered to the subject in a therapeutically effective amount, as that term is defined above. Dosages of pharmaceutically active agents can be determined by methods known in the art, *see, e.g., Remington's*

Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa). The therapeutically effective dosage of any specific agent will vary somewhat from agent to agent, and patient to patient, and will depend upon the condition of the patient and the route of delivery. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the agent. Toxicity concerns at the higher level can restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the agent. A dosage from about 10 mg/kg to about 50 mg/kg can be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg can be employed for intramuscular injection. Particular dosages are about 1 μmol/kg to 50 μmol/kg, and more particularly to about 22 μmol/kg and to 33 μmol/kg of the agent for intravenous or oral administration, respectively.

[0129] In particular embodiments of the invention, more than one administration (*e.g.*, two, three, four, or more administrations) can be employed over a variety of time intervals (*e.g.*, hourly, daily, weekly, monthly, *etc.*) to achieve therapeutic effects.

[0130] The present invention finds use in veterinary and medical applications. Suitable subjects include both avians and mammals, with mammals being preferred. The term "mammal" as used herein includes, but is not limited to, humans, primates, bovines, ovines, caprines, equines, felines, canines, lagomorphs, *etc.* Human subjects include neonates, infants, juveniles, and adults. The subject may be one in need of the methods of the invention, e.g., a subject that has or is suspected of having cancer. The subject may be a laboratory animal, e.g., an animal model of a disease.

[0131] Non-limiting embodiments of the invention include the following.

[0132] Embodiment 1. A chimeric binding agent comprising a first domain that specifically binds to an antigen on an epithelial cancer cell expressing at least one mesenchymal cell marker and a second domain that mediates antibody-directed cellular cytotoxicity (ADCC) by engaging a myeloid-derived cell that accumulates in mesenchymal tumors.

[0133] Embodiment 2. The chimeric binding agent of embodiment 1, wherein the myeloid-derived cell is a macrophage, dendritic cell, or granulocyte, such as a neutrophil, basophil, eosinophil, or mast cell.

[0134] Embodiment 3. The chimeric binding agent of embodiment 1 or 2, wherein the epithelial cancer cell is a late stage epithelial cancer cell.

[0135] Embodiment 4 The chimeric binding agent of embodiment 3, wherein the epithelial cancer cell has at least partially transitioned to a mesenchymal cell.

[0136] Embodiment 5. The chimeric binding agent of any one of embodiments 1-4, wherein the epithelial cancer cell is chemotherapy resistant or refractory.

[0137] Embodiment 6. The chimeric binding agent of any one of embodiments 1-5, wherein the first domain is an antibody domain.

[0138] Embodiment 7. The chimeric binding agent of any one of embodiments 1-6, wherein the second domain is an antibody domain.

[0139] Embodiment 8. The chimeric binding agent of any one of embodiments 1-7, wherein the first domain is a humanized or human antibody domain.

[0140] Embodiment 9. The chimeric binding agent of any one of embodiments 1-8, wherein the second domain is a humanized or human antibody domain.

[0141] Embodiment 10. The chimeric binding agent of any one of embodiments 1-9, which is a chimeric antibody or an antigen-binding fragment thereof.

[0142] Embodiment 11. The chimeric binding agent of any one of embodiments 1-10, wherein the first domain specifically binds an integrin.

[0143] Embodiment 12. The chimeric binding agent of embodiment 11, wherein the integrin is integrin αv.

[0144] Embodiment 13. The chimeric binding agent of embodiment 11, wherein the integrin is integrin β3.

[0145] Embodiment 14. The chimeric binding agent of embodiment 11, wherein the integrin is integrin αvβ3.

[0146] Embodiment 15. The chimeric binding agent of any one of embodiments 1-14, wherein the first domain specifically binds an antigen on the surface of a cancer cell, including receptors on the surface of epithelial-like tumor cells (such as EGFR, HER2, EpCAM, E-cadherin, ZO-1, integrin α6β4) or mesenchymal-like tumor cells (such as integrin αvβ3, integrin β1, integrin αvβ6, N-cadherin, OB-cadherin, syndecan-1).

[0147] Embodiment 16. The chimeric binding agent of any one of embodiments 1-14, wherein the first domain specifically binds a neoantigen that has not been previously recognized by the immune system.

[0148] Embodiment 17. The chimeric binding agent of any one of embodiments 1-16, wherein the first domain comprises a Fab domain of an antibody.

[0149] Embodiment 18. The chimeric binding agent of embodiment 17, wherein the first domain comprises a Fab domain of an IgG antibody.

[0150] Embodiment 19. The chimeric binding agent of embodiment 18, wherein the first domain comprises a Fab domain of an IgG4 antibody.

[0151] Embodiment 20. The chimeric binding agent of embodiment 19, wherein the first domain comprises the amino acid sequence of the light chain of hLM609-hIgG4-S228P (SEQ ID NO:2) or a sequence at least 90% identical thereto and the Fab portion of the heavy chain of hLM609-hIgG4-S228P (SEQ ID NO:3) or a sequence at least 90% identical thereto.

[0152] Embodiment 21. The chimeric binding agent of embodiment 19, wherein the first domain comprises the amino acid sequence of the Fab portion of the heavy chain of LM609_7 (SEQ ID NO:5) or a sequence at least 90% identical thereto and the light chain of LM609_7 (SEQ ID NO:6) or a sequence at least 90% identical thereto, the Fab portion of the heavy chain of JC7U (SEQ ID NO:7) or a sequence at least 90% identical thereto and the light chain of JC7U (SEQ ID NO:8), or a sequence at least 90% identical thereto.

[0153] Embodiment 22. The chimeric binding agent of any one of embodiments 1-19, wherein the first domain further specifically binds a second antigen.

[0154] Embodiment 23. The chimeric binding agent of embodiment 22, wherein the first domain is a bispecific antibody domain.

[0155] Embodiment 24. The chimeric binding agent of embodiment 22 or 23, wherein the second antigen is an immune checkpoint molecule, such as PD-1, PD-L1, or CTLA-4.

[0156] Embodiment 25. The chimeric binding agent of embodiment 22 or 23, wherein the second antigen is a cancer stem cell marker, such as CD133, CD44, CD90, CD117, CD166, or CD105, or an effector cell antigen.

[0157] Embodiment 26. The chimeric binding agent of embodiment 22 or 23, wherein the second antigen is an effector cell antigen.

[0158] Embodiment 27. The chimeric binding agent of any one of embodiments 1-26, wherein the second domain engages macrophages.

[0159] Embodiment 28. The chimeric binding agent of any one of embodiments 1-27, wherein the second domain does not significantly engage natural killer cells.

[0160] Embodiment 29. The chimeric binding agent of any one of embodiments 1-28, wherein the second domain does not significantly engage lymphocytes.

[0161] Embodiment 30. The chimeric binding agent of any one of embodiments 1-29, wherein the second domain specifically binds a protein on the surface of the myeloid-derived cell.

[0162] Embodiment 31. The chimeric binding agent of embodiment 30, wherein the second domain specifically binds an Fc-gamma receptor.

[0163] Embodiment 32. The chimeric binding agent of embodiment 30, wherein the second domain specifically binds Fc-gamma receptor I (FcγRI, CD64).

[0164] Embodiment 33. The chimeric binding agent of any one of embodiments 1-32, wherein the second domain comprises an Fc domain of an antibody.

[0165] Embodiment 34. The chimeric binding agent of embodiment 33, wherein the second domain comprises an Fc domain of an IgG antibody.

[0166] Embodiment 35. The chimeric binding agent of embodiment 34, wherein the second domain comprises an Fc domain of an IgG4 antibody.

[0167] Embodiment 36. The chimeric binding agent of embodiment 33, wherein the second domain comprises an Fc domain of an IgA or IgE antibody.

[0168] Embodiment 37. The chimeric binding agent of any one of embodiments 33-36, wherein the second domain further comprises a hinge domain of an antibody.

[0169] Embodiment 38. The chimeric binding agent of embodiment 37, wherein the second domain comprises the amino acid sequence of the heavy chain Fc domain and hinge domain of hLM609-hIgG4-S228P (SEQ ID NO:4) or a sequence at least 90% identical thereto.

[0170] Embodiment 39. The chimeric binding agent of any one of embodiments 1-38, wherein the amino acid sequence comprises a S228P mutation (Eu numbering system) in the hinge region.

[0171] Embodiment 40. The chimeric binding agent of embodiment 39, comprising the amino acid sequence of the hLM609-hIgG4-S228P heavy chain (SEQ ID NO:1) and light chain (SEQ ID NO:2) or a sequence at least 90% identical thereto.

[0172] Embodiment 41. The chimeric binding agent of embodiment 39 or 40, wherein the amino acid sequence comprises a mutation selected from:

a) S239D/A330L/I332E;

b) I332E;

c) G236A/S239D/I332E;

d) G236A;

e) N297A/E382V/M428I;

f) M252Y/S254T/T256E;

g) Q295R/L328W/A330V/P331A/I332Y/E382V/M428I;

h) L234A/L235A/P329G;

i) M428L/N434S;

j) L234A/L235A/P331S;

k) L234A/L235A/P329G/M252Y/S254T/T256E;

l) S298A/E333A/K334/A;

m) S239D/I332E;

n) G236A/S239D/A330L/I332E;

o) S239D/I332E/G236A;

p) L234Y/G236W/S298A;

q) F243L/R292P/Y300L/V305I/P396L;

r) K326W/E333S;

s) K326A/E333A;

t) K326M/E333S;

u) C221D/D222C;

v) S267E/H268F/S324W;

w) H268F/S324W;

x) E345R

y) R435H;

z) N434A;

aa) M252Y/S254T/T256E;

ab) M428L/N434S;

ac) T252L/T/253S/T254F;

ad) E294delta/T307P/N434Y;

ae) T256N/A378V/S383N/N434Y;

af) E294delta

ag) L235E;

ah) L234A/L235A;

ai) S228P/L235E;

aj) P331S/L234E/L225F;

ak) D265A;

al) G237A;

am) E318A;

an) E233P;

ao) G236R/L328R;

ap) H268Q/V309L/A330S/P331S;

aq) L234A/L235A/G237A/P238S/H268A/A330S/P331S;

ar) A330L;

as) D270A;

at) K322A;

au) P329A;

av) P331A;

aw V264A;

ax) F241A;

ay) N297A or G or N

az) S228P/F234A/L235A; or

ba) any combination of a) to az).

[0173] Embodiment 42. A polynucleotide encoding the chimeric binding agent of any one of embodiments 1-40.

[0174] Embodiment 43. A vector comprising the polynucleotide of embodiment 42.

[0175] Embodiment 44. A host cell comprising the polynucleotide of embodiment 42 or the vector of embodiment 43.

[0176] Embodiment 45. A composition comprising the chimeric binding agent of any one of embodiments 1-41 and a carrier.

[0177] Embodiment 46. A pharmaceutical composition comprising the chimeric binding agent of any one of embodiments 1-41 and a pharmaceutically acceptable carrier.

[0178] Embodiment 47. The pharmaceutical composition of embodiment 46, further comprising an additional therapeutic agent.

[0179] Embodiment 48. The pharmaceutical composition of embodiment 47, wherein the additional therapeutic agent is a chemotherapeutic agent.

[0180] Embodiment 49. A kit comprising the chimeric binding agent of any one of embodiments 1-41.

[0181] Embodiment 50. A method of targeting a myeloid-derived cell that accumulates in tumors to a cancer cell expressing at least one cell marker, comprising contacting the cancer cell and the myeloid-derived cell with an effective amount of the chimeric binding agent of any one of embodiments 1-41.

[0182] Embodiment 51. The method of embodiment 50, wherein the cancer cell expresses the at least one cell marker due to cellular stress.

[0183] Embodiment 52. The method of embodiment 50, wherein the cancer cell expresses the at least one cell marker due to undergoing an epithelial to mesenchymal transition.

[0184] Embodiment 53. A method of targeting a myeloid-derived cell that accumulates in mesenchymal tumors to an epithelial cancer cell expressing at least one mesenchymal cell marker, comprising contacting the cancer cell and the myeloid-derived cell with an effective amount of the chimeric binding agent of any one of embodiments 1-41.

[0185] Embodiment 54. The method of embodiment 53, wherein the myeloid-derived cell is a macrophage, dendritic cell, or granulocyte, such as a neutrophil, basophil, eosinophil, or mast cell.

[0186] Embodiment 55. A method of treating a cancer expressing at least one cell marker in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent of any one of embodiments 1-41 or the pharmaceutical composition of any one of embodiments 46-48 to the subject, thereby treating the cancer.

[0187] Embodiment 56. A method of treating an epithelial cell cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent of any one of embodiments 1-41 or the pharmaceutical composition of any one of embodiments 46-48 to the subject, thereby treating the epithelial cell cancer.

[0188] Embodiment 57. A method of treating a cancer in a subject in need thereof, comprising the steps of:

a) selecting a subject having cancer cells that are enriched for an antigen specifically bound by the chimeric binding agent of any one of embodiments 1-41 and enriched for myeloid-derived cells that accumulates in mesenchymal tumors; and

b) administering a therapeutically effective amount of the chimeric binding agent of any one of embodiments 1-41 or the pharmaceutical composition of any one of embodiments 46-48 to the subject, thereby treating the cancer.

[0189] Embodiment 58. A method of treating an epithelial cell cancer in a subject in need thereof, comprising the steps of:

a) selecting a subject having epithelial cancer cells that are enriched for an antigen specifically bound by the chimeric binding agent of any one of embodiments 1-41 and enriched for myeloid-derived cells that accumulate in mesenchymal tumors; and

b) administering a therapeutically effective amount of the chimeric binding agent of any one of embodiments 1-41 or the pharmaceutical composition of any one of embodiments 46-48 to the subject, thereby treating the epithelial cell cancer.

[0190] Embodiment 59. The method of embodiment 58, wherein step a) comprises obtaining a sample of the cancer from the subject and measuring the level of antigen and myeloid-derived cells in the sample.

[0191] Embodiment 60. The method of any one of embodiments 55-59, wherein the myeloid-derived cell is a macrophage, dendritic cell, or granulocyte, such as a neutrophil, basophil, eosinophil, or mast cell.

[0192] Embodiment 61. The method of embodiment 60, wherein the epithelial cell cancer is a late stage epithelial cell cancer.

[0193] Embodiment 62. The method of embodiment 61, wherein one or more of the epithelial cells in the cancer have at least partially transitioned to mesenchymal cells.

[0194] Embodiment 63. The method of any one of embodiments 58-62, wherein the epithelial cell cancer is or has become chemotherapy resistant or refractory.

[0195] Embodiment 64. The method of any one of embodiments 55-63, wherein the cancer is a carcinoma such as a cancer of the gastrointestinal tract, breast, lungs (e.g., non-small cell lung cancer), colon, prostate, or bladder.

[0196] Embodiment 65. The method of any one of embodiments 55-64, further comprising administering to the subject a CD47 blocking agent and/or an immune checkpoint inhibitor and/or an EGFR inhibitor.

[0197] Embodiment 66. The method of any one of embodiments 55-64, wherein the method does not comprise administering to the subject a CD47 blocking agent.

[0198] Embodiment 67. The method of any one of embodiments 58-66, wherein the epithelial cell cancer expresses CD47.

[0199] Embodiment 68. The method of any one of embodiments 58-66, wherein the epithelial cell cancer does not express CD47.

[0200] Embodiment 69. The method of any one of embodiments 55-68, further comprising administering to the subject an additional cancer therapeutic agent or treatment.

[0201] Embodiment 70. The method of any one of embodiments 55-69, wherein the chimeric binding agent or pharmaceutical composition is administered to the subject intravenously, subcutaneously, or intramuscularly or is injected in situ into or near the cancer.

[0202] Embodiment 71. The method of any one of embodiments 55-70, further comprising the step of isolating myeloid-derived cells from the subject, contacting the myeloid-derived cells with the chimeric binding agent or pharmaceutical composition, and administering the contacted myeloid-derived cells to the subject.

[0203] Embodiment 72. The method of any one of embodiments 55-71, wherein the subject is a human.

[0204] The present invention is more particularly described in the following examples that are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

Example 1

Expression of integrin β3 positively correlates with macrophage markers across multiple cancers

[0205] During cancer progression, the tumor microenvironment becomes dramatically altered with the appearance of various stromal and immune cells that influence the malignant behavior of the tumor (Coussens, 2002; Ruffell, 2015). Accordingly, it is important to consider what immune cells are available when targeting tumors with therapeutic antibodies. Although the enrichment of integrin αvβ3 in tumor cells is a driver of an aggressive, drug resistant tumor phenotype (Desgrosellier, 2009; Seguin, 2014a), the impact of αvβ3-positive tumor cells on the tumor immune microenvironment has not been well defined. As reported in Figure 1A of Wettersten *et al., Cancer Res. 79*:5048 (2019), we queried multiple TCGA datasets to identify whether β3-expressing tumors may be enriched for certain immune effector cell types that could contribute to antibody-mediated killing. This analysis reveals that mRNA expression of *ITGB3* positively correlates (rho≥0.3) with marker sets for macrophages (MΦ), dendritic cells (DC), and neutrophils (NΦ), but not with NK cells (NK) for certain types of solid tumors. For example, *ITGB3* mRNA expression positively correlates with macrophage markers for kidney, breast, GBM, lung, stomach, prostate, pancreas, esophageal, and colorectal cancers, while no correlation is observed for renal papillary, sarcoma, thyroid, melanoma, and ovarian cancers. Also, *ITGB3* positively correlates with other immune cell types such as mast cells, T cells and B cells, but this relationship is observed for a limited number of tumor types. Interestingly, no correlation between *ITGB3* and immune cell markers is observed for thyroid, melanoma, kidney papillary, and sarcoma despite these cancers having the highest median expression of *ITGB3* across the TCGA pan-cancer dataset.

[0206] As reported in Figure 1B of Wettersten *et al., Cancer Res. 79*:5048 (2019), the positive correlation of integrin β3 and immune cell type markers was validated for an independent tumor sample set of 10 frozen lung adenocarcinoma biopsies analyzed using the NanoString nCounter platform. Even for this modest sample size, tumors with above median *ITGB3* expression were enriched for markers characterizing macrophages, dendritic cells, and neutrophils (but not NK cells) compared with tumors having below median *ITGB3* expression. Consistent with the analysis of TCGA datasets, there is a strong positive correlation between

ITGB3 and these marker sets. Together, these data suggest that β3-positive epithelial cancers may be enriched for multiple cell types that could serve as effector cells for antibody-mediated therapy.

[0207] As reported in Figure 1C of Wettersten *et al., Cancer Res. 79*:5048 (2019), to further validate the positive correlation of the enrichment of macrophages with β3 expression on tumor cells at the protein level for a variety of genetically and histologically distinct solid tumor types, we performed immunohistochemical staining for a series of commercially available tumor microarray slides. This analysis reveals that integrin β3 protein expression on tumor cells positively correlates with the presence of macrophage markers CD68 and CD163 for lung, prostate, colorectal, kidney, and glioblastoma tumors. High magnification images confirm that individual areas with integrin β3 staining on tumor cells are enriched for cells that stain positive for the macrophage markers. Notably, the percent of tumors with positive tumor cell expression of β3 ranges from 29-54% among the array slides examined, indicating there is a significant portion of β3+ tumors across this diverse population of tumor types, grades, and stages. Together, these findings indicate that tumors with high tumor cell expression of integrin β3 are particularly enriched for TAMs, a component of the tumor microenvironment that contributes to tumor progression (Pathria, 2019), and that these cells might prove important when targeting tumors with certain therapeutic antibodies.

Example 2

Tumor cell expression of integrin β3 is enriched after tumors acquire resistance to the EGFR inhibitor erlotinib in vivo

[0208] An enrichment of TAMs has been observed following cancer therapy, including the EGFR inhibitor erlotinib (Chung, 2012), and we previously reported that integrin αvβ3 is upregulated during the acquisition of erlotinib resistance in lung cancers in mice and for the BATTLE trial in man (Seguin, 2014b). Accordingly, in Figure 2B of Wettersten *et al., Cancer Res. 79*:5048 (2019), we showed that αvβ3-negative HCC827 human EGFR mutant lung tumors that have acquired resistance to erlotinib in vivo not only gain αvβ3 as they become drug resistant, but they also become enriched for TAMs.

Example 3

An anti-αvβ3 monoclonal antibody triggers macrophage-mediated tumor cell killing

[0209] Considering the co-enrichment of TAMs and integrin αvβ3-expressing tumor cells, we reasoned that exploiting this relationship could provide a basis for a therapeutic strategy to

treat αvβ3+ cancers. We further reasoned that therapeutically targeting integrin αvβ3 may provide a new opportunity to treat tumors that gain expression of αvβ3 as a means to evade the effects of the EGFR inhibitor erlotinib. To test this hypothesis, we used a function blocking monoclonal antibody we previously developed, LM609, which recognizes integrin αvβ3 on human but not mouse cells (Cheresh, 1987) and served as the parent antibody for a fully humanized version, Vitaxin/etaracizumab (Delbaldo, 2008; Gutheil, 2000).

[0210] LM609 was tested for its ability to block the growth of tumors that achieve erlotinib resistance by virtue of increase expression of integrin αvβ3. First, the ability of LM609 to delay the onset of erlotinib resistance was tested. Briefly, HCC827 (5×10^6 tumor cells in 100 µl of PBS) αvβ3-negative human EGFR mutant lung cancer cells were subcutaneously injected to the right flank of female nu/nu mice (Charles River, 088, 8-10 weeks old). Tumors were measured with calipers twice per week. Animals with a tumor volume of 250-700 mm^3 were randomly assigned into groups treated with combinations of Captisol (oral, six times/week), PBS (i.p., twice/week), LM609 (i.p., 10 mg/kg, twice/week), or erlotinib (oral, 6.25 mg/kg, six times/week). Vehicle-treated mice were sacrificed on day 15 due to large tumor size, and erlotinib groups on day 50. Tumors were placed into liquid nitrogen, OCT compound, or 10% formalin. As reported in Figure 2C of Wettersten *et al., Cancer Res. 79*:5048 (2019), LM609 alone has no effect on the growth of HCC827 xenograft tumors prior to the development of drug resistance due to lack of the αvβ3 antigen. While mice treated with erlotinib alone showed an initial reduction in tumor size, this is followed by an eventual tumor re-growth and gain in αvβ3 expression. In contrast, the combination of erlotinib plus LM609 prolonged drug sensitivity and prevented the appearance of integrin β3 on tumor cells.

[0211] Next, LM609 was tested for its ability to re-sensitize resistant tumors to the effects of erlotinib. To generate erlotinib-resistant tumors in vitro, HCC827 or PC9 human lung cancer cells (5×10^6 tumor cells in 100 µl of PBS) were subcutaneously injected to the right flank of female nu/nu mice (Charles River, 088, 8-10 weeks old) and tumors were measured with calipers twice per week. Animals with a tumor volume of 100-200 mm^3 were randomly assigned into groups treated with combinations of Captisol (oral, six times/week) or erlotinib (oral, 6.25 mg/kg, six times/week). For each of the individual tumors shown in Figure S3 of Wettersten *et al., Cancer Res. 79*:5048 (2019)}, vehicle-treated erlotinib sensitive (HCC827-P and PC9-P) and erlotinib-resistant (HCC827-R18 and PC9-R4L) cells were isolated. A gain of αvβ3 expression on the cell surface of the erlotinib-resistant tumor cells was confirmed by flow cytometry. When the HCC827-R18 and PC9-R4L erlotinib resistant cell lines were injected subcutaneously into recipient mice, systemic treatment with LM609 (i.p., 10 mg/kg,

twice/week) was able to re-sensitize resistant tumors to the growth inhibitory effects of erlotinib (**FIG. 1**).

[0212] Finally, we considered whether the anti-tumor activity of LM609 might relate to the co-enrichment of TAMs and integrin αvβ3-expressing tumor cells. As reported in Figure 2A of Wettersten *et al., Cancer Res. 79*:5048 (2019), we found that αvβ3-expressing human lung and pancreatic xenograft tumors growing in nude mice were highly sensitive to LM609, and that this effect could be completely blocked by macrophage depletion using clodronate liposomes, demonstrating that TAMs play a critical role in the anti-tumor efficacy of this tumor-targeted antibody. Successful depletion of macrophages by clodronate treatment was confirmed by staining tumor sections for the mouse macrophage marker F4/80. Thus, macrophages are required for the anti-tumor activity of LM609.

Example 4

LM609 induces macrophage-mediated antibody-dependent cell-mediated cytotoxicity (ADCC)

[0213] To confirm that the mechanism of action for LM609 is macrophage dependent, we asked whether LM609 can kill tumor cells in vitro using macrophages isolated from mouse tumors or bone marrow or human blood.

[0214] TAMs were isolated from tumor tissues as described (Kaneda, 2016). Tumors were dissociated in HBSS containing collagenase IV (Sigma, C5138), hyaluronidase (Sigma, H2654), dispase ll (Roche, 04942078001), and DNase IV (Millipore, D5025) at 37°C for 15 minutes. Cell suspensions were filtered through 70 μm cell strainers and washed with PBS. Single cell suspensions (10^6 cells/100 μL in 5% BSA in PBS) were incubated with Mouse BD Fc Block™ (BD Biosciences, 553142, 1:50) for 10 minutes at 4°C and fluorescently labeled antibodies, CD11b (eBioscience, 17-0112-81, 1:100), and Ly-6G (eBioscience, 25-5931-81, 1:100), for one hour at 4°C. TAMs (CD11b-positive, Ly-6G-negative) were sorted.

[0215] Mouse bone marrow-derived macrophages (BMDMs) were aseptically harvested from euthanized 8-10 week-old female C57BL/6 mice by flushing leg bones with RPMI, filtering through 70 μm cell strainers, and incubating in Red Blood Cell Lysing Buffer Hybri-Max™ (Sigma, R7757). Cells were incubated with mouse M-CSF (Peprotech, 315-02) for 7 days before ADCC assays.

[0216] Human peripheral blood mononuclear cells (PBMCs) and macrophages were isolated using leukoreduction system chambers (LRSC) purchased from the San Diego Blood Bank. PBMCs were isolated from LRSC using Histopaque-1083 (Sigma, 10831) following the

manufacturer's protocol. To obtain macrophages, PBMCs were incubated in tissue culture plates with human M-CSF (Peprotech, 300-25) for 5 days.

[0217] We used the isolated macrophages as effector cells in an antibody-dependent cellular-cytotoxicity (ADCC) assay. Briefly, target cells (i.e., tumor cells) stained with CFSE Cell Division Tracker Kit (BioLegend, 423801) were co-cultured with effector cells (i.e., TAMs) with or without isotype IgG or LM609 for 5-16 hr at 37°C, stained with PI, and flow cytometry was performed on BD LSRFortessa™. The ratio of dead target cells (PI-positive) to the total target cell population (CFSE-positive) was calculated as described (Bracher, 2007).

[0218] As reported in Figure 3A-3B from Wettersten *et al., Cancer Res. 79*:5048 (2019), LM609 showed robust ADCC activity using TAMs isolated from mouse Lewis lung carcinoma (LLC) tumors grown in immune-competent C57BL6 mice or immune-compromised athymic nude mice. The antibody could also kill tumor cells using bone marrow derived macrophages (BMDM) isolated from healthy mice, as well as human monocyte-derived macrophages isolated from healthy donor blood.

[0219] To our surprise, LM609-mediated ADCC was not achieved with mouse NK cells or peripheral blood mononuclear cells (PBMCs) isolated from human blood, immune effector cell types to which antibodies are commonly engineered for optimal binding (Listinsky, 2013; Yu, 2017). In fact, NK cells are not correlated with β3 expression in tumors. These findings were reported in Figure 3E of Wettersten *et al., Cancer Res. 79*:5048 (2019).

[0220] Binding of the antibody to Fc receptors on macrophages is critical for its killing capacity, since there was no macrophage-mediated killing in the presence of an antibody blocking of the Fc receptors CD16, CD32, and CD64, and a form of LM609 lacking the Fc portion (Fab LM609) could not trigger macrophage-mediated killing. These findings were reported in Figures 3C-3D of Wettersten *et al., Cancer Res. 79*:5048 (2019).

[0221] Monoclonal antibodies can direct macrophages to induce tumor cell killing through two primary mechanisms, processes known as antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC). In Figure 3F of Wettersten *et al., Cancer Res. 79*:5048 (2019), we show that LM609 induced macrophage ADCC, but not ADCP or direct killing, and this required integrin β3 expression. The lack of an ADCP response is consistent with high tumor cell expression of CD47, the "don't eat me" signal (Chao, 2012) that tumor cells often exploit to evade phagocytosis. Macrophage-mediated ADCC but not ADCP or direct killing was also observed for additional αvβ3-expressing tumor cell lines representing tumor types for which *ITGB3* expression is linked to macrophage

enrichment, including lung, pancreas, brain, and kidney cancer, as reported in Figure 3G of Wettersten *et al., Cancer Res. 79*:5048 (2019).

[0222] Together, these findings suggest that the anti-tumor activity of LM609 involves the opsonization of αvβ3-expressing tumor cells with the monoclonal antibody, followed by the subsequent engagement of macrophage Fc receptors to induce killing.

Example 5

A new humanized form of LM609 designed for preferential engagement of macrophages

[0223] According to in vitro ADCC assays, the mouse monoclonal antibody LM609 is able to selectively engage macrophages but not NK cells to mediate ADCC, and we reasoned that a humanized form of LM609 may be created that retains this functional distinction.

[0224] Antibody Fc engineering and glycoengineering strategies to enhance binding to NK cells for the purposes of triggering ADCC include changes to promote Fc binding to the only Fc receptor expressed on NK cells, CD16 (FcγRIII). However, our findings suggest that αvβ3-expressing tumors that are mesenchymal, stem-like, and drug-resistant contain high levels of macrophages, dendritic cells, and neutrophils (but not NK cells) as reported in Wettersten *et al., Cancer Res. 79*:5048 (2019). Thus, designing an anti-αvβ3 antibody to induce ADCC that requires NK cell engagement represents a mismatch between the antigen (αvβ3) and the types of effector cells that are present within αvβ3-expressing tumors. We therefore reasoned that if anti-αvβ3 could be engineered to recruit macrophages, this new antibody could show improved anti-tumor efficacy by more strongly triggering ADCC.

[0225] Our design goal was to create a new humanized form of LM609 that preferentially engages macrophages more than other immune effector cell types. LM609 is a mouse monoclonal IgG1κ antibody that recognizes human integrin αvβ3 (**FIG. 2**). Several humanized forms of LM609 have previously been generated as hIgG1 isotypes (**FIG. 3** and **FIG. 4**). Since hIgG4 binds only to FcγRI/CD64 but not other Fc receptors, we created a new humanized form of LM609 that involved switching the isotype of etaracizumab/Vitaxin from a hIgG1 isotype (**FIG. 3**) to a hIgG4-S228P isotype (**FIG. 5**). The S228P (Eu numbering system) mutation in the antibody hinge region was included to prevent Fab-arm exchange as previously reported (Reddy, 2000). **FIG. 6** shows the amino acid sequence alignment comparing humanized LM609 hIgG1 vs. hIgG4-S228P forms.

[0226] Isotype switching to hIgG4 has been previously utilized for the generation of cancer therapeutics, although the rationale for this change is to create an antibody that lacks

engagement of ADCC effector cells, most notably NK cells and monocytes. In contrast, macrophages are not widely considered to be mediators of ADCC. Considering that the mouse monoclonal antibody LM609 was able to recruit macrophages to induce ADCC, but not phagocytosis, our utilization of isotype switching to hIgG4 represents an unconventional and unexpected strategy to engage macrophages for ADCC.

[0227] Whereas NK cells utilize only FcγRIII/CD16 to engage antibody Fc regions, macrophages can utilize FcγRI/CD64. Using a cell-based ADCC reporter bioassay, we show that hLM609-hIgG4-S228P is able to strongly activate FcγRI on effector cells, while the hIgG1 and hIgG1-I332E isotypes show lower levels of engagement (**Fig. 7**). In contrast, the hIgG1 isotype can strongly activate FcγRIII and the hIgG1-I332E mutation enhances this as expected (**Fig. 7**). Notably, hLM609-hIgG4 produced no activation of FcγRIII on effector cells, confirming that the hIgG4 isotype primarily interacts with FcγRI. While conventional thinking may suggest that isotype switching to hIgG4-S228P would ablate all effector cell engagement, we show here that hLM609-hIgG4-S228P is able to selectively engage and activate the Fc receptor expressed on macrophages, FcγRI/CD64.

[0228] We next confirmed that isotype switching to hIgG4 did not alter the ability of humanized LM609 to block integrin αvβ3-mediated cell adhesion. Each antibody was tested for its ability to prevent the adhesion of integrin αvβ3-expressing tumor cells to the αvβ3-mediated adhesion to fibrinogen as well as the β1-integrin mediated adhesion to type I collagen. **FIG. 8** shows both IgG1 and IgG4 forms of humanized LM609 blocked adhesion to fibrinogen without disrupting β1-mediated adhesion to collagen.

[0229] Next, we confirmed that the hIgG4 form of humanized LM609 is unable to engage NK cells, as predicted by the inability of IgG4 to bind to the only Fc receptor expressed by NK cells, FcγRIIIA/CD16. For an in vitro ADCC assay using CD16-expressing human NK cells, we confirmed that the hIgG4-S228P form of humanized LM609 is not able to engage NK cells to mediate ADCC (**FIG. 9A**). In contrast, the hIgG4-S228P form of humanized LM609 (but not the hIgG1-WT form) engaged primary human macrophages to induce ADCC for H1975 human lung cancer cells with endogenous expression of β3 (**FIG. 9B**). Macrophage-mediated killing activity for the hIgG4-S228P form of humanized LM609 was further confirmed using macrophages isolated from three individual healthy blood donors with the polymorphic variants in CD16/CD32 as shown (**FIG. 9C**). Furthermore, both LM609 and hLM609-IgG4-S228P are able to induce ADCC utilizing human macrophages as effector cells (**FIG. 10A**). Unlike hLM609-hIgG1, the hLM609-IgG4-S228P isotype is not able to utilize NK cells for tumor cell killing (**FIG. 10B**). This distinction could produce a therapeutic advantage by

achieving matching between the antigen (integrin αvβ3) and the types of effector cells that are particularly enriched in αvβ3-expressing tumors, such as macrophages.

[0230] As observed for LM609, the hIgG4-S228P form of humanized LM609 was able to engage mouse bone marrow-derived macrophages to induce ADCC in vitro (**FIG. 11**). Having established that the mouse monoclonal antibody LM609 kills αvβ3-expressing tumor cells by recruiting macrophages for ADCC, we next compared the anti-tumor activity of LM609 and hLM609-hIgG4-S228P in mice. In fact, both antibodies produced equivalent anti-tumor activity (**FIG. 12**), suggesting that the humanized form and isotype switching to enable macrophage engagement was sufficient to mimic the activity of the mouse monoclonal antibody. We next compared the anti-tumor activity of hLM609-hIgG1 (the isotype that engages NK cells) to hLM609-hIgG4-S228P (the isotype that engages macrophages). Importantly, the antibody affinity for these isotype variants is equivalent, as shown by their ability to block αvβ3-dependent cell adhesion (**Fig. 8**). For a fast-growing human tumor xenograft in mice, the anti-tumor activity for hLM609-hIgG4-S228P is superior to that of hLM609-hIgG1 (**FIG. 13**), suggesting that the ability to selectively engage macrophages provides a therapeutic advantage for αvβ3-expressing tumors with abundant macrophages but not NK cells.

[0231] We next compared the tumor accumulation of hLM609-hIgG1 to hLM609-hIgG4-S228P. hLM609-hIgG4-S228P was able to localize to the tumor to a greater degree than hLM609-hIgG1 (**Fig. 14**). Without being bound by theory, it is thought that hLM609-hIgG4-S228P, because it engages fewer effector cells overall, is better able to locate to the tumor where primarily macrophages are located. In contrast, hLM609-hIgG1, which engages a wider variety of immune cells, may engage effector cells in the blood, lymph node, spleen, etc. and thus may not be readily available to localize to the tumor.

[0232] Together, these findings indicate that a hIgG4-S228P form of humanized LM609 mimics the functional activity of mouse monoclonal LM609. Specifically, these antibodies are able to preferentially engage macrophages to induce killing of integrin αvβ3-expressing tumor cells. This antibody design strategy reflects the goal of matching the tumor cell antigen (αvβ3) with the appropriate ADCC-inducing effector cell (macrophages). By preventing the antibody from broadly engaging immune effector cell types that are not enriched within the tumor microenvironment, we propose the antibody is better able to accumulate in the tumor upon binding to αvβ3 on tumor cells and/or CD64/FcγRI on macrophages.

[0233] Some therapeutic antibodies induce tumor cell killing via ADCC. This occurs when the antibody Fc region engages Fc receptors on immune effector cells to trigger the release of

cytotoxic granules that induce tumor cell killing. Since this scenario typically involves antibody binding to CD16 on NK cells, many antibody glycoengineering and Fc engineering strategies have been designed to promote this interaction. Because IgG4 has high affinity for CD64, but weak affinities for all other receptors, IgG4 is generally considered to be a poor inducer of Fc-mediated effector functions. Therefore, isotype switching to IgG4 is an unexpected approach to enhance effector cell mediated killing of tumor cells.

[0234] For example, the FDA has approved three hIgG4 tumor-therapeutic antibodies, pembrolizumab (KEYTRUDA), nivolumab (OPDIVO), and cemiplimab (LIBTAYO), all of which target the immune checkpoint molecule PD-1 that is expressed mainly on activated T and NK cells. These antibodies work by neutralizing T cell inhibition, that is, preventing the immunosuppressive consequences when PD-1 (on T and NK cells) binds to PD-L1 (on tumor cells). The IgG4 subclass allows the antibody to block the function of PD-1 without engaging additional immune effector cells. As is common for hIgG4 antibodies, all three anti-PD-1 antibodies contain the S228P mutation to stabilize the hIgG4 antibody structure at the hinge region. Based on knowledge in the art, an IgG4-S228P antibody is predicted to block the function of the target antigen without any effector cell engagement.

[0235] We reported in Wettersten *et al., Cancer Res. 79*:5048 (2019) that macrophage engagement is required for the anti-tumor activity of a mouse monoclonal antibody recognizing integrin αvβ3, LM609. Mechanistically, we determined that blocking all Fc receptors (CD16, CD32, and CD64) could prevent the ability of LM609 to induce ADCC in vitro. However, LM609 induced macrophage-ADCC was independent of CD64, since LM609 (and mouse IgG1 antibodies) cannot bind to CD64. While LM609 triggered potent anti-tumor activity by selectively engaging macrophages, the inability of LM609 to bind CD64 suggested that CD64 engagement was not critical.

[0236] Being phagocytotic cells, macrophages are widely known to induce ADCP, and this is generally understood to involve CD16 and CD32 engagement. As such, ADCP could be enhanced by isotype switching to IgG2, which has high affinity to CD32 that is mainly expressed in macrophages. However, the efficacy of such an antibody would be limited by expression of the CD47 "don't eat me" signal on tumor cells. It has been less frequently reported that macrophages can also induce ADCC, with this activity linked to CD16. Thus, it could not have been predicted that macrophage ADCC would be triggered by an IgG4-CD64 interaction.

[0237] Antibodies for cancer therapy include a number that recognize the epithelial-like tumor cell antigens EGFR, Her2, and EpCAM. Engineering of such antibodies can enhance

ADCC by promoting the engagement of NK cells via CD16 binding to the antibody Fc. However, cancer therapy and progression can eventually induce an EMT or enrichment with cancer stem cells that involves not only the loss of epithelial markers, but the exclusion or inactivation of NK cells and CD8+ T cells. Thus, late-stage, mesenchymal-like, stem-like tumors become refractory to epithelial-targeting monoclonal antibodies that utilize NK cells for tumor killing.

[0238] One hallmark of EMT in cancer is the switch of tumor immune content from immune-hot to immune-cold. Although it is unclear if they are a cause or an effect of EMT, tumor-associated macrophages are highly immunosuppressive and act to exclude T cells and NK cells, creating an immune-cold tumor microenvironment. The advantage of the present invention is the ability to achieve "antigen-effector cell matching" to induce tumor cell killing by 1) recognizing a stem/mesenchymal marker on the tumor cell surface (integrin $\alpha v\beta 3$), and 2) engaging tumor-associated macrophages to induce ADCC.

[0239] The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

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Sequences:

hLM609-hIgG4-S228P (humanized LM609)

Heavy chain **(SEQ ID NO:1)**

```
QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYDMSWVRQA PGKGLEWVAK VSSGGGSTYY 60
LDTVQGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHL HGSFASWGQG TTVTVSSAST 120
KGPSVFPLAP CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 180
SLSSVVTVPS SSLGTKTYTC NVDHKPSNTK VDKRVESKYG PPCPPCPAPE FLGGPSVFLF 240
PPKPKDTLMI SRTPEVTCVV VDVSQEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV 300
SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP SQEEMTKNQV 360
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSRLTVD KSRWQEGNVF 420
SCSVMHEALH NHYTQKSLSL SLGK                                       444
```

Light chain **(SEQ ID NO:2)**

```
EIVLTQSPAT LSLSPGERAT LSCQASQSIS NFLHWYQQRP GQAPRLLIRY RSQSISGIPA 60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SGSWPLTFGG GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                            214
```

Fab domain of heavy chain (**SEQ ID NO:3**)

```
QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYDMSWVRQA PGKGLEWVAK VSSGGGSTYY 60
LDTVQGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHL HGSFASWGQG TTVTVSSAST 120
KGPSVFPLAP CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 180
SLSSVVTVPS SSLGTKTYTC NVDHKPSNTK VDKRV                          215
```

Fc and hinge domain of heavy chain (**SEQ ID NO:4**)

```
ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY 60
VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK 120
AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL 180
DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK           229
```

shLM609-hIgG1-WT (super-humanized LM609_7)

Fab domain of heavy chain (**SEQ ID NO:5**)

```
QVQLQESGPG LVKPSQTLSL TCTVSGASIS RGGYYWSWIR QYPGKGLEWI GYIHSHSGST 60
YYNPSLKSRV TIAIDTSKNQ LSLRLTSVTA ADTAVYYCAR HNYGSFAYWG QGTLVTVSSA 120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG 180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKV                        217
```

Light chain (**SEQ ID NO:6**)

```
ELVMTQSPEF QSVTPKETVT ITCRASQDIG NSLHWYQQKP GQSPKLLIKY ASQPVFGVPS 60
RFRGSGSGTD FTLTISRLEP EDFAVYYCQQ SNSWPHTFGQ GTKLEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                           214
```

shLM609-hIgG1-WT (super-humanized JC7U)

Fab domain of heavy chain (**SEQ ID NO:7**)

```
QVQLQESGPG LVKPSQTLSL TCTVSGASIS RGGYRWSWIR QYPGKGLEWI GYIHSHSGST 60
YYNPSLKSRV TIAIDTSKNQ LSLRLTSVTA ADTAVYYCAR QNLGSFAYWG QGTLVTVSSA 120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG 180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKV                        217
```

Light chain (**SEQ ID NO:8**)

```
ELVMTQSPEF QSVTPKETVT ITCRASQDIG NSLHWYQQKP GQSPKLLIKY ASQPVFGVPS 60
RFRGSGSGTD FTLTISRLEP EDFAVYYCQQ SQFWPHTFGQ GTKLEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                           214
```

hLM609-hIgG1-WT (humanized LM609)

Heavy chain (**SEQ ID NO:9**)

```
QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYDMSWVRQA PGKGLEWVAKVSSGGGSTYY 60
LDTVQGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHL HGSFASWGQG TTVTVSSAST 120
KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 180
SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK VDKRVEPKSC DKTHTCPPCP APELLGGPSV 240
FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 300
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK 360
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG 420
NVFSCSVMHE ALHNHYTQKS LSLSPGK                                    447
```

Light chain (**SEQ ID NO:10**)

```
EIVLTQSPAT LSLSPGERAT LSCQASQSIS NFLHWYQQRP GQAPRLLIRY RSQSISGIPA 60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SGSWPLTFGG GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                            214
```

mAb LM609-mIgG1-kappa

Heavy chain (**SEQ ID NO:11**)

```
MNFGLRLIFL VLTLKGVKCE VQLVESGGGL VKPGGSLKLS CAASGFAFSS YDMSWVRQIP 60
EKRLEWVAKV SSGGGSTYYL DTVQGRFTIS RDNAKNTLYL QMSSLNSEDT AMYYCARHNY 120
GSFAYWGQGT LVTVSAAKTT PPSVYPLAPG SAAQTNSMVT LGCLVKGYFP EPVTVTWNSG 180
SLSSGVHTFP AVLQSDLYTL SSSVTVPSST WPSETVTCNV AHPASSTKVD KKIVPRDCGC 240
KPCICTVPEV SSVFIFPPKP KDVLTITLTP KVTCVVVDIS KDDPEVQFSW FVDDVEVHTA 300
QTQPREEQFN STFRSVSELP IMHQDWLNGK EFKCRVNSAA FPAPIEKTIS KTKGRPKAPQ 360
VYTIPPPKEQ MAKDKVSLTC MITDFFPEDI TVEWQWNGQP AENYKNTQPI MDTDGSYFVY 420
SKLNVQKSNW EAGNTFTCSV LHEGLHNHHT EKSLSHSPGK                      460
```

Light chain (**SEQ ID NO:12**)

```
MVFTPQILGL MLFWISASRG DIVLTQSPAT LSVTPGDSVS LSCRASQSIS NHLHWYQQKS 60
HESPRLLIKY ASQSISGIPS RFSGSGSGTD FTLSINSVET EDFGMYFCQQ SNSWPHTFGG 120
GTKLEIKRAD AAPTVSIFPP SSEQLTSGGA SVVCFLNNFY PKDINVKWKI DGSERQNGVL 180
NSWTDQDSKD STYSMSSTLT LTKDEYERHN SYTCEATHKT STSPIVKSFN RNEC       234
```

hLM609-hIgG4-S228P (humanized LM609)

Heavy chain encoding sequence (**SEQ ID NO:13**)

gcggccgccatgaattttggactgaggctgattttcctggtgctgaccctgaaaggcgtccagtgtcaggtccaactggtcgaatcgg
ggggggggagttgtccaacctgggagaagcctgcggctatcatgcgctgcatcgggatttacatttagctcgtatgatatgagctggg
tcaggcaagcccccggaaagggactggaatgggtcgcgaaagtcagctctgggggagggagcacctactatctggacacggtcca
aggacgattcacaattagcagagacaattcgaaaaatacactatacctgcaaatgaatagcctccgggccgaggatacggcggtct
actactgcgctcgccacttgcacggatcatttgcatcatgggggcagggtaccactgtcacggtctcgagcgctagcaccaagggcc
cctccgtgttccccctggcccttgctcccggtccacctccgagtctaccgccgctctgggctgcctggtgaaagactacttccccgag
cctgtgaccgtgagctggaactctggcgccctgacctccggcgtgcacaccttccctgccgtgctgcaatcctccggcctgtactccct
gtcctccgtggtgacagtgccctcctccagcctgggcaccaagacctacacctgtaacgtggaccacaagccctccaacaccaaggt
ggacaagcgggtggaatctaaatacggccctccctgcccccccctgccctgcccctgaatttctgggcggaccttccgtgtttctgttcc
ccccaaagcccaaggacaccctgatgatctcccggaccccgaagtgacctgcgtggtggtggacgtgtcccaggaagatccaga
ggtgcagttcaactggtatgttgacggcgtggaagtgcacaacgccaagaccaagcccagagaggaacagttcaactccacctac
cgggtggtgtccgtgctgaccgtgctgcaccaggactggctgaacggcaaagagtacaagtgcaaggtgtccaacaagggcctgc
cctccagcatcgaaaagaccatctccaaggccaagggccagccccgcgagccccaggtgtacaccctgcccccctagccaggaaga
gatgaccaagaaccaggtgtccctgacctgtctggtgaaaggcttctacccctccgacattgccgtggaatgggagtccaacggcca
gcccgagaacaactacaagaccacccccccctgtgctggactccgacggctccttcttcctgtactctcggctgacagtggataagtcc
cggtggcaggaaggcaacgtgttctcctgcagcgtgatgcacgaggccctgcacaaccactatacccagaagtccctgtccctgag
cctgggcaagtgatgaaagctt

Light chain encoding sequence (**SEQ ID NO:14**)

gcggccgccatgaattttggactgaggctgattttcctggtgctgaccctgaaaggcgtccagtgtgagatcgtcctcacccaatcgc
cggcgacgctgagcctctctcccggagagcgggcgaccttgagctgccaagcgagccaatcaatctccaatttcttgcactggtatc
aacaaaggcccggacaagcaccgaggctgctgataagatataggagccaatcgatctccgggatacccgcacgatttagcggaag
cggatcgggcaccgattttacgctaacgatttcgagcctggagccggaggactttgcggtctattactgccaacaatcgggaagctg
gccgctgacatttggaggaggtaccaaggtcgagatcaagcgtacggtcgcggcgccttctgtgttcattttccccccatctgatgaa
cagctgaaatctggcactgcttctgtggtctgtctgctgaacaacttctaccctagagaggccaaagtccagtggaaagtggacaat
gctctgcagagtgggaattcccaggaatctgtcactgagcaggactctaaggatagcacatactccctgtcctctactctgacactga
gcaaggctgattacgagaaacacaaagtgtacgcctgtgaagtcacacatcaggggctgtctagtcctgtgaccaaatccttcaata
ggggagagtgctgatagtaaaagctt

hLM609-hIgG1-WT (humanized LM609)

Heavy chain encoding sequence (**SEQ ID NO:15**)

gcggccgccatgaattttggactgaggctgattttcctggtgctgaccctgaaaggcgtccagtgtcaggtccaactggtcgaatcgg
ggggggggagttgtccaacctgggagaagcctgcggctatcatgcgctgcatcgggatttacatttagctcgtatgatatgagctggg
tcaggcaagcccccggaaagggactggaatgggtcgcgaaagtcagctctgggggagggagcacctactatctggacacggtcca
aggacgattcacaattagcagagacaattcgaaaaatacactatacctgcaaatgaatagcctccgggccgaggatacggcggtct
actactgcgctcgccacttgcacggatcatttgcatcatgggggcagggtaccactgtcacggtctcgagcgctagcacaaagggcc
ctagtgtgtttcctctggctccctcttccaaatccacttctggtggcactgctgctctgggatgcctggtgaaggattactttcctgaacc
tgtgactgtctcatggaactctggtgctctgacttctggtgtccacactttccctgctgtgctgcagtctagtggactgtactctctgtca
tctgtggtcactgtgccctcttcatctctgggaacccagacctacatttgtaatgtgaaccacaaaccatccaacactaaagtggaca
aaagagtggaacccaaatcctgtgacaaaacccacacctgcccaccttgtcctgcccctgaactgctgggaggaccttctgtgtttct
gttcccccccaaaccaaaggataccctgatgatctctagaacccctgaggtgacatgtgtggtggtggatgtgtctcatgaggaccct
gaggtcaaattcaactggtacgtggatggagtggaagtccacaatgccaaaaccaagcctagagaggaacagtacaattcaacct
acagagtggtcagtgtgctgactgtgctgcatcaggattggctgaatggcaaggaatacaagtgtaaagtctcaaacaaggccctg
cctgctccaattgagaaaacaatctcaaaggccaagggacagcctagggaaccccaggtctacaccctgccaccttcaagagagg
aaatgaccaaaaaccaggtgtccctgacatgcctggtcaaaggcttctacccttctgacattgctgtggagtgggagtcaaatggac
agcctgagaacaactacaaaacaaccccccctgtgctggattctgatggctctttctttctgtactccaaactgactgtggacaagtct
agatggcagcaggggaatgtcttttcttgctctgtcatgcatgaggctctgcataaccactacactcagaaatccctgtctctgtctcc
cgggaaatgatagtaaaagctt

CLAIMS

What is claimed is:

1. A chimeric binding agent comprising a first domain that specifically binds to integrin αvβ3 on an epithelial cancer cell expressing at least one mesenchymal cell marker and a second domain that mediates antibody-directed cellular cytotoxicity (ADCC) by engaging a macrophage that accumulates in mesenchymal tumors.

2. The chimeric binding agent of claim 1, wherein the epithelial cancer cell is a late stage epithelial cancer cell.

3 The chimeric binding agent of claim 2, wherein the epithelial cancer cell has at least partially transitioned to a mesenchymal cell.

4. The chimeric binding agent of any one of claims 1-3, wherein the epithelial cancer cell is chemotherapy resistant or refractory.

5. The chimeric binding agent of any one of claims 1-4, wherein the first domain is an antibody domain.

6. The chimeric binding agent of any one of claims 1-5, wherein the second domain is an antibody domain.

7. The chimeric binding agent of any one of claims 1-6, wherein the first domain is a humanized or human antibody domain.

8. The chimeric binding agent of any one of claims 1-7, wherein the second domain is a humanized or human antibody domain.

9. The chimeric binding agent of any one of claims 1-8, which is a chimeric antibody or an antigen-binding fragment thereof.

10. The chimeric binding agent of any one of claims 1-9, wherein the first domain comprises a Fab domain of an antibody.

11. The chimeric binding agent of claim 10, wherein the first domain comprises a Fab domain of an IgG antibody.

12. The chimeric binding agent of claim 11, wherein the first domain comprises a Fab domain of an IgG4 antibody.

13. The chimeric binding agent of claim 12, wherein the first domain comprises the amino acid sequence of the light chain of hLM609-hIgG4-S228P (SEQ ID NO:2) or a sequence at least 90% identical thereto and the Fab portion of the heavy chain of hLM609-hIgG4-S228P (SEQ ID NO:3) or a sequence at least 90% identical thereto.

14. The chimeric binding agent of claim 13, wherein the first domain comprises the amino acid sequence of the Fab portion of the heavy chain of LM609_7 (SEQ ID NO:5) or a sequence at least 90% identical thereto and the light chain of LM609_7 (SEQ ID NO:6) or a sequence at least 90% identical thereto, the Fab portion of the heavy chain of JC7U (SEQ ID NO:7) or a sequence at least 90% identical thereto and the light chain of JC7U (SEQ ID NO:8), or a sequence at least 90% identical thereto.

15. The chimeric binding agent of any one of claims 1-12, wherein the first domain further specifically binds a second antigen.

16. The chimeric binding agent of claim 15, wherein the first domain is a bispecific antibody domain.

17. The chimeric binding agent of claim 15 or 16, wherein the second antigen is an immune checkpoint molecule, such as PD-1, PD-L1, or CTLA-4.

18. The chimeric binding agent of claim 15 or 16, wherein the second antigen is a cancer stem cell marker, such as CD133, CD44, CD90, CD117, CD166, or CD105, or an effector cell antigen.

19. The chimeric binding agent of claim 15 or 16, wherein the second antigen is an effector cell antigen.

20. The chimeric binding agent of any one of claims 1-19, wherein the second domain does not significantly engage natural killer cells.

21. The chimeric binding agent of any one of claims 1-20, wherein the second domain does not significantly engage lymphocytes.

22. The chimeric binding agent of any one of claims 1-21, wherein the second domain specifically binds a protein on the surface of the myeloid-derived cell.

23. The chimeric binding agent of claim 22, wherein the second domain specifically binds an Fc-gamma receptor.

24. The chimeric binding agent of claim 22, wherein the second domain specifically binds Fc-gamma receptor I (FcγRI, CD64).

25. The chimeric binding agent of any one of claims 1-24, wherein the second domain comprises an Fc domain of an antibody.

26. The chimeric binding agent of claim 25, wherein the second domain comprises an Fc domain of an IgG antibody.

27. The chimeric binding agent of claim 26, wherein the second domain comprises an Fc domain of an IgG4 antibody.

28. The chimeric binding agent of claim 26, wherein the second domain comprises an Fc domain of an IgA or IgE antibody.

29. The chimeric binding agent of any one of claims 25-28, wherein the second domain further comprises a hinge domain of an antibody.

30. The chimeric binding agent of claim 29, wherein the second domain comprises the amino acid sequence of the heavy chain Fc domain and hinge domain of hLM609-hIgG4-S228P (SEQ ID NO:4) or a sequence at least 90% identical thereto.

31. The chimeric binding agent of any one of claims 1-30, wherein the amino acid sequence comprises a S228P mutation (Eu numbering system) in the hinge region.

32. The chimeric binding agent of claim 31, comprising the amino acid sequence of the hLM609-hIgG4-S228P heavy chain (SEQ ID NO:1) and light chain (SEQ ID NO:2) or a sequence at least 90% identical thereto.

33. The chimeric binding agent of claim 31 or 32, wherein the amino acid sequence comprises a mutation selected from:

a) S239D/A330L/I332E;
b) I332E;
c) G236A/S239D/I332E;
d) G236A;
e) N297A/E382V/M428I;
f) M252Y/S254T/T256E;
g) Q295R/L328W/A330V/P331A/I332Y/E382V/M428I;
h) L234A/L235A/P329G;
i) M428L/N434S;
j) L234A/L235A/P331S;
k) L234A/L235A/P329G/M252Y/S254T/T256E;
l) S298A/E333A/K334/A;
m) S239D/I332E;
n) G236A/S239D/A330L/I332E;
o) S239D/I332E/G236A;
p) L234Y/G236W/S298A;
q) F243L/R292P/Y300L/V305I/P396L;
r) K326W/E333S;
s) K326A/E333A;
t) K326M/E333S;
u) C221D/D222C;

v) S267E/H268F/S324W;
w) H268F/S324W;
x) E345R
y) R435H;
z) N434A;
aa) M252Y/S254T/T256E;
ab) M428L/N434S;
ac) T252L/T/253S/T254F;
ad) E294delta/T307P/N434Y;
ae) T256N/A378V/S383N/N434Y;
af) E294delta
ag) L235E;
ah) L234A/L235A;
ai) S228P/L235E;
aj) P331S/L234E/L225F;
ak) D265A;
al) G237A;
am) E318A;
an) E233P;
ao) G236R/L328R;
ap) H268Q/V309L/A330S/P331S;
aq) L234A/L235A/G237A/P238S/H268A/A330S/P331S;
ar) A330L;
as) D270A;
at) K322A;
au) P329A;
av) P331A;
aw V264A;
ax) F241A;
ay) N297A or G or N
az) S228P/F234A/L235A; or
ba) any combination of a) to az).

34. A polynucleotide encoding the chimeric binding agent of any one of claims 1-33.

35. A vector comprising the polynucleotide of claim 34.

36. A host cell comprising the polynucleotide of claim 34 or the vector of claim 35.

37. A composition comprising the chimeric binding agent of any one of claims 1-33 and a carrier.

38. A pharmaceutical composition comprising the chimeric binding agent of any one of claims 1-33 and a pharmaceutically acceptable carrier.

39. The pharmaceutical composition of claim 38, further comprising an additional therapeutic agent.

40. The pharmaceutical composition of claim 39, wherein the additional therapeutic agent is a chemotherapeutic agent.

41. A kit comprising the chimeric binding agent of any one of claims 1-33.

42. A method of targeting a macrophage to a cancer cell expressing integrin αvβ3, comprising contacting the cancer cell and the macrophage with an effective amount of the chimeric binding agent of any one of claims 1-33.

43. The method of claim 42, wherein the cancer cell expresses integrin αvβ3 due to cellular stress.

44. The method of claim 42, wherein the cancer cell expresses integrin αvβ3 due to undergoing an epithelial to mesenchymal transition.

45. A method of targeting a macrophage that accumulates in mesenchymal tumors to an epithelial cancer cell expressing at least one mesenchymal cell marker, comprising contacting the cancer cell and the macrophage with an effective amount of the chimeric binding agent of any one of claims 1-33.

46. A method of treating a cancer expressing integrin αvβ3 in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the cancer.

47. A method of treating an epithelial cell cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the epithelial cell cancer.

48. A method of treating a cancer in a subject in need thereof, comprising the steps of:
a) selecting a subject having cancer cells that are enriched for integrin αvβ3 and enriched for macrophages; and
b) administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the cancer.

49. A method of treating an epithelial cell cancer in a subject in need thereof, comprising the steps of:
a) selecting a subject having epithelial cancer cells that are enriched for integrin αvβ3 and enriched for macrophages that accumulate in mesenchymal tumors; and
b) administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the epithelial cell cancer.

50. The method of claim 48 or 49, wherein step a) comprises obtaining a sample of the cancer from the subject and measuring the level of integrin αvβ3 and macrophages in the sample.

51. The method of claim 49, wherein the epithelial cell cancer is a late stage epithelial cell cancer.

52. The method of claim 49, wherein one or more of the epithelial cells in the cancer have at least partially transitioned to mesenchymal cells.

53. The method of any one of claims 49-52, wherein the epithelial cell cancer is or has become chemotherapy resistant or refractory.

54. The method of any one of claims 46-53, wherein the cancer is a carcinoma such as a cancer of the gastrointestinal tract, breast, lungs (e.g., non-small cell lung cancer), colon, prostate, or bladder.

55. The method of any one of claims 42-54, further comprising administering to the subject a CD47 blocking agent and/or an immune checkpoint inhibitor and/or an EGFR inhibitor.

56. The method of any one of claims 42-54, wherein the method does not comprise administering to the subject a CD47 blocking agent.

57. The method of any one of claims 46-56, wherein the epithelial cell cancer expresses CD47.

58. The method of any one of claims 46-56, wherein the epithelial cell cancer does not express CD47.

59. The method of any one of claims 42-58, further comprising administering to the subject an additional cancer therapeutic agent or treatment.

60. The method of any one of claims 42-59, wherein the chimeric binding agent or pharmaceutical composition is administered to the subject intravenously, subcutaneously, or intramuscularly or is injected in situ into or near the cancer.

61. The method of any one of claims 42-60, further comprising the step of isolating macrophages from the subject, contacting the macrophages with the chimeric binding agent or pharmaceutical composition, and administering the contacted macrophages to the subject.

62. The method of any one of claims 42-61, wherein the subject is a human.

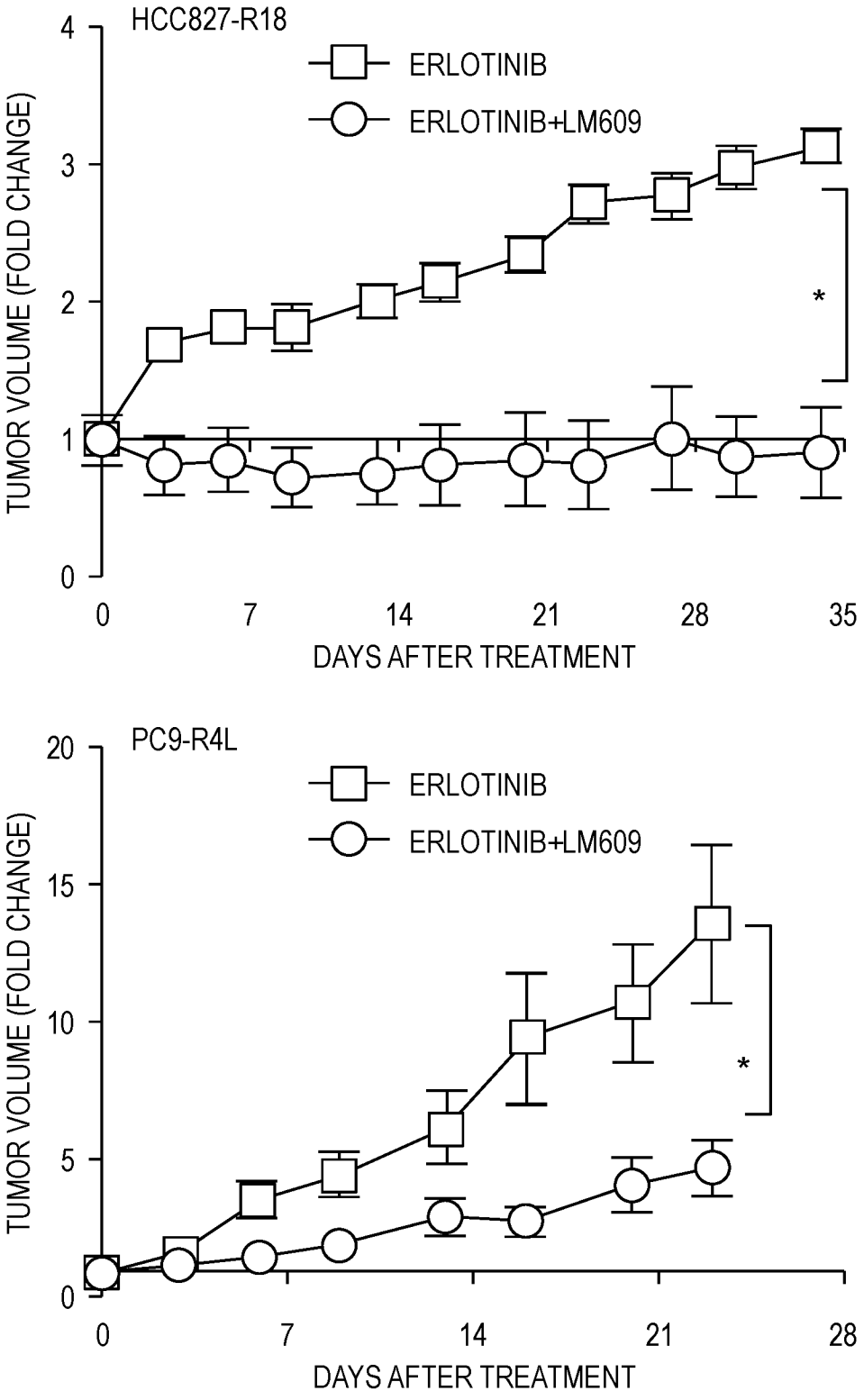

HCC827-R18
ERLOTINIB
ERLOTINIB+LM609
TUMOR VOLUME (FOLD CHANGE)
0
1
2
3
4
*
0
7
14
21
28
35
DAYS AFTER TREATMENT
PC9-R4L
ERLOTINIB
ERLOTINIB+LM609
TUMOR VOLUME (FOLD CHANGE)
0
5
10
15
20
*
0
7
14
21
28
DAYS AFTER TREATMENT

FIG. 1

mAb LM609-mIgG1

H-GAMMA-1		HEAVY CHAIN: AMINO ACID SEQUENCE (460 AA) SIGNAL PEPTIDE-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-CONSTANT REGION-STOP CODON
VH	1-117	MNFGLRLIFLVLTLKGVKCEVQLVESGGGLVKPGGSLKLSCAASGFAFSSYDMSWVRQIPEKRLEWV AKVSSGGGSTYYLDTVQGRFTISRDNAKNTLYLQMSSLNSEDTAMYYCARHNYGSFAYWGQGTLVTV SA
CH1	118-215	AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVPSSTWPSETVTCNVAHPASSTKVDKKI
HINGE		VPRDCGCKPCICT
CH2	231-340	VPEVSSVFIFPPKPKDVLTITL TPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR VNSAAFPAPIEKTISKTK
CH3	341-445	GRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAE NYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSP
CS	341-445	GK
L-KAPPA		LIGHT CHAIN: AMINO ACID SEQUENCE (234 AA) SIGNAL PEPTIDE-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-CONSTANT REGION-STOP CODON
V-KAPPA	1-107	MVFTPQILGLMLFWISASRGDIVLTQSPATLSVTPGDSVSLSCRASQSISNHLHWYQQKSHESPRLL IKYASQSISGIPSRFSGSGSGTDFTLSINSVETEDFGMYFCQQSNSWPHTFGGGTKLEIK
C-KAPPA	108-214	RADAAPT VSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTL TKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

FIG. 2

hLM609-hIgG1-WT

IMGT.ORG CHAIN ID: INN 8862_H

H-GAMMA-1 (VH (1-117)[D1]+CH1 (118-215)[D2]+CH2 (231-340)[D3]+CH3 (341-445)[D4])

```
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVAKVSSGGGSTYY  60
LDTVQGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHLHGSFASWGQGTTVTVSSAST 120
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY 180
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV 240
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY 300
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK 360
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG 420
NVFSCSVMHEALHNHYTQKSLSLSPGK                                   447
```

IMGT.ORG CHAIN ID: INN 8862_L

L-KAPPA (V-KAPPA (1-107)[D1]+C-KAPPA (108-214)[D2])

```
EIVLTQSPATLSLSPGERATLSCQASQSISNFLHWYQQRPGQAPRLLIRYRSQSISGIPA  60
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQSGSWPLTFGGGTKVEIKRTVAAPSVFIFPP 120
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT 180
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                            214
```

FIG. 3

hLM609_7

FAB DOMAIN OF HEAVY CHAIN

QVQLQESGPG LVKPSQTLSL TCTVSGASIS RGGYYWSWIR QYPGKGLEWI GYIHSHSGST 60
YYNPSLKSRV TIAIDTSKNQ LSLRLTSVTA ADTAVYYCAR HNYGSFAYWG QGTLVTVSSA 120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG 180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKV 217

LIGHT CHAIN

ELVMTQSPEF QSVTPKETVT ITCRASQDIG NSLHWYQQKP GQSPKLLIKY ASQPVFGVPS 60
RFRGSGSGTD FTLTISRLEP EDFAVYYCQQ SNSWPHTFGQ GTKLEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC 214

JC7U

FAB DOMAIN OF HEAVY CHAIN

QVQLQESGPG LVKPSQTLSL TCTVSGASIS RGGYRWSWIR QYPGKGLEWI GYIHSHSGST 60
YYNPSLKSRV TIAIDTSKNQ LSLRLTSVTA ADTAVYYCAR QNLGSFAYWG QGTLVTVSSA 120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG 180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKV 217

LIGHT CHAIN

ELVMTQSPEF QSVTPKETVT ITCRASQDIG NSLHWYQQKP GQSPKLLIKY ASQPVFGVPS 60
RFRGSGSGTD FTLTISRLEP EDFAVYYCQQ SQFWPHTFGQ GTKLEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC 214

FIG. 4

hLM609-hIgG4-S228P

H-GAMMA-1 (VH (1-117)[D1]+CH1 (118-215)[D2]+CH2 (231-340)[D3]+CH3 (341-445)[D4])
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVAKVS
SGGGSTYYLDTVQGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHLHGSF
ASWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSN
TKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
GNVFSCSVMHEALHNHYTQKSLSLSLGK

L-KAPPA (V-KAPPA (1-107)[D1]+C-KAPPA (108-214)[D2])
EIVLTQSPATLSLSPGERATLSCQASQSISNFLHWYQQRPGQAPRLLIRYRS
QSISGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSGSWPLTFGGGTKV
EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC

FIG. 5

SCORE	EXPECT	METHOD	IDENTITIES	POSITIVES	GAPS
816 BITS(2109)	0.0	COMPOSITIONAL MATRIX ADJUST.	418/447 (94%)	427/447 (95%)	3/447 (0%)

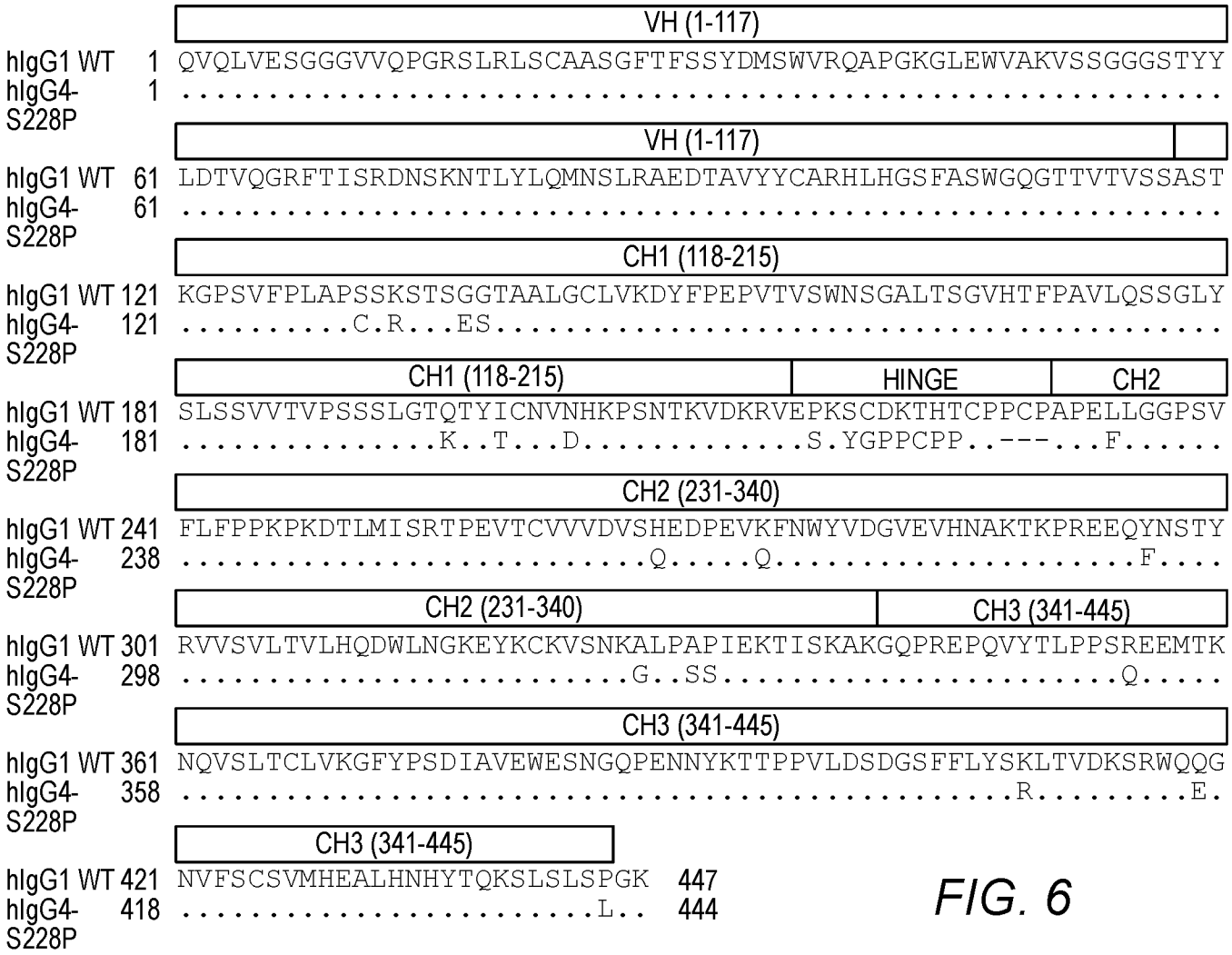


FIG. 6

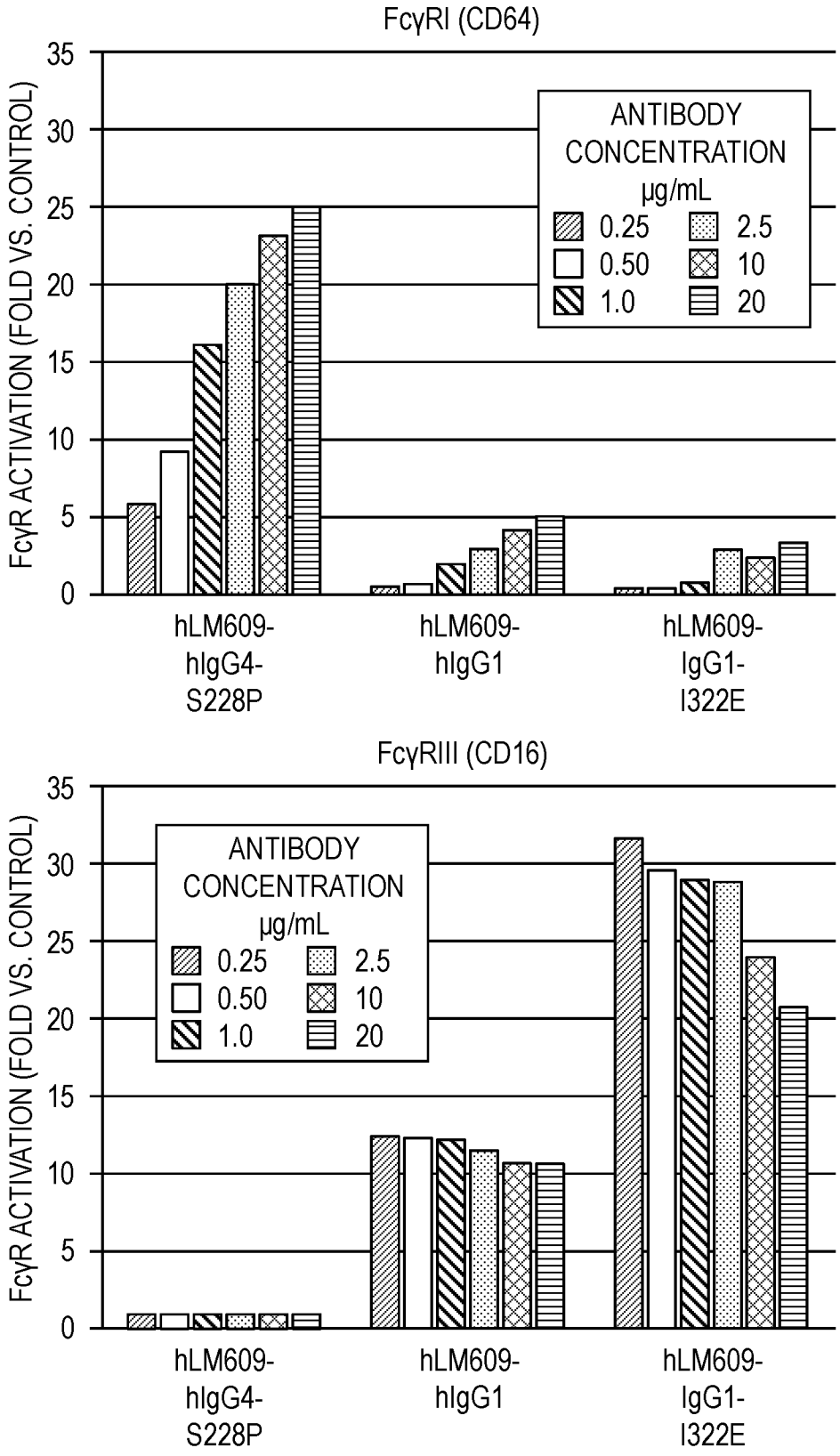

FcγRI (CD64)
FcγR ACTIVATION (FOLD VS. CONTROL)
35
30
25
20
15
10
5
0
ANTIBODY CONCENTRATION µg/mL
0.25
0.50
1.0
2.5
10
20
hLM609-hIgG4-S228P
hLM609-hIgG1
hLM609-IgG1-I322E
FcγRIII (CD16)
FcγR ACTIVATION (FOLD VS. CONTROL)
35
30
25
20
15
10
5
0
ANTIBODY CONCENTRATION µg/mL
0.25
0.50
1.0
2.5
10
20
hLM609-hIgG4-S228P
hLM609-hIgG1
hLM609-IgG1-I322E

FIG. 7

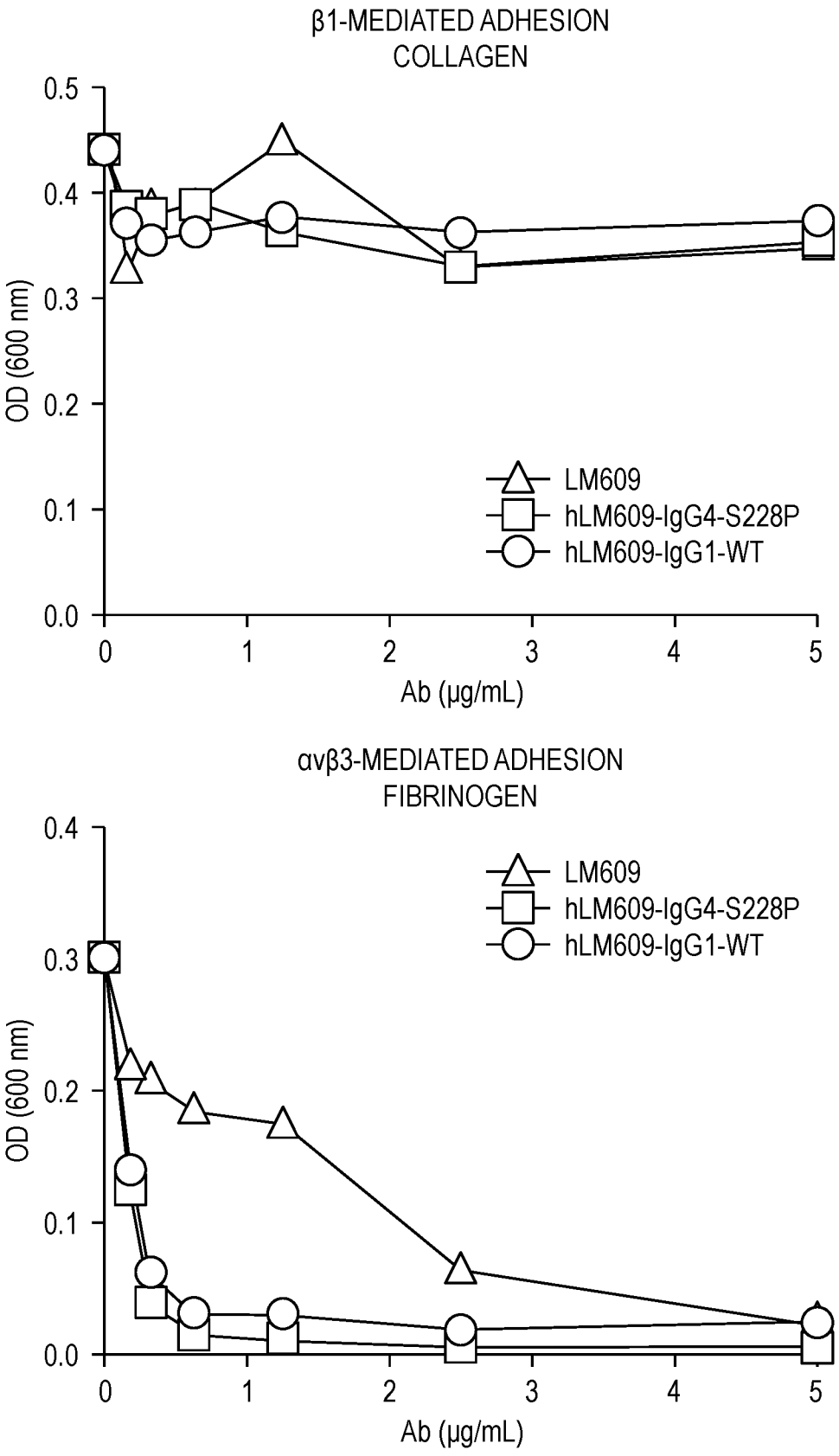

β1-MEDIATED ADHESION
COLLAGEN
OD (600 nm)
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
Ab (μg/mL)
LM609
hLM609-IgG4-S228P
hLM609-IgG1-WT
αvβ3-MEDIATED ADHESION
FIBRINOGEN
OD (600 nm)
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
Ab (μg/mL)
LM609
hLM609-IgG4-S228P
hLM609-IgG1-WT

FIG. 8

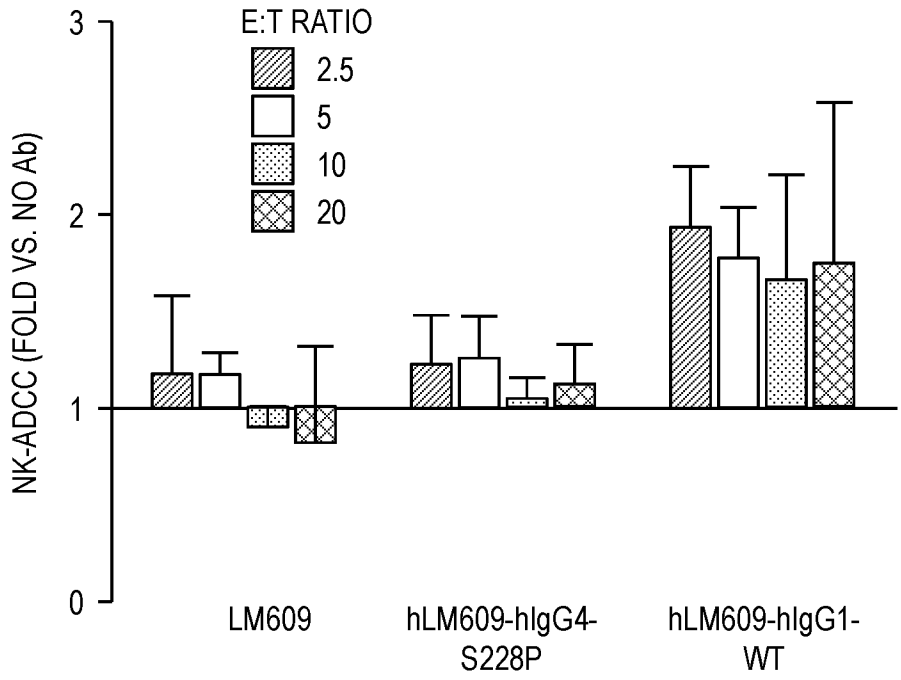

E:T RATIO
2.5
5
10
20
NK-ADCC (FOLD VS. NO Ab)
3
2
1
0
LM609
hLM609-hIgG4-S228P
hLM609-hIgG1-WT

FIG. 9A

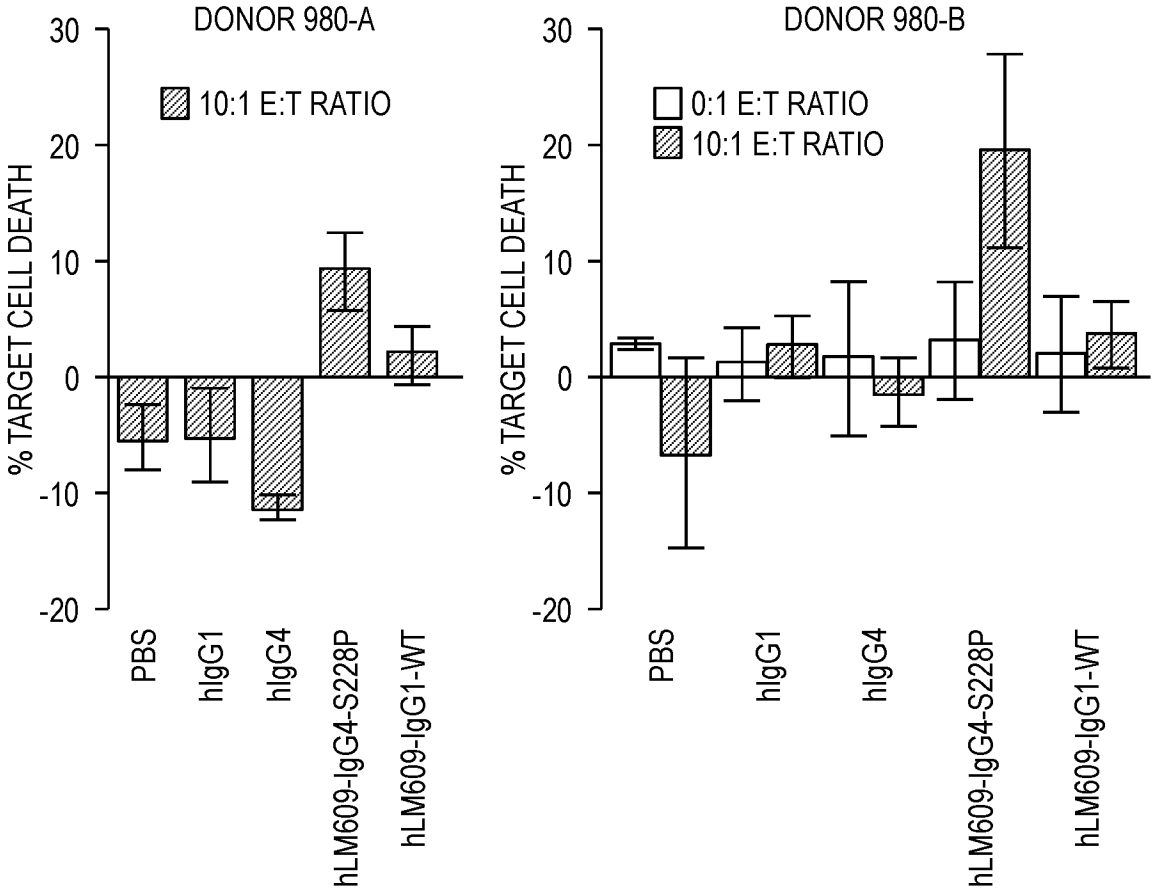
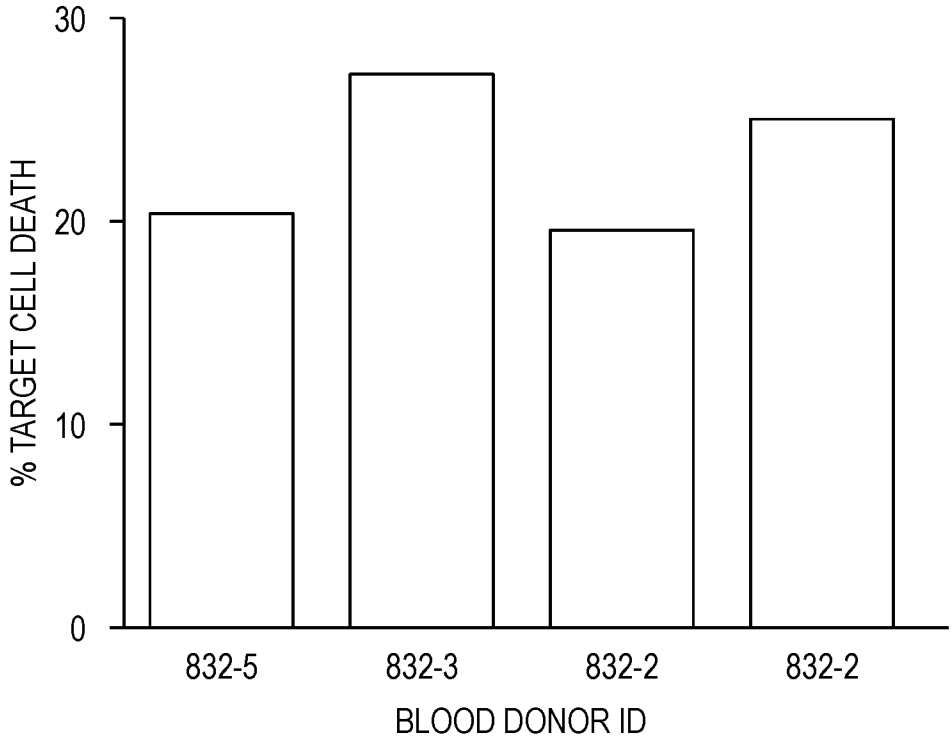

DONOR 980-A
10:1 E:T RATIO
% TARGET CELL DEATH
30
20
10
0
-10
-20
PBS
hIgG1
hIgG4
hLM609-IgG4-S228P
hLM609-IgG1-WT
DONOR 980-B
0:1 E:T RATIO
10:1 E:T RATIO
% TARGET CELL DEATH
PBS
hIgG1
hIgG4
hLM609-IgG4-S228P
hLM609-IgG1-WT

FIG. 9B



MACROPHAGE-ADCC FOR hLM609-hIgG4-S228P

DONOR POLYMORPHIC VARIANTS:

	CD32	CD16
832-2	H131 (HI)	F158 (LO)
832-3	H131 (HI)	F158/V158 (HET)
832-5	R131 (LO)	F158 (LO)

FIG. 9C

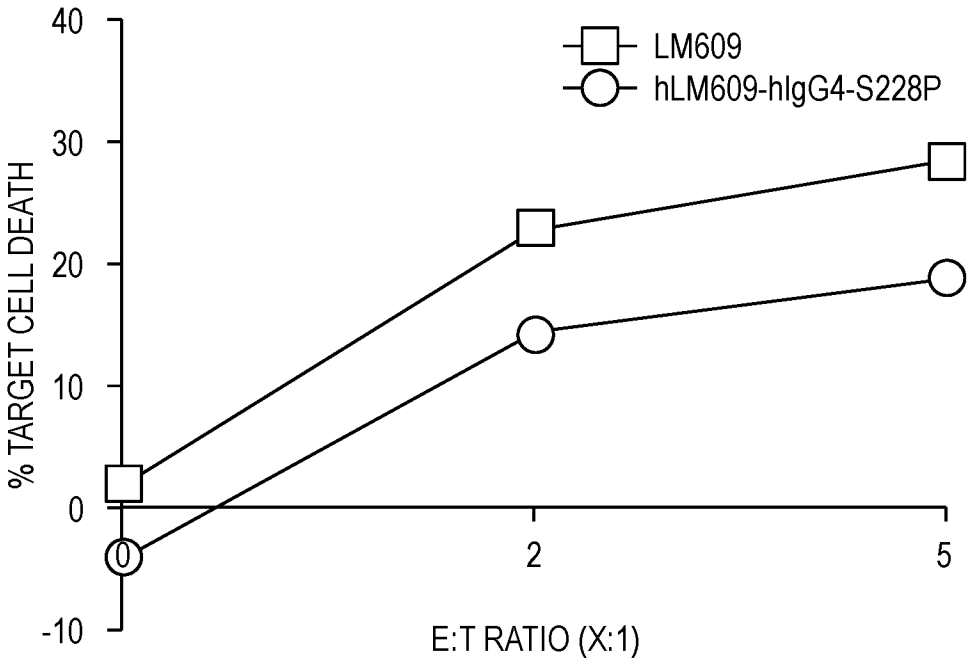


FIG. 10A

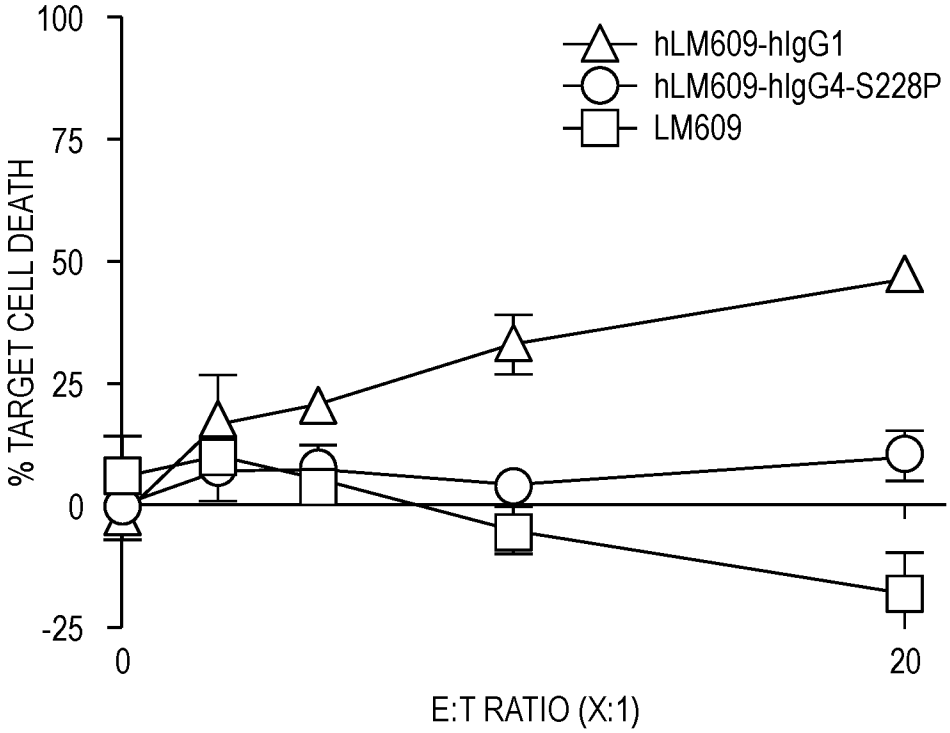


FIG. 10B

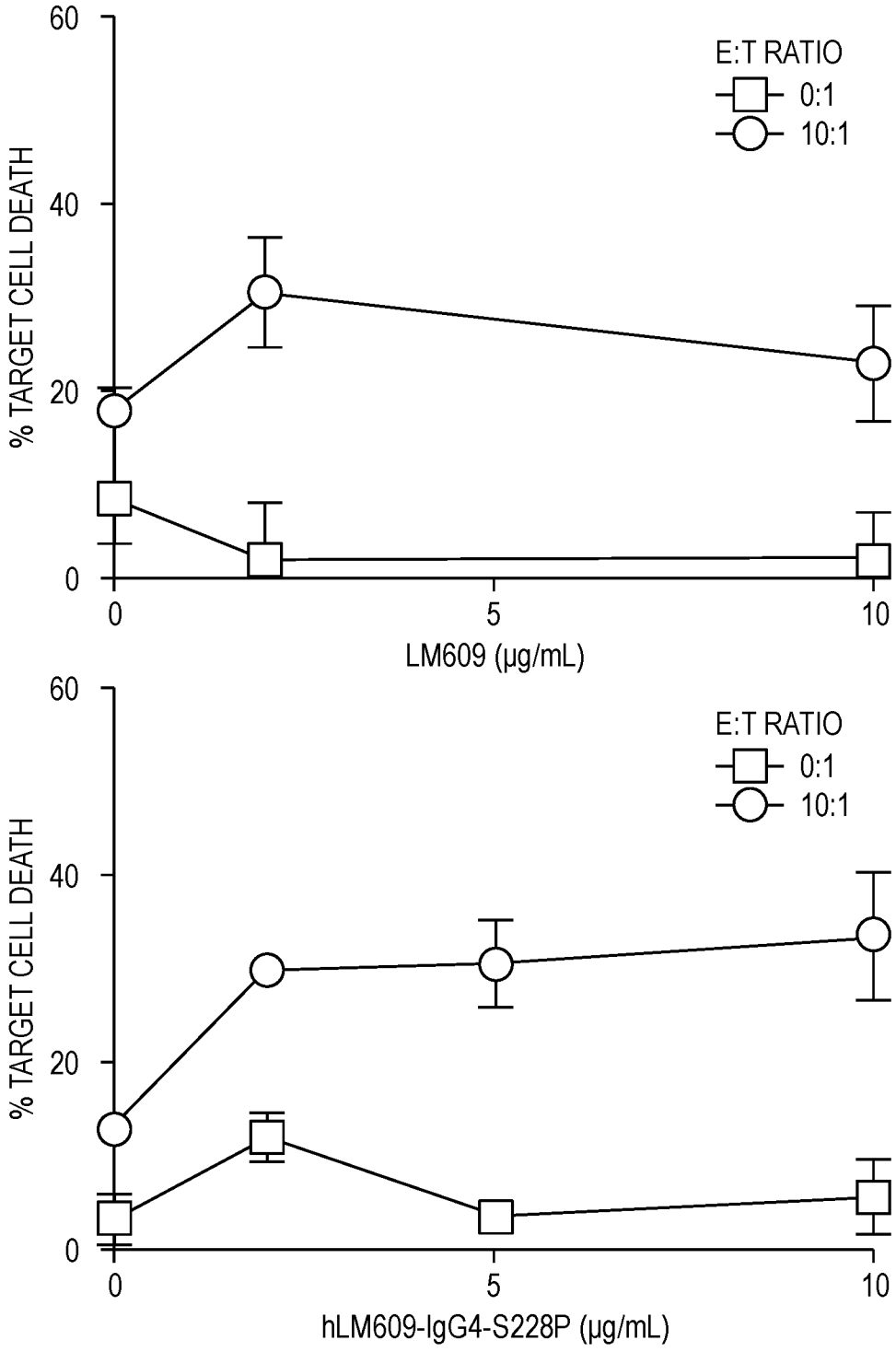

60
40
20
0
% TARGET CELL DEATH
E:T RATIO
0:1
10:1
0
5
10
LM609 (µg/mL)
60
40
20
0
% TARGET CELL DEATH
E:T RATIO
0:1
10:1
0
5
10
hLM609-IgG4-S228P (µg/mL)

FIG. 11

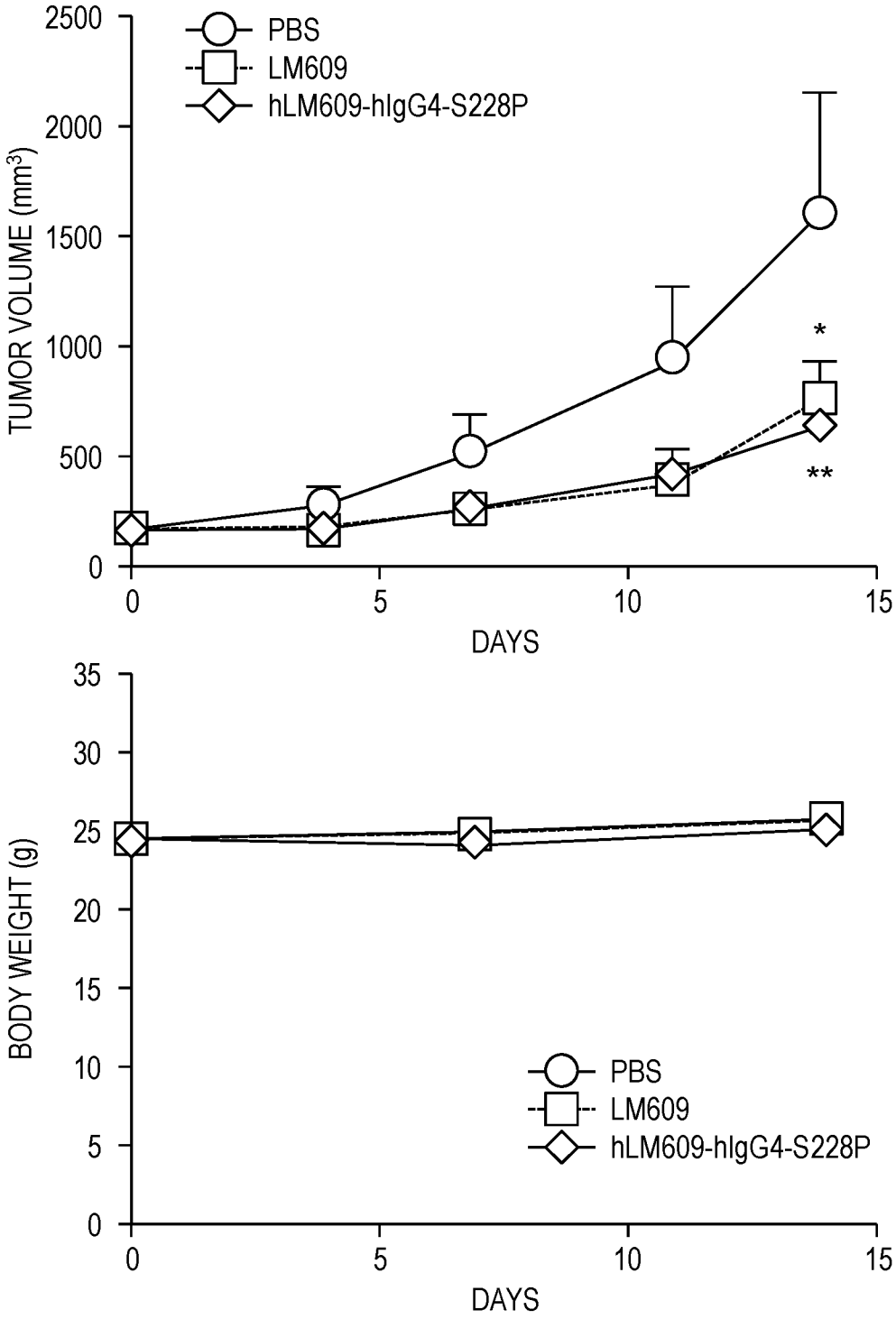

PBS
LM609
hLM609-hIgG4-S228P
TUMOR VOLUME (mm3)
2500
2000
1500
1000
500
0
0
5
10
15
DAYS
*
**
BODY WEIGHT (g)
35
30
25
20
15
10
5
0
0
5
10
15
DAYS
PBS
LM609
hLM609-hIgG4-S228P

FIG. 12

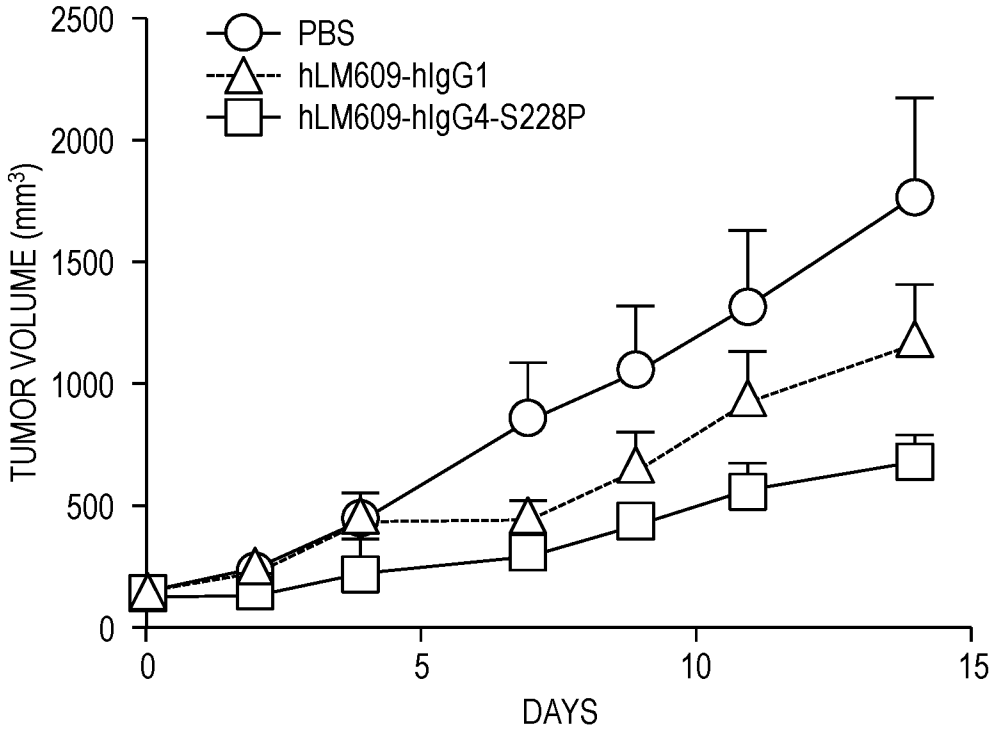

PBS
hLM609-hIgG1
hLM609-hIgG4-S228P
TUMOR VOLUME (mm3)
2500
2000
1500
1000
500
0
0
5
10
15
DAYS

FIG. 13

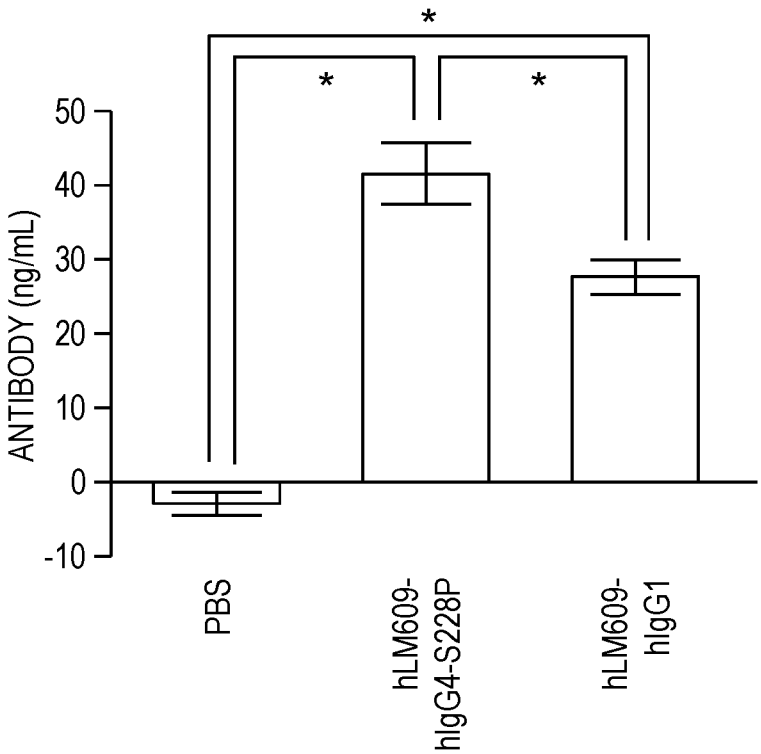

*
*
*
ANTIBODY (ng/mL)
50
40
30
20
10
0
-10
PBS
hLM609-hIgG4-S228P
hLM609-hIgG1

FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/028775

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; **C07K 16/32**(2006.01)i; **C07K 16/30**(2006.01)i; **A61K 45/06**(2006.01)i; **A61P 35/00**(2006.01)i; **A61K 39/00**(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 16/28(2006.01); A61K 39/395(2006.01); C07K 14/435(2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: chimeric, integrin, αvβ3, epithelial cancer, antibody-directed cellular cytotoxicity (ADCC), macrophage, mesenchymal tumor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WETTERSTEN, H. I. et al., "Arming Tumor-associated macrophages to reverse epithelial cancer progression", Cacer Research, 2019, Vol. 79, No. 19, pp 5048-5059 abstract, pages 5049, 5057, table 4, figure 4	1-4
A	MULGREW, K. et al., "Direct targeting of αvβ3 integrin on tumor cells with a monoclonal antibody, Abegrin", Molecular Cancer Therapeutics, 2006, Vol. 5, No. 12, pp 3122-3129 the whole document	1-4
A	MITJANS, F. et al., "An anti-αv-integrin antibody that blocks integrin function inhibits the development of a human melanoma in nude mice", Journal of Cell Science, 1995, Vol. 108, pp 2825-2838 the whole document	1-4
A	WETTERSTEN, H. I. et al. "Targeting integrin αvβ3-expressing cancer stem cells to manipulate tumor-associated macrophages", Cancer Research, 2017, Vol. 77. No. 13, Abstract Number : 3966 the whole document	1-4
A	WO 2018-183894 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 04 October 2018 (2018-10-04) the whole document	1-4

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2021	**10 August 2021**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office **189 Cheongsa-ro, Seo-gu, Daejeon** **35208, Republic of Korea**	**Jung, Da Won**
Facsimile No. **+82-42-481-8578**	Telephone No. **+82-42-481-5373**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/028775

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/028775

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **46-62**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 46-62 pertain to a method for treatment of the human body by surgery or therapy(PCT Article 17(2)(a)(i) and Rule 39.1(iv)).

2. ☑ Claims Nos.: **11-14, 16, 23-24, 26-28, 30, 32, 35-36, 39-40, 43-44, 51-52**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 11-14, 16, 23-24, 26-28, 30, 32, 35-36, 39-40, 43-44, 51-52 are regarded to be unclear because they refer to claims which do not comply with PCT Rule 6.4(a).

3. ☑ Claims Nos.: **5-10, 15, 17-22, 25, 29, 31, 33-34, 37-38, 41-42, 45-50, 53-62**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2021/028775

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2018-183894 A1	04 October 2018	CN 110662559 A	07 January 2020
		EP 3600422 A1	05 February 2020
		JP 2020-512978 A	30 April 2020
		JP 2021-095423 A	24 June 2021
		KR 10-2019-0133205 A	02 December 2019
		US 2020-0109205 A1	09 April 2020