(54) Title: USE OF TGF ALPHA FOR THE TREATMENT OF DISEASES AND DISORDERS

(57) Abstract: The invention provides a methods of a disease or disorder in a subject by inducing a TGF alpha immune response. The TGF-alpha immune response is induced using a TGF-alpha polypeptide or biologically active fragment, a vaccine, a genetic construct or a transformed cell, for example.

USE OF TGF ALPHA FOR THE TREATMENT OF DISEASES AND DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 USC §119(e) to U.S. Application Serial No. 62/460,616 filed February 17, 2017. The disclosure of the prior application is considered part of and is incorporated by reference in the disclosure of this application in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, name TODARO1100_1WO_Sequence_Listing.txt, was created on February 15, 2018, and is 18 kb. The file can be assessed using Microsoft Word on a computer that uses Windows OS.

BACKGROUND OF THE INVENTION

[0003] The invention relates generally to growth factors and more specifically to the use of TGF alpha to induce a TGF-alpha specific immune response in a subject.

BACKGROUND INFORMATION

[0004] The epithelial growth factor receptor EGFR is a transmembrane protein, member of the ErbB family of receptors. Upon binding of one of its specific ligands, the receptor is activated which triggers the receptor's dimerization and auto-phosphorylation, leading to the activation of signaling pathways responsible for cell proliferation, epithelial differentiation and development. Many cancers present EGFR somatic mutation responsible for its duplication or constant activation, resulting in uncontrolled cell division. Along with other growth factors, transforming growth factor TGF alpha (TGF-alpha) and the epithelial growth factor (EGF) are ligands for the EGF receptor; however, TGF-alpha is a much more potent ligand than EGF.

[0005] TGF polypeptides are a unique class of proteins distinguishable from other growth factors such as EGF from the standpoint of both biological properties and chemical structure. These TGFs, in turn, possess a variety of properties of value or potential value in the health sciences field including potent, but reversible, cell growth or mitogenic properties which find use in cell repair including, for example, wound healing and ulcer therapy. Additionally, the production of TGF polypeptides, or elevated levels of production, are characteristic of, if not essential to, the morphologic transformation of certain cell lines in both human and murine tissue and/or fluids; therefore, the TGF polypeptides or antigenic fragments thereof are of value in differentiating normal cells from tumor cells and

antibodies raised thereto have application in both the diagnosis and treatment of malignancies. Further, realization that certain TGF polypeptides specifically interact with and produce their biological effects through cellular membrane EGF receptors raises the possibility, once the basic TGF polypeptide structure is determined, of correlating its structure with the structure of EGF to develop oligopeptides having chemical characteristics to allow binding to the EGF receptors without concomitant phenotypic transformation of the cell. Oligopeptides having this characteristic EGF receptor binding ability find application in treatment of malignancies, since the oligopeptide will interfere or compete with TGF for available receptor sites and thereby interrupt the expression of the transformed properties of the cell.

[0006] When applied to untransformed, non-neoplastic cells growing in culture, TGF-alpha induces changes including a) loss of density-dependent inhibition of cell growth in monolayer culture, b) overgrowth of cells in monolayer culture, c) change in cellular shape, indicating the acquisition of a neoplastic phenotype, and d) acquisition of anchorage-independence, with the resultant ability to grow in soft agar, which highlights cell growth and mitogenic properties. TGF-alpha and EGF share some properties, specifically, the growth factors are both heat-stable, acid-stable peptides, which are sensitive to reducing agents and proteases. Additionally, both TGF-alpha and EGF specifically interact with, and produce biological effects through, EGF receptors with TGF-alpha competing with EGF for binding to the EGF receptor. However, TGF-alpha is distinguishable from EGF, in that EGF does not induce anchorage-independent growth of cells in culture and has only a slight effect on the phenotype of cultured cells, whereas TGF-alpha produces a more pronounced phenotypic alteration in cultured cells and confers on them the ability to behave as transformed cells.

[0007] When the sequence of TGF-alpha was determined it was found to have 50 amino acids, 13 of which were identical to epidermal growth factor (EGF). It was found that TGF-alpha and EGF could bind to this same receptor on cells, but TGF-alpha was much more potent than EGF. The sequences of both were found to depend for their activity by forming 3 disulfide bonds with exactly the same three dimensional pattern. TGF-alpha was primarily produced by tumor cells and could stimulate normal cells to change their morphology to behave like tumor cells. EGF first recorded in large concentration in mouse salivary glands. TGF-alpha was primarily in tumor cells; however, it also was expressed in embryos.

[0008] Recent reports have shown that EGF has activity when tested with individuals that have lung cancer. However, the effect is not large; most patients still show progressive disease.

[0009] Immunotherapy, which aims to, actively or passively, induce, enhance or suppress an immune response in a subject, is of great interest in cancer therapy in that it is a powerful tool to stimulate the existing immune response and to target and eliminate cancer cells specifically. Specifically, EGF vaccines, e.g. CIMAVax, have been developed that have successfully treated some cancers. Composed of a recombinant human EGF conjugated to a protein carrier, the vaccine aims to immunize lung cancer patients with EGF to induce the production of antibodies targeting self-EGF. Without targeting cancer cells directly, the vaccine targets EGFR's ligand EGF and show some efficiency at reducing EGF concentration in the blood. EGF is one of the main EGFR ligand, along with TGF-alpha. However TGF alpha is a much more potent ligand to EGFR than EGF. Given the efficiency of the existing EGF vaccine, and in regards of the more efficient binding of TGF-alpha to EGFR as compared to EGF, it is suggested that the anti-TGF alpha immune response induced by the TGF-alpha polypeptide or biologically active fragment thereof of the present invention would be more efficient at treating cancer.

[0010] The increasing knowledge of cancer and immune disorders indicates that the future for treatment is targeted therapies. In this regard, there is an increasing need for the identification of new targets and for the development of new targeted therapies.

SUMMARY OF THE INVENTION

[0011] The present invention is based on the seminal discovery that TGF-alpha may be a more potent ligand for the EGF receptor as compared to EGF and therefore is useful to induce a TGF-alpha specific immune response in a subject. Specifically, the present invention is based on the induction of an TGF-alpha specific immune response for the treatment of cancer or other diseases and disorders related to TGF-alpha producing cells and/or TGF-alpha ligand/receptor interactions. Additionally, the present invention provides TGF-alpha polypeptides or biologically active fragments thereof, genetic constructs including nucleic acid sequences encoding TGF-alpha polypeptides, vaccines and genetically modified cells that induce a TGF-alpha immune response. TGF-alpha polypeptides and nucleic acid sequences encoding them are known in the art and included herein.

[0012] In one embodiment, the present invention provides a method of treating a TGF-alpha related disease or disorder in a subject comprising administering to the subject a

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therapeutically effective amount of a TGF-alpha polypeptide or a biologically active fragment thereof. In one exemplary aspect, the TGF-alpha polypeptide or biologically active fragment thereof has at least 75% identity to an amino acid sequence of any of SEQ ID NOs: 5-8, which are splice variants of TGF-alpha or SEQ ID NO:10, the mature protein.

[0013] In another aspect, the TGF-alpha polypeptide or biologically active fragment thereof is a fusion protein. In an additional aspect, the TGF-alpha polypeptide or biologically active fragment thereof is genetically modified. In a further aspect, the TGF-alpha polypeptide or biologically active fragment thereof comprises a heterologous sequence. In an additional aspect, the TGF-alpha related disease or disorder is cancer or an immune disorder. In one aspect, the method further comprises the administration of a checkpoint inhibitor or other therapeutic agent, such as a chemotherapeutic agent. In one aspect, the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4.

[0014] In another embodiment, the present invention provides a method of treating a TGF-alpha related disease or disorder in a subject comprising inducing the *in vivo* production of TGF-alpha antibodies in the subject. In one aspect, the method comprises the administration of a TGF-alpha polypeptide or a biologically active fragment thereof. In certain aspects, the TGF-alpha polypeptide has at least 75% sequence identity to the amino acid sequence of any of SEQ ID NOs:5-8 or 10. In an additional aspect, the TGF-alpha polypeptide or a biologically active fragment thereof is a fusion protein. In a further aspect, the TGF-alpha polypeptide or a biologically active fragment thereof is genetically modified or comprises a heterologous sequence. In another aspect, the method comprises the administration of a TGF-alpha vaccine.

[0015] In an additional embodiment, the present invention provides a vaccine for administration to a subject to produce an immune response against TGF-alpha comprising a TGF-alpha polypeptide or a biologically active fragment thereof and optionally an adjuvant. The vaccine composition optionally includes a checkpoint inhibitor, an anti-cancer agent or combinations thereof or other therapeutic molecules.

[0016] In a further embodiment, the present invention provides a vaccine for administration to a subject to produce an immune response against TGF-alpha comprising a nucleic acid molecule encoding a TGF-alpha polypeptide or a biologically active fragment thereof and optionally an adjuvant. The vaccine composition optionally includes a checkpoint inhibitor, an anti-cancer agent or combinations thereof or other therapeutic molecules.

[0017] In one embodiment, the present invention provides an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a nucleic acid

sequence encoding a TGF-alpha protein or biologically active fragment thereof, a transmembrane domain and an intracellular signaling domain. In an aspect, the transmembrane domain comprises a transmembrane domain of a protein selected from the TGF-alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In an additional aspect, the nucleic acid sequence encoding the TGF-alpha polypeptide or biologically active fragment thereof is connected to the transmembrane domain by a linker or hinge region. A chimeric antigen receptor (CAR) T cell immunotherapeutic agent is an exemplary CAR.

[0018] In another aspect, the present invention provides for a chimeric antigen receptor (CAR) polypeptide, wherein the CAR comprises a TGF-alpha protein or biologically active fragment thereof, a transmembrane domain and an intracellular signaling domain.

[0019] In an additional aspect, the present invention provides for a method of providing anti-tumor antibodies in a subject, comprising administering to the subject an immune cell comprising a nucleic acid molecule encoding a TGF-alpha polypeptide or biologically active fragment thereof or a TGF-alpha polypeptide or biologically active fragment thereof.

[0020] In a further aspect, the present invention provides a pharmaceutical composition comprising a TGF-alpha polypeptide or biologically active fragment thereof and a pharmaceutically acceptable carrier. The pharmaceutical composition optionally includes a checkpoint inhibitor, an anti-cancer agent or combinations thereof or other therapeutic molecules.

BRIEF DESCIPTION OF THE DRAWINGS

[0021] Figure 1 shows a diagram of four TGF-alpha amino acid sequence splice variants.

[0022] Figure 2 shows a sequence comparison of four TGF-alpha amino acid splice variants (SEQ ID NOs:5-8).

DETAILED DESCRIPTION OF THE INVENTION

[0023] The present invention is based on the seminal discovery that TGF-alpha may be a more potent ligand for the EGF receptor as compared to EGF and therefore may be useful to induce a TGF-alpha specific immune response in a subject. Specifically, the present invention is based on the induction of a TGF-alpha specific immune response for the treatment of cancer or other diseases related to TGF-alpha producing cells and/or TGF-alpha ligand/receptor interactions. Additionally, the present invention provides TGF-alpha polypeptides or biologically active fragments thereof, genetic constructs including nucleic

acid sequences encoding TGF-alpha polypeptides, vaccines and genetically modified cells that induce a TGF-alpha immune response.

[0024] Before the present compositions and methods are described, it is to be understood that this invention is not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims.

[0025] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0026] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0027] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

[0028] In one embodiment, the present invention provides a method of treating a TGF-alpha related disease or disorder in a subject comprising administering to the subject a therapeutically effective amount of a TGF-alpha polypeptide or a biologically active fragment thereof.

[0029] Transforming growth factor alpha (TGF-alpha or TGF α) is a member of the epidermal growth factor (EGF) family. The human *TGF A* gene encodes four protein-coding splice variants (see SEQ ID NOs:5-8) which are encoded by four propeptides or precursor protein nucleic acid sequences (see SEQ ID NOs:1-4). The propeptides are then cleaved to form the mature TGF-alpha protein (see SEQ ID NOs:9-10). TGF-alpha is a mitogenic polypeptide that is a ligand for the epidermal growth factor receptor EGFR. TGF-alpha is a membrane anchored-growth factor. The precursor is composed of an extracellular domain containing a hydrophobic transmembrane domain, 50 amino acids of the mature TGF-alpha

(of which 13 are identical to EGF), and a 35-residue-long cytoplasmic domain. This structure is shared by all the members of the EGF/TGF-alpha family. Both the soluble form (cleaved from the integral membrane glycoprotein by a protease) and the membrane anchored TGF-alpha can bind to and activate EGFR. Both EGF and TGF-alpha bind to EGFR, which induces receptor dimerization and auto-phosphorylation leading to the activation of signaling pathways responsible for cell proliferation, epithelial differentiation and development; however, the ligand recognition involves different amino acid motifs. Additionally, TGF-alpha is a more potent ligand for EGFR compared to EGF. It is to be noted that TGF-alpha and TGF-beta are not structurally or genetically related to one another and act through different receptor mechanisms. There are four different nucleotide and amino acid sequence - identified for TGF-alpha corresponding to the four protein-coding splice variants of TGF-alpha (see Figures 1 and 2 and Table 4). It is understood that other nucleic acid or amino acid sequences known to those of skill in the art as TGF-alpha are included in the invention. SEQ ID NOs:1-10 are provided herein as exemplary molecules and are not meant to be limiting. TGF-alpha sequences are well known to those of skill in the art.

[0030] The terms "peptide" and "polypeptide" are used interchangeably herein and refer to any chain of at least two amino acids, linked by covalent chemical bound. As used herein polypeptide can refer to the complete amino acid chain forming a protein or to a fragment thereof.

[0031] The term "fragment" refers to a continuous element. For example, a part of a structure such as an amino acid sequence or protein refers to a continuous element of said structure. A portion, a part or a fragment of a structure preferably comprises one or more functional properties of said structure. For example, a portion, a part or a fragment of an epitope, peptide or protein is preferably immunologically equivalent to the epitope, peptide or protein it is derived from. In the context of the present invention, a "fragment" of a structure such as an amino acid sequence preferably comprises portions of TGF-alpha polypeptide.

[0032] The term "biologically active fragment" as used herein refers to a fragment of a polypeptide which still contains a specific biological activity of said polypeptide. In the context of the present invention, a "biologically active fragment" of TGF-alpha is a fragment of the TGF-alpha polypeptide that is able to induce an immune response in a subject. e.g. bind to EGFR or induce the production of anti-TGF-alpha antibodies.

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[0033] The amino acid sequence of the TGF-alpha polypeptides and biologically active fragments may comprise 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 5-8 and 10 (mature species). The nucleic acid sequence encoding TGF-alpha polypeptides and biologically active fragments may comprise 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 1-4 and 9 (mature species). The amino acid sequence of the TGF-alpha polypeptides or biologically active fragments and/or nucleic acid sequence encoding such may be genetically modified by modifying, mutating, inserting or deleting one or more amino acids or nucleotides. Methods for genetically modifying amino acids or nucleic acids are known generally in the art and include error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, Gene Site Saturation Mutagenesis™ (GSSM™), synthetic ligation reassembly (SLR) or a combination thereof. In another aspect, the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof. Additionally, TGF-alpha polypeptides or biologically active fragments or the nucleic acid sequence encoding such may further comprise heterologous amino acid or nucleic acid sequence.

[0034] The terms "fusion molecule" and "fusion protein" are used interchangeably and are meant to refer to a biologically active polypeptide, e.g., a TGF alpha or an antibody or a fragment thereof (e.g., Fc region), with or without a further effector molecule usually a protein or peptide sequence covalently linked (i.e. fused) by recombinant, chemical or other suitable method. If desired, the fusion molecule can be used at one or several sites through a peptide linker sequence. Alternatively, the peptide linker may be used to assist in construction of the fusion molecule. Specifically preferred fusion molecules are fusion proteins. Generally fusion molecule also can include conjugate molecules.

[0035] Fc-Fusion proteins (also known as Fc chimeric fusion protein, Fc-Ig, Ig-based Chimeric Fusion protein and Fc-tag protein) are composed of the Fc domain of IgG

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genetically linked to a peptide or protein of interest. Fc-Fusion proteins have become valuable reagents for in vivo and in vitro research. The Fc-fused binding partner can range from a single peptide, a ligand that activates upon binding with a cell surface receptor, signaling molecules, the extracellular domain of a receptor that is activated upon dimerization or as a bait protein that is used to identify binding partners in a protein microarray. One of the most valuable features of the Fc domain in vivo, is it can dramatically prolong the plasma half-life of the protein of interest, which for bio-therapeutic drugs, results in an improved therapeutic efficacy; an attribute that has made Fc-Fusion proteins attractive bio-therapeutic agents.

[0036] TGF alpha Fc fusion proteins of the invention include the human TGF alpha polypeptide or a biologically active fragment thereof, fused at the 3' end to a human Ig Fc sequence. The Fc fusion proteins would be produced in host cells grown in appropriate medium and purified by affinity chromatography columns. Control human Ig protein from will be used. Suitable cells for the production of the protein fusion are well known but skilled artisans in the art, and can for example be 293T cells, transfected with an expression vector coding for human a TGF alpha or a biologically active fragment thereof, a linker, and a human Ig Fc.

[0037] The Fc fragment of the fusion protein of the invention could be selected from the group consisting of IgA, IgG, IgD, IgE and IgM. In a preferred embodiment the protein fusion of the invention would be comprises human TGF alpha protein or a biologically active fragment thereof and an Fc protein of an IgG. Specifically it could be selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

[0038] The term "linker", as used herein, refers to a short peptide sequence that occur between protein domains or protein fragment to be fused. Linkers are often composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers are used when it is necessary to ensure that two adjacent domains do not sterically interfere with one another. As described in Chen X, et al., (Fusion Protein Linkers: Property, Design and Functionality. Advanced drug delivery reviews. 2013;65(10):1357-1369. doi:10.1016/j.addr.2012.09.039) many linkers are well known in the art and the choice of a linker depends on several criteria including the type of fusion protein and the type of linker (rigid or flexible). The linker of the present invention would be a short flexible sequence to increase stability and folding of the fusion protein.

[0039] The terms "treatment" and "treating" are used interchangeably herein with the term "therapeutic method" and refers to both 1) therapeutic treatments or measures that cure,

slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic conditions or disorder, and 2) prophylactic/ preventative measures. Those in need of treatment may include individuals already having a particular medical disorder as well as those who may ultimately acquire the disorder (*i.e.*, those needing preventive measures). Treatment includes monotherapy approaches or combination therapy, for example, use of a TGF-alpha vaccine alone or in combination with other therapeutic regimens. TGF-alpha therapy can be prior to, simultaneously with, or following other therapies, e.g., immunosuppressive therapy, chemotherapy, radiotherapy and the like. TGF-alpha therapy described herein can be prior to or following tumor resection, for example.

[0040] The terms "effective dose" , "therapeutically efficient dose", "effective amount", therapeutically effective amount" and the like mean the amount of the subject compound that results in either amelioration of symptoms in a subject or a desired biological outcome (biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician). Specifically, such amount should be sufficient to induce a TGF-alpha immune response, e.g., induce the production of anti-TGF-alpha antibodies.

[0041] The terms "administration of" and or "administering" should be understood to mean providing a composition in a therapeutically effective amount to the individual in need of treatment. Administration route is not specifically limited and can include oral, intravenous, intramuscular, infusion, intrathecal, intradermal, subcutaneous, sublingual, buccal, rectal, vaginal, ocular, optic route, nasal, inhalation, nebulization, cutaneous, topical, transdermal, intraperitoneal or intratumoral administrations.

[0042] As used herein the term "TGF-alpha related disease or disorder" refers to any condition that would benefit from treatment with a TGF-alpha polypeptide of biologically active fragment thereof, vaccine or transformed cell. Examples of diseases and disorders include cancer, immune and inflammatory diseases and disorders.

[0043] In some aspects, the TGF-alpha related disease or disorder is cancer or an immune disorder.

[0044] The term "cancer" refers to a group diseases characterized by abnormal and uncontrolled cell proliferation starting at one site (primary site) with the potential to invade and to spread to others sites (secondary sites, metastases) which differentiate cancer (malignant tumor) from benign tumor. Virtually all the organs can be affected, leading to more than 100 types of cancer that can affect humans. Cancers can result from many causes including genetic predisposition, viral infection, exposure to ionizing radiation, exposure

environmental pollutant, tobacco and or alcohol use, obesity, poor diet, lack of physical activity or any combination thereof.

[0045] "Cancer cell" or "tumor cell", and grammatical equivalents refer to the total population of cells derived from a tumor or a pre-cancerous lesion, including both non tumorigenic cells, which comprise the bulk of the tumor population, and tumorigenic stem cells (cancer stem cells).

[0046] Exemplary cancers include, but are not limited to: Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor, Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood: Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primary; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-Cell Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma. Childhood Brain Stem; Glioma. Childhood Visual Pathway and Hypothalamic; Hairy Cell Leukemia; Head and Neck

Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS— Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's; Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non-Hodgkin's, Childhood; Lymphoma, Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Malignant Thymoma; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplasia Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood', Pancreatic Cancer, Islet Cell; Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma;

Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland'Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (OsteosarcomaVMalignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T-Cell Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma, Malignant; Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor.

[0047] In certain aspects, the method is directed at treating cancers selected from lung, stomach, prostate, colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital tract. In a preferred embodiment, the invention involves treating cancers that are epithelial tumors such as colon, stomach, lung (e.g., NSCLC), and head and neck cancer.

[0048] In another aspect, the method further comprises administering to the subject a chemotherapeutic agent and/or radiation either prior to, simultaneously with or following treatment with the TGF alpha polypeptide or biologically active fragment thereof.In some aspects several cancer treatments can be used in "combination therapy", or "in combination". The phrases "combination therapy", "combined with" and the like refer to the use of more than one medication or treatment simultaneously to increase the response. The TGF-alpha polypeptide or biologically active fragment thereof, vaccine or genetically modified or transformed cell of the present invention might for example be used in combination with other drugs or treatment in use to treat cancer. Specifically the

administration of TGF-alpha polypeptide or biologically active fragment thereof, vaccine or transformed cell to a subject can be in combination with chemotherapy, radiation, or administration of a therapeutic antibody (e.g., Erbitux, Tarceva, Iressa and Tykerb) for example. Such therapies can be administered prior to, simultaneously with, or following administration of TGF alpha polypeptide or biologically active fragment thereof, vaccine or transformed cell thereof. TGF-alpha therapy can be prior to, simultaneously with, or following other therapies, e.g., immunosuppressive therapy, chemotherapy, radiotherapy and the like. TGF-alpha therapy described herein can be prior to or following tumor resection, for example.

[0049] The term "chemotherapeutic agent" as used herein refers to any therapeutic agent having antineoplastic effect and used to treat cancer. In certain aspects, a chemotherapeutic agent, is a cytotoxic drug, an immunotherapeutic agent or radiation.

[0050] Examples of chemotherapeutic agents include, but are not limited to, Actinomycin, Azacitidine, Azathioprine, Bleomycin, Bortezomib, Carboplatin, Capecitabine, Cisplatin, Chlorambucil, Cyclophosphamide, Cytarabine, Daunorubicin, Docetaxel, Doxifluridine, Doxorubicin, Epirubicin, Epothilone, Etoposide, Fiuorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Imatinib, Irinotecan, Mechlorethamine, Mercaptopurine, Methotrexate, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Teniposide, Tioguanine, Topotecan, Valrubicin, Vinblastine, Vincristine, Vindesine, Vinorelbine, panitumamab, Erbitux (cetuximab), matuzumab, IMC-IIF 8, TheraCIM hR3, denosumab, Avastin (bevacizumab), Humira (adalimumab), Herceptin (trastuzumab), Remicade (infliximab), rituximab, Synagis (palivizumab), Mylotarg (gemtuzumab oxogamicin), Raptiva (efalizumab), Tysabri (natalizumab), Zenapax (dacliximab), NeutroSpec (Technetium (99mTc) fanolesomab), tocilizumab, ProstaScint (Indium-Ill labeled Capromab Pendetide), Bexxar (tositumomab), Zevalin (ibritumomab tiuxetan (IDEC-Y2B8) conjugated to yttrium 90), Xolair (omalizumab), MabThera (Rituximab), ReoPro (abciximab), MabCampath (alemtuzumab), Simulect (basiliximab), LeukoScan (sulesomab), CEA-Scan (arcitumomab), Verluma (nofetumomab), Panorex (Edrecolomab), alemtuzumab, CDP 870, natalizumab, Gilotrif (afatinib), Lynparza (olaparib), Perjeta (pertuzumab), Otdivo (nivolumab), Bosulif (bosutinib), Cabometyx (cabozantinib), Ogivri (trastuzumab-dkst), Sutent (sunitinib malate), Adcetris (brentuximab vedotin), Alecensa (alectinib), Calquence (acalabrutinib), Yescarta (ciloleucel), Verzenio (abemaciclib), Keytruda (pembrolizumab), Aliqopa (copanlisib), Nerlynx (neratinib), Imfinzi (durvalumab), Darzalex (daratumumab), Tecentriq (atezolizumab), Avelumab (Bavencio), Durvalumab (Imfinzi), Iplimumab (Yervoy) and

Tarceva (erlotinib). Examples of immunotherapeutic agent include, but are not limited to, interleukins (Il-2, Il-7, Il-12), cytokines (Interferons, G-CSF, imiquimod), chemokines (CCL3, CCl26, CXCL7), immunomodulatory imide drugs (thalidomide and its analogues), MGA271, lirilumab, and BMS-986016.

[0051] Checkpoint inhibitor therapy is a form of cancer treatment currently that uses immune checkpoints which affect immune system functioning. Immune checkpoints can be stimulatory or inhibitory. Tumors can use these checkpoints to protect themselves from immune system attacks. Checkpoint therapy can block inhibitory checkpoints, restoring immune system function. Checkpoint proteins include programmed cell death 1 protein (PDCD1, PD-1; also known as CD279) and its ligand, PD-1 ligand 1 (PD-L1, CD274), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), A2AR (Adenosine A2A receptor), B7-H3 (or CD276), B7-H4 (or VTCN1), BTLA (B and T Lymphocyte Attenuator, or CD272), IDO (Indoleamine 2,3-dioxygenase), KIR (Killer-cell Immunoglobulin-like Receptor), LAG3 (Lymphocyte Activation Gene-3), TIM-3 (T-cell Immunoglobulin domain and Mucin domain 3), and VISTA (V-domain Ig suppressor of T cell activation).

[0052] Programmed cell death protein 1, also known as PD-1 and CD279 (cluster of differentiation 279), is a cell surface receptor that plays an important role in down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. PD-1 is an immune checkpoint and guards against autoimmunity through a dual mechanism of promoting apoptosis (programmed cell death) in antigen-specific T-cells in lymph nodes while simultaneously reducing apoptosis in regulatory T cells (anti-inflammatory, suppressive T cells).

[0053] PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family. PD-L1 protein is upregulated on macrophages and dendritic cells (DC) in response to LPS and GM-CSF treatment, and on T cells and B cells upon TCR and B cell receptor signaling, whereas in resting mice, PD-L1 mRNA can be detected in the heart, lung, thymus, spleen, and kidney. PD-L1 is expressed on almost all murine tumor cell lines, including PA1 myeloma, P815 mastocytoma, and B16 melanoma upon treatment with IFN-γ. PD-L2 expression is more restricted and is expressed mainly by DCs and a few tumor lines.

[0054] CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152 (cluster of differentiation 152), is a protein receptor that, functioning as an immune checkpoint, downregulates immune responses. CTLA4 is constitutively expressed in regulatory T cells but only upregulated in conventional T cells after activation - a phenomenon which is particularly notable in cancers. CTLA4 is a member of the

immunoglobulin superfamily that is expressed by activated T cells and transmits an inhibitory signal to T cells. CTLA4 is homologous to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA-4 binds CD80 and CD86 with greater affinity and avidity than CD28 thus enabling it to outcompete CD28 for its ligands. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. CTLA4 is also found in regulatory T cells and contributes to its inhibitory function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4.

[0055] There are several checkpoint inhibitors that are currently used to treat cancer. PD-1 inhibitors include Pembrolizumab (Keytruda) and Nivolumab (Opdivo). PD-L1 inhibitors include Atezolizumab (Tecentriq), Avelumab (Bavencio) and Durvalumab (Imfinzi). CTLA-4 inhibitors include Iplimumab (Yervoy). There are several other checkpoint inhibitors being developed including an anti B7-H3 antibody (MGA271), an anti-KIR antibody (Lirilumab) and an anti-LAG3 antibody (BMS-986016).

[0056] In certain aspects the chemotherapeutic agent is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and/or iplimumab. In one aspect, the method further comprising the administration of a checkpoint inhibitor. In another aspect, the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4.

[0057] The compositions of the invention can be used in combination with one or more other anti-cancer therapies, for example, the one or more other anticancer therapies are selected from the group consisting of a cytotoxic chemotherapy, an anti-cancer vaccine, an anti-tumor vaccine, an anti-immunocytokine therapy, an immunocytokine therapy, a checkpoint inhibitor, and a chimeric antigen receptor (CAR) T cell immunotherapeutic agent, and gene transfer therapy.

[0058] In an additional aspect, the cancer is a tumor. In certain aspects, the tumor is resected prior to treatment. In yet another aspect, the subject is a human.

[0059] As used herein, the term "modulating an immune response" refers to either enhancing or inhibiting an immune response. In some aspects, the TGF-alpha polypeptide or biologically active fragment thereof of the present invention induce or enhance an immune response.

[0060] As used herein, the term "modulating TGF-alpha signaling" refers to either increasing or decreasing TGF-alpha signaling. In some aspects, the TGF-alpha polypeptide of biologically active fragment thereof, vaccine or transformed cell of the present invention decreases TGF-alpha signaling.

[0061] An immune disease or disorder is a dysfunction of the immune system. These disorders can be characterized in several different ways: by the component(s) of the immune system affected; by whether the immune system is overactive or underactive and by whether the condition is congenital or acquired. Immune diseases and disorders comprise autoimmune diseases or disorders, characterized by the dysfunction of the adaptive immune system, where adaptive immune B and T cells have lost their ability to differentiate self from non-self, and inflammatory diseases or disorders characterized by the dysfunction of the innate immune system, where innate immune cells inappropriately secrete inflammatory molecules such as cytokines.

[0062] Immune and inflammatory diseases and disorders include, but are not limited to, Acute disseminated encephalomyelitis (ADEM), Addison's disease, Agammaglobulinemia, Alopecia areata, Amyotrophic lateral sclerosis (aka Lou Gehrig's disease), Ankylosing Spondylitis, Antiphospholipid syndrome, Antisynthetase syndrome, Arthritis, Asthma; Atherosclerosis Atopic allergy, Atopic dermatitis, Autoimmune aplastic anemia, Autoimmune cardiomyopathy, Autoimmune enteropathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune pancreatitis, Autoimmune peripheral neuropathy, Autoimmune polyendocrine syndrome, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune urticaria, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Behçet's disease, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous pemphigoid, Cancer, Castleman's disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy, Chronic inflammatory demyelinating polyneuropathy, Chronic obstructive pulmonary disease, Chronic recurrent multifocal osteomyelitis, Churg-Strauss syndrome, Cicatricial pemphigoid, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Contact dermatitis, Cranial arteritis, CREST syndrome, Crohn's disease, Cushing's Syndrome, Cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Discoid lupus erythematosus, Diverticulitis, Dressler's syndrome, Drug-induced lupus, Eczema, Endometriosis, Eosinophilic fasciitis, Eosinophilic gastroenteritis, Eosinophilic pneumonia, Epidermolysis bullosa acquisita, Erythema nodosum, Erythroblastosis fetalis, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibrosing alveolitis (or Idiopathic pulmonary fibrosis), Gastritis, Gastrointestinal pemphigoid, Glomerulonephritis, Goodpasture's

syndrome, graft versus host disease, Gout , Graves' disease, Guillain-Barré syndrome, Hashimoto's encephalopathy, Hashimoto's thyroiditis, Henoch-Schonlein purpura, Hepatitis, Herpes gestationis aka Gestational Pemphigoid, Hidradenitis suppurativa, Hughes-Stovin syndrome, Hypogammaglobulinemi, Idiopathic inflammatory demyelinating diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura, IgA nephropathy, Inclusion body myositis, Interstitial cystitis, Irritable bowel syndrome, Juvenile idiopathic arthritis aka Juvenile rheumatoid arthritis, Kawasaki's disease, Lambert-Eaton myasthenic syndrome, Laryngitis, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Linear IgA disease, Lupoid hepatitis aka Autoimmune hepatitis, Lupus erythematosus, Majeed syndrome, Microscopic colitis, Microscopic polyangiitis, Miller-Fisher syndrome, Mixed connective tissue disease, Morphea, Mucha-Habermann disease aka Pityriasis lichenoides et varioliformis acuta, Multiple sclerosis, Myasthenia gravis, Myositis, Myopathies, Ménière's disease, Narcolepsy, Nephritis, Neuromyelitis optica, Neuromyotonia, Occular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Ord's thyroiditis, Palindromic rheumatism, PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Pars planitis, Parsonage-Turner syndrome, Pelvic inflammatory disease, Pemphigus vulgaris, Perivenous encephalomyelitis, Pernicious anaemia, Pharyngitis, Pleurisy, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progressive inflammatory neuropathy, Prostatitis, Psoriasis, Psoriatic arthritis, Pure red cell aplasia, Pyoderma gangrenosum, Rasmussen's encephalitis, Raynaud phenomenon, Reiter's syndrome, Relapsing polychondritis, Restless leg syndrome, Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis, Scleroderma, Seborrheic dermatitis, Serum Sickness, Sinusitis, Sjögren's syndrome, Splenitis, Spondyloarthropathy, Stiff person syndrome, Still's disease, Subacute bacterial endocarditis (SBE), Susac's syndrome, Sweet's syndrome, Sydenham chorea, Sympathetic ophthalmia, Systemic lupus erythematosus, Takayasu's arteritis, Temporal arteritis, Thrombocytopenia, thyroiditis, Tolosa-Hunt syndrome, Transverse myelitis, Ulcerative colitis, Undifferentiated spondyloarthropathy, Urticarial vasculitis, Vasculitis, Vitiligo, Wegener's granulomatosis, Familial Mediterranean fever (FMF), Hyperimmunoglobulinemia D with recurrent fever (HIDS), TNF receptor associated periodic syndrome (TRAPS), Muckle–Wells syndrome (CAPS, urticaria deafness amyloidosis), Familial cold urticarial, Neonatal onset multisystem inflammatory disease,

Periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA syndrome), Blau syndrome, Pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA), Deficiency of the interleukin-1–receptor antagonist (DIRA), Allergic reactions, Crohn's disease and Gout.

[0063] In certain aspects, the immune disorder is Rheumatoid arthritis, Systemic lupus erythematosus, Celiac disease, Crohn's disease, Inflammatory bowel disease, Sjogren's syndrome, Polymyalgia rheumatic, Psoriasis, Multiple sclerosis, Ankylosing spondylitis, Type 1 diabetes, Alopecia areata, Vasculitis, Temporal arteritis, Graves' disease, or Hashimoto's thyroiditis.

[0064] In another aspect, the method further comprises administering to the subject an immune modulator either prior to, simultaneously with or following treatment with the TGF-alpha polypeptide or biologically active fragment thereof.

[0065] The term "immune modulator" as used herein refers to any therapeutic agent that modulates the immune system. Examples of immune modulators include eicosanoids, cytokines, prostaglandins, interleukins, chemokines, checkpoint regulators, TNF superfamily members, TNF receptor superfamily members and interferons. Specific examples of immune modulators include PGI2, PGE2, PGF2, CCL14, CCL19, CCL20, CCL21, CCL25, CCL27, CXCL12, CXCL13, CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, CXCL10, IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL15, IL17, IL17, INF-α, INF-β, INF-ε, INF-γ, G-CSF, TNF-α, CTLA, CD20, PD1, PD1L1, PD1L2, ICOS, CD200, CD52, LTα, LTαβ, LIGHT, CD27L, 41BBL, FasL, Ox40L, April, TL1A, CD30L, TRAIL, RANKL, BAFF, TWEAK, CD40L, EDA1, EDA2, APP, NGF, TNFR1, TNFR2, LTβR, HVEM, CD27, 4-1BB, Fas, Ox40, AITR, DR3, CD30, TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, RANK, BAFFR, TACI, BCMA, Fn14, CD40, EDAR XEDAR, DR6, DcR3, NGFR-p75, and Taj. Other examples of immune modulators include tocilizumab (Actemra), CDP870 (Cimzia), enteracept (Enbrel), adalimumab (Humira), Kineret, abatacept (Orencia), infliximab (Remicade), rituzimab (Rituxan), golimumab (Simponi), Avonex, Rebif, ReciGen, Plegridy, Betaseron, Copaxone, Novatrone, natalizumab (Tysabri), fingolimod (Gilenya), teriflunomide (Aubagio), BG12, Tecfidera, and alemtuzumab (Campath, Lemtrada).

[0066] A used herein, the term "subject," including grammatical variations thereof, refers to any individual or patient to which the subject methods are performed. Generally the subject is human, although as will be appreciated by those in the art, the subject may be an animal. Thus other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs,

etc., and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of subject.

[0067] In a further aspect, the administration of the TGF-alpha polypeptide or biologically active fragment thereof induces the *in vivo* production of anti-TGF-alpha antibodies.

[0068] As used herein the terms "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. "Antibody," as used herein, refers to an immunoglobulin or immunologically active portions thereof, and encompasses any polypeptide comprising an antigen-binding site regardless of the source, species of origin, method of production, and characteristics. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgC3, IgG4, IgA, and IgA2. It includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, or antibody fragments. An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')2, Fc, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdfv) and fragments including either a VL or VH domain. Also includes is a Fc fragment, antigen-Fc fusion proteins, and Fc-targeting moiety

[0069] In a specific embodiment, the administration of the TGF-alpha polypeptide or biologically active fragment thereof, vaccine or transformed cell of the present invention can be intravenous, oral, intramuscular, subcutaneous, intrathecal, infusion, transdermal, sublingual, buccal, rectal, vagina, ocular, optic, nasal, inhalation, nebulization, or cutaneous administration.

[0070] In another embodiment, the present invention provides a method of treating a TGF-alpha related disease or disorder in a subject comprising inducing the *in vivo* production of anti-TGF-alpha antibodies in the subject. In one aspect, the method comprises the administration of a TGF-alpha polypeptide or a biologically active fragment thereof. In certain aspects, the TGF-alpha polypeptide or biologically active fragment thereof has at least 75% sequence identity to the amino acid sequence of any of SEQ ID NOs:5-8 or 10. In an additional aspect, the TGF-alpha polypeptide or a biologically active fragment thereof is a fusion protein. In a further aspect, the TGF-alpha polypeptide or a biologically active fragment thereof is genetically modified or comprises a heterologous sequence. In another aspect, the method comprises the administration of a TGF-alpha vaccine. In certain aspects,

the vaccine is a DNA, RNA or peptide vaccine. In an additional aspect, the method comprises administering a cell transformed with a nucleic acid molecule encoding a TGF-alpha polypeptide or a biologically active fragment thereof. In one aspect, the TGF-alpha related disease or disorder is cancer or an immune disorder. In a further aspect, the method comprises administering to the subject a chemotherapeutic agent, radiation or immune modulator either prior to, simultaneously with or following treatment with the TGF-alpha polypeptide or biologically active fragment thereof, TGF-alpha vaccine or transformed cell. In certain aspects, the chemotherapeutic agent is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and/or iplimumab. In certain aspects, the immune modulator is tocilizumab (Actemra), CDP870 (Cimzia), enteracept (Enbrel), adalimumab (Humira), Kineret, abatacept (Orencia), infliximab (Remicade), rituzimab (Rituxan), golimumab (Simponi), Avonex, Rebif, ReciGen, Plegridy, Betaseron, Copaxone, Novatrone, natalizumab (Tysabri), fingolimod (Gilenya), teriflunomide (Aubagio), BG12, Tecfidera, and alemtuzumab (Campath, Lemtrada). In one aspect, the method further comprising the administration of a checkpoint inhibitor. In another aspect, the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4. In one aspect, the cancer is lung, stomach, prostate, colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, or urogenital tract. In an additional aspect, the immune disorder is Rheumatoid arthritis, Systemic lupus erythematosus, Celiac disease, Crohn's disease, Inflammatory bowel disease, Sjogren's syndrome, Polymyalgia rheumatic, Psoriasis, Multiple sclerosis, Ankylosing spondylitis, Type 1 diabetes, Alopecia areata, Vasculitis, Temporal arteritis, Graves' disease or Hashimoto's thyroiditis.

[0071] In an additional embodiment, the present invention provides a vaccine for administration to a subject to produce an immune response against TGF-alpha comprising a TGF-alpha polypeptide or a biologically active fragment thereof and optionally an adjuvant.

[0072] In a further embodiment, the present invention provides a vaccine for administration to a subject to produce an immune response against TGF-alpha comprising a nucleic acid molecule encoding a TGF-alpha polypeptide or a biologically active fragment thereof and optionally an adjuvant. In one aspect, the nucleic acid is DNA or RNA.

[0073] The term "vaccine" refers to a biological preparation that provides an immune response to an antigen. A vaccine typically contains an agent that resembles a disease-causing agent and is often made from weakened or killed forms it, its toxins, or one of its

surface proteins. Vaccine stimulates the immune system to recognize the agent as a threat, destroy it, and to further recognize and destroy it in the future. Vaccines can be prophylactic or therapeutic (e.g., cancer vaccines). The term "cancer vaccine" refers to any preparation capable of being used as an inoculation material or as part of an inoculation material, that will provide a treatment for, inhibit and/or convey immunity to cancer and/or tumor growth. A vaccine may be a peptide vaccine, a DNA vaccine or a RNA vaccine for example.

[0074] A peptide/polypeptide vaccine comprising a TGF-alpha polypeptide or biologically active fragment thereof is within the scope of the invention. In one aspect, the vaccine comprises an immunogenic amount of the comprising a TGF-alpha polypeptide or biologically active fragment thereof immunogens of the present invention. comprising a TGF-alpha polypeptide or biologically active fragment thereof of the invention may be conveniently formulated into vaccine compositions comprising one or more of the peptides alone or in association with a pharmaceutically acceptable carrier.

[0075] The vaccine comprises and can be made by providing immunogenic amounts of the peptides alone or in a pharmaceutically acceptable vehicle or carrier. Carriers include water, saline, dextrose, and glycerol, for example. The vaccine can optionally further comprise additional immune-stimulatory molecules and adjuvants or mixture of adjuvants. One of ordinary skill in the art would be able to identify vehicles, carriers, other antigens or immunogens, and immunomodulators, such as adjuvants or cytokines, appropriate for the present invention. Additional additives would also be readily apparent to one of skill in the art, such as wetting agents or preservatives.

[0076] The term "adjuvant" refers to a substance used in vaccine preparation to enhance the immune response to an immunogen. There are multiples adjuvant know in the art, however in the U.S, the FDA only approved the use of aluminum salts such as aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), or mixed aluminum salts, AS04 (combination of aluminum hydroxide and monophosphoryl lipid A, MPL), and AS03 (an oil-in-water emulsion) as adjuvants for vaccines.

[0077] A DNA or RNA vaccine is also within the scope of the present invention. One aspect of the invention is a DNA or RNA vaccine comprising DNA or RNA encoding immunoreactive peptides or compositions of the present invention, i.e. TGF-alpha polypeptides or biologically active fragments. Methods for making DNA or RNA sequences suitable for DNA or RNA vaccines are known in the art. One of ordinary skill would be able to determine appropriate promoters or other regulatory sequences which may be used in the DNA or RNA construct encoding the immunoreactive compositions. DNA or RNA

vaccines may further comprise other components as in the vaccines and compositions described above and below, such as carriers and agents which increase levels of immunity, such as liposomes. DNA or RNA vaccines may be administered by routes similar to other vaccines. Administration of a DNA or RNA vaccine results in expression of antigens which produce a protective immune response.

[0078] The phase "immune response" refers to the body's response originating from the immune system activation by antigens. This process involved several types of immune cells, including antigen presenting cells (APCs), B cells and T cells lymphocytes. Specifically the recombinant TGF-alpha polypeptide or biologically active fragment thereof of the vaccine of the present invention is recognize by the APCs, which activate B cells lymphocytes. With T-helper lymphocytes support, B cells expand, differentiate into mature B cells and ultimately produce anti-TGF-alpha antibodies that uniquely recognize and neutralize autologous TGF-alpha. The term "immunize" refers to eliciting an immune response in an animal, both a humoral immune response and a cellular immune response leading to an immune memory to maintain immunity against said antigen. Specifically, a TGF-alpha immune response refers to an immune response in which anti-TGF-alpha antibodies are produce.

[0079] The term "recombinant" or "recombinant TGF-alpha" refers to TGF-alpha proteins resulting from the expression of recombinant DNA (DNA molecules synthesized using laboratory methods of genetic recombination i.e. molecular cloning of the nucleic acid sequence coding for the protein or portion of protein of interest into a plasmid vector.

[0080] In one embodiment, the present invention provides an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a nucleic acid sequence encoding a TGF-alpha protein or biologically active fragment thereof, a transmembrane domain and an intracellular signaling domain. In an aspect, the transmembrane domain comprises a transmembrane domain of a protein selected from the TGF-alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In an additional aspect, the nucleic acid sequence encoding the TGF-alpha polypeptide or biologically active fragment thereof is connected to the transmembrane domain by a linker or hinge region.

[0081] The nucleic acid construct of the present invention may be introduced into a cell to be altered thus allowing expression of the chimeric protein within the cell. A variety of cells are known in the art and suitable for recombinant proteins expression. Examples of typical

cell used for recombinant protein expression include, but are not limited to, the bacteria *E. coli*, or the yeast *S. cerevisiae*.

[0082] In another aspect, the present invention provides for a chimeric antigen receptor (CAR) polypeptide, wherein the CAR comprises a TGF-alpha protein or biologically active fragment thereof, a transmembrane domain and an intracellular signaling domain. In one aspect, the transmembrane domain comprises a transmembrane domain of a protein selected from TGF-alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In an additional aspect, the nucleic acid encoding the TGF-alpha polypeptide or biologically active fragment thereof is connected to the transmembrane domain by a linker or hinge region.

[0083] In an additional aspect, the present invention provides for a method of providing anti-tumor immunity in a subject, comprising administering to the subject an immune cell comprising a nucleic acid molecule encoding a TGF-alpha polypeptide or biologically active fragment thereof or a TGF-alpha polypeptide or biologically active fragment thereof.

[0084] Immunotherapies include the use of adoptive transfer of genetically engineered T cells, modified to recognize and eliminate cancer cells specifically. T cells can be genetically modified to stably express on their surface chimeric antigen receptors (CAR). CAR are synthetic proteins comprising of a signaling endodomain, consisting of an intracellular domain, a transmembrane domain, and an extracellular domain. Upon interaction with the target cancer cell expressing the antigen, the chimeric antigen receptor triggers an intracellular signaling leading to T-cell activation and to a cytotoxic immune response against tumor cells. Such therapies have been shown to be efficient against relapsed/refractory disease. Additionally, CAR-T cells can be engineered to include co-stimulatory receptor that enhance the T-cell-mediated cytotoxic activity. Furthermore, CAR-T cells can be engineered to produce and deliver protein of interest in the tumor microenvironment.

[0085] The isolated nucleic acid molecule encoding a CAR of the polypeptide comprising a TGF alpha protein or biologically active fragment thereof of the present invention will be inserted into a host cell, through the introduction of a vector encoding said polypeptide into said host cell.

[0086] The term "nucleic acid" or "nucleic acid construct" is used herein to refer to a recombinant nucleic acid construct that is manipulated by human intervention. A recombinant nucleic acid construct can contain two or more nucleotide sequences that are

linked in a manner such that the product is not found in a cell in nature. In particular, the two or more nucleotide sequences can be operatively linked, such as a gene encoding a protein of interest, one or more protein tags, functional domains and the like.

[0087] The term "vector" or "cloning vector" or "expression vector" is used herein to refer to a recombinant nucleic acid construct that is manipulated by human intervention. Vectors derived from retrovirus such as lentivirus are suitable tools to achieve long-term gene transfer since they allow long term and stable integration of a transgene and its propagation in daughter cells. The expression vector of the present invention will typically comprise the nucleic acid sequence encoding the CAR polypeptide operably linked to a promoter for the regulation of the expression of said nucleic acid, as well as the typical sequences contained into cloning vectors such as transcription and translation terminators, initiation sequences, an origin of replication functional in at least the target organism, convenient restriction endonucleases, and one or more selectable markers. The cloning vector of the present invention can be selected from the group comprising plasmid, phagemid, phage derivative, virus, and cosmid. Viruses that are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses.

[0088] As used herein, a promoter is intended to mean a polynucleotide sequence capable of facilitating transcription of genes in operable linkage with the promoter. Several types of promoters are well known in the art and suitable for use with the present invention, for example constitutive promoters that allows for unregulated expression in mammalian cells, such as the cytomegalovirus (CMV) promoter. Alternatively, the nucleic acid may include one or more inducible promoters. An inducible promoter is a promoter that, in the absence of an inducer (such as a chemical and/or biological agent), does not direct expression, or directs low levels of expression of an operably linked gene (including cDNA), and, in response to an inducer, its ability to direct expression is enhanced. Exemplary inducible promoters include, for example, promoters that respond to heavy metals, to thermal shocks, to hormones, and those that respond to chemical agents, such as glucose, lactose, galactose or antibiotic.

[0089] The vector of the present invention may be physically introduced into a host cell to be altered thus allowing expression of the recombinant CAR protein. A variety of methods are known in the art and suitable for introduction of nucleic acid into a cell, including viral (transduction) and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, transformation, transfection, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock,

magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion. Other methods of transfection include proprietary transfection reagents such as Lipofectamine ™, Dojindo Hilymax ™, Fugene ™, jetPEI ™, Effectene ™ and DreamFect ™.

[0090] The term " host cells", as used herein, refers to the immune cells isolated from a subject in need of the treatment. Prior to expansion and genetic modification, the immune cells can be obtained from several sources including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from an site of infection, ascites, pleural effusion spleen tissue, and tumors. Typically immune cells are isolated from a unit of blood collected from a subject using a number of techniques known to the skilled artisan, such as Ficoll ™ separation. The isolated immune cell is selected from the group consisting of an autologous T cell, an allogenic T cell, a T helper cell, a cytotoxic T cell, an effector T cell, a suppressor T cell, a NK cell, a NKT cell or a T cell progenitor cell. T cell population can be isolated and enriched from blood samples using various techniques known in the art (for example U.S. Patent No. 9, 745, 368).

[0091] Generally, the T cells of the invention are activated and expanded *ex vivo*, using methods known to the skilled in the art, as described for examples in U.S. Patents Nos.: 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,687,041; and U.S. Patent Application No. 20060121005.

[0092] Upon isolation from a subject in need, the immune cells, genetically engineered to express a vector encoding a CAR polypeptide comprising a TGF alpha protein of biologically active fragment thereof, activated and expanded *ex vivo*, would be administered to the recipient to provide a therapeutic benefit, i.e. induce the production of anti-TGF alpha antibodies thereby inhibiting cancer cells' uncontrolled TGF alpha-mediated proliferation.

[0093] Alternatively, the CAR-T cell encoding a CAR polypeptide comprising a TGF alpha protein of biologically active fragment thereof and inducing the production of anti-TGF alpha antibodies could be administered in combination with other anti-tumor therapy to enhance the efficacy of the treatment.

[0094] In a further aspect, the present invention provides a pharmaceutical composition comprising a TGF-alpha polypeptide or biologically active fragment thereof, vaccine or transformed cell and a pharmaceutically acceptable carrier.

[0095] By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0096] The compositions for administration will commonly comprise a solution of the peptide or peptide conjugate dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of fusion protein in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

[0097] Thus, a typical pharmaceutical composition of the present invention for intravenous administration would be about 0.1 to 10 mg per patient per day. Dosages from 0.1 up to about 100 mg per patient per day may be used. Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as REMINGTON'S PHARMACEUTICAL SCIENCE, 19TH ED., Mack Publishing Company, Easton, Pa. (1995).

[0098] Presented below are examples discussing the rational, the potential use of TGF-alpha polypeptides or biologically active fragments and the generation and use of a TGF-alpha vaccine contemplated for the discussed applications.

[0099] The following examples are provided to further illustrate the embodiments of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1
HUMAN CANCER CELLS SECRETE TRANSFORMING GROWTH FACTOR ALPHA

[0100] Human cancer cell lines were developed and cultured from lung carcinoma, colon carcinoma, ovary carcinoma, brain carcinoma, and melanoma lesion patient samples. To assess the transforming potential of cancer cell secretion, supernatants collected from the

cultured cancer cell lines were applied to previously cultured normal cells. Cancer cell line derived supernatants transformed cultured normal cells to behave like tumor cells. The cancer cell line derived supernatants were purified and sequenced. The results identified two major growth factors: transforming growth factor TGF-alpha (TGF α) and transforming growth factor beta (TGF-β, which is structurally unrelated to TGF-alpha). The sequence of TGF-alpha was found to have 50 amino acids, 13 of which are identical to epidermal growth factor (EGF).

[0101] TGF-alpha and EGF both bind to the EGF receptor (EGFR), but TGF-alpha is a much more potent ligand for binding EGFR than EGF. Like EGF, TGF-alpha is a hormone-like protein produced and excreted by many tumors. It was found that upon binding to the EGF receptor, that TGF-alpha, unlike EGF, stimulated cell division, promoted the anchorage-independent growth, thus facilitating tumor growth.

[0102] The production of TGF-alpha by transformed cells and the responses of normal cells to the addition of TGF-alpha to the culture medium suggested a TGF-alpha-induced autocrine growth stimulation by rebinding of the released factors at the cell surface. As such, inducing an immune response against TGF-alpha may be a potent cancer therapeutic rationale.

EXAMPLE 2

EVALUATION OF TGF-ALPHA LEVELS AND BIOACTIVITY IN PATIENTS' URINE

[0103] Many human tumor cell lines express high levels of both TGF-alpha and EGFR. TGF-alpha is primarily found in tumor cells and embryos as well as in many human cancers such as carcinomas of lung, colon, stomach, and head and neck. TGF-alpha presence was evaluated in the urine of 53 subjects comprising 18 apparently health controls, 3 patients with benign conditions (villous colic adenoma, fibrocystic breast and 38 weeks pregnancy), and 32 patients with confirmed cancer diagnostic (13 lung carcinomas, 7 gastrointestinal carcinomas, 3 urogenital carcinomas, 5 breast carcinomas and 4 lymphoid cancers). As detailed in Table 1, substantially increased levels of TGF-alpha were found in urine from cancer patients as compared to matched controls. Only one apparently healthy control presented increased TGF-alpha urine concentration (6%), the only benign condition presenting elevated TGF-alpha urine concentration was in the pregnancy context, while 81% of the cancer patients had more than a two-fold increase in TGF-alpha urine concentration as compared to the normal value. Interestingly this value ranged from 57%

for gastrointestinal carcinomas to up to 100% for certain cancer types (urogenital and breast).

[0104] This result indicated that using urine as a convenient and readily available material to that can be used to detect TGF-alpha concentration. Moreover, it illustrated that TGF-alpha measurement might be a useful diagnostic marker to detect certain types of cancer.

[0105] Table 1: Detection of TGF-alpha antigen in urine

PATIENT GROUP	OVERALL POSITIVE
Apparently healthy controls	1/18 (6%)
Patients with benign conditions	1/3 (33%)
colon (villous adenoma)	0/1 (0%)
breast (fibrocystic)	0/1 (0%)
pregnancy (normal, 38 wks)	1/1 (100%)
Patients with cancer	26/32 (81%)
Lung	11/13 (85%)
Gastrointestinal	4/7 (57%)
Urogenital	3/3 (100%)
Breast	5/5 (100%)
Lymphoid	3/4 (75%)
Cut off > 2 x average normal value	

[0106] Further, TGF-alpha bioactivity was evaluated in the urine of 50 subjects comprising 25 normal controls, and 25 patients with confirmed cancer diagnostic (5 lung carcinomas, 5 breast carcinomas, 5 colon carcinomas, 5 melanomas and 5 leukemia). As detailed in Table 3, substantially increased bioactivity of TGF-alpha was found in urine from cancer patients as compared to matched controls. Only one control presented an increased TGF-alpha urine bioactivity (4%), while an average of 64% of the cancer patients had a significantly higher TGF-alpha urine bioactivity as compared to the normal value. Interestingly this value ranged from 0% for leukemia to 80% for lung and breast cancers, and up to 100% for melanomas.

[0107] This result indicated that urine is a convenient and readily available material that can be used for the bio-characterization of TGF-alpha bioactivity. Moreover, it illustrated that TGF-alpha bioactivity can be correlated with presence of certain cancers and that TGF-alpha bioactivity measurement might be a useful diagnostic marker to detect certain types of cancer.

Table 2: Detection of TGF bioactivity in urine of cancer patients

DIAGNOSIS	NO. POSITIVE/NO. TESTED
Lung Cancer	4/5 (80%)
Breast Cancer	4/5 (80%)
Colon Cancer	3/5 (60%)
Melanomas	5/5 (100%)
Leukemia	0/5 (0%)
Normal Conditions	1/25 (4%)

EXAMPLE 3

EVALUATION THE EFFECT OF TGF-ALPHA TREATMENT ON TUMOR CELL GROWTH

[0108] In order to assess the effects of TGF-alpha on tumor cell growth (stimulating or inhibiting effects), and to compare them to the effects of EGF, Human squamous carcinoma A431 cells, known for their abnormally high levels of EGFR, were cultured and exposed to various doses of EGF or TGF-alpha. Tumor cell growth in soft agar, spreading and regular growth were evaluated. Interestingly, TGF-alpha was found more efficient at allowing normal cells to grow in soft agar as compared to EGF. TGF-alpha was also found more efficient to allow the cells to spread apart from one another due to increased ruffling of the cell membrane.

[0109] It was also found that, as illustrated in Table 3, human tumor cells that have an abnormally high number of EGF receptors on the cell surface (>5 x 105, i.e. A431 cells) are actually inhibited and not stimulated by the higher doses of TGF alpha.

[0110] Table 3: Effect of TGF-alpha and EGF on the growth of human tumor cell line A431

	Concentration	Cell Numbers/Day 7 (1×10^5)
EGF	0.1 µg/ml	12
	1.0 µg/ml	46
	10 µg/ml	62
TGFα	0.1 µg/ml	75
	1.0 µg/ml	90
	10 µg/ml	<20

EXAMPLE 4

EVALUATION OF TGF-ALPHA ANTIBODIES EFFECT ON IN VITRO COLONY FORMATION AND INVASIVENESS

[0111] To further evaluate the effect of modulating TGF-alpha on tumor cells growth, clonogenic assays (or colony formation assays), invasion assays and migration assays will be performed to show that clearing TGF-alpha from the culture media using anti-TGF-alpha

antibodies will result in an effective inhibition of clonogenic colony formation of cancer cells and an inhibition of invasion and migration by the cancer cells.

[0112] During the clonogenic assay, the effectiveness of anti-TGF-alpha antibodies on the survival and proliferation of human cancer cells will be assessed and compare to the efficacy of anti-EGF antibodies and anti-EGFR antibodies and control IgG to inhibit cell proliferation. The cells will be treated before being plated in tissue culture dishes and allowed to grow. After the period of time required for the cells treated with the control IgG to grow and form colonies, all plates will be fixed, colonies stained and counted. The percentage of surviving cells which will have grown into colonies will be evaluated. Comparative cell survival curves will be generated to compare the effect of anti-TGF-alpha antibodies to control IgG, anti-EGF antibodies and anti-EGFR antibodies on cell survival.

[0113] Comparison of the number of colonies formed following IgG treatment to the number of colonies formed after anti-TGF-alpha treatment is expected to show that anti-TGF-alpha antibodies are potent inhibitors of clonogenic colony formation of human cancer cells. Further, it is expected that anti-TGF-alpha antibodies will be a more potent inhibitor of colony formation that anti EGF antibodies or anti-EGFR antibodies.

[0114] The effectiveness of anti-TGF-alpha antibodies on migratory and invasive properties of human cancer cells will be assessed by invasion and migration assays, and the efficacy of anti-TGF-alpha antibodies will be compared to anti-EGF and anti-EGFR antibodies, as well as to control IgG. The cells will be treated before being plated in tissue culture dishes and allowed to migrate through a porous membrane and invade an extracellular matrix. After the period of time required for the cells treated with the control IgG migrate and invade the extracellular matrix, all plates will be fixed, cells stained and counted. The percentage of migrating and invasive cells will be evaluated and comparative percentages of migratory and invasive cells will be established to determine the effect of anti-TGF-alpha antibodies as compared to control IgG, anti-EGF antibodies and anti-EGFR antibodies.

[0115] Comparison of the number of migrating and invasive cells after the control IgG treatment to the number of migrating and invasive cells after anti-TGF-alpha treatment is expected to show that anti-TGF-alpha antibodies are potent inhibitors of human cancer cell migration and invasion. Further, it is expected that anti-TGF-alpha antibodies will be a more potent cancer cell migration and invasion inhibitor than anti EGF antibodies or anti-EGFR antibodies.

EXAMPLE 5

USE OF ANTI-TGF-ALPHA ANTIBODIES IN MOUSE XENOGRAFT TUMOR MODELS

[0116] Immunodeficient or syngeneic mice will be subcutaneously transplanted with human tumor cells known to produce TGF-alpha following a xenograft model approach. Once the cells have developed into palpable tumors of approximately 3 mm mean diameter, each animal (5 in each group) will receive one injection weekly, for 3 weeks, of 0.5 mg/mouse of an antibody suspension.

[0117] Several commercially available antibodies directed against TGF-alpha (monoclonal and polyclonal, of mouse origin, and recognizing the human molecule) will be used in order to determine which TGF-alpha epitopes induce the more efficient tumor-inhibitory therapeutic response.

[0118] As such, the xenograph models will be used to assess the greater efficacy of different TGF-alpha antibodies and epitopes. Specifically, 4 groups of 5 mice will be used, receiving respectively a control IgG, anti-TGF-alpha antibodies, anti-EGF antibodies, or anti-EGFR antibodies. Control IgG, anti-TGF-alpha, anti-EGF, and anti-EGFR-antibodies will be purchased from the same company (Life Span BioSciences Inc.). This approach is expected to identify any TGF-alpha epitopes that have greater tumor inhibition efficacy.

[0119] To assess the best delivery method, in alternative experiments the antibody injections would be performed either intraperitoneally or intratumorally.

EXAMPLE 6

IN VIVO EVALUATION OF THERAPEUTIC EFFICACY OF TGF-ALPHA ANTIBODIES FOR THE INHIBITION OF CANCER CELL GROWTH

[0120] The efficacy of TGF-alpha antibodies to inhibit the growth of human cancer cells known to produce TGF-alpha will be evaluated. Four groups of 5 xenograph mice described above will be injected with control IgG antibodies, anti-TGF-alpha antibodies, anti-EGF antibodies, and anti-EGFR antibodies. In alternative experiments, the mice used would be either immunodeficient or syngeneic mice, and the antibodies injections would be received either intraperitoneally or intratumorally.

[0121] Comparison received of the control IgG group to the anti-TGF-alpha antibody group is expected to show that anti-TGF-alpha antibodies are highly efficient for the inhibition of the growth of subcutaneously injected human cancer cells known to produce TGF-alpha. Additionally, the comparison of the control IgG group to the anti-EGF and anti-EGFR antibody groups is expected to show the efficacy of using either anti-EGF or anti-EGFR

antibodies to inhibit the growth of subcutaneously injected human cancer cells known to produce TGF-alpha (and thus to activate the EGFR pathway).

[0122] Furthermore, comparison of the anti TGF-alpha antibody group to either the anti-EGF or the anti-EGFR antibody groups it is expected to show that anti-TGF-alpha antibodies will be more efficient than both anti-EGF and anti-EGFR antibodies to inhibit the growth of human cancer cells known to produce TGF-alpha.

EXAMPLE 7

IN VIVO EVALUATION OF THERAPEUTIC EFFICACY OF TGF-ALPHA ANTIBODIES IN COMBINATION WITH PD-1 ANTIBODIES

[0123] The efficacy of TGF-alpha antibodies in combination with anti-PD-1antibodies to inhibit the growth of human cancer cells known to produce TGF-alpha will be evaluated. Eight groups of 5 xenograph mice (described above) will be treated with control IgG antibodies, anti-TGF-alpha antibodies, anti-EGF antibodies, anti-EGFR antibodies, control IgG antibodies + anti-PD-1 antibodies, anti-TGF-alpha antibodies + anti-PD-1 antibodies, anti-EGF antibodies + anti-PD-1 antibodies, and anti-EGFR antibodies + anti-PD-1 antibodies, respectively. In alternative experiments, the mice used would be either immunodeficient or syngeneic mice, and the antibodies injections would be received either intraperitoneally or intratumorally.

[0124] Comparison of the control IgG group to the anti TGF-alpha antibody group is expected to show that anti-TGF-alpha antibodies are highly efficient to inhibit the growth of human cancer cells known to produce TGF-alpha. Comparison of the control IgG group to the anti-EGF and anti-EGFR antibody groups is expected to show the efficacy of using either anti-EGF or anti-EGFR antibodies to inhibit the growth of human cancer cells (and thus to activate the EGFR pathway). Comparison of the control IgG group and the control IgG + anti-PD-1group is expected to show the efficacy of the anti-PD-1 antibodies to inhibit the growth of human cancer cells. Comparison of the anti-EGF and anti-EGFR antibody groups and the anti-EGF + anti-PD-1 antibody and anti-EGFR+ anti-PD-1 antibody groups is expected to demonstrate the benefits of combining anti-PD-1 antibodies to anti-EGF or anti-EGFR antibodies, and therefore enhancing the efficacy the inhibition of the growth of human cancer cells.

[0125] Furthermore, comparison of the anti-TGF-alpha antibody group to either the anti-EGF or the anti-EGFR groups is expected to show that anti-TGF-alpha antibodies are more efficient than both anti-EGF and anti-EGFR antibodies at inhibiting the growth of human cancer cells known to produce TGF-alpha.

[0126] Moreover, comparison of the combination anti TGF-alpha + anti-PD-1 antibody group to either the combination anti-EGF + anti-PD-1 antibody or anti-EGFR+ anti-PD-1 antibody groups is expected to show that anti-TGF-alpha antibodies combined with anti-PD-1 antibodies are more efficient than both anti-EGF and anti-EGFR antibodies either alone or in combination with anti-PD-1 antibodies to inhibit the growth human cancer cells.

[0127] Ultimately, the experiments will highlight the benefits of combining anti-PD-1 antibodies to anti-TGF-alpha antibodies to synergize their effects and induce a more efficient inhibition of the growth of human cancer cells.

EXAMPLE 8
DEVELOPMENT OF A TGF-ALPHA VACCINE INDUCING HIGH TITER OF TGF-ALPHA ANTIBODIES

[0128] Based on the preclinical data collected using the injection of commercially available anti-TGF-alpha antibodies, an epitope of the protein would be isolated as the best antigen to be used in a vaccine to induce an immune response against autologous TGF-alpha.

[0129] This new vaccine would be capable of inducing a high titer of antibodies directed against TGF-alpha, there by inhibiting the TGF-alpha induced autocrine stimulatory pathway.

[0130] Following the methods know in the art for vaccine production, a viral vector, derived from example from a retrovirus, an adenovirus, an adeno-associated virus (AAV), a poxvirus or a herpes virus, encoding at least one antigen and at least one co-stimulatory molecule will be used. The antigen used would be recombinant TGF-alpha polypeptide or a fragment thereof to reflect the best epitopes identified using commercially available antibodies.

[0131] The vaccine would then be prepared to comprise a vector encoding the recombinant TGF-alpha or fragment and optionally a carrier protein, and an adjuvant to enhance the immune response induced by the vaccine.

[0132] This highly immunoreactive vaccine would be engineered to be capable of inducing high titer of TGF-alpha antibodies in its recipient, leading to the clearance of autologous TGF-alpha from the recipient circulation. This would be highly efficient to inhibit the TGF-alpha/EGFR interaction and the proliferation signaling pathway associated with it which is responsible for the autocrine growth stimulation leading to tumor growth in human cancers known to produce TGF-alpha.

[0133] Table 4: TGF alpha sequences

| SEQ ID NO.: 1 TGF-ALPHA splice variant 1 nucleic acid sequence | GTCAGCTGTGCCCCGGTCGCCGAGTGGCGAGGAGGTGACGGTAGCCGCCTT CCTATTTCCGCCCGGCGGGCAGCGCTGCGGGGCGAGTGCCAGCAGAGAGGC GCTCGGTCCTCCCTCCGCCCTCCCGCGCCGGGGGCAGGCCCTGCCTAGTCTG CGTCTTTTTCCCCCGCACCGCGGCGCCGCTCCGCCACTCGGGCACCGCAGGT AGGGCAGGAGGCTGGAGAGCCTGCTGCCCGCCCGCCCGTAAAATGGTCCCC TCGGCTGGACAGCTCGCCCTGTTCGCTCTGGGTATTGTGTTGGCTGCGTGCC AGGCCTTGGAGAACAGCACGTCCCCGCTGAGTGCAGACCCGCCCGTGGCTG CAGCAGTGGTGTCCCATTTTAATGACTGCCCAGATTCCCACACTCAGTT **CTGCTTCCATGGAACCTGCAGGTTTTGGTGCAGGAGGACAAGCCAGC ATGTGTCTGCCATTCTGGGTACGTTGGTGCACGCTGTGAGCATGCGGA CCTCCTGGCCGTGGTGGCTGCCAGCCAGAAGAAGCAGGCCATCACCGCCT** TGGTGGTGGTCTCCATCGTGGCCCTGGCTGTCCTTATCATCACATGTGTGCT GATACACTGCTGCCAGGTCCGAAAACACTGTGAGTGGTGCCGGGCCCTCAT CTGCCGGCACGAGAAGCCCAGCGCCCTCCTGAAGGGAAGAACCGCTTGCTG CCACTCAGAAACAGTGGTCTGAAGAGCCCAGAGGAGGAGTTTGGCCAGGTG GACTGTGGCAGATCAATAAAGAAAGGCTTCTTCAGGACAGCACTGCCAGAG ATGCCTGGGTGTGCCACAGACCTTCCTACTTGGCCTGTAATCACCTGTGCAG CCTTTTGTGGGCCTTCAAAACTCTGTCAAGAACTCCGTCTGCTTGGGGTTATT CAGTGTGACCTAGAGAAGAAATCAGCGGACCACGATTTCAAGACTTGTTAA AAAAGAACTGCAAAGAGACGGACTCCTGTTCACCTAGGTGAGGTGTGTGCA GCAGTTGGTGTCTGAGTCCACATGTGTGCAGTTGTCTTCTGCCAGCCATGGA TTCCAGGCTATATATTTCTTTTTAATGGGCCACCTCCCCACAACAGAATTCTG CCCAACACAGGAGATTTCTATAGTTATTGTTTTCTGTCATTTGCCTACTGGGG AAGAAAGTGAAGGAGGGGAAACTGTTTAATATCACATGAAGACCCTAGCTT TAAGAGAAGCTGTATCCTCTAACCACGAGACCCTCAACCAGCCCAACATCTT CCATGGACACATGACATTGAAGACCATCCCAAGCTATCGCCACCCTTGGAG ATGATGTCTTATTTATTAGATGGATAATGGTTTTATTTTTAATCTCTTAAGTC AATGTAAAAAGTATAAAACCCCTTCAGACTTCTACATTAATGATGTATGTGT TGCTGACTGAAAAGCTATACTGATTAGAAATGTCTGGCCTCTTCAAGACAGC TAAGGCTTGGGAAAAGTCTTCCAGGGTGCGGAGATGGAACCAGAGGCTGGG TTACTGGTAGGAATAAAGGTAGGGGTTCAGAAATGGTGCCATTGAAGCCAC AAAGCCGGTAAATGCCTCAATACGTTCTGGGAGAAAACTTAGCAAATCCAT CAGCAGGGATCTGTCCCCTCTGTTGGGGAGAGAGGAAGAGTGTGTGTGTCT ACACAGGATAAACCCAATACATATTGTACTGCTCAGTGATTAAATGGGTTCA CTTCCTCGTGAGCCCTCGGTAAGTATGTTTAGAAATAGAACATTAGCCACGA GCCATAGGCATTTCAGGCCAAATCCATGAAAGGGGGACCAGTCATTTATTTT CCATTTGTTGCTTGGTTGGTTTGTTGCTTTATTTTTAAAAGGAGAAGTTTAA CTTTGCTATTTATTTTCGAGCACTAGGAAAACTATTCCAGTAATTTTTTTTTC CTCATTTCCATTCAGGATGCCGGCTTTATTAACAAAAACTCTAACAAGTCAC CTCCACTATGTGGGTCTTCCTTTCCCCTCAAGAGAAGGAGCAATTGTTCCCC TGAGCATCTGGGTCCATCTGACCCATGGGGCCTGCCTGTGAGAAACAGTGG GTCCCTTCAAATACATAGTGGATAGCTCATCCCTAGGAATTTTCATTAAAAT TTGGAAACAGAGTAATGAAGAAATAATATATAAACTCCTTATGTGAGGAAA TGCTACTAATATCTGAAAAGTGAAAGATTTCTATGTATTAACTCTTAAGTGC ACCTAGCTTATTACATCGTGAAAGGTACATTTAAAATATGTTAAATTGGCTT GAAATTTTCAGAGAATTTTGTCTTCCCCTAATTCTTCTTCCTTGGTCTGGAAG AACAATTTCTATGAATTTTCTCTTTATTTTTTTTTATAATTCAGACAATTCTAT GACCCGTGTCTTCATTTTTGGCACTCTTATTTAACAATGCCACACCTGAAGC ACTTGGATCTGTTCAGAGCTGACCCCCTAGCAACGTAGTTGACACAGCTCCA GGTTTTTAAATTACTAAAATAAGTTCAAGTTTACATCCCTTGGGCCAGATAT GTGGGTTGAGGCTTGACTGTAGCATCCTGCTTAGAGACCAATCAACGGACA CTGGTTTTTAGACCTCTATCAATCAGTAGTTAGCATCCAAGAGACTTTGCAG AGGCGTAGGAATGAGGCTGGACAGATGGCGGAAGCAGAGGTTCCCTGCGA AGACTTGAGATTTAGTGTCTGTGAATGTTCTAGTTCCTAGGTCCAGCAAGTC ACACCTGCCAGTGCCCTCATCCTTATGCCTGTAACACACATGCAGTGAGAGG CCTCACATATACGCCTCCCTAGAAGTGCCTTCCAAGTCAGTCCTTTGGAAAC CAGCAGGTCTGAAAAGAGGCTGCATCAATGCAAGCCTGGTTGGACCATTG TCCATGCCTCAGGATAGAACAGCCTGGCTTATTTGGGGATTTTTCTTCTAGA AATCAAATGACTGATAAGCATTGGATCCCTCTGCCATTTAATGGCAATGGTA |

	GTCTTTGGTTAGCTGCAAAAATACTCCATTTCAAGTTAAAAATGCATCTTCT AATCCATCTCTGCAAGCTCCCTGTGTTTCCTTGCCCTTTAGAAAATGAATTGT TCACTACAATTAGAGAATCATTTAACATCCTGACCTGGTAAGCTGCCACACA CCTGGCAGTGGGGAGCATCGCTGTTTCCAATGGCTCAGGAGACAATGAAAA GCCCCCATTTAAAAAAATAACAAACATTTTTTAAAAGGCCTCCAATACTCTT ATGGAGCCTGGATTTTTCCCACTGCTCTACAGGCTGTGACTTTTTTAAGCAT CCTGACAGGAAATGTTTTCTTCTACATGGAAAGATAGACAGCAGCCAACCC TGATCTGGAAGACAGGGCCCCGGCTGGACACACGTGGAACCAAGCCAGGG ATGGGCTGGCCATTGTGTCCCCGCAGGAGAGATGGGCAGAATGGCCCTAGA GTTCTTTTCCCTGAGAAAGGAGAAAAAGATGGGATTGCCACTCACCCACCC ACACTGGTAAGGGAGGAGAATTTGTGCTTCTGGAGCTTCTCAAGGGATTGT GTTTTGCAGGTACAGAAAACTGCCTGTTATCTTCAAGCCAGGTTTTCGAGGG CACATGGGTCACCAGTTGCTTTTTCAGTCAATTTGGCCGGGATGGACTAATG AGGCTCTAACACTGCTCAGGAGACCCCTGCCCTCTAGTTGGTTCTGGGCTTT GATCTCTTCCAACCTGCCCAGTCACAGAAGGAGGAATGACTCAAATGCCCA AAACCAAGAACACATTGCAGAAGTAAGACAAACATGTATATTTTTAAATGT TCTAACATAAGACCTGTTCTCTCTAGCCATTGATTTACCAGGCTTTCTGAAA GATCTAGTGGTTCACACAGAGAGAGAGAGAGTACTGAAAAAGCAACTCCTC TTCTTAGTCTTAATAATTTACTAAAATGGTCAACTTTTCATTATCTTTATTAT AATAAACCTGATGCTTTTTTTTAGAACTCCTTACTCTGATGTCTGTATATGTT GCACTGAAAAGGTTAATATTTAATGTTTTAATTTATTTTGTGTGGTAAGTTA ATTTTGATTTCTGTAATGTGTTAATGTGATTAGCAGTTATTTTCCTTAATATC TGAATTATACTTAAAGAGTAGTGAGCAATATAAGACGCAATTGTGTTTTTCA GTAATGTGCATTGTTATTGAGTTGTACTGTACCTTATTTGGAAGGATGAAGG AATGAATCTTTTTTTCCTAAATCAA
SEQ ID NO.: 2 TGF-ALPHA splice variant 2 nucleic acid sequence	ACTCGGGCACCGCAGGTAGGGCAGGAGGCTGGAGAGCCTGCTGCCCGCCCG CCCGTAAAATGGTCCCCTCGGCTGGACAGCTCGCCCTGTTCGCTCTGGGTAT TGTGTTGGCTGCGTGCCAGGCCTTGGAGAACAGCACGTCCCCGCTGAGTGA CCCGCCCGTGGCTGCAGCAGT**GGTGTCCCATTTTAATGACTGCCCAGATT CCCACACTCAGTTCTGCTTCCATGGAACCTGCAGGTTTTTGGTGCAGGA GGACAAGCCAGCATGTGTCTGCCATTCTGGGTACGTTGGTGCACGCTG TGAGCATGCGGACCTCCTGGCCGTGGTGGCTGCCAGCCAGAAGAAGCAG** GCCATCACCGCCTTGGTGGTGGTCTCCATCGTGGCCCTGGCTGTCCTTATCA TCACATGTGTGCTGATACACTGCTGCCAGGTCCGAAAACACTGTGAGTGGTG CCGGGCCCTCATCTGCCGGCACGAGAAGCCCAGCGCCCTCCTGAAGGGAAG AACCGCTTGCTGCCACTCAGAAACAGTGGTCTGAAGAGCCCAGAGGAGGAG TTTGGCCAGGTGGACTGTGGCAGATCAATAAAGAAAGGCTTCTTCAGGACA GCACTGCCAGAGATGCCTGGGTGTGCCACAGACCTTCCTACTTGGCCTGTAA TCACCTGTGCAGCCTTTTGTGGGCCTTCAAAACTCTGTCAAGAACTCCGTCT GCTTGGGGGTTATTCAGTGTGACCTAGAGAAGAAATCAGCGGACCACGATTT CAAGACTTGTTAAAAAAGAACTGCAAAGAGACGGACTCCTGTTCACCTAGG TGAGGTGTGTGCAGCAGTTGGTGTCTGAGTCCACATGTGTGCAGTTGTCTTC TGCCAGCCATGGATTCCAGGCTATATATTTCTTTTTAATGGGCCACCTCCCC ACAACAGAATTCTGCCCAACACAGGAGATTTCTATAGTTATTGTTTTCTGTC ATTTGCCTACTGGGGAAGAAAGTGAAGGAGGGGAAACTGTTTAATATCACA TGAAGACCCAGCTTTAAGAGAAGCTGTATCCTCTAACCACGAGACCCTCA ACCAGCCCAACATCTTCCATGGACACATGACATTGAAGACCATCCCAAGCT ATCGCCACCCTTGGAGATGATGTCTTATTTATTAGATGGATAATGGTTTTATT TTTAATCTCTTAAGTCAATGTAAAAAGTATAAAACCCCTTCAGACTTCTA
SEQ ID NO.: 3 TGF-ALPHA splice variant 3 nucleic acid sequence	GCCCGCCTGGGAGAGTGGAGAGAAGCGAGTAGTCTCAATGGGTTGGCGCCT GGGAGGCAAGGGGAGTCATTAAGAATGACAAAGAGGGGACTGTCCGGTCG AGCGCCGAGGCTTCCCCGGGAAAGTGGCTGCCTGGGCCGTGCGTGAGAACA CCTGGGAAGGGGAAGCGGAGTGTGAACGGGCTTGAACTTTAATCCGCCGCA GCTGTGTGTCTGCTCAGACACATGCCTACACCTACCTGCTTACCTGGCTGCC TCCAAGCCCGGCTCAGCCGGCGTCGCTGGCGTGTAGACTGGGCCGCCTCGG AACGGGGGCCGGCGTCTAACCCTCTGGCCGGGTCTTGCCTGCGCCCCTTTGG CACCCCCTGCCCACCTCTAAGCCCCAGCGACGAGCAGAGATGTTCAAGATC GGCAGAGGCGCCCTCGATCTCTTTTCAGAGCTCTTGAGCTTTGGGGGTATTG TGTTGGCTGCGTGCCAGGCCTTGGAGAACAGCACGTCCCCGCTGAGTGCAG

	ACCCGCCCGTGGCTGCAGCAGTGGTGTCCCATTTTAATGACTGCCCAGAT **TCCCACACTCAGTTCTGCTTCCATGGAACCTGCAGGTTTTTGGTGCAGG** **AGGACAAGCCAGCATGTGTCTGCCATTCTGGGTACGTTGGTGCACGCT** **GTGAGCATGCGGACCTCCTGGCCGTGGTGGCTGCCAGCCAGAAGAAGCA** GGCCATCACCGCCTTGGTGGTGGTCTCCATCGTGGCCCTGGCTGTCCTTATC ATCACATGTGTGCTGATACACTGCTGCCAGGTCCGAAAACACTGTGAGTGGT GCCGGGCCCTCATCTGCCGGCACGAGAAGCCCAGCGCCCTCCTGAAGGGAA GAACCGCTTGCTGCCACTCAGAAACAGTGGTCTGAAGAGCCCAGAGGAGGA GTTTGGCCAGGTGGACTGTGGCAGATCAATAAAGAAAGGCTTCTTCAGGAC AGCACTGCCAGAGATGCCTG
SEQ ID NO.: 4 TGF-ALPHA splice variant 4 nucleic acid sequence	AACACCTGGGAAGGGGAAGCGGAGTGTGAACGGGCTTGAACTTTAATCCGC CGCAGCTGTGTGTCTGCTCAGACACATGCCTACACCTACCTGCTTACCTGGC TGCCTCCAAGCCCGGCTCAGCCGGCGTCGCTGGCGTGTAGACTGGGCCGCCT CGGAACGGGGGCCGGCGTCTAACCCTCTGGCCGGGTCTTGCCTGCGCCCCTT TGGCACCCCCTGCCCACCTCTAAGCCCCAGCGACGAGCAGAGATGTTCAAG ATCGGCAGAGGCGCCCTCGATCTCTTTTCAGAGCTCTTGAGCTTTGGGGGTA TTGTGTTGGCTGCGTGCCAGGCCTTGGAGAACAGCACGTCCCCGCTGAGTGA **CCCGCCCGTGGCTGCAGCAGTGGTGTCCCATTTTAATGACTGCCCAGATT** **CCCACACTCAGTTCTGCTTCCATGGAACCTGCAGGTTTTTGGTGCAGGA** **GGACAAGCCAGCATGTGTCTGCCATTCTGGGTACGTTGGTGCACGCTG** **TGAGCATGCGGACCTCCTGGCCGTGGTGGCTGCCAGCCAGAAGAAGCAG** GCCATCACCGCCTTGGTGGTGGTCTCCATCGTGGCCCTGGCTGTCCTTATCA TCACATGTGTGCTGATACACTGCTGCCAGGTCCGAAAACACTGTGAGTGGTG CCGGGCCCTCATCTGCCGGCACGAGAAGCCCAGCGCCCTCCTGAAGGGAAG AACCGCTTGCTGCCACTCAGAAACAGTGGTCTGAAGAGCCCAGAGGAGGAG TTTGGCCAGGTGGACTGTGGCAGATCAATAAAGAAAGGCTTCTTCAGGACA GCACTGCCAGAGATGCCTGGGTGTGCCACAGACCTTCCTACTTGGCCTGTAA TCACCTGTGCAGCCTTTTGTGGGCCTTCAAAACTCTGTCAAGAACTCCGTCT GCTTGGGGTTATTCAGTGTGACCTAGAGAAG
SEQ ID NO.: 5 TGF-ALPHA splice variant 1 amino acid sequence	MVPSAGQLALFALGIVLAACQALENSTSPLSADPPVAAA**VVSHFNDCPDSHTQ** **FCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLA**VVAASQKKQAITAL VVVSIVALAVLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACCHSET VV
SEQ ID NO.: 6 TGF-ALPHA splice variant 2 amino acid sequence	MVPSAGQLALFALGIVLAACQALENSTSPLSDPPVAAA**VVSHFNDCPDSHTQF** **CFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLA**VVAASQKKQAITALV VVSIVALAVLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACCHSETV V
SEQ ID NO.: 7 TGF-ALPHA splice variant 3 amino acid sequence	MFKIGRGALDLFSELLSFGGIVLAACQALENSTSPLSADPPVAAA**VVSHFNDCP** **DSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLA**VVAASQKKQ AITALVVVSIVALAVLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTAC CHSETVV
SEQ ID NO.: 8 TGF-ALPHA splice variant 4 amino acid sequence	MFKIGRGALDLFSELLSFGGIVLAACQALENSTSPLSDPPVAAA**VVSHFNDCPD** **SHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLA**VVAASQKKQA ITALVVVSIVALAVLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACC HSETVV
SEQ ID NO.:9 TGF-ALPHA Mature nucleic acid sequence	**GTGTCCCATTTTAATGACTGCCCAGATTCCCACACTCAGTTCTGCTTCC** **ATGGAACCTGCAGGTTTTTGGTGCAGGAGGACAAGCCAGCATGTGTCT** **GCCATTCTGGGTACGTTGGTGCACGCTGTGAGCATGCGGACCTCCTGG** **CCGTG**
SEQ ID NO:10 TGF-ALPHA Mature amino acid sequence	**VVSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLA**

[0134] Although the invention has been described with reference to the above examples, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1.   A composition for administration to a subject to produce an immune response against TGF-alpha comprising a TGF-alpha polypeptide or a biologically active fragment thereof.

2.   The composition of claim 1, further comprising a checkpoint inhibitor.

3.   The composition of claim 1, wherein the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4.

4.   A composition for administration to a subject to produce an immune response against TGF-alpha comprising a nucleic acid molecule encoding a TGF-alpha polypeptide or a biologically active fragment thereof.

5.   The composition of claim 4, wherein the nucleic acid is DNA or RNA.

6.   The composition of claims 1 or 4, further comprising an adjuvant.

7.   An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a nucleic acid sequence encoding a TGF-alpha protein or biologically active fragment thereof, a transmembrane domain and an intracellular signaling domain.

8.   The nucleic acid molecule of claim 7, wherein the transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the TGF-alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

9.   The nucleic acid molecule of claim 7, wherein the nucleic acid sequence encoding the TGF-alpha polypeptide or biologically active fragment is connected to the transmembrane domain by a linker or hinge region.

10.   A chimeric antigen receptor (CAR) polypeptide, wherein the CAR comprises a TGF-alpha protein or biologically active fragment thereof, a transmembrane domain and an intracellular signaling domain.

11.   The polypeptide of claim 10, wherein the transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the TGF-alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

12.   The polypeptide of claim 10, wherein a nucleic acid encoding the TGF-alpha polypeptide or biologically active fragment is connected to the transmembrane domain by a linker or hinge region.

13.   A method of providing anti-tumor immunity in a subject, comprising administering to the subject an immune cell comprising the nucleic acid molecule of any of claims 7-9 or the polypeptide of any of claims 10-12.

14.   A pharmaceutical composition comprising a TGF-alpha polypeptide or biologically active fragment thereof and a pharmaceutically acceptable carrier.

15.   The composition of claim 14, further comprising a checkpoint inhibitor.

16.   The composition of claim 15, wherein the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4.

17.   A method of treating a TGF-alpha related disease or disorder thereof in a subject comprising administering to the subject a therapeutically effective amount of a TGF alpha polypeptide or a biologically active fragment thereof.

18.   The method of claim 17, wherein the TGF-alpha related disease or disorder is cancer or an immune disorder.

19.   The method of claim 18, wherein the cancer is selected from the group consisting of lung, stomach, prostate, colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital tract.

20.   The method of claim 19, wherein the cancer is colon, stomach, lung or head and neck cancer.

21.   The method of claim 18, wherein the immune disorder is selected from the group consisting of Rheumatoid arthritis, Systemic lupus erythematosus, Celiac disease, Crohn's disease, Inflammatory bowel disease, Sjogren's syndrome, Polymyalgia rheumatic, Psoriasis, Multiple sclerosis, Ankylosing spondylitis, Type 1 diabetes, Alopecia areata, Vasculitis, Temporal arteritis, Graves' disease and Hashimoto's thyroiditis.

22.   The method of claim 17, wherein the TGF-alpha polypeptide or biologically active fragment thereof has at least 75% identity to the amino acid sequence of any of SEQ ID NOs: 5-8 or 10.

23.   The method of claim 17, wherein the TGF-alpha polypeptide or biologically active fragment thereof is a fusion protein.

24.   The method of claim 17, wherein the TGF-alpha polypeptide or biologically active fragment thereof is genetically modified.

25.   The method of claim 17, wherein the TGF-alpha polypeptide or biologically active fragment thereof comprises a heterologous sequence.

26.   The method of claim 17, further comprising administering to the subject a chemotherapeutic agent, radiation and/or an immune modulator either prior to, simultaneously with or following treatment with the TGF-alpha polypeptide or biologically active fragment thereof.

27.   The method of claim 26, wherein the chemotherapeutic agent is selected from the group consisting of Actinomycin, Azacitidine, Azathioprine, Bleomycin, Bortezomib, Carboplatin, Capecitabine, Cisplatin, Chlorambucil, Cyclophosphamide, Cytarabine, Daunorubicin, Docetaxel, Doxifluridine, Doxorubicin, Epirubicin, Epothilone, Etoposide, Fluorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Imatinib, Irinotecan, Mechlorethamine, Mercaptopurine, Methotrexate, Mitoxantrone, Oxaliplatin, Paclitaxel, Pemetrexed, Teniposide, Tioguanine, Topotecan, Valrubicin, Vinblastine, Vincristine, Vindesine, Vinorelbine, panitumamab, Erbitux, matuzumab, IMC-IIF 8, TheraCIM hR3, denosumab, Avastin, Humira, Herceptin, Remicade, rituximab, Synagis, Mylotarg, Raptiva, Tysabri, Zenapax, NeutroSpec, tocilizumab, ProstaScint, Bexxar, Zevalin, Xolair, MabThera, ReoPro, MabCampath, Simulect, LeukoScan, CEA-Scan (, Verluma, Panorex, alemtuzumab, CDP 870, natalizumab, MGA271, lirilumab, BMS-986016, pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and iplimumab.

28.   The method of claim 27, wherein the chemotherapeutic agent is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and/or iplimumab.

29.   The method of claim 26, wherein the immune modulator is selected from the group consisting of tocilizumab, CDP870, enteracept, adalimumab, Kineret, abatacept, infliximab, rituzimab, golimumab, Avonex, Rebif, ReciGen, Plegridy, Betaseron, Copaxone, Novatrone, natalizumab, fingolimod, teriflunomide, BG12, Tecfidera, and alemtuzumab.

30.   The method of claim 17, further comprising the administration of a checkpoint inhibitor.

31.   The method of claim 30, wherein the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4.

32.   The method of claim 18, wherein the cancer is a tumor.

33.   The method of claim 32, wherein the tumor is resected prior to treatment.

34.   The method of claim 17, wherein the subject is a human.

35.   The method of claim 17, wherein the administration of the TGF-alpha polypeptide or biologically active fragment thereof induces the *in vivo* production of anti-TGF-alpha antibodies.

36.   The method of claim 17, wherein the TGF-alpha polypeptide or biologically active fragment thereof is administered by intravenous, oral, intramuscular, subcutaneous, intrathecal, infusion, transdermal, sublingual, buccal, rectal, vagina, ocular, optic, nasal, inhalation, nebulization, cutaneous, intraperitoneal or intratumoral administration.

37.   A method of treating a TGF-alpha related disease or disorder in a subject comprising inducing the *in vivo* production of anti-TGF-alpha antibodies in the subject.

38.   The method of claim 37, comprising the administration of a TGF-alpha polypeptide or a biologically active fragment thereof.

39.   The method of claim 38, wherein the TGF-alpha polypeptide has at least 75% sequence identity to the amino acid sequence of any of SEQ ID NOs:5-8 or 10.

40.   The method of claim 38, wherein the TGF-alpha polypeptide or a biologically active fragment thereof is a fusion protein.

41.   The method of claim 38, wherein the TGF-alpha polypeptide or a biologically active fragment thereof is genetically modified or comprises a heterologous sequence.

42.   The method of claim 37, comprising the administration of a TGF-alpha vaccine.

43.   The method of claim 42, wherein the vaccine is a DNA, RNA or peptide vaccine.

44.   The method of claim 37, comprising administering a cell transformed with a nucleic acid molecule encoding a TGF-alpha polypeptide or a biologically active fragment thereof.

45.   The method of claim 44, wherein the cell is transformed with a nucleic acid sequence having at least 75% sequence identity to of any of SEQ ID NOs: 1-4 or 9.

46.   The method of claim 37, wherein the TGF-alpha related disease or disorder is cancer or an immune disorder.

47.   The method claim 37 further comprising administering to the subject a chemotherapeutic agent, radiation or an immune modulator either prior to, simultaneously with or following treatment with the TGF-alpha polypeptide or biologically active fragment thereof, TGF-alpha vaccine or transformed cell.

48.   The method of claim 47, wherein the chemotherapeutic agent is pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and/or iplimumab.

49.   The method of claim 47, wherein the immune modulator is selected from the group consisting of tocilizumab, CDP870, enteracept, adalimumab, Kineret, abatacept, infliximab, rituzimab, golimumab, Avonex, Rebif, ReciGen, Plegridy, Betaseron, Copaxone, Novatrone, natalizumab, fingolimod, teriflunomide, BG12, Tecfidera, and alemtuzumab.

50.   The method of claim 37, further comprising the administration of a checkpoint inhibitor.

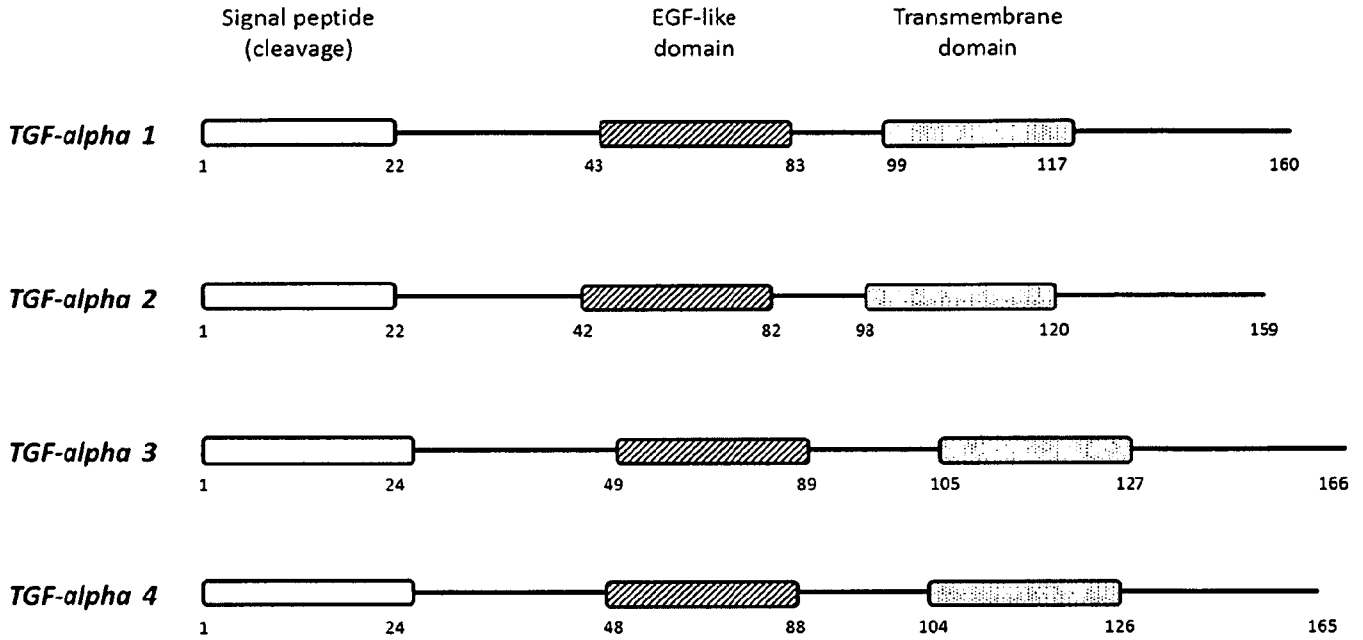51.   The method of claim 50, wherein the checkpoint inhibitor inhibits PD-1, PD-L1, PD-L2 or CTLA-4.

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FIGURE 1

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TGF-alpha 1   ------------------------------------MVPSAGQLALFALGIVLAACQALENSTSPLSADPPVAAAV
TGF-alpha 2   ------------------------------------MVPSAGQLALFALGIVLAACQALENSTSPLS-DPPVAAAV
TGF-alpha 3   MFKIGRGALDLFSELLSFG-----------------------GIVLAACQALENSTSPLSADPPVAAAV
TGF-alpha 4   MFKIGRGALDLFSELLSFG-----------------------GIVLAACQALENSTSPLS-DPPVAAAV

TGF-alpha 1   VSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLAVVAASQKKQAITALVVVSIVALA
TGF-alpha 2   VSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLAVVAASQKKQAITALVVVSIVALA
TGF-alpha 3   VSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLAVVAASQKKQAITALVVVSIVALA
TGF-alpha 4   VSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLAVVAASQKKQAITALVVVSIVALA

TGF-alpha 1   VLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACCHSETVV
TGF-alpha 2   VLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACCHSETVV
TGF-alpha 3   VLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACCHSETVV
TGF-alpha 4   VLIITCVLIHCCQVRKHCEWCRALICRHEKPSALLKGRTACCHSETVV
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FIGURE 2