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(54) Title: Inhibition of CXCL12 in Cancer Immunotherapy

(57) Abstract: A method is described for increasing effector T-cell accumulation in cancer cell-containing sites of a tumor, comprising administering to a subject in need thereof a pharmaceutically effective amount of a CXCL12-targeted immune response agent. In some embodiments, the CXCL12-targeted immune response agent is administered with another molecule that inhibits or destroys the tumor cell.

FIGURE 1A

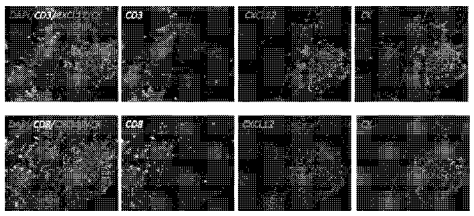
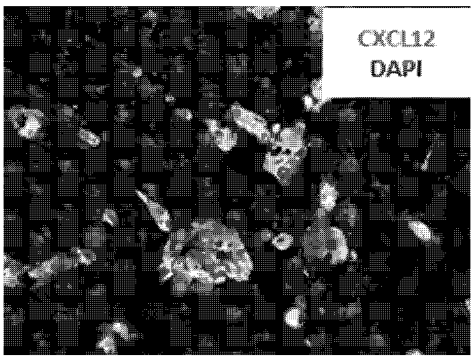


FIGURE 1B



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Inhibition of CXCL12 in Cancer Immunotherapy

[0001] The present invention is concerned with therapy of tumors. In particular, the invention is concerned with reducing or preventing immune suppression and increasing T-cell recruitment and accumulation in the cancerous tumor microenvironment, in order to overcome the exclusion and death of CD3+ T-cells, and preferably CD3+ effector T-cells from the tumor and the suppression of anti-tumor T-cell activity.

Introduction

[0002] Immunotherapy of cancer has made recent progress by focusing on overcoming T-cell immunological checkpoints with blocking monoclonal antibodies to CTLA-4 and the PD-1/PD-L1 receptor/ligand pair, leading to noteworthy results in cancer patients (1-6). Many patients, however, did not respond to these immunological checkpoint antagonists for reasons that are not understood. For example, patients with pancreatic ductal adenocarcinoma (PDA), the fourth most common cause of cancer-related deaths in the United States, had no objective responses to α-CTLA-4 (7) or α-PD-L1 monoclonal antibodies (5).

[0003] Cancer is the second leading cause of death in the United States, exceeded only by heart disease. Despite recent advances in cancer diagnosis and treatment, surgery and radiotherapy may be curative if a cancer is found early, but current drug therapies for metastatic disease are mostly palliative and seldom offer a long-term cure. Even with new chemotherapies entering the market, the need continues for new drugs effective in monotherapy or in combination with existing agents as first line therapy, and as second and third line therapies in treatment of resistant tumors.

[0004] Cancer cells are by definition heterogeneous. For example, within a single tissue or cell type, multiple mutational 'mechanisms' may lead to the development of cancer. As such, heterogeneity frequently exists between cancer cells taken from tumors of the same type that have originated in different individuals and even between cancer cells from different regions of a tumor in a single individual. Frequently observed mutational 'mechanisms' associated with some cancers may differ between one tissue type and another (e.g., frequently observed mutational 'mechanisms' leading to colon cancer may differ from frequently observed 'mechanisms' leading to leukemias). It is therefore often difficult to predict whether a particular cancer will respond to a particular chemotherapeutic agent. (*Cancer Medicine,* 5th Edition, Bast et al. eds., B. C. Decker Inc., Hamilton, Ontario).

[0005] Recent efforts in treating cancer focus on targeted therapeutics or treatments that specifically inhibit vital signaling pathways. However, drug resistance and cancer progression invariably develop. Accordingly, new compounds and methods for treating cancer are needed. The present invention addresses these needs.

[0006] CXCL12 is a chemokine that localizes to human PDA. However, there are mixed reports linking CXCL12 to cancer. It has been suggested that antagonizing CXCL12, or its receptor, CXCR4, increases T-cell trafficking across the blood-brain barrier and improves survival rates from West Nile virus disease (McCandless et al.) and it has been speculated that anti-CXCL12 therapy might be useful for the treatment of ovarian cancer, because CXCL12 inhibition leads to a reduction in FoxP3+ regulatory T-cells in ovarian tumors (Righi et al., *Cancer Res. 2011 Aug 15; 71(16):5522-34*). However, the art concerning CXCL12 is unclear, with some reports indicating that CXCL12 expression impairs immune control in a tumor established with the B16 tumor cell line (20; Righi et al.; Vianello *et al., J Immunol. 2006 Mar 1;176(5):2902-14*), while other studies indicate that CXCL12 expression enhances immune control (Nomura et al., *Int J Cancer. 2001 Mar 1;91(5):597-606;* Fushimi et al., *Cancer Res. 2006 Apr 1;66(7):3513-22;* Williams et al., *Mol Cancer. 2010 Sep 17;9:250; and* Dannussi-Joannopoulos et al., *Blood. 2002 Sep 1;100(5):1551-8*). Thus, it is not clear whether CXCL12 has any role in the development of cancer.

[0007] The present invention addresses the continued need to improve and develop new cancer treatments.

Summary of the Invention

[0008] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject.

[0009] The present invention relates to a method of inducing an immune response to an epithelial tumor characterized by bound CXCL12 comprising administering to a subject with the tumor one or more CXCL12-targeted immune response agents; wherein administration of the CXCL12-targeted immune response agent enhances or induces a tumor antigen-specific immune response against the epithelial tumor.

[0010] In preferred embodiments, the CXCL12-targeted immune response agent is an anti-CXCL12 antibody. In further preferred embodiments, the anti-CXCL12 antibody belongs to immunoglobulin isotypes that bind to Fc receptors that mediate phagocytosis by macrophages or that mediate antibody-dependent cell-mediated cytotoxicity by natural killer cells. Examples of anti-CXCL12 antibody immunoglobulin isotypes include, but are not limited to: the IgG1 and IgG3 immunoglobulin isotypes. In further preferred embodiments, the anti-CXCL12 antibody belongs to the human IgG1 or IgG3 immunoglobulin isotypes. In further preferred embodiments, the anti-CXCL12 antibody triggers the antibody-dependent elimination of epithelial tumor cells. One example of an anti-CXCL12 antibody includes, but is not limited to an anti–SDF-1 antibody.

[0011] In a preferred embodiment, the method also comprises administration of an anti-CD47 antibody. In a further preferred embodiment, the anti-CD47 antibody disrupts the interaction between CD47 on cancer cells with SIRPα on phagocytic cells. In a further preferred embodiment, the anti-CD47 antibody enhances the antibody-dependent elimination of epithelial tumor cells. Thus, in preferred embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with an anti-CD47 antibody.

[0012] In a further preferred embodiment, the CXCL12-targeted immune response agent is a bispecific antibody. These bispecific antibodies include, but are not limited to: a bispecific T-cell engager (BiTE) antibody, a dual-affinity retargeting molecule (DART), a CrossMAb antibody, a DutaMab™ antibody, a DuoBody antibody; a Triomab, a TandAb, a bispecific NanoBody, a Tandem scFv, a diabody, a single chain diabody, a HSA body, a (scFv)2 HSA Antibody, an scFv-IgG antibody, a Dock and Lock bispecific antibody, a DVD-IgG antibody, a TBTI DVD-IgG, an IgG-fynomer, a Tetravalent bispecific tandem IgG antibody, a dual-targeting domain antibody, a chemically linked bispecific (Fab')2 molecule, a crosslinked mAb, a Dual-action Fab IgG (DAF-IgG), an orthoFab-IgG, a bispecific CovX-Body, a bispecific hexavalent trimerbody, 2 scFv linked to diphtheria toxin, and an ART-Ig.

[0013] In a further preferred embodiment, the CXCL12-targeted immune response agent is a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12. In a further preferred embodiment, the CAR T-cell causes apoptosis of the epithelial tumor cells.

[0014] In a further preferred embodiment, the CXCL12-targeted immune response agent is a T-cell preloaded with bispecific antibody. In a further preferred embodiment, the T-cell preloaded with bispecific antibody is polyclonally-activated.

[0015] In a preferred embodiment, the anti-CXCL12 antibody or fragment thereof is fused to a heterologous molecule, substance, or agent. In a further preferred embodiment, the heterologous molecule, substance, or agent binds to a cytokine. Examples of such a cytokine include, but are not limited to: IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, GM-CSF, TNF, IFN-α, IFN-β, IFN-γ, or FasL. In general, the cytokine is a pro-inflammatory cytokine that promotes inflammatory responses. In a further preferred embodiment, the anti-CXCL12 antibody or fragment thereof is fused to two molecules that bind to cytokines. In some embodiments, the two molecules may bind the same cytokine, while in other embodiments, the two molecules may be distinct and may bind different cytokines. In general, any cytokine or combination of cytokines capable of stimulating an immune response (pro-inflammatory cytokines) may bind to the heterologous molecule, substance, or agent, to be delivered to tumor cells characterized by CXCL12.

[0016] In another preferred embodiment, the anti-CXCL12 antibody or fragment thereof is fused to a radionuclide. Examples of such radionuclides include, but are not limited to ^{131}Iodine, ^{90}γYttrium, ^{177}Lutetium, ^{188}Rhenium, ^{67}Copper, ^{211}Astatine, ^{213}Bismuth, ^{125}Iodine, or ^{111}Indium.

[0017] In a further preferred embodiment, the anti-CXCL12 antibody or fragment thereof is fused to a toxin. Examples of such toxins include, but are not limited to Pseudomonas exotoxin, staphylococcal enterotoxin A, ricin A-chain, or plant ribosome-inactivating proteins such as saporin. Additional examples of toxins include, but are not limited to, diphtheria toxin and pokeweed antiviral toxin. Other plant and bacterial toxins are contemplated for attachment to the anti-CXCL12 antibody or fragment thereof.

[0018] In a preferred embodiment, the anti-CXCL12 antibody or fragment thereof is fused to a pro-apoptotic protein. Examples of such pro-apoptotic proteins include, but are not limited to caspase-3, FOXP3, or death ligand TNF-related apoptosis-inducing ligand (TRAIL). Additional examples of pro-apoptotic proteins include, but are not limited to: bad, bax, bcl-2, bcl-w, BID, BIM, Caspase 8, CD40, CD40 Ligand, cIAP-2, Cytochrome-C, DR6, Fas, Fas Ligand, HSP27, HSP60, HSP70, HTRA, IGF-1, IGF-2, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, IGF-1sR, livin, p21, p27, p53, SMAC, Survivin, sTNFRI, sTNFRII, TNF alpha, TNF beta, TRAIL R1, TRAIL R2, TRAIL R3, TRAIL R4, and XIAP.

[0019] In a preferred embodiment, the anti-CXCL12 antibody or fragment thereof is fused to a chemotherapeutic agent/anti-cancer compound. Examples of chemotherapeutic agents/anti-cancer compounds are provided herein.

[0020] In another preferred embodiment, the anti-CXCL12 targeting agent is used to deliver a second molecule to the tumor cells that inhibits the growth of or eliminates the tumor cells. The second molecule can be a pro-apoptotic protein that triggers apoptosis, a radionuclide or toxin that kills tumor cells, or any heterologous molecule, substance or agent that binds to a chemokine or cytokine that stimulates the immune system to identify and eliminate the tumor cells. In some embodiments, the anti-CXCL12 targeting agent is fused/covalently bound to a pro-apoptotic protein, to a radionuclide or toxin, or to a chemotherapeutic agent. In other embodiments, the anti-CXCL12 targeting agent forms a non-covalent complex with a pro-apoptotic protein, a radionuclide or toxin, a chemotherapeutic agent, or a cytokine or chemokine.

[0021] In some embodiments, the anti-CXCL12 targeting agent is a bispecific antibody or fragment thereof that binds to CXCL12 and also binds to a cytokine or chemokine that activates the immune system to inhibit the growth of or eliminate the tumor cells. Cytokines include, but are not limited to: IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, IL-27, GM-CSF, TNF, IFN-α, IFN-β, IFN-γ, or FasL.

[0022] In preferred embodiments, the anti-CXCL12 targeting agent and the second molecule are administered in a therapeutically effective amount to a patient in need thereof. The second molecule can be a pro-apoptotic protein, a radionuclide or toxin, a chemotherapeutic or any heterologous molecule, substance or agent capable of binding to a chemokine or cytokine to activate the immune system to eliminate the tumor cell. For example, the anti-CXCL12 targeting agent may be an anti-CXCL12 antibody conjugated to a chemotherapeutic agent to form an antibody-drug conjugate (ADC) for delivery to the tumor cells, may be a bispecific antibody that binds to CXCL12 and a cytokine to activate the immune system to eliminate the tumor cells, may be an anti-CXCL12 antibody conjugated to radionuclides to eliminate the tumor cells, etc.

[0023] In an even further preferred embodiment, the method inhibits cancer cell growth. In an even further preferred embodiment, the method eliminates cancer cells. In an even further preferred embodiment, the method reduces tumor mass. In a further preferred embodiment, the tumor is resistant to immunotherapy.

[0024] In preferred embodiments, the tumor is an adenocarcinoma, sarcoma, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterine cancer, ovarian cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, esophageal cancer, pancreas cancer, pancreatic ductal adenocarcinoma (PDA), renal cancer, stomach cancer, multiple myeloma or cerebral cancer.

[0025] In further embodiments, the method also comprises administering a checkpoint antagonist. Thus, in preferred embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with a checkpoint antagonist, including for example, an antibody directed to a checkpoint protein.

[0026] In further preferred embodiments, the method also comprises administering a PD-1 signaling inhibitor. In further preferred embodiments, the PD-1 signaling inhibitor is a PD-1 antagonist. In further preferred embodiments, the PD-1 antagonist is an anti-PD-1 antibody. In further preferred embodiments, the PD-1 signaling inhibitor is a PD-L1 antagonist. In further preferred embodiments, the PD-L1 antagonist is an anti-PD-L1 antibody. Thus, in preferred embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with a PD-1 signaling inhibitor, and preferably with a PD-1 antagonist, including for example, an anti-PD-1 antibody, or a PD-L1 antagonist, including for example, an anti-PD-L1 antibody.

[0027] In further embodiments, the method also comprises administering a CTLA-4 antagonist. In further embodiments, the CTLA-4 antagonist is an anti-CTLA-4 antibody. Thus, in preferred embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with a CTLA-4 antagonist, including for example, an anti-CTLA-4 antibody.

[0028] In further embodiments, the method also comprises administering a TIM-3 antagonist. In even further embodiments, the TIM-3 antagonist is an anti-TIM-3 antibody. Thus, in preferred embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with a TIM-3 antagonist, including for example, an anti-TIM-3 antibody.

[0029] In further embodiments, the method also comprises administering a LAG3 antagonist. In even further embodiments, the LAG3 antagonist is an anti-LAG3 antibody. Thus, in preferred embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with a LAG3 antagonist, including for example, an anti-LAG3 antibody.

[0030] In other preferred embodiments, the method also comprises administering an agonist to a T-cell co-receptor. Examples of such agonists to a T-cell co-receptor, include, but are not limited to an agonistic antibody to a T-cell co-receptor. Examples of such T-cell co-receptors, include, but are not limited to, 4-1BB (CD137) and ICOS (CD278). Thus, in preferred

embodiments, the CXCL12-targeted immune response agent of the present invention is administered in combination with an agonist to a T-cell co-receptor, and preferably to an agonistic antibody to a T-cell co-receptor, and even more preferably, an agonistic antibody to 4-1BB (CD137) or ICOS (CD278).

[0031] In other preferred embodiments, the anti-CD47 antibody, the PD-1 signaling inhibitor, the PD-1 antagonist, the anti-PD-1 antibody, the PD-L1 antagonist, the anti-PD-L1 antibody, the CTLA-4 antagonist, the anti-CTLA-4 antibody, the TIM-3 antagonist, the anti-TIM-3 antibody, the LAG3 antagonist, the anti-LAG3 antibody, the T-cell co-receptor agonist, the T-cell co-receptor agonistic antibody, the agonistic antibody to 4-1BB (CD137), the agonistic antibody to ICOS (CD278) and/or the checkpoint antagonist acts synergistically with the CXCL12-targeted immune response agent.

[0032] In other preferred embodiments, the method also comprises administering other anti-cancer therapies. In these embodiments, other anti-cancer therapies include, but are not limited to: chemotherapeutic agents, radiation therapy, cancer therapy, immunotherapy, or cancer vaccines. Examples of such immunotherapies include, but are not limited to adoptive T-cell therapies or cancer vaccine preparations designed to induce T lymphocytes to recognize tumor cells.

[0033] In other preferred embodiments, the cancer vaccine recognizes one or more tumor antigens expressed on the cancer cells. Examples of such tumor antigens include, but are not limited to MAGE-A1, MAGE-A2, MAGE- A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-I, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, BAGE-I, RAGE- 1, LB33/MUM-1, PRAME, NAG, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1/CT7, MAGE-C2, NY-ESO-I, LAGE-I, SSX-I, SSX-2(HOM-MEL-40), SSX-3, SSX-4, SSX-5, SCP-I and XAGE, melanocyte differentiation antigens, p53, ras, CEA, MUC1, PMSA, PSA, tyrosinase, Melan-A, MART-1, gp100, gp75, alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR alpha fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomerase, GnTV, Herv-K-mel, NA-88, SP17, and TRP2-Int2, (MART-I), E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6,

p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, alpha.-fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\170K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, tyrosinase related proteins, TRP-1, TRP-2, or mesothelin.

[0034] In other preferred embodiments, the anti-cancer therapy includes, but is not limited to: aspirin, sulindac, curcumin, alkylating agents including: nitrogen mustards, such as mechlor-ethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); thylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2´-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; epipodophylotoxins such as etoposide and teniposide; antibiotics, such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase, cytokines such as interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, TNF-beta and GM-CSF, anti-angiogenic factors, such as angiostatin and endostatin, inhibitors of FGF or VEGF such as soluble forms of receptors for angiogenic factors, including soluble VGF/VEGF receptors, platinum coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p´-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone

propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; non-steroidal antiandrogens such as flutamide; kinase inhibitors, histone deacetylase inhibitors, methylation inhibitors, proteasome inhibitors, monoclonal antibodies, oxidants, anti-oxidants, telomerase inhibitors, BH3 mimetics, ubiquitin ligase inhibitors, stat inhibitors and receptor tyrosin kinase inhibitors such as imatinib mesylate (marketed as Gleevac or Glivac) and erlotinib (an EGF receptor inhibitor) now marketed as Tarveca; inhibitors of PI-3 kinase, including PI-3 kinasedelta; and anti-virals such as oseltamivir phosphate, Amphotericin B, and palivizumab.

[0035] In other preferred embodiments, the CXCL12-targeted immune response agent and the PD-1 signaling inhibitor and/or the anti-cancer therapy are administered simultaneously, separately, or sequentially.

[0036] In further preferred embodiments, the patient is a human. In other preferred embodiments, the "patient" or "subject suitable for treatment" may be a mammal, such as a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orangutan, gibbon), or a human. In other embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g. murine, primate, porcine, canine, or rabbit animals) may be employed.

[0037] In embodiments of the invention, the method is effective to reduce the growth rate and immune evasion of a tumor.

[0038] In another aspect, there is provided a method for treating a cancer comprising administering to a subject in need thereof a pharmaceutically effective amount of a CXCL12-targeted immune response agent. In one preferred embodiment, the cancer is an epithelial tumor characterized by bound CXCL12. In a further embodiment, the cancer is a pancreatic tumor. In one further embodiment, the pancreatic tumor is a pancreatic ductal adenocarcinoma (PDA). In a further embodiment, the cancer is colorectal cancer. In a further embodiment, the cancer is ovarian cancer. In an even further embodiment, the cancer is non-small cell lung cancer (NSCLC).

[0039] In another aspect, there is provided the use of a CXCL12-targeted immune response agent for inducing an immune response to the cancer cells of an epithelial tumor characterized by bound CXCL12.

[0040] In another aspect, there is provided the use of a CXCL12-targeted immune response agent bound to a second molecule. The second molecule may be a pro-apoptotic protein, a radionuclide or toxin, any heterologous molecule, substance or agent capable of binding to a chemokine or cytokine, or a chemotherapeutic agent. In some aspects, the CXCL12-targeted immune response agent is a bispecific antibody or fragment thereof that binds CXCL12 and one or more cytokines. In preferred embodiments, the cytokine(s) induce an immune response to the epithelial tumor cells characterized by bound CXCL12. In preferred embodiments, the cytokine is a pro-inflammatory cytokine. In still other preferred embodiments, the cytokine is IL-12. In still other embodiments, IL-12 synergizes with other cytokines, including, but not limited to, IL-2, IL-6, IL-7, IL-10, IL-15, IL-17, IL-18, IL-21, GM-CSF, TNF, IFN-α, IFN-β, IFN-γ, or FasL, etc., to stimulate the immune system and/or enhance the production of tumor-infiltrating effector cells.

[0041] In another aspect, there is provided the use of a CXCL12-targeted immune response agent for the treatment of a tumor. In one embodiment, the tumor is an epithelial tumor characterized by bound CXCL12. In another embodiment, the tumor is a pancreatic tumor. In an even further embodiment, the tumor is a pancreatic ductal adenocarcinoma (PDA). In a further embodiment, the tumor is a colorectal tumor. In a further embodiment, the tumor is an ovarian tumor. In another aspect, the invention provides a method of promoting T-cell infiltration into cancerous tumor tissue characterized by bound CXCL12 by administering a CXCL12-targeted immune response agent to the individual.

[0042] In another preferred embodiment, the invention provides the use of a CXCL12-targeted immune response agent in the manufacture of a medication for inducing an immune response to an epithelial tumor, preferably, an epithelial tumor characterized by bound CXCL12.

Brief Description of the Figures

[0043] Embodiments are illustrated by way of example and not limitation in the figures of the accompanying drawings, in which like references indicate similar elements and in which:

[0044] **Figure 1(A)** shows CXCL12 association with cancer cells of mouse and human cancers. Confocal microscopy of serial sections of an orthotopic mouse NSCLC stained with CD3 (white), CD8 (white), CXCL12 (red), and cytokeratin (green). CD3+ T cells are excluded from cytokeratin-positive cancer cells, which are associated with CXCL12.

[0045] **Figure 1**(B) shows staining of human NSCLC with anti-CXCL12, demonstrating that the chemokine also associates with cancer cells of the human form of the disease.

Detailed Description of the Invention

A. Definitions

[0046] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art, such as in the arts of peptide chemistry, cell culture and phage display, nucleic acid chemistry and biochemistry. Standard techniques are used for molecular biology, genetic and biochemical methods (see Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel et al., Short Protocols in Molecular Biology (1999) 4th ed., John Wiley & Sons, Inc.), which are incorporated herein by reference.

[0047] As used herein, "T-cells" or "CD3+ T-cells" are defined as those lymphocyte lineage cells that express the cell surface marker CD3, which includes CD4+ T-cells, CD8+ T-cells, and Foxp3+ regulatory T-cells, and gamma-delta T cells. "Effector CD3+ T-cells" are defined as those mature T-cell population groups that assist with the activity of other immune cells by releasing T-cell cytokines or have direct cytotoxic function. Such cells include CD4+ T-cells, CD8+ T-cells, and Foxp3+ regulatory T-cells.

[0048] As used herein, CXCL12 is a chemokine also known as stroma-derived factor (SDF-1/CXCL12), is a primary ligand of chemokine receptor CXCR4, and plays significant roles in promoting tumor progression. CXCL12 is constitutively expressed in numerous key organs including lung, liver, kidney, and bone marrow. Many tumors are also associated with high levels

of CXCL12 expression. The interaction of CXCL12 and CXCR4 induces intracellular signaling transduction and previous studies have shown that CXCL12/CXCR4 pathway is correlated to oncogenic events such as cancer cell survival, the communication within tumor microenvironment, metastasis, angiogenesis, and evasion of anti-cancer immune responses. Thus, the CXCL12/CXCR4 axis represents a promising therapeutic target for cancers. Developing an antibody against CXCL12 can directly target the tumors that are physically associated with CXCL12 and therefore may distinguish normal epithelial cells from those of a malignant organ origin. Currently, Zong et al has developed a high-affinity humanized monoclonal antibody specific for CXCL12 in preclinical stage (hu30D8) and has shown antitumor effects in xenograft and orthotopic tumor models (Zong et al., *Clin Cancer Res*; 19(16): 4433-45, 2013).

[0049] As used herein, a "CXCL12-targeted immune response agent" is an exogenous factor, such as a pharmaceutical compound, biological molecule or modified biological material that enhances or induces tumor antigen-specific immune responses, including direct cytotoxicity and Fc receptor-mediated phagocytosis and cytotoxicity, by binding specifically to CXCL12. In some embodiments, the CXCL12-targeted immune response agent may be fused to (e.g., covalently attached) or non-covalently attached to a pro-apoptotic protein, a radionuclide or toxin, or a small molecule/chemotherapeutic agent capable of inhibiting or eliminating the tumor cells. In other embodiments, the CXCL12-targeted immune response agent may be a bispecific antibody that binds to CXCL12 and a cytokine or chemokine to activate the immune system to destroy the tumor cells.

[0050] Suitable CXCL12-targeted immune response agents may be identified using standard *in vitro* or *ex vivo* assays, such as by staining tissue sections with an anti-CXCL12 antibody.

[0051] Preferred examples of CXCL12-targeted immune response agents include, but are not limited to, anti-CXCL12 antibodies, bispecific antibodies, CAR T-cells engineered to express a chimeric antigen receptor against CXCL12, and T-cells preloaded with bispecific antibodies. In some embodiments, T-cells are preloaded with bispecific antibodies that deliver a cytokine to the tumor cell.

[0052] As used herein, "anti-CXCL12 antibody" is defined as a monoclonal antibody that exclusively recognizes the antigen, CXCL12, and thereby elicits immune responses, such as Fc receptor-mediated phagocytosis and antibody-dependent cell-mediated cytotoxicity. Preferred examples of anti-CXCL12 antibodies include, but are not limited to, MAB310 (R&D Systems) and hu30D8.

[0053] As used herein, "bispecific antibodies" are recombinant monoclonal antibodies and antibody-like molecules that combine the specificities of two distinct antibodies in one molecule. Thus, they can therefore simultaneously target two distinct antigens. In the present invention, one of the antigens targeted by the bispecific antibody is CD3 which would cause the bispecific antibody to bind to T-cells. In the present invention, the other antigen targeted by the bispecific antibodies is CXCL12 which would cause the bispecific antibody to bind to CXCL12-coated cancer cells.

[0054] Preferred examples of bispecific antibodies include, but are not limited to, bispecific T-cell engager (BiTE) antibodies, dual-affinity retargeting molecules (DARTs), CrossMAb antibodies, DutaMab™ antibodies, DuoBody antibodies; Triomabs, TandAbs, bispecific NanoBodies, T-cells preloaded with bispecific antibodies, polyclonally-activated T-cells preloaded with bispecific antibodies, Tandem scFvs, diabodies, single chain diabodies, HSA bodies, (scFv)2 HSA antibodies, scFv-IgG antibodies, Dock and Lock bispecific antibodies, DVD-IgG antibodies, TBTI DVD IgG antibodies, IgG-fynomers, Tetravalent bispecific tandem IgG antibodies, dual-targeting domain antibodies, chemically linked bispecific (Fab')2 molecules, crosslinked mAbs, dual-action Fab IgG antibodies (DAF-IgGs), orthoFab-IgG antibodies, bispecific CovX-Bodies, bispecific hexavalent trimerbodies, 2 scFv linked to diphtheria toxin antibodies, and ART-Igs.

[0055] As used herein, Dual-Affinity Retargeting (DART) platform technology is a type of bispecific antibody developed by MacroGenics. The platform is capable of targeting multiple different epitopes with a single recombinant molecule and is specifically engineered to accommodate various region sequences in a "plug-and-play" fashion. In this technology, a proprietary covalent linkage is developed and thus, the molecule possesses exceptional stability, optimal heavy and light chain pairing, and predictable antigen recognition. The DART platform is believed to reduce the probability for immunogenicity.

[0056] As used herein, Cross monoclonal antibodies (CrossMAbs) are a type of bispecific antibody invented by Roche. The purpose of this technology is to create a bispecific antibody that closely resembles a natural IgG mAb as a tetramer consisting of two light chain-heavy chain pairs, and to solve the problem of light chain mispairing. This technology is believed to prevent unspecific binding of the light chain to its heavy counterpart thereby prevent unwanted side products. In addition, this method leaves the antigen-binding regions of the parental antibodies intact and thus can convert any antibodies into a bispecific IgG.

[0057] As used herein, a DutaMab is a type of bispecific antibody invented by Dutalys (recently acquired by Roche). This platform differs by developing fully human bispecific antibodies that show high affinity in each arm and simultaneously bind both targets. DutaMabs are also believed to possess excellent stability and good manufacturing properties.

[0058] Duobody antibodies are a type of bispecific antibodies created by Genmab. This platform generates stable bispecific human IgG1 antibodies and is able to fully retain IgG1 structure and function. Two parental IgG1 monoclonal antibodies are first separately produced, each containing single matched mutations in the third constant domain. Subsequently, these IgG1 antibodies are purified according to standard processes for recovery and purification. After production and purification (post-production), the two antibodies are recombined under tailored laboratory conditions resulting in a bispecific antibody product with a very high yield (typically >95%) (Labrijn et al, *PNAS* 2013;110(13):5145-5150). The Duobody platform is believed to have minimal immunogenicity and can combine any antigen binding sequence derived from any antibody-generating platform to generate a bispecific product.

[0059] As used herein, CAR T-cells, also known as chimeric antigen receptor T-cells, are produced by using adoptive cell transfer technique. T-cells are first collected from patients' blood and recombinant receptors are introduced into these T-cells using genetic engineering methods such as retroviruses. CAR T-cells are then infused into the patient, the tumor-associated antigen is recognized by the CAR T-cell, and is destroyed. Thus, CAR T-cells enhance tumor specific immunosurveillance. The structure of CAR most commonly incorporates a single-chain variable fragment (scFv) derived from a monoclonal antibody that links to intracellular signaling domains and forms a single chimeric protein. In the present invention, the CAR T-cell is developed specifically against CXCL12.

[0060] As used herein, CD47, also known as Integrin Associated Protein, is a transmembrane receptor that belongs to the immunoglobulin superfamily and is ubiquitously expressed on the surface of normal and solid tumor cells. CD47 is also involved in numerous normal and pathological processes including immunity, apoptosis, proliferation, migration, and inflammation. Previous studies have demonstrated the expression of CD47 on various cancer cells and revealed its role in promoting cancer progression. By binding with signal regulatory protein (SIRPα), the primary ligand of CD47 expressed on phagocytic cells (dendritic cells, macrophages, and neutrophils), CD47 prohibits phagocytosis and thus allows tumor cells to evade immune surveillance. Thus, CD47 appears as an important therapeutic target for cancer

treatments. Anti-CD47 monoclonal antibodies for clinical uses are currently being developed by Stanford University (phase I, cancer treatment), by the Ukraine Antitumor Center (phase I, cancer treatment), and by Vasculox, Inc. (Preclinical, organ transplantation).

[0061] As used herein, "anti-CD47 antibody" is defined as a monoclonal antibody that exclusively recognizes and binds to the antigen, CD47. Binding prevents the interaction between CD47 and SIRPα, a protein on phagocytes, thereby reversing the inhibition of phagocytosis normally caused by the CD47/ SIRPα interaction. When coupled with a cancer antigen-specific antibody, the anti-CD47 antibody eliminates the "don't eat me signal" and allows the cancer antigen-specific antibody to more efficiently induce a tumor antigen-specific immune response.

[0062] As used herein, antibody-dependent cell-mediated cytotoxicity is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies.

[0063] In the present invention, term "antibody," refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. Examples of molecules which are described by the term "antibody" in this application include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab' fragments, F(ab')2, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term "single chain Fv" or "scFv" as used herein refers to a polypeptide comprising a VL domain of antibody linked to a VH domain of an antibody.

[0064] Antibodies of the invention include, but are not limited to, monoclonal, multispecific, bi-specific, human, humanized, mouse, or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, antiidiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgC2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0065] In the present invention, a "PD-1 signaling inhibitor" is an exogenous factor, such as a pharmaceutical compound or molecule that inhibits or prevents the activation of PD-1 by its ligand PD-L1 and thereby blocks or inhibits PD-1 signaling in cells within the cancerous tumor.

A PD-1 signaling inhibitor is defined broadly as any molecule that prevents the negatively regulation by PD-1 of T-cell activation.

[0066] Preferred examples of a PD-1 signaling inhibitor include, but are not limited to, a PD-1 antagonist and/or a PD-L1 antagonist.

[0067] In the present invention, a "PD-1 antagonist" is defined as a molecule that inhibits PD-1 signaling by binding to or interacting with PD-1 to prevent or inhibit the binding and/or activation of PD-1 by PD-L1, thereby inhibiting PD-1 signaling and/or enhancing T-cell activation. Preferred examples of a PD-1 antagonist, include, but are not limited to an anti-PD-1 antibody, which are well known in the art. See, Topalian, et al. NEJM 2012.

[0068] In the present invention, a "PD-L1 antagonist" is defined as a molecule that inhibits PD-1 signaling by binding to or inhibiting PD-L1 from binding and/or activating PD-1, thereby inhibiting PD-1 signaling and/or enhancing T-cell activation. Preferred examples of a PD-L1 antagonist, include, but are not limited to an anti-PD-L1 antibody which are well known in the art. See, Brahmer, et al. NEJM 2012.

[0069] In the present invention, a "CTLA-4 antagonist" is defined as a molecule that inhibits CTLA-4 signaling by binding to or inhibiting CTLA-4 from binding and/or activating to B7 molecules, known in the art to be present on antigen-presenting cells, thereby preventing interactions of B7 molecules with the co-stimulatory molecule CD28, and inhibiting T-cell function. Preferred embodiments of a CTLA-4 antagonist, include, but are not limited to anti-CTLA-4 antibodies.

[0070] In the present invention, a "LAG3 antagonist" is defined as a molecule that inhibits LAG3 signaling by binding to or inhibiting LAG3 from binding and/or activating MHC molecules and any other molecule, known in the art to be present on antigen-presenting cells, thereby preventing LAG3 interactions and promoting T-cell function. Preferred embodiments of a LAG3 antagonist, include, but are not limited to anti-LAG3 antibodies.

[0071] In the present invention, a "TIM-3 antagonist" is defined as a molecule that inhibits the CD8+ and CD4+ Th1-specific cell surface protein, TIM-3, which, when ligated by galectin-9, for example, causes T-cell death. Preferred embodiments of a TIM-3 antagonist, include, but are not limited to anti-TIM-3 antibodies that block interaction with its ligands.

[0072] In the present invention, a PD-1 antagonist, a CTLA-4 antagonist, a TIM-3 antagonist, and a LAG3 antagonist are T-cell checkpoint antagonists. Other examples of checkpoint

antagonists are well known in the art. Blocking CXCR4 with any CXCR4 signaling inhibitor, leads to the uncovering of the anti-cancer effects of the T-cell checkpoint antagonists.

[0073] In the present invention, a "T-cell co-receptor" is a cell surface receptor that binds to ligands on antigen-presenting cells that are distinct from the peptide-MHC complex that engages the T-cell receptor. Ligation of T-cell co-receptors enhance the antigen-specific activation of the T-cell by recruiting intracellular signaling proteins (e.g., NFkappaB and PI3-kinase) inside the cell involved in the signaling of the activated T lymphocyte. Preferred embodiments of a T-cell co-receptor antagonist, include, but are not limited to anti-T-cell co-receptor antibodies, such as, for example, antibodies directed to 4-1BB(CD137) and ICOS (CD278).

[0074] In preferred embodiments, the CXCL12-targeted immune response agent of the present invention, whether it be an anti-CXCL12 antibody, a bispecific antibody, a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12, or a T-cell preloaded with bispecific antibodies, has synergistic activity with a PD-1 signaling inhibitor of the present invention. In preferred embodiments, the CXCL12-targeted immune response agent is for example, MAB310 or hu30D8 and has synergistic activity with a PD-1 signaling inhibitor of the present invention, such as, for example, an anti-PD-1 antibody or an anti-PD-L1 antibody.

[0075] In other preferred embodiments, the CXCL12-targeted immune response agent has synergistic activity with one or more cytokines, including but not limited to: IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, IL-27, GM-CSF, TNF, IFN-α, IFN-β, IFN-γ, or FasL. Cytokines may stimulate the immune system to inhibit the growth of or identify and eliminate tumor cells.

[0076] As used herein, a cytokine is a secreted protein that is involved in cell signalling, including regulation of the immune system. Cytokines are typically produced by immune cells, including macrophages, B lymphocytes, T lymphocytes and mast cells, and other cells such as endothelial cells, fibroblasts, and various stromal cells. A given cytokine may be produced by different types of cells. Some cytokines are responsible for activating immune responses against tumors while other cytokines downregulate the immune system to maintain homeostasis and promote self-tolerance. Cytokines include interleukins, lymphokines, monokines, interferons (IFN), colony stimulating factors (CSF), chemokines, and a variety of other proteins. As used herein, chemokines are a subset of cytokines that activate the immune system to attract immune cells to the site of inflammation. Chemokines are generally pro-inflammatory cytokines.

[0077] As used herein, a pro-apoptotic protein is a protein that activates cellular process leading to apoptosis. Examples of such pro-apoptotic proteins include, but are not limited to caspase-3, FOXP3, or death ligand TNF-related apoptosis-inducing ligand (TRAIL). Additional examples of pro-apoptotic proteins include, but are not limited to, bad, bax, bcl-2, bcl-w, BID, BIM, Caspase 8, CD40, CD40 Ligand, cIAP-2, Cytochrome-C, DR6, Fas, Fas Ligand, HSP27, HSP60, HSP70, HTRA, IGF-1, IGF-2, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, IGF-1sR, livin, p21, p27, p53, SMAC, Survivin, sTNFRI, sTNFRII, TNF alpha, TNF beta, TRAIL R1, TRAIL R2, TRAIL R3, TRAIL R4, and XIAP.

[0078] As used herein, a toxin is a molecule capable of killing a cell, typically by inhibiting protein synthesis. Toxins include but are not limited to bacterial toxins, such as diphtheria toxins, staphylococcal enterotoxin A, and Pseudomonas exotoxins, plant toxins (e.g., ricin, abrin, mistletoe lectin, modeccin, hemitoxins or class I ribosome inactivating proteins such as PAP, pokeweed antiviral toxin, saporin, bryodin 1, bouganin, and gelolin), etc. (Kreitman 2006). Toxins typically have poor selectivity, and thus, need to be conjugated to a molecule having specificity for a tumor cell, e.g., such as an anti-CXCL12 antibody or fragment thereof, in order to target and kill cancer cells, while leaving noncancerous cells intact and functional. Once bound to the tumor cell, the anti-CXCL12 antibody-toxin conjugate is internalized, and the toxin localizes to the cytoplasm to kill the cell. Toxins may be derived from both plant and bacterial sources. Toxins may be genetically fused or chemically conjugated to the CXCL12-targeted immune response agent.

[0079] As used herein, a radionuclide is a radioactive atom that emits excess nuclear energy (radiation) which damages cells and triggers apoptosis. Examples of such radionuclides include, but are not limited to ^{131}Iodine, $^{90}\gamma$Yttrium, ^{177}Lutetium, ^{188}Rhenium, ^{67}Copper, ^{211}Astatine, ^{213}Bismuth, ^{125}Iodine, or ^{111}Indium. By attaching the radionucleotide to a CXCL12-targeted immune response agent, the radionuclide can deliver a toxic dose of radiation specifically to tumor cells characterized by CXCL12. Radionuclides not only destroy the target cell, but also adjacent tumor cells that may lack CXCL12.

[0080] In accordance with present invention embodiments as described herein, toxins, pro-apoptotic proteins, chemokines or cytokines, radionuclides, and chemotherapeutics in combination with the CXCL12-targeted immune response agent have the capacity to eliminate both a primary tumor site as well as cancer that has spread throughout the body, including malignant cell populations that may be undetectable by diagnostic imaging.

[0081] In preferred embodiments, the CXCL12-targeted immune response agent of the present invention, whether it be an anti-CXCL12 antibody, a bispecific antibody, a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12, or a T-cell preloaded with bispecific antibodies, has synergistic activity with a CTLA-4 antagonist of the present invention. In preferred embodiments, the CXCL12-targeted immune response agent is for example, MAB310 or hu30D8 and has synergistic activity with a CTLA-4 antagonist of the present invention, such as, for example, an anti-CTLA-4 antibody.

[0082] In preferred embodiments, the CXCL12-targeted immune response agent of the present invention, whether it be an anti-CXCL12 antibody, a bispecific antibody, a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12, or a T-cell preloaded with bispecific antibodies, has synergistic activity with a LAG3 antagonist of the present invention. In preferred embodiments, the CXCL12-targeted immune response agent is for example, MAB310 or hu30D8 and has synergistic activity with a LAG3 antagonist of the present invention, such as, for example, an anti-LAG3 antibody.

[0083] In preferred embodiments, the CXCL12-targeted immune response agent of the present invention, whether it be an anti-CXCL12 antibody, a bispecific antibody, a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12, or a T-cell preloaded with bispecific antibodies, has synergistic activity with a TIM-3 antagonist of the present invention. In preferred embodiments, the CXCL12-targeted immune response agent is for example, MAB310 or hu30D8 and has synergistic activity with a TIM-3 antagonist of the present invention, such as, for example, an anti-TIM-3 antibody.

[0084] In preferred embodiments, the CXCL12-targeted immune response agent of the present invention, whether it be an anti-CXCL12 antibody, a bispecific antibody, a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12, or a T-cell preloaded with bispecific antibodies, has synergistic activity with a checkpoint antagonist of the present invention. In preferred embodiments, the CXCL12-targeted immune response agent is for example, MAB310 or hu30D8 and has synergistic activity with a checkpoint antagonist of the present invention and those known in the art.

[0085] In preferred embodiments, the CXCL12-targeted immune response agent of the present invention, whether it be an anti-CXCL12 antibody, a bispecific antibody, a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12, or a T-cell preloaded with bispecific antibodies, has synergistic activity with a T-cell co-receptor antagonist of the present

invention. In preferred embodiments, the CXCL12-targeted immune response agent is for example, MAB310 or hu30D8 and has synergistic activity with a T-cell co-receptor antagonist of the present invention, such as, for example, an anti-T-cell co-receptor antibody, for example, an anti-4-1BB (CD137) antibody or an anti-ICOS (CD278)antibody.

[0086] In the present invention, a "tumor" is defined as a population of heterogeneous cells, collectively forming a mass of tissue in a subject resulting from the abnormal proliferation of malignant cancer cells. In some preferred embodiments, the tumor may comprise of p53+ (Gene ID; 2191, reference sequence NP_004451.2 GI:16933540) cancer cells. In other embodiments, the tumors are p53 negative. Thus, a "tumor" will contain both normal or "non-cancerous" cells and "cancer" or "cancerous" cells. A tumor typically comprises or is associated with p53+ and/or FAP+ stromal cells and/or inflammatory/immune cells. The cancer cells are often grouped together in "nests", separated by stromal regions containing extracellular matrix (e.g., collagen), immune cells and FAP+ fibroblastic cells.

[0087] The presence of FAP+ stromal cells in a cancerous tumour may be identified using routine techniques, including protein based methods, such as fluorescence microscopy and immunohistology or nucleic acid based methods, such as RT-PCR. Kraman *et al. Science*. 330, 827-30 (2010).

[0088] In the present invention, "proximity" is defined as the distance between the CD3+ T-cells, and even more preferably effector CD3+ T-cells, and the cancer cells within a tumor. For example, one way to measure "proximity" is to cross-section the tumor, such as a PDA tumor, and then stain the tumor with a cancer detecting antibody, such as anti-p53 (loss-of-heterozygosity at the p53 locus cancer cells may be have mutant p53 protein detectable) and anti-CD3epsilon (T-cells are +). The section is then subjected to ARIOL scanning. An instrument then evaluates the image, and calculates for each p53+ cell the distance to the nearest CD3+ cell. A histogram can then be constructed. Preferably, increases in the proximity of the T-cells among the cancer cells is increased by at least 2 fold (distance between cancer cell and nearest T-cell is decreased by 2 fold), 3 fold (distance between cancer cell and nearest T-cell is decreased by 3 fold), 4 fold (distance between cancer cell and nearest T-cell is decreased by 4 fold) or 5 fold (distance between cancer cell and nearest T-cell is decreased by 5 fold). An alternative assay is to delineate the areas of the tumor that contain cancers cells, identified by monoclonal appropriate antibodies, and calculate the density of CD3+ T cells contained within these areas and outside these areas, which are taken as stromal regions.

[0089] When effector CD3+ T-cells are in close proximity to the cancerous tumor cell, effector response ensues. Otherwise, if a T cell that has a TCR specific for a cancer cell antigen cannot come into contact with the cancer cell, the T cell will not be activated and its anti-cancer functions are not elicited, allowing the tumor to evade the immune recognition.

[0090] In the present invention, "frequency" is defined as the quantitative increase in T-cells and even more preferably effector CD3+ T-cells that are found among the cancer cells in the tumor microenvironment. Preferably, increases in frequency of the T-cells among the cancer cells is increased by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 300%.

[0091] Examples of tumors include, but are not limited to, sarcomas, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, esophageal cancer, pancreas cancer, renal cancer, stomach cancer, multiple myeloma and cerebral cancer. Preferred embodiments of tumors are adenocarcinomas. In some embodiments, the cancer may be pancreatic cancer, for example pancreatic ductal adenocarcinoma.

B. T-cell Exclusion in Tumors

[0092] "T-cell exclusion" in a tumor is defined as those tumor evasion mechanisms known in the art where effector CD3+ T-cell subsets are prevented from being recruited to and accumulating among cancer cells within the tumor microenvironment. Tumor evasion mechanisms include, but are not limited to: (1) immunologic barriers within the tumor microenvironment, including a failure of immunosurveillance in the tumor, (2) non-functional antigen presenting cells, and (3) dysfunctional CD4+ T-cells, CD8+ T-cells, and excessive numbers of Foxp3+ regulatory T-cells, A model of human PDA was developed to replicate a failure of immunosurveillance in the tumor. This failure is attributable to local immunosuppression mediated by the FAP+ stromal cell, which manifests as exclusion and likely death of T-cells from regions of the tumor containing PDA cells and involves its production of CXCL12.

[0093] In the prior art, expression of CXCL12 is associated with both impairment and promotion of immune control of growth of tumors. The art that demonstrates impairment of immune control (Righi et al.) indicates that this results from the recruitment of FoxP3+ regulatory T-cells to the tumor by expression of CXCL12. Surprisingly, it was observed that CXCL12 expression results in exclusion of all T-cells, including CD4+ T-cells, CD8+ T-cells,

and Foxp3+ regulatory T-cells. The use of CXCL12-targeted immune response agents are expected to overcome this exclusion, thus eliciting tumor antigen-specific immune responses against the tumor cells.

[0094] Accordingly, the present invention provides a method for recruitment of CD3+ T-cell subsets, including CD4+ T-cells, CD8+ T-cells, and Foxp3+ regulatory T-cells, to cancer cell-containing regions of a tumor in a subject, and methods for treating tumors by restoring immunological control of tumor growth. In this manner, the present invention overcomes the problem of T-cell exclusion and allows effector CD3+ T-cell subsets to accumulate and recruit to the cancer cells in order to carry out their endogenous function of eliminating the cancer cells.

[0095] Therefore, the described method herein increases the recruitment of effector CD3+ T-cell accumulation in the sites of a tumor that contain cancer cells, comprising administering to a subject in need thereof a pharmaceutically effective amount of a CXCL12-targeted immune response agent.

[0096] The efficacy of the present invention is based on the observation that FAP+ stromal cells secrete CXCL12 which becomes associated with the cancer cells within the tumor. Administration of a CXCL12-targeted immune response agent as described herein, such as for example an anti-CXCL12 antibody, a bispecific antibody, or a CAR T-cell, results in CD3+ effector T-cell recruitment to the cancer cell-containing sites of the tumor and elimination of the cancer cells.

[0097] For example, in one preferred embodiment of the present invention, anti-CXCL12 antibodies, bispecific antibodies and CAR T-cells, are examples of CXCL12-targeted immune response agents that can be used to recruit CD3+ T-cells to the cancer cell-containing sites of tumors and restore immunological regulation of the cancerous tumor cells. This restoration of immunological surveillance of the cancerous tumor leads to the elimination of the cancerous cells.

[0098] In one preferred example, the described invention increases T-cell accumulation and recruitment to the cancerous tumor cells, such as PDA, to reduce the tumor growth and overcome tumor evasion mechanisms. For example, like most solid tumors, PDA tumors contain stromal cells that express fibroblast activation protein (FAP). FAP+ stromal cells are found in both PDA and other tumors and are known to secrete CXCL12. One tumor evasion strategy is for cancer cells to bind CXCL12 and suppress local immune regulation of the tumor by excluding effector T-cells from accumulating amongst the cancer cells. In the presence of a CXCL12-targeted

immune response agent, such as, for example, anti-CXCL12 antibodies, bispecific antibodies or CAR T-cells, immune regulation of the tumor is restored.

[0099] Specifically, it is the recruitment of CD3+ T-cells that accumulate to the cancerous cells, such as PDA cells, when in the presence of a CXCL12-targeted immune response agent, such as, for example, anti-CXCL12 antibodies, bispecific antibodies or CAR T-cells, and these T-cells restore immunological regulation of the tumor.

[00100] As a result, this invention provides a method to treat a cancer comprised subject, such as a subject who contains PDA, by administering to a subject in need thereof a pharmaceutically effective amount of a CXCL12-targeted immune response agent. Manufacture and medication of a CXCL12-targeted immune response agent is able to reduce immune suppression, increase infiltration of effector T-cells amongst the cancer cells, restore immunological regulation of the tumor, and effectively reduce and eliminate cancer cells, preferably, in a tumor comprised of FAP+ stromal cells.

[00101] This invention relates to the use of CXCL12-targeted immune response agents to reduce or abolish tumor immunosuppression in an individual with cancer. The CXCL12-targeted immune response agent described here can be used to initiate or enhance immune responses against cancer cells in a subject, in particular cell-mediated immune responses.

[00102] The CXCL12-targeted immune response agent as described herein reduces the ability of the cancerous tumor to suppress immune responses, for example by excluding CD3+ T-cell subsets, such that immune responses to the tumor are more effective in the subject. This may have a beneficial therapeutic effect on the cancerous tumor of a human patient.

[00103] In some embodiments, the anti-CXCL12 targeting agent can be used to deliver a second molecule to the tumor cells in order to eliminate or inhibit the growth of the tumor cells. In some cases, the second molecule is a pro-apoptotic protein that triggers apoptosis, a toxin or radionuclide that destroys the tumor cells, a chemotherapeutic agent/anti-cancer compound or any other heterologous molecule, substance, or agent capable of binding to a chemokine or cytokine that triggers the immune system to eliminate the tumor cell.

[00104] In some embodiments, the anti-CXCL12 targeting agent is a bidirectional antibody molecule or fragment thereof that binds CXCL12 and also binds to a cytokine. In some cases, the bidirectional antibody may bind to endogenously expressed cytokine(s). In other cases, the bidirectional antibody may be pre-loaded with a particular cytokine before infusion into the patient.

[00105] Cytokines stimulate effector cells and stromal cells at the tumor site, thereby, enhancing tumor cell recognition by cytotoxic effector cells (Lee et al., 2011), and have broad anti-tumor activity. For example, IL-12 regulates both innate and adaptive immune responses, induces anti-cancer effects, and synergizes with other cytokines for increased immunoregulatory and anti-tumor activities (Lee et al., 2011).

[00106] In preferred embodiments, the cytokine is IL-12. IL-12 may be delivered to the tumor via a bispecific antibody or any other molecule that can bind to IL-12 to deliver IL-12 to the site of the tumor. Other cytokines include IL-2, IL-6, IL-7, IL-10, IL-15, IL-17, IL-18, IL-21, GM-CSF, TNF, IFN-α, IFN-β, IFN-γ, or FasL. In preferred embodiments, cytokines are pro-inflammatory.

[00107] IL-12 may inhibit tumor growth by activating the immune system. For example, IL-12 is known to activate T and natural killer (NK) cells, as well as facilitate presentation of tumor antigens through the upregulation of class I and II major histocompatibility (MHC) molecules, and to generate T helper type I (Th1) immune responses (Weiss et al., 2007). IL-12 also is known to exert potent anti-tumor effects in solid tumors, leukemias, lymphomas and melanomas, as well as induce expression of IFN-γ in developing T cells, thereby priming these T-cells to stably produce IFN-γ (*Id.*).

[00108] In some embodiments, the CXCL12-targeted immune response agent as described herein may be administered in combination with a cytokine, such as IL-12. The CXCL12-targeted immune response agent as described herein may bind endogenous cytokine, acting to increase the local concentration of the cytokine, e.g., IL-12. In some embodiments, the CXCL12-targeted immune response agent is a bispecific antibody that binds both CXCL12 and a cytokine such as IL-12. In some embodiments, inhibition of CXCL12 alleviates suppression of immune responses (specifically, the exclusion of T cells from the tumor microenvironment), while delivery of IL-12 stimulates the immune system. In some embodiments, the CXCL12-targeted immune response agent and IL-12 act synergistically to activate the immune system to inhibit the growth of or eliminate tumor cells.

[00109] IL-12 is known to be associated with side effects including toxicity. Thus, by binding IL-12 to the CXCL12-targeted immune response agent, which can specifically deliver IL-12 to the tumor site, IL-12 can be delivered in a sufficient amount to activate the immune system while reducing or eliminating toxic side effects. Likewise, any other cytokine having toxic side effects may be bound to the CXCL12-targeted immune response agent to reduce toxicity.

[00110] Further, the CXCL12-targeted immune response agent as described herein may be administered in combination with multiple cytokines. Cytokines may synergize with other cytokine(s) to reduce immunosuppressive effects from administration of a particular cytokine. For example, repeated administration of IL-12 attenuated other cytokines involved in stimulation of the immune system, including INF-γ (Weiss et al. 2007). Thus, IL-12 may be coadministered with IL-2 to overcome the inhibition of INF-γ production. IL-12 may synergize with cytokines to elicit higher levels of T and NK cell activation and T cell proliferation than if administered individually. For example, IL-12 in combination with IL-2 reportedly induced IFN-γ, TNFα and GM-CSF production at a higher rate in T and NK cells than with IL-12 alone (*Id.*).

[00111] In other embodiments, the anti-CXCL12 antibody or fragment thereof and IL-12 is coadministered with IL-15; the anti-CXCL12 antibody or fragment thereof and IL-12 is coadministered with IL-7; the anti-CXCL12 antibody or fragment thereof and IL-12 is coadministered with IL-21; the anti-CXCL12 antibody or fragment thereof and IL-12 is coadministered with IL-18; the anti-CXCL12 antibody or fragment thereof and IL-12 is coadministered with GM-CSF; or the anti-CXCL12 antibody or fragment thereof and IL-12 co-administered with INF-α (Weiss et al., 2007). Coadministration can alleviate attenuation of cytokines needed for immune system activation as well as synergize with each other to activate the immune system.

[00112] In another preferred embodiment, the anti-CXCL12 antibody or fragment thereof or any other molecule capable of binding to CXCL12 is fused to a radionuclide. Examples of such radionuclides include, but are not limited to ^{131}Iodine, ^{90}γYttrium, ^{177}Lutetium, ^{188}Rhenium, ^{67}Copper, ^{211}Astatine, ^{213}Bismuth, ^{125}Iodine, or ^{111}Indium. The radionuclides emit radiation causing destruction of the tumor cell.

[00113] In a further preferred embodiment, the anti-CXCL12 antibody is fused to a toxin. Examples of such toxins include, but are not limited to Pseudomonas exotoxin, staphylococcal enterotoxin A, ricin A-chain, or plant ribosome-inactivating protein saporin. Other bacterial toxins include diphtheria toxins and other plant toxins include abrin, mistletoe lectin, modeccin, hemitoxins, PAP, pokeweed antiviral toxin, saponin, bryodin 1, bouganin, and gelolin, etc. For example, the toxins may block protein synthesis leading to the destruction of the tumor cell.

[00114] In a preferred embodiment, the anti-CXCL12 antibody is fused to a pro-apoptotic protein. Examples of such pro-apoptotic proteins include, but are not limited to caspase-3,

FOXP3, or death ligand TNF-related apoptosis-inducing ligand (TRAIL). Other pro-apoptotic proteins include but are not limited to bad, bax, bcl-2, bcl-w, BID, BIM, Caspase 8, CD40, CD40 Ligand, cIAP-2, Cytochrome-C, DR6, Fas, Fas Ligand, HSP27, HSP60, HSP70, HTRA, IGF-1, IGF-2, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, IGF-1sR, livin, p21, p27, p53, SMAC, Survivin, sTNFRI, sTNFRII, TNF alpha, TNF beta, TRAIL R1, TRAIL R2, TRAIL R3, TRAIL R4, and XIAP. Such pro-apoptotic proteins, when triggered, lead to the death of the tumor cell.

[00115] Provided in this description are methods of cancer immunotherapy in an individual in need thereof, which comprise administering to the individual a CXCL12-targeted immune response agent as described herein in an amount effective to treat the cancer, for example by increasing the effectiveness of the host immune response against the cancer in the individual.

[00116] Also provided herein are methods of reducing immune suppression in a cancerous tumor in an individual and/or increasing the effectiveness of an immune response, preferably a cell-mediated immune response, to a cancerous tumor in an individual, comprising administering a CXCL12-targeted immune response agent to the individual, as described herein.

[00117] Also provided herein are methods of increasing the sensitivity of a cancerous tumor in an individual to host immune responses, the method comprising administering a CXCL12-targeted immune response agent as described in the present invention to an individual.

[00118] Also provided herein are methods for increasing T-cell accumulation and recruitment, preferably effector CD3+ T-cell accumulation in the sites of a cancerous tumor that contain cancer cells, the method comprising administering a CXCL12-targeted immune response agent as described herein, such as, for example, anti-CXCL12 antibodies, bispecific antibodies or CAR T-cells. A CXCL12-targeted immune response agent can be used to increase T-cell accumulation and recruitment at the cancer containing sites in a tumor. The present invention also relates to the use of a CXCL12-targeted immune response agent in the manufacture of a medication for use in increasing T-cell accumulation at the cancer containing sites in a tumor.

[00119] We have observed that, before treatment, most T-cells are found in the stromal regions of the tumor, such as, for example colorectal cancer and/or ovarian cancer. This distribution of T-cells is believed to be at least partially responsible for the inability of the immune response to the cancer cells to control tumor growth. The administration of a CXCL12-targeted immune response agent increases the accumulation of effector T-cells at in the cancer cell regions of the tumor.

[00120] Tumor therapy, as referred to herein, includes therapies which reduce the rate of tumor growth, that is slow down, but do not necessarily eliminate tumor growth.

[00121] Reduction in the rate of tumor growth can be, for example, a reduction in at least 10%, 20%, 30%, 40%, 50%, 75%, 100%, 150%, 200% or more of the rate of growth of a tumor. For example, the rate of growth can be measured over 1, 2, 3, 4, 5, 6 or 7 days, or for longer periods of one or more weeks.

[00122] In some embodiments, the invention may result in the arrest of tumor growth, or the reduction in tumor size or the elimination of a tumor.

[00123] Cancer cells within the tumor in the subject may be immunologically distinct from normal somatic cells in the subject (for example, the tumor may be immunogenic; alternatively, even if it is not immunogenic, it may present different immunological determinants(s) from somatic cells). For example, the cancer cells may be capable of eliciting a systemic immune response in the subject against one or more antigens expressed by the cancer cells. The antigens that elicit the immune response may be tumor antigens or may be shared by normal cells.

[00124] In embodiments, the tumor, although presenting different antigenic determinants, is hidden from the immune system of a subject and displays tumor evasion characteristics. For example, the tumor may exclude immune cells, thus lowering its immunological visibility and sensitivity, and/or preventing the immune system from acting to attack the tumor.

[00125] CD8+ T-cells that are specific for cancer cells within the cancerous tumor may be present in the subject.

[00126] In embodiments, CD8+ T-cells may be recruited to the cancerous tumor. In some embodiments, the cancer cells may express one or more antigens that are not expressed by normal somatic cells in the subject (i.e. tumor antigens). Tumor antigens are known in the art and may elicit immune responses in the subject. In particular, tumor antigens may elicit T-cell-mediated immune responses against cancer cells in the subject i.e. the tumor antigens may be recognized by CD8+ T-cells in the subject.

[00127] Tumor antigens expressed by cancer cells in a cancerous tumor may include, for example, cancer-testis (CT) antigens encoded by cancer-germ line genes, such as MAGE-A1, MAGE-A2, MAGE- A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-I, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, BAGE-I, RAGE- 1, LB33/MUM-1, PRAME, NAG, MAGE-Xp2

(MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE- C1/CT7, MAGE-C2, NY-ESO-I, LAGE-I, SSX-I, SSX-2(HOM-MEL-40), SSX-3, SSX-4, SSX-5, SCP-I and XAGE and immunogenic fragments thereof (Simpson et al., Nature Rev (2005) 5, 615-625, Gure et al., Clin Cancer Res (2005) 11, 8055-8062; Velazquez et al., Cancer Immun (2007) 7, 1 1 ; Andrade et al., Cancer Immun (2008) 8, 2; Tinguely et al., Cancer Science (2008); Napoletano et al., Am J of Obstet Gyn (2008) 198, 99 e91-97).

[00128] Other tumor antigens that may be expressed include, for example, overexpressed or mutated proteins and differentiation antigens particularly melanocyte differentiation antigens such as p53, ras, CEA, MUC1, PMSA, PSA, tyrosinase, Melan-A, MART-1, gp100, gp75, alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR.alpha. fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, GnTV, Herv-K-mel, NA-88, SP17, and TRP2-Int2, (MART-I), E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, alpha.-fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\170K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, and TPS and tyrosinase related proteins such as TRP-1, TRP-2, and mesothelin.

[00129] Other tumor antigens that may be expressed include out-of-frame peptide-MHC complexes generated by the non-AUG translation initiation mechanisms employed by “stressed” cancer cells (Malarkannan et al. Immunity 1999).

[00130] Other tumor antigens that may be expressed are well-known in the art (see for example WO00/20581; Cancer Vaccines and Immunotherapy (2000) Eds Stern, Beverley and Carroll, Cambridge University Press, Cambridge) The sequences of these tumor antigens are readily available from public databases but are also found in WO 1992/020356 A1, WO 1994/005304 A1, WO 1994/023031 A1, WO 1995/020974 A1, WO 1995/023874 A1 & WO 1996/026214 A1.

[00131] A subject suitable for treatment as described above may be a mammal, such as a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline

(e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orangutan, gibbon), or a human.

[00132] In some embodiments, the subject is a human. In other embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (*e.g.* murine, primate, porcine, canine, or rabbit animals) may be employed.

[00133] In some embodiments, the subject may have minimal residual disease (MRD) after an initial cancer treatment.

[00134] A subject with cancer may display at least one identifiable sign, symptom, or laboratory finding that is sufficient to make a diagnosis of cancer in accordance with clinical standards known in the art. Examples of such clinical standards can be found in textbooks of medicine such as Harrison's Principles of Internal Medicine, 15th Ed., Fauci AS et al., eds., McGraw-Hill, New York, 2001. In some instances, a diagnosis of a cancer in a subject may include identification of a particular cell type (e.g. a cancer cell) in a sample of a body fluid or tissue obtained from the subject.

C. Formulations

[00135] A suitable serum concentration of CXCL12-targeted immune response agent for the induction of an immune response to an epithelial tumor characterized by bound CXCL12 may be readily determined from *in vivo* assays.

[00136] The CXCL12-targeted immune response agent may be administered together with other anti-cancer therapies, such as conventional chemotherapeutic agents, radiation therapy or cancer immunotherapy. For example, the CXCL12-targeted immune response agent is administered together with an anti-cancer compound. The CXCL12-targeted immune response agent and the anti-cancer compound may be separate compounds or molecules or they may be covalently or non-covalently linked in a single compound, molecule, particle or complex.

[00137] An anti-cancer compound may be any anti-cancer drug or medicament which has activity against cancer cells. Suitable anti-cancer compounds for use in combination with CXCR4 as disclosed herein may include aspirin, sulindac, curcumin, alkylating agents including: nitrogen mustards, such as mechlor-ethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); thylenimines/methylmelamine such as thriethylenemelamine (TEM),

triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2´-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; epipodophylotoxins such as etoposide and teniposide; antibiotics, such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase, cytokines such as interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, TNF-beta and GM-CSF, anti-angiogenic factors, such as angiostatin and endostatin, inhibitors of FGF or VEGF such as soluble forms of receptors for angiogenic factors, including soluble VGF/VEGF receptors, platinum coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p´-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; non-steroidal antiandrogens such as flutamide; kinase inhibitors, histone deacetylase inhibitors, methylation inhibitors, proteasome inhibitors, monoclonal antibodies, oxidants, anti-oxidants, telomerase inhibitors, BH3 mimetics, ubiquitin ligase inhibitors, stat inhibitors and receptor tyrosin kinase inhibitors such as imatinib mesylate (marketed as Gleevac or Glivac) and erlotinib (an EGF receptor inhibitor) now marketed as Tarveca; and anti-virals such as oseltamivir phosphate, Amphotericin B, and palivizumab.

[00138] While it is possible for CXCL12-targeted immune response agents and anti-cancer compounds to be administered alone, it is preferable to present the compounds in the same or separate pharmaceutical compositions (e.g. formulations).

[00139] A pharmaceutical composition may comprise, in addition to the CXCL12-targeted immune response agent and/or an anti-cancer compound, one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilizers, preservatives, lubricants, or other materials well known to those skilled in the art. Suitable materials will be sterile and pyrogen-free, with a suitable isotonicity and stability. Examples include sterile saline (e.g. 0.9% NaCl), water, dextrose, glycerol, ethanol or the like or combinations thereof. Such materials should be non-toxic and should not interfere with the efficacy of the active compound. The precise nature of the carrier or other material will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below. Suitable materials will be sterile and pyrogen free, with a suitable isotonicity and stability. Examples include sterile saline (e.g. 0.9% NaCl), water, dextrose, glycerol, ethanol or the like or combinations thereof. The composition may further contain auxiliary substances such as wetting agents, emulsifying agents, pH buffering agents or the like.

[00140] Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

[00141] The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

[00142] In some embodiments, one or both of the CXCL12-targeted immune response agent and anti-cancer compound may be provided in a lyophilized form for reconstitution prior to administration. For example, lyophilized reagents may be re-constituted in sterile water and mixed with saline prior to administration to a subject

[00143] The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[00144] Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, lozenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

[00145] Optionally, other therapeutic or prophylactic agents may be included in a pharmaceutical composition or formulation.

[00146] Increasing immune response to tumors as described herein may be useful in immunotherapy for the treatment of cancer.

[00147] Treatment may be any treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition or delay of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, cure or remission (whether partial or total) of the condition, preventing, delaying, abating or arresting one or more symptoms and/or signs of the condition or prolonging survival of a subject or patient beyond that expected in the absence of treatment.

[00148] Treatment as a prophylactic measure (i.e. prophylaxis) is also included. For example, a subject susceptible to or at risk of the occurrence or re-occurrence of cancer may be treated as described herein. Such treatment may prevent or delay the occurrence or re-occurrence of cancer in the subject.

[00149] In particular, treatment may include inhibiting cancer growth, including complete cancer remission, and/or inhibiting cancer metastasis. Cancer growth generally refers to any one of a number of indices that indicate change within the cancer to a more developed form. Thus, indices for measuring an inhibition of cancer growth include a decrease in cancer cell survival, a decrease in tumor volume or morphology (for example, as determined using computed tomographic (CT), sonography, or other imaging method), a delayed tumor growth, a destruction of tumor vasculature, improved performance in delayed hypersensitivity skin test, an increase in the activity of cytolytic T-lymphocytes, and a decrease in levels of tumor-specific antigens. Increasing immune response to tumors in a subject may improve the capacity of the subject to resist cancer growth, in particular growth of a cancer already present the subject and/or decrease the propensity for cancer growth in the subject.

[00150] CXCL12-targeted immune response agents may be administered as described herein in therapeutically-effective amounts.

[00151] The term "therapeutically-effective amount" as used herein, pertains to that amount of an active compound, or a combination, material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio.

[00152] It will be appreciated that appropriate dosages of the active compounds can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the administration. The selected dosage level will depend on a variety of factors including, but not limited to, the route of administration, the time of administration, the rate of excretion of the active compound, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of active compounds and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve concentrations of the active compound at a site of therapy without causing substantial harmful or deleterious side-effects.

[00153] In general, a suitable dose of the active compound is in the range of about 100 μg to about 250 mg per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

[00154] For example, a CXCL12-targeted immune response agent as described herein, such as such as, for example, anti-CXCL12 antibodies, bispecific antibodies, or CAR T-cells may be administered by continuous intravenous infusion in an amount sufficient to maintain the serum concentration at a level that inhibits tumor growth. Other CXCL12-targeted immune response agents described herein can also be used in this same manner.

[00155] Administration *in vivo* can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals). Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the physician.

[00156] Administration of anti-cancer compounds and the CXCL12-targeted immune response agent may be simultaneous, separate or sequential. By "simultaneous" administration, it is meant that the anti-cancer compounds and the CXCL12-targeted immune response agents are administered to the subject in a single dose by the same route of administration.

[00157] By "separate" administration, it is meant that the anti-cancer compounds and the CXCL12-targeted immune response agent are administered to the subject by two different routes of administration which occur at the same time. This may occur for example where one agent is administered by infusion or parenterally and the other is given orally during the course of the infusion or parenteral administration.

[00158] By "sequential" it is meant that the anti-cancer compounds and the CXCL12-targeted immune response agent are administered at different points in time, provided that the activity of the first administered agent is present and ongoing in the subject at the time the second agent is administered. For example, the anti-cancer compounds may be administered first, such that an immune response against a tumor antigen is generated, followed by administration of the CXCL12-targeted immune response agent, such that the immune response at the site of the tumor is enhanced, or *vice versa*. Preferably, a sequential dose will occur such that the second of the two agents is administered within 48 hours, preferably within 24 hours, such as within 12, 6, 4, 2 or 1 hour(s) of the first agent.

[00159] Multiple doses of the CXCL12-targeted immune response agent may be administered, for example 2, 3, 4, 5 or more than 5 doses may be administered after administration of the anti-cancer compounds. The administration of the CXCL12-targeted immune response agent may continue for sustained periods of time after administration of the anti-cancer compounds. For example treatment with the CXCL12-targeted immune response agent may be continued for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month or at least 2 months. Treatment with the CXCL12-targeted immune response agent may be continued for as long as is necessary to achieve complete tumor rejection.

[00160] Multiple doses of the anti-cancer compounds may be administered, for example 2, 3, 4, 5 or more than 5 doses may be administered after administration of the CXCL12-targeted immune response agent. The administration of the anti-cancer compounds may continue for sustained periods of time after administration of the CXCL12-targeted immune response agent. For example treatment with the anti-cancer compounds may be continued for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month or at least 2 months. Treatment with the anti-

cancer compounds may be continued for as long as is necessary to achieve complete tumor rejection.

[00161] The active compounds or pharmaceutical compositions comprising the active compounds may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); and parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly. Usually administration will be by the intravenous route, although other routes such as intraperitoneal, subcutaneous, transdermal, oral, nasal, intramuscular or other convenient routes are not excluded.

[00162] The pharmaceutical compositions comprising the active compounds may be formulated in suitable dosage unit formulations appropriate for the intended route of administration.

[00163] Formulations suitable for oral administration (e.g. by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

[00164] A tablet may be made by conventional means, e.g., compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g. povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g. lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, silica); disintegrants (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g. sodium lauryl sulfate); and preservatives (e.g. methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.

Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

[00165] Formulations suitable for parenteral administration (e.g. by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilizers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 μg/ml, for example from about 10 ng/ml to about 1 μg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

[00166] Compositions comprising anti-cancer compounds and/or CXCL12-targeted immune response agents may be prepared in the form of a concentrate for subsequent dilution, or may be in the form of divided doses ready for administration. Alternatively, the reagents may be provided separately within a kit, for mixing prior to administration to a human or animal subject.

[00167] The CXCL12-targeted immune response agent may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the individual circumstances. For example, CXCL12-targeted immune response agents as described herein may be administered in combination with one or more additional active compounds.

[00168] In still other embodiments, the CXCL12-targeted immune response agents as described herein may be administered in combination with one or more additional cytokines. Cytokines include, but are not limited to: IL-2, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, GM-CSF, TNF, IFN-α, IFN-β, IFN-γ, or FasL.

[00169] In some embodiments, the treatment of a subject using a CXCL12-targeted immune response agent as described herein may further comprise administering one or more additional immunotherapeutic agents to the subject.

[00170] An immunotherapeutic agent may facilitate or enhance the targeting of cancer cells by the immune system, in particular T-cells, through the recognition of antigens expressed by the cancer cells.

[00171] Suitable agents include cancer vaccine preparations designed to induce T lymphocytes (T-cells) recognizing a localized region of an antigen or epitope specific to the tumor cell.

[00172] A cancer vaccine is an agent, a cell-based agent, molecule, or immunogen which stimulates or elicits an endogenous immune response in a subject or subject against one or more tumor antigens. Suitable cancer vaccines are known in the art and may be produced by any convenient technique.

[00173] The use of tumor antigens to generate immune responses is well-established in the art (see for example; Kakimi K, et al. Int J Cancer. 2011 Feb 3; Kawada J, Int J Cancer. 2011 Mar 16; Gnjatic S, et al. Clin Cancer Res. 2009 Mar 15;15(6):2130-9; Yuan J, et al. Proc Natl Acad Sci U S A. 2008 Dec 23;105(51):20410-5; Sharma P, et al. J Immunother. 2008 Nov-Dec;31(9):849-57; Wada H, et al. Int J Cancer. 2008 Nov 15;123(10):2362-9; Diefenbach CS, et al. Clin Cancer Res. 2008 May 1;14(9):2740-8; Bender A, et al. Cancer Immun. 2007 Oct 19;7:16; Odunsi K, et al. Proc Natl Acad Sci U S A. 2007 Jul 31;104(31):12837-42; Valmori D, et al. Proc Natl Acad Sci U S A. 2007 May 22;104(21):8947-52; Uenaka A, et al. Cancer Immun. 2007 Apr 19;7:9; Kawabata R, et al. Int J Cancer. 2007 May 15 ; 120(10):2178-84; Jäger E, et al. Proc Natl Acad Sci U S A. 2006 Sep 26;103(39):14453-8; Davis ID Proc Natl Acad Sci U S A. 2005 Jul 5;102(27):9734; Chen Q, Proc Natl Acad Sci U S A. 2004 Jun 22;101(25):9363-8; Jäger E, Proc Natl Acad Sci U S A. 2000 Oct 24;97(22):12198-203; Carrasco J, et al. J Immunol. 2008 Mar 1;180(5):3585-93; van Baren N, et al. J Clin Oncol. 2005 Dec 10;23(35):9008-21; Kruit WH, et al. Int J Cancer. 2005 Nov 20;117(4):596-604; Marchand M, et al. Eur J Cancer. 2003 Jan;39(1):70-7; Marchand M et al. Int J Cancer. 1999 Jan 18;80(2):219-30; Atanackovic D, et al. Proc Natl Acad Sci U S A. 2008 Feb 5;105(5):1650-5).

[00174] Cancer cells from the subject may be analyzed to identify a tumor antigen expressed by the cancer cells. For example, a method as described herein may comprise the step of identifying a tumor antigen which is displayed by one or more cancer cells in a sample obtained from the subject. A cancer vaccine comprising one or more epitopes of the identified tumor antigen may

then be administered to the subject whose cancer cells express the antigen. The vaccine may induce or increase an immune response, preferably a T-cell mediated immune response, in the subject against the cancer cells expressing the identified tumor antigen.

[00175] The cancer vaccine may be administered before, at the same time, or after the CXCL12-targeted immune response agent is administered to the subject as described here.

[00176] Adoptive T-cell therapy involves the administration to a subject of tumor-specific T-cells to a subject. Preferably, the T-cells were previously isolated from the subject and expanded *ex vivo*. Suitable adoptive T-cell therapies are well known in the art (J. Clin Invest. 2007 June 1; 117(6): 1466–1476.) For example, adoptive T-cell therapy using CAR T-cells (chimeric antigen receptor) would be greatly improved if used in combination with a CXCL12-targeted immune response agent. CAR T-cells must migrate into a tumor to get in proximity to the cancer cells within the tumor in order to mediate their killing activity. The present invention, such as such as, for example, AMD3100, BMS-936564/MDX-1338, AMD11070, or LY2510924, used in combination with CAR T-cells may improve this type of immunotherapy.

[00177] In some embodiments, the treatment of an individual using a CXCL12-targeted immune response agent may further comprise administering one or more tumor therapies to treat the cancerous tumor. Such therapies include, for example, tumor medicaments, radiation and surgical procedures.

[00178] A tumor medicament is an agent which is administered to a subject for the purpose of treating a cancer. Suitable medicaments for the treatment of tumors are well known in the art.

[00179] Suitable medicaments for use in combination with CXCL12-targeted immune response agent as disclosed herein may include aspirin, sulindac, curcumin, alkylating agents including: nitrogen mustards, such as mechlor-ethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); thylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2´-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs

such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; epipodophylotoxins such as etoposide and teniposide; antibiotics, such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase, cytokines such as interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha, TNF-beta and GM-CSF, anti-angiogenic factors, such as angiostatin and endostatin, inhibitors of FGF or VEGF such as soluble forms of receptors for angiogenic factors, including soluble VGF/VEGF receptors, platinum coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p´-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; non-steroidal antiandrogens such as flutamide; kinase inhibitors, histone deacetylase inhibitors, methylation inhibitors, proteasome inhibitors, monoclonal antibodies, oxidants, anti-oxidants, telomerase inhibitors, BH3 mimetics, ubiquitin ligase inhibitors, stat inhibitors and receptor tyrosin kinase inhibitors such as imatinib mesylate (marketed as Gleevac or Glivac) and erlotinib (an EGF receptor inhibitor) now marketed as Tarveca; and anti-virals such as oseltamivir phosphate, Amphotericin B, and palivizumab.

[00180] Additionally, other T-cell checkpoint antagonists, like Lag-3, or inhibitors of IDO1/IDO2 (indoleamine 2,3-dioxygenase) could also be used in combination with the present invention. These enzymes catabolize tryptophan in the tumor microenvironment, which impairs T-cell function. By using a CXCL12-targeted immune response agent, such as for example, anti-CXCL12 antibodies, bispecific antibodies, or CAR T-cells, in combination with a T-cell checkpoint antagonist may synergistically increase cancer cell killing within a tumor.

[00181] Various embodiments are disclosed above for a CXCL12-targeted immune response agent. Aspects and embodiments of the invention relating to a CXCL12-targeted immune response agent and optionally one or more other agents disclosed above include disclosure of the administration of the compounds or agents separately (sequentially or simultaneously) or in combination (co-formulated or mixed). For each aspect or embodiment, the specification further

discloses a composition comprising the CXCL12-targeted immune response agent and optionally one or more other agents co-formulated or in admixture with each other and further discloses a kit or unit dose containing the CXCL12-targeted immune response agent. Optionally, such compositions, kits or doses further comprise one or more carriers in admixture with the agent or co-packaged for formulation prior to administration to an individual.

[00182] Immunosuppression has previously been shown to result from the exclusion and/or death of T-cells from the microenvironment of the cancerous tumor (U.S. Appl. No. 14/620,463, incorporated by reference in its entirety herein). Inhibition of CXCR4 signaling using a CXCR4 signaling inhibitor as described in the referenced application, such as, for example, AMD3100, BMS-936564/MDX-1338, AMD11070, or LY2510924, as described therein, overcomes this exclusion and exposes cancer cells in the tumor to T-cells. In other aspects of the invention, methods of treatment may comprise the administration of a CXCR4 signaling inhibitor in combination with a CXCL12-targeted immune response agent, as described above, for the treatment of cancer. The CXCR4 signaling inhibitor and CXCL12-targeted immune response agent may be administered in the absence of the PD-1 signaling inhibitor.

[00183] Suitable CXCL12-targeted immune response agents, immunotherapeutic agents and methods of treatment are described *mutatis mutandis* above.

[00184] Various embodiments are also disclosed above for combinations of a PD-1 signaling inhibitor and a CXCL12-targeted immune response agent. Aspects and embodiments of the invention relating to combinations of a PD-1 signaling inhibitor and a CXCL12-targeted immune response agent and optionally one or more other agents disclosed above include disclosure of the administration of the compounds or agents separately (sequentially or simultaneously) or in combination (co-formulated or mixed). For each aspect or embodiment, the specification further discloses a composition comprising the PD-1 signaling inhibitor and CXCL12-targeted immune response agent and optionally one or more other agents co-formulated or in admixture with each other and further discloses a kit or unit dose containing the PD-1 signaling inhibitor and CXCL12-targeted immune response agent packaged together, but not in admixture. Optionally, such compositions, kits or doses further comprise one or more carriers in admixture with one or both agents or co-packaged for formulation prior to administration to a subject.

[00185] Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

[00186] Other aspects and embodiments of the invention provide the aspects and embodiments described above with the term "comprising" replaced by the term "consisting of" and the aspects and embodiments described above with the term "comprising" replaced by the term "consisting essentially of".

[00187] "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each (i) A, (ii) B and (iii) A and B, just as if each is set out individually.

[00188] It is to be understood that the application discloses all combinations of any of the above aspects and embodiments described above with each other, unless the context demands otherwise. Similarly, the application discloses all combinations of the preferred and/or optional features either singly or together with any of the other aspects, unless the context demands otherwise.

[00189] Modifications of the above embodiments, further embodiments and modifications thereof will be apparent to the skilled person on reading this disclosure, and as such these are within the scope of the present invention.

[00190] All documents and sequence database entries mentioned in this specification are incorporated herein by reference in their entirety for all purposes.

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Claims

1. A method of inducing an immune response to an epithelial tumor characterized by bound CXCL12 comprising administering to a subject with the tumor one or more CXCL12-targeted immune response agents; wherein administration of the CXCL12-targeted immune response agent enhances or induces a tumor antigen-specific immune response against the epithelial tumor.

2. The method of claim 1, wherein the CXCL12-targeted immune response agent is an anti-CXCL12 antibody.

3. The method of claim 2, wherein the anti-CXCL12 antibody belongs to immunoglobulin isotypes that bind to Fc receptors that mediate phagocytosis by macrophages or that mediate antibody-dependent cell-mediated cytotoxicity by natural killer cells.

4. The method of either claim 2 or 3, wherein the anti-CXCL12 antibody belongs to the IgG1 or IgG3 immunoglobulin isotypes.

5. The method of any one of claims 1-4, wherein the anti-CXCL12 antibody triggers the antibody-dependent elimination of epithelial tumor cells

6. The method of any one of claims 1-5, wherein the anti-CXCL12 antibody is hu30D8.

7. The method of any one of claims 2-6, wherein the method also comprises administration of an anti-CD47 antibody.

8. The method of claim 7, wherein the anti-CD47 antibody disrupts the interaction between CD47 on cancer cells with SIRPα on phagocytic cells.

9. The method of claims 7 or 8, wherein the anti-CD47 antibody enhances the antibody-dependent elimination of epithelial tumor cells.

10. The method of claim 1, wherein the CXCL12-targeted immune response agent is a bispecific antibody.

11. The method of claim 10, wherein the bispecific antibody is selected from the list consisting of: a bispecific T-cell engager (BiTE) antibody, a dual-affinity retargeting molecule (DART), a CrossMAb antibody, a DutaMab™ antibody, a DuoBody antibody; a Triomab, a TandAb, a

bispecific NanoBody, Tandem scFv, a diabody, a single chain diabody, a HSA body, a (scFv)2 HSA Antibody, an scFv-IgG antibody, a Dock and Lock bispecific antibody, a DVD-IgG antibody, a TBTI DVD-IgG, an IgG-fynomer, a Tetravalent bispecific tandem IgG antibody, a dual-targeting domain antibody, a chemically linked bispecific (Fab')2 molecule, a crosslinked mAb, a Dual-action Fab IgG (DAF-IgG), an orthoFab-IgG, a bispecific CovX-Body, a bispecific hexavalent trimerbody, 2 scFv linked to diphtheria toxin, and an ART-Ig.

12. The method of claims 10 or 11, wherein the bispecific antibody binds to CXCL12 and a chemokine.

13. The method of claim 1, wherein the CXCL12-targeted immune response agent is a CAR T-cell engineered to express a chimeric antigen receptor against CXCL12.

14. The method of claim 13, wherein the CAR T-cell causes apoptosis of the epithelial tumor cells.

15. The method of claim 1, wherein the CXCL12-targeted immune response agent is a T-cell preloaded with bispecific antibody.

16. The method of claim 15, wherein the T-cell preloaded with bispecific antibody is polyclonally-activated.

17. The method of any one of claims 1-16, wherein cancer cell growth is inhibited.

18. The method of any one of claims 1-17, wherein cancer cells are eliminated.

19. The method of any one of claims 1-18, wherein tumor mass is reduced.

20. The method of any one of claims 1-19 wherein the tumor is resistant to immunotherapy.

21. The method of any one of claims 1-20, wherein the tumor is a adenocarcinoma, sarcoma, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovarian cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, esophageal cancer, pancreas cancer, pancreatic ductal adenocarcinoma (PDA), renal cancer, stomach cancer, multiple myeloma or cerebral cancer.

22. The method of any one of claims 1-21, wherein the method also comprises administering a checkpoint antagonist.

23. The method of claim 22, wherein the checkpoint antagonist is synergistic with the CXCL12-targeted immune response agent.

24. The method of either claim 22 or 23, wherein the checkpoint antagonist is a PD-1 signaling inhibitor.

25. The method of claim 24, wherein the PD-1 signaling inhibitor is a PD-1 antagonist.

26. The method of claim 25, wherein the PD-1 antagonist is an anti-PD-1 antibody.

27. The method of claim 24, wherein the PD-1 signaling inhibitor is a PD-L1 antagonist.

28. The method of claim 27, wherein the PD-L1 antagonist is an anti-PD-L1 antibody.

29. The method of claim 22 or 23, wherein the checkpoint antagonist is a CTLA-4 antagonist, TIM-3 antagonist, or a LAG3 antagonist.

30. The method of claim 1-21, wherein the method also comprises administering a T-cell co-receptor agonist.

31. The method of claim 30, wherein the T-cell co-receptor agonist is an anti-T-cell co-receptor antibody.

32. The method of claim 31, wherein the anti-T-cell co-receptor antibody is an anti-4-1BB (CD137) antibody or an anti-ICOS (CD278) antibody.

33. The method of any one of claims 30-32, wherein the T-cell co-receptor agonist is synergistic with the CXCL12-targeted immune response agent.

34. The method of any one of claims 1-33, wherein the method also comprises administering other anti-cancer therapies.

35. The method of claim 34, wherein the anti-cancer therapies are selected from chemotherapeutic agents, radiation therapy, cancer therapy, immunotherapy, or cancer vaccines.

36. The method of claim 35, wherein the immunotherapy is selected from adoptive T-cell therapies or cancer vaccine preparations designed to induce T lymphocytes to recognize tumor cells.

37. The method of claim 36, wherein the cancer vaccine recognizes one or more tumor antigens expressed on the cancer cells.

38. The method of claim 37, wherein the tumor antigen is selected from MAGE-A1, MAGE-A2, MAGE- A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-I, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, BAGE-I, RAGE- 1, LB33/MUM-1, PRAME, NAG, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE- C1/CT7, MAGE-C2, NY-ESO-I, LAGE-I, SSX-I, SSX-2(HOM-MEL-40), SSX-3, SSX-4, SSX-5, SCP-I and XAGE, melanocyte differentiation antigens, p53, ras, CEA, MUC1, PMSA, PSA, tyrosinase, Melan-A, MART-1, gp100, gp75, alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR alpha fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomerase, GnTV, Herv-K-mel, NA-88, SP17, and TRP2-Int2, (MART-I), E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, alpha.-fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\170K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, tyrosinase related proteins, TRP-1, TRP-2, or mesothelin.

39. The method of claim 35, wherein the anti-cancer therapy is selected from: aspirin, sulindac, curcumin, alkylating agents including: nitrogen mustards, such as mechlor-ethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); thylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2´-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin

(pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; epipodophylotoxins such as etoposide and teniposide; antibiotics, such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase, cytokines such as interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, TNF-beta and GM-CSF, anti-angiogenic factors, such as angiostatin and endostatin, inhibitors of FGF or VEGF such as soluble forms of receptors for angiogenic factors, including soluble VGF/VEGF receptors, platinum coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p´-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; non-steroidal antiandrogens such as flutamide; kinase inhibitors, histone deacetylase inhibitors, methylation inhibitors, proteasome inhibitors, monoclonal antibodies, oxidants, anti-oxidants, telomerase inhibitors, BH3 mimetics, ubiquitin ligase inhibitors, stat inhibitors and receptor tyrosin kinase inhibitors such as imatinib mesylate (marketed as Gleevac or Glivac) and erlotinib (an EGF receptor inhibitor) now marketed as Tarveca; and anti-virals such as oseltamivir phosphate, Amphotericin B, and palivizumab.

40. The method of any one of claims 34-39 wherein the CXCL12-targeted immune response agent and the anti-cancer therapy are administered simultaneously.

41. The method of any one of claims 34-39 wherein the CXCL12-targeted immune response agent and the anti-cancer therapy are administered separately.

42. The method of any one of claims 34-39 wherein the CXCL12-targeted immune response agent and the anti-cancer therapy are administered sequentially.

43. The method of any one of claims 1-42, wherein the patient is a human.

44. The method of any one of claims 1-43, wherein the CXCL12-targeted immune response agent delivers another molecule that inhibits the growth of or kills the epithelial tumor.

45. The method of any one of claims 1-44, wherein the other molecule is a cytokine, a toxin, a pro-apoptotic protein, or a chemotherapeutic agent.

46. The method of any one of claims 1-45, wherein the CXCL12-targeted immune response agent is covalently attached to the toxin, pro-apoptotic protein, or chemotherapeutic agent.

FIGURE 1A

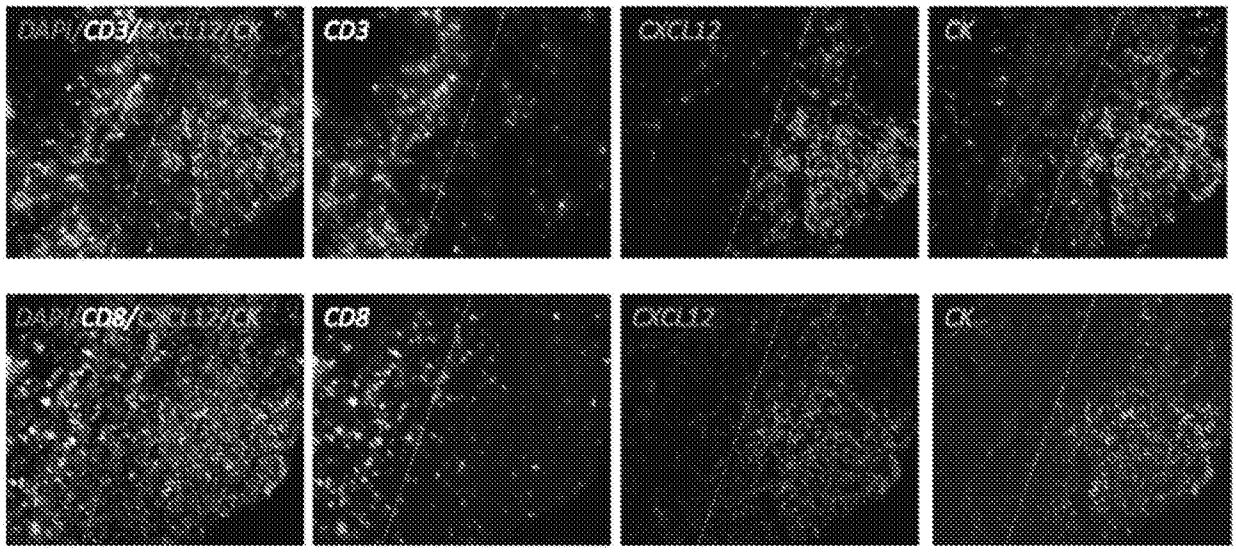
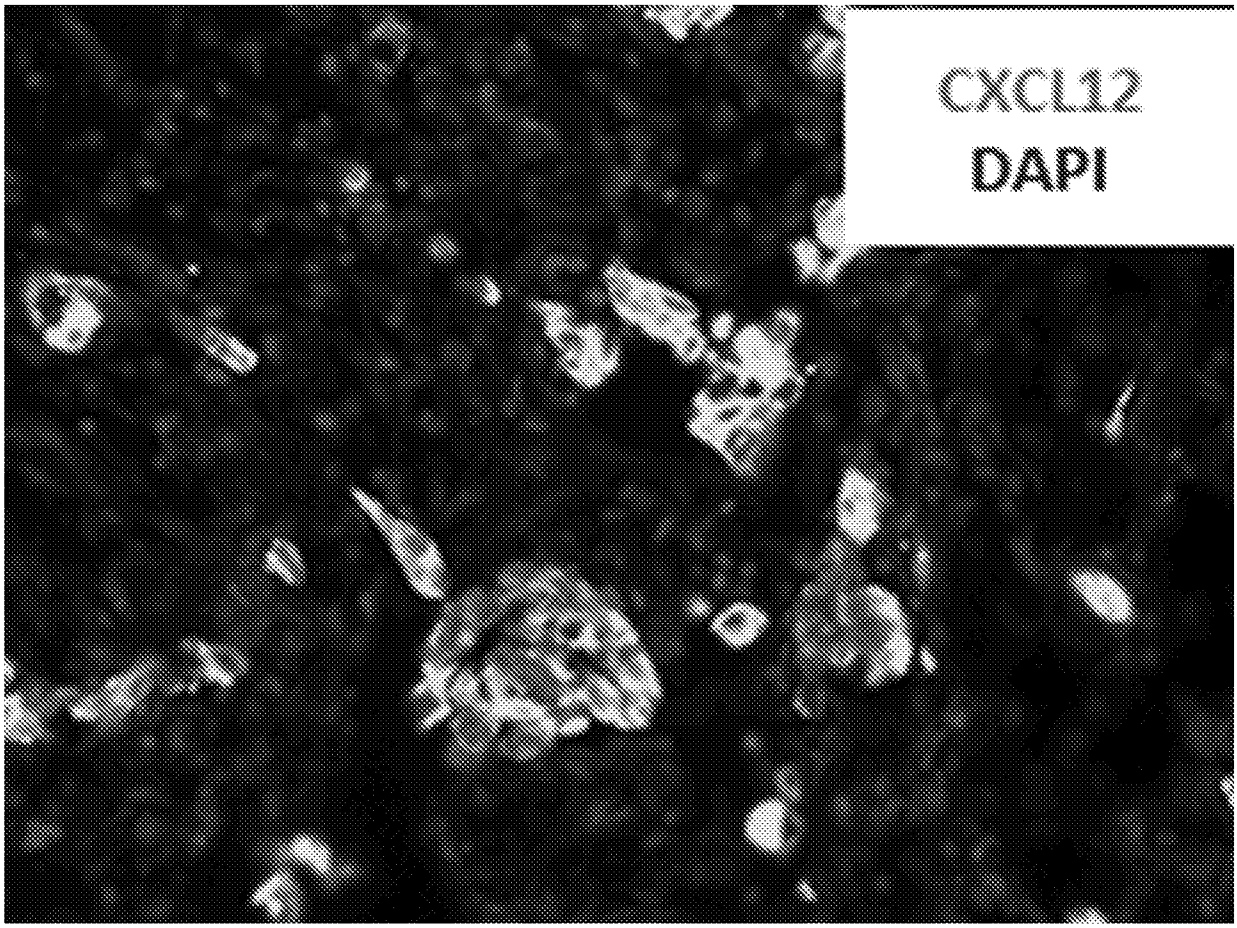


FIGURE 1B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/044254

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24
ADD.

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C. ZHONG ET AL: "Development and Preclinical Characterization of a Humanized Antibody Targeting CXCL12", CLINICAL CANCER RESEARCH, vol. 19, no. 16, 15 August 2013 (2013-08-15), pages 4433-4445, XP055260304, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0943 paragraph "Generation and characterization of humanized 30D8" ----- -/--	1-6, 17-19,21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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30 September 2016	10/10/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PHILLIPS RODERICK J ET AL: "The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases", AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, AMERICAN LUNG ASSOCIATION, NEW YORK, NY, US, vol. 167, no. 12, 15 June 2003 (2003-06-15), pages 1676-1686, XP002481676, ISSN: 1073-449X, DOI: 10.1164/RCCM.200301-071OC see paragraph "Depletion of CXCL12 inhibits NSCLC metastases", see paragraph "Depletion of CXCL12 in vivo does not alter primary tumor size or tumor-derived angiogenesis"	1-46

X	ORIMO AKIRA ET AL: "Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion", CELL, CELL PRESS, US, vol. 121, no. 3, 6 May 2005 (2005-05-06), pages 335-348, XP009138583, ISSN: 0092-8674, DOI: 10.1016/J.CELL.2005.02.034 [retrieved on 2005-05-05] figure 6	1,17-19, 21
