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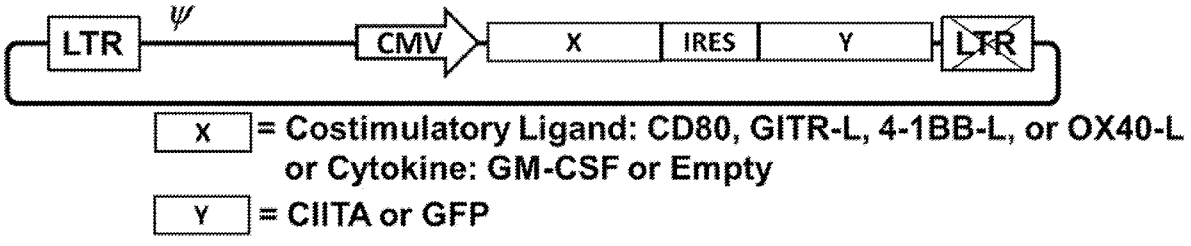

Figure 1

(57) Abstract: Provided are modified cancer cells that are modified to co-express class II trans-activator (CIITA), and an immuno-stimulatory molecule. The immuno-stimulatory molecule is OX-40-ligand or 4-lBB-Ligand. Methods of making the cells are provided by introducing polynucleotides encoding the CIITA and the immune-stimulatory molecule into cancer cells. Methods of stimulating humoral and cell-mediated immune responses by administering the modified cancer cells, or polynucleotides encoding the CIITA and immune-stimulatory molecules are also provided. These approaches can be used to stimulate an immune response against any of a wide variety of cancer antigens.

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MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II-EXPRESSING CANCER CELL VACCINE AND METHODS OF USE FOR PRODUCING INTEGRATED IMMUNE RESPONSES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application no. 62/701,791, filed July 22, 2018, the disclosure of which is incorporated herein by reference.

FIELD

[0002] The present disclosure relates generally to prophylaxis and therapy of cancer, and more specifically to compositions and methods for improving immune responses to cancer.

BACKGROUND

[0003] Tumor antigen-specific CD4+ T cells, CD8+ T cells and B cells play cooperative roles in antitumor immunity. At the tumor site, CD8+ T cells, also known as cytotoxic T cells, are considered to be the main effector cells to destroy cancer cells. CD4+ T cells, also known as helper T cells, help the activation, function and maintenance of CD8+ T cells through activation of antigen-presenting cells and/or secreting cytokines. CD4+ T cells also help activation of B cells to induce antibody secretion by expressing CD40-ligand (CD40L) which binds to CD40 molecule on B cells, and secreting cytokines that induce antibody class-switching. B cells produce tumor antigen-specific antibodies that bind to tumor antigen proteins to form antigen-antibody complex, sometimes referred to as an "immune complex". Immune complexes are efficiently captured by antigen-presenting cells and at the same time activate antigen-presenting cells (APCs) through binding to Fc receptors. Subsequently, activated antigen-presenting cells cross-present tumor antigen proteins to CD4+ and CD8+ T cells. Because of the distinct and collaborative antitumor functions by CD4+ T cells, CD8+ T cells and B cells, a strategy which would establish integrated CD4+ T cells, CD8+ T cells and antibody-secreting B cells would be a promising immunotherapy for cancer patients.

[0004] T cells destroy cancer cells by recognizing tumor antigen protein-derived peptides presented on MHC molecules on cancer cells. However, it is known that some cancer cells escape from T cell-mediated killing by eliminating MHC molecules from their surface. Antibodies that bind on cell surface of cancer cells destroy cancer cells through

antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) irrespective of MHC expression (or in a MHC-independent manner).

[0005] CD4+ helper T cells are considered to play a central role in inducing integrated antitumor immune response, because they help both CD8+ T cells and B cells. Generally, activation of CD4+ T cells requires antigen-presenting cells that capture and cross-present extracellular proteins such as tumor antigen proteins. Recently, we have discovered a unique CD4+ T-cell subset which directly recognizes MHC class II (MHC-II)-expressing cancer cells. This CD4+ T-cell subset, which we named "tumor-recognizing CD4+ T cells (TR-CD4 cells)", enhanced function of tumor antigen-specific CD8+ T cells by directly recognizing cancer cells without the need for antigen-presenting cells. Therefore, TR-CD4 cells are expected to efficiently provide help to other immune cells to enhance antitumor immunity at the tumor site. However, there is no presently known method to efficiently induce TR-CD4 cells in the body. Thus, there is an oncoming and unmet need for compositions and methods to improve immune responses to cancer, and other immunogenic agents. The present disclosure is related to these needs.

BRIEF SUMMARY

[0006] The present disclosure provides compositions and methods that are useful for stimulating and/or enhancing immune responses, including but not necessarily limited to immune responses to peptide antigens. In embodiments, cell-mediated immunity, humoral immunity, or both are stimulated and/or enhanced by using the compositions and methods of this disclosure.

[0007] The disclosure in certain aspects comprises compositions for use in vaccination. In embodiments, the disclosure provides cellular vaccine compositions comprising modified cancer cells that are engineered to overexpress class II trans-activator (CIITA) gene, and an immuno-stimulatory molecule. The immuno-stimulatory molecules described in this disclosure include GM-CSF, CD80, GITR-Ligand, OX-40-ligand, and 4-1BB-Ligand. In one embodiment, CD86 may be used. In embodiments, modified cancer cells express 4-BB-ligand and/or OX40-ligand, as described further below. In alternative embodiments, the disclosure includes using polynucleotides that encode the CIITA protein, and the immune-stimulatory agents, such as in expression vectors, as the agents that are delivered to an individual. In embodiments, as an alternative to the CIITA gene, the disclosure includes engineering cancer cells to increase expression of MHC II alpha and beta chains.

[0008] Using relevant mouse models, vaccines described herein are demonstrated to induce potent and long-lasting antitumor CD8+ T cells, compared to cancer cells expressing CIITA or the co-stimulatory ligand alone. Further, cellular vaccines described herein induce production of cytotoxic antibodies against cell surface molecules on cancer cells. Therefore, the vaccines described herein are expected to provide protective immunity against MHC-expressing cancers by T cell-mediated cytotoxicity, but also MHC-loss immune escape variants, by antibody-mediated cytotoxicity.

[0009] It will be recognized by those skilled in the art that the term MHC as used herein is extendable to human applications via the MHC human equivalent, referred to in the art as leukocyte antigen gene complex (HLA).

[0010] As will be recognized by the non-limiting examples presented with this disclosure, in order to induce TR-CD4 cells, we expressed MHC-II on cell surface of murine cancer cell lines by retrovirally overexpressing MHC class II transactivator (CIITA) gene, which is a master regulator of MHC class II-mediated antigen presentation. To enhance immunogenicity of MHC-II-expressing cancer cells, an immuno-stimulatory gene was also co-overexpressed. In contrast to the parental cancer cells or cells that expressing CIITA-alone, some engineered cancer cell lines co-expressing CIITA and an immuno-stimulatory gene, particularly 4-1BB-ligand (BB-L), induced strong and long-lasting antitumor immune response in syngeneic mice. Cancer cells that co-express CIITA+BB-L, but which do not express BB-L alone, induced circulating antibodies that specifically bind on surface of cancer cells and kill cancer cells. Cancer-specific antibodies induced by CIITA+BB-L-expressing cancer cells protected mice against MHC-loss cancer cell growth. These findings show that engineered cancer cells that co-express CIITA+BB-L are suitable for use as vaccines to induce integrated T-cell and antibody response for protection against MHC-expressing and MHC-loss cancers.

BRIEF DESCRIPTION OF THE FIGURES

[0011] **Figure 1. Generation of murine cancer cell lines co-expressing CIITA and immuno-stimulatory genes.** CIITA and/or immunostimulatory gene (CD80, GM-CSF, GITR-Ligand, 4-1BB-Ligand, and OX40-Ligand) were cloned into a bi-cistronic retroviral transfer plasmid (pQCXIX, purchased from Clontech). Retroviral particles were produced by co-transfection of GP2-293 packaging cell line (Clontech) of the transfer plasmid and the pVSV-G envelope-expressing plasmid (Clontech). Murine cancer cell lines were engineered to express CIITA and/or an immuno-stimulatory gene by retroviral transduction.

[0012] Figure 2. Immunogenicity of engineered cancer cells. Effect of expression of CIITA and an immuno-stimulatory genes on growth of a murine lymphoma cell line, EL4, in syngeneic (C57BL/6) mice. Mice were subcutaneously injected with EL4 cells that were engineered to express indicated gene(s). Tumor volume was calculated from diameters as 0.5 × (shorter diameter)^2 × (longer diameter). Expression of CIITA alone did not alter tumor growth of EL4. Co-expression of CIITA and an immune stimulatory gene significantly delayed tumor growth. In particular 4-1BB-L and OX40-L induced spontaneous complete regression in all mice. Whereas expression of 4-1BB-L alone induced complete regression, OX-40L alone only partially delayed tumor growth.

[0013] Figure 3. Induction of memory CD8+ T-cell response by engineered cancer cells. (A) Experimental approach. To investigate long-term antitumor memory immune response, mice were first inoculated with EL4 engineered with 4-1BB-L alone, CIITA+4-1BB-L, or CIITA+OX40-L. Two months after complete regression, mice were subcutaneously re-challenged with the parental EL4 and tumor growth was monitored. (B) Growth of the parental EL4 after rechallenge. Only some mice that rejected EL4 expressing 4-1BB-L alone or CIITA+OX40-L showed protection upon rechallenge. In contrast, all mice that initially received EL4-expressing CIITA+4-1BB-L rejected rechallenged parental EL4. (C) To investigate memory CD8+ T-cell responses, mice were first inoculated with the indicated engineered EL4. Immediately and one month after complete regression, EL4-specific CD8+ T cells in the spleen were investigated by coculture with the parental EL4 and measure cytokine production by intracellular cytokine staining assay. (D) Immediately after tumor regression (Day 20), mice that received EL4 expressing 4-1BB-L alone and CIITA+4-1BB-L showed similar EL4-specific CD8+ T cells. Mice that received CIITA+OX40-L showed decreased EL4-specific CD8+ T cells. One month after (Day 50), whereas mice that received EL4 expressing 4-1BB-L alone and CIITA+OX40-L showed decrease in EL4-specific CD8+ T cells compared to those at Day 20, percentage of EL4-specific CD8+ T cells in mice received EL4 expressing CIITA+4-1BB-L was maintained.

[0014] Figure 4. Induction of antibody response by engineered cancer cells. (A) Experimental schema. To investigate protective antibody response, mice were first inoculated with EL4 engineered with 4-1BB-L alone, CIITA+4-1BB-L, or CIITA+OX40-L. Two months after complete regression, mice were subcutaneously re-challenged with EL4 engineered to silence MHC class I expression by disrupting b2m gene by CRISPR/Cas9 technology (b2m-/- EL4) and tumor growth was monitored. (B) Growth of MHC-loss EL4 (b2m-/- EL4) after rechallenge. Mice that initially rejected EL4-expressing 4-1BB-L alone or

CIITA+OX40-L showed no or partial protection, respectively, against MHC-loss EL4. In contrast, all mice that initially received EL4-expressing CIITA+4-1BB-L rejected rechallenged MHC-loss EL4. (C) To investigate induction of antibodies against cell surface molecules on cancer cells, sera were collected from mice after they rejected engineered EL4 expressing 4-1BB-L alone, CIITA+4-1BB-L, or CIITA+OX40-L. The parental EL4 were first incubated with diluted serum and were stained with fluorescently labelled anti-mouse IgG antibody. Fluorescent intensity measured by flow cytometry is shown. (D) Fluorescent intensity was compared between treatment groups. Mice that rejected EL4 expressing CIITA+4-1BB-L or to the lesser extent EL4 expressing CIITA+OX40-L developed serum antibodies that bound on EL4. (E) The same sera from CIITA+4-1BB-L expressing EL4 rejected mice in (C) was used to stain irrelevant control cells such as activated murine T cells, B16F10 murine melanoma cell line and MC38 murine colon cancer cell line, indicating no cross-reactivity other than EL4. (F) Cytotoxicity by antibodies induced by engineered cancer cells. The parental EL4 were first loaded with fluorescent Calcein AM reagent, incubated with diluted serum, and were incubated with the rabbit complement. Cytotoxicity was calculated from fluorescent level in the supernatant.

[0015] Figure 5. Effect of therapeutic vaccination on tumor growth. (A) Experimental schema. Mice were first subcutaneously inoculated with EL4-expressing CIITA or MHC-loss EL4. On days 3, 10, and 17 mice were vaccinated with irradiated CIITA-EL4 or CIITA+4-1BB-L-EL4, or untreated. (B) Growth of CIITA-expressing EL4. There is no significant effect by vaccination with CIITA-EL4, tumor growth was significantly inhibited by CIITA+4-1BB-L-EL4. Two out of 5 mice completely rejected tumors. (C) Mice were first subcutaneously inoculated with MHC-loss EL4. On days 3, 10, and 17 mice were vaccinated with irradiated CIITA+4-1BB-L-EL4, or untreated. Mice that were vaccinated with CIITA+4-1BB-L-EL4 showed delayed tumor growth and 2 out of 7 mice completely rejected tumors. (D) Survival of mice in (C).

[0016] Figure 6. Confirmation in other murine tumor models. (A) Mice were subcutaneously inoculated with MC38 colon cancer and B16F10 melanoma cell lines that were engineered to express the indicated genes. In both murine tumor models, co-expression of CIITA and 4-1BB-L induced spontaneous rejection. (B) Serum from mice in (A) were used to stain the parental MC38 and B16F10. Only mice that rejected engineered cancer cells expressing CIITA+4-1BB-L induced significant antibodies that bound on cell surface of cancer cells. (C) Induction of ovarian tumor-reactive antibody response by vaccination. Naïve mice were vaccinated with engineered murine ovarian cancer cell line, ID8, expressing

CIITA+4-1BB-L or CIITA+OX40-L on days 0 and 7. Nineteen days after the second vaccination, sera were collected and used to stain the parental ID8 cell line. Both mice that were vaccinated with CIITA+4-1BB-L-ID8 induced ID8-reactive antibodies, whereas half of mice that received CIITA+OX40-L-ID8 induced significant ID8-reactive antibodies.

DETAILED DESCRIPTION

[0017] Unless defined otherwise herein, all technical and scientific terms used in this disclosure have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains.

[0018] Every numerical range given throughout this specification includes its upper and lower values, as well as every narrower numerical range that falls within it, as if such narrower numerical ranges were all expressly written herein.

[0019] The disclosure includes all steps and compositions of matter described herein in the text and figures of this disclosure, including all such steps individually and in all combinations thereof, and includes all compositions of matter including but not necessarily limited to vectors, cloning intermediates, cells, cell cultures, progeny of the cells, and the like.

[0020] The disclosure includes but is not limited to engineered immunogenic cancer cells described herein, cancer vaccines made using the immunogenic cancer cells, methods of making the immunogenic cancer cells, immunogenic compositions, polynucleotides, and methods for the treatment of cancer. The disclosure includes all polynucleotides disclosed herein, their complementary sequences, and reverse complementary sequences. For any reference to a polynucleotide or amino acid sequence by way of a database entry, the polynucleotide and amino acid sequence presented in the database entry is incorporated herein as it exists on the effective filing date of this application or patent.

[0021] As discussed above, cancer cells express an array of immunogenic antigens that are recognized by T cells and B cells. Therefore, the present disclosure utilizes modified cancer cells as potent vaccines to induce polyvalent immune response.

[0022] In embodiments, the disclosure comprises modifying cancer cells as described herein, and comprises the modified cancer cells themselves, and compositions, such as pharmaceutical compositions, comprising the cancer cells. In embodiments, the cancer cells are of any cancer type, including solid and liquid tumors. In embodiments, cancer cells modified according to this disclosure include but are not necessarily limited to breast cancer, prostate cancer, pancreatic cancer, lung cancer, liver cancer, ovarian cancer, cervical cancer,

colon cancer, esophageal cancer, stomach cancer, bladder cancer, brain cancer, testicular cancer, head and neck cancer, melanoma, skin cancer, any sarcoma, including but not limited to fibrosarcoma, angiosarcoma, adenocarcinoma, and rhabdomyosarcoma, and any blood cancer, including all types of leukemia, lymphoma, or myeloma.

[0023] In embodiments, a cellular vaccine composition described herein is administered to an individual who has cancer, or previously had cancer, or is at risk for developing cancer. The cancer can be any of the aforementioned types. In embodiments, modified cancer cells for use as vaccines of this disclosure comprise cancer cells from a cancer cell line. In embodiments, modified cancer cells for use as vaccines of this disclosure comprise cancer cells from an individual, and are modified such that they express or overexpress CIITA and one or more co-stimulatory molecules or immuno-stimulatory cytokines, as described herein, and are provided to the same individual as a cancer therapy. In embodiments, allogenic cancer cells are modified and used in the methods described herein. In embodiments, the modified cancer cells are the same cancer type as a cancer against which a therapeutic immune response is generated in an individual.

[0024] In embodiments, the individual may be vaccinated with one or more antigens that are expressed by the modified cancer cells (or the cancer cells that are targeted using polynucleotides, as described herein). In embodiments, a tumor or cancer cell lysate may be used as the vaccination. In embodiments, immunological protection elicited by methods of the present disclosure (with or without subsequent vaccination) can be durable, and last for days, weeks or months, or longer, including but not limited to after vaccination, and such vaccinations can be effective to elicit protection after a single dose, or multiple doses. Booster vaccinations can be used according to schedules that are known in the art and can be adapted for use with methods of this disclosure when provided the benefit of this specification, and include such approaches as a Prime-Boost strategy.

[0025] With respect to immune responses that are stimulated and/or enhanced as described herein, for induction of TR-CD4 cells by cancer cell-based vaccines, cancer cells need to express MHC-II (or HLA, in the case of humans). However, not all cancer cells constitutively express cell surface MHC-II. For instance, none of murine cancer cell lines, including but not necessarily limited to EL4 lymphoma, B16F10 melanoma, MC38 colon cancer, and ID8 ovarian cancers, express constitutive MHC-II.

[0026] In order to express MHC-II on cell surfaces of murine cancer cell lines, we retrovirally overexpressed MHC class II transactivator (CIITA) gene, which is a master regulator of MHC class II-mediated antigen presentation. Thus, in embodiments, each cancer

cell modified for use as a vaccine as described herein will either be modified such that it expresses CIITA if it did not previously express it, or will be modified such that it expresses more CIITA, relative to the amount expressed prior to being modified according to this disclosure. Those skilled in the art will recognize that CIITA is also referred to as C2TA, NLRA, MHC2TA, and CIITAIV.

[0027] Instead of using the CIITA gene, overexpression of MHC class II alpha and beta chain genes are expected to induce cell surface MHC class II expression. Thus, in embodiments, engineering of cancer cells using recombinant molecular biology approaches, such as by direction introduction of MHC alpha and beta chain encoding polynucleotides, is considered to be an alternative approach to provide modified cancer cell vaccines that will function in a manner similar to cancer cells modified as otherwise described herein. In certain embodiments, the disclosure provides for increasing MHC or HLA expression by introducing polynucleotides directly, or to produce modified cancer cells, using polynucleotides that encode HLA class II alpha and beta chains. HLA class II alpha and beta chains for any particular individual can be determined using techniques that are well established in the art. In embodiments, preexisting cancer cells that are matched to an individual's HLA type can be used. Alternatively, any biological sample from an individual that comprises nucleated cells can be tested to determine the HLA type of the individual, and suitable polynucleotides encoding the pertinent HLA class II alpha and beta chains can be designed and produced, and used in embodiments of this disclosure. In embodiments, the HLA class II alpha chains are for HLA-DM, HLA-DMA, HLA-DO, HLA-DOA, HLA-DP, HLA-DPA1, HLA-DQ, HLA-DQA1, HLA-DQA2, HLA-DR or HLA-DRA, or any subtype of these HLA types. In embodiments, the HLA class II beta chains are for HLA-DMB, HLA-DOB, HLA-DPB1, HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB3, HLA-DRB4, or HLA-DRB5, or any subtype of these HLA types.

[0028] Representative and non-limiting examples of murine and human amino acid sequences of CIITA, and co-expressed proteins, as well as DNA sequences encoding them, are provided below. The disclosure includes using nucleotide and amino acid sequences that are different from those provided here, so long as the modified cancer cells function to enhance immune responses relative to unmodified cancer cells. In embodiments, the cancer cells express CIITA and co-stimulatory molecules or immuno-stimulatory cytokines described herein that are identical to the amino acid sequences described below, or have at from 70-99% amino acid identity with the pertinent sequences. The disclosure includes using proteins with amino acid insertions, deletions, and substitutions, provided they retain their

intended function. All polynucleotide sequences encoding the proteins described herein are encompassed by this disclosure, and are not to be limited by those presented below.

[0029] Examples of this disclosure combine engineered expression or overexpression of CIITA with one or a combination of G-CSF, CD80, GITR-Ligand, OX-40-ligand, and 4-1BB-Ligand. However, it is demonstrated that co-expression of CIITA with 4-1BB-L is superior to the other co-expressed proteins. Thus, in embodiments, the disclosure provides compositions and methods for use as cancer vaccines that comprise modified cancer cells that are engineered by recombinant molecular biology approaches to express CIITA and an immuno-stimulatory that is preferably 4-1BB-L, although the other immuno-stimulatory factors are included within the scope of this disclosure.

[0030] In embodiments, use of a cellular cancer vaccine described herein comprises a cancer therapy. In embodiments, use of a cellular cancer vaccine described herein produces a durable memory response, including but not necessarily limited to a durable CD8+ T cell memory response. In embodiments, a single administration of a cellular vaccine composition described herein produced an immune response that lasts at least from at least one month, to at least one year, or for at least one year, or will provide life-long protection, and thus for use in humans or non-human animals can last for decades. Thus, human and veterinary uses are included.

[0031] In embodiments, use of a cellular cancer vaccine or related polynucleotide as described herein produces any one or any combination of results, which can be compared to any suitable reference: improved activation of T cells, increase of TR-CD4+ T cells, improved CD8+ memory cell production and/or persistence, improved production of anti-cancer antibodies, improved inhibition of tumor growth, and improved survival time. In embodiments, a vaccination of this disclosure prevents formation of tumors, or limits growth of an existing tumor, or eradicates existing tumors. In embodiments, the reference is obtained by cancer cells that express a different immune-stimulatory molecule than the immune-stimulatory molecule that was a component of an improved immune response. In embodiments, the ability of a vaccine described herein to improve response to rechallenge with cancer cells is improved.

[0032] Vectors encoding the CIITA and or the co-stimulatory molecules can be any suitable vector or other polynucleotide. One or more vectors or polynucleotides can be used. In non-limiting embodiments, retroviral vectors may be used. Figure 1 provides a non-limiting embodiment of a suitable vector. In embodiments, a sequence encoding, or designed to encode CIITA once integrated, is used alone in a vector. In embodiments, a sequence

encoding, or designed to encode a co-stimulatory molecule once integrated is used alone in a vector. In embodiments, a single vector encodes or is designed to encode both the CIITA and co-stimulatory molecule. Thus, in embodiments, the disclosure comprises polycistronic vectors. In embodiments, the CIITA and the sequence encoding the co-stimulatory molecule are separated by, for example, and internal ribosome entry sequence (IRES).

[0033] In embodiments, the cancer cell vaccines, or polynucleotides encoding the proteins described herein, are used concurrently or sequentially with conventional chemotherapy, or radiotherapy, or another immunotherapy, or before or after a surgical intervention, such as a tumor resection. In embodiments, the cancer cell vaccines, or polynucleotides encoding the proteins that are recombinantly expressed by the cancer cell vaccines, are used in single, or multiple doses. In embodiments, the cancer vaccines are provided only once, or weekly, monthly, every 3 months, every 6 months, yearly, or in a pre-determined interval of years.

[0034] Cancer cell vaccines described herein can be administered to an individual in need thereof using any suitable route, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In embodiments, an amount of cancer cells administered comprises an effective dose. In embodiments, an effective dose comprises sufficient cells to produce one or more effects described herein, including any cell-mediated response, or humoral response, or a combination thereof, which is effective to inhibit growth of cancer, and/or generate an anti-cancer memory response. In embodiments, 10^4 to 10^9 modified cancer cells are introduced. In embodiments, a cancer cell composition of this disclosure for use as a vaccine comprises isolated cells modified as described herein, wherein all or some of the cancer cells are modified. In embodiments, the disclosure includes compositions comprising cells, wherein from 1-100% of the cells are modified cancer cells. In embodiments, the disclosure provides compositions comprising cancer cells, wherein 1-100% of the cancer cells are modified cancer cells. Those skilled in the art will recognize that retention of cancer cell morphology is a solution is pertinent to the modified cancer cell phenotype. In embodiments, modified cancer cells can be included in a pharmaceutical composition. Modified cancer cells and/or polynucleotides of the present disclosure can be provided in pharmaceutical compositions by combining them with any suitable pharmaceutically acceptable carriers, excipients and/or stabilizers. Examples of pharmaceutically acceptable carriers, excipients and stabilizer can be found in Remington:

The Science and Practice of Pharmacy (2005) 21st Edition, Philadelphia, PA. Lippincott Williams & Wilkins, the disclosure of which is incorporated herein by reference.

[0035] In embodiments, one or more recombinant polynucleotide described herein for use in making the cellular vaccine formulations, or another therapeutic polynucleotide, can be used as the agent that is delivered to the individual, and thus the polynucleotides themselves may comprise a therapeutic agent. In embodiments, a composition delivered to an individual according to this disclosure can be a cell-free composition. In embodiments, a combination of modified cancer cells, and polynucleotides that are not in cells, can be used.

[0036] In embodiments, if a therapeutic agent used in a method of this disclosure is a polynucleotide, it can be administered to the individual as a naked polynucleotide, in combination with a delivery reagent, or as a recombinant plasmid or viral vector which comprises and/or expresses the polynucleotide agent. In one embodiment, the proteins are encoded by a recombinant oncolytic virus, which can specifically target cancer cells, and which may be non-infective to non-cancer cells, and/or are eliminated from non-cancer cells if the oncolytic virus enters the non-cancer cells. Examples of recombinant oncolytic viruses that can be used with this disclosure include but are not limited to recombinant vaccinia virus (rOVV). In embodiments, one or more polynucleotides described herein can be delivered via a modified virus comprising a modified viral capsid or other protein that is targeted to, and thus will bind with specificity, to one or more ligands that are preferentially or exclusively expressed by cancer cells. In embodiments, separate polynucleotides encoding distinct proteins described herein can be used. In embodiments, one or more polynucleotides described herein can be injected directly into a tumor.

[0037] Polynucleotide therapeutic agents of this disclosure can be combined if desired with a delivery agent. Suitable delivery reagents for administration include but are not limited to Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; or polycations (e.g., polylysine), liposomes, or combinations thereof.

[0038] Therapy or inhibition of cancer as described herein may be combined with any other anti-cancer approach, such as surgical interventions and conventional chemotherapeutic agents. In embodiments, cancer treatment according to this disclosure can be combined with administration of one or more immune checkpoint inhibitors. In embodiments, the checkpoint inhibitors comprise an anti-programmed cell death protein 1 (anti-PD-1) checkpoint inhibitor, or an anti-Cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) checkpoint inhibitor. There are numerous such checkpoint inhibitors known in the art. For example, anti-PD-1

agents include Pembrolizumab and Nivolumab. An anti-PD-L1 example is Avelumab. An anti-CTLA-4 example is Ipilimumab.

[0039] In certain non-limiting demonstrations in the examples below, immunogenicity of engineered cancer cells is analyzed using syngeneic C57BL/6 mice in with modified lymphoma, colon cancer cells, melanoma and ovarian cancer cell lines, all of which demonstrate co-expression of CIITA and 4-1BB-L is an effective approach to stimulating potent anticancer responses. Thus, and without intending to be bound by any particular theory, it is expected that the approaches described herein, and particularly co-expression of CIITA with 4-1BB-L, will be broadly applicable to a wide variety of cancer types, and will function with the same or similar efficacy in humans, given that clinically relevant mouse models are used to demonstrate aspects of the disclosure.

[0040] Aspects of the disclosure are demonstrated by the following examples, which are intended to illustrate but not limit the disclosure.

EXAMPLES

[0041] Immunogenicity of the engineered cancer cells was investigated by introducing them into syngeneic C57BL/6 mice.

[0042] In an EL4 lymphoma model, overexpression of CIITA alone did not change tumor growth as compared to the parental EL4. In contrast, co-expression of CIITA and immuno-stimulatory molecules significantly delayed tumor growth. In particular, EL4 co-expressing OX40-L+CIITA or 4-1BB-L+CIITA was completely rejected. In this model, 3 groups that received EL4 overexpressing OX40-L+CIITA, 4-1BB-L+CIITA, and 4-1BB-L alone showed complete tumor elimination in all mice (Figure 2).

[0043] In order to evaluate induction of long-term memory T-cell response by the engineered cancer cells, mice that rejected EL4 overexpressing OX40-L+CIITA, 4-1BB-L+CIITA, or 4-1BB-L alone were rechallenged with the parental EL4 (Figure 3A). Only a fraction of mice that rejected EL4 overexpressing 4-1BB-L alone or OX40-L+CIITA were resistant to the rechallenge (Figure 3B). In contrast, all mice that rejected 4-1BB-L+CIITA rejected rechallenged EL4. 4-1BB-L-EL4 and 4-1BB-L+CIITA-EL4 induced comparable EL4-specific CD8+ T-cell response at early phase of immune response (Figure 3D LEFT). In contrast, CD8+ T cells induced by 4-1BB-L+CIITA were maintained at later time point, compared to significant decrease in 4-1BB-L alone group (Figure 3D RIGHT). Mice that rejected OX40-L+CIITA developed fewer EL4-specific CD8+ T cells at earlier time point and further decreased at later time point (Figure 3D LEFT and RIGHT).

[0044] In order to determine if the engineered cancer cells induce antitumor antibodies, mice that rejected EL4 overexpressing OX40-L+CIITA, 4-1BB-L+CIITA, and 4-1BB-L alone were rechallenged with EL4 that were engineered by CRISPR/Cas9 gene-editing to silence β2m gene and thus express no MHC molecule (MHC-loss EL4) (Figure 4A). As shown in Figure 4B, all mice that rejected 4-1BB+CIITA-expressing EL4 were resistant to rechallenge with MHC-loss EL4, whereas those rejected EL4 expressing 4-1BB-L alone or OX40-L+CIITA showed no or partial resistance, respectively (Figure 4B). The presence of circulating EL4-reactive antibodies was tested by incubating the parental EL4 in diluted serum and by detecting immunoglobulin (IgG) bound on EL4 by fluorescent anti-mouse IgG antibody. EL4-expressing 4-1BB-L+CIITA induced significantly higher EL4-binding IgG response than EL4 expressing 4-1BB-L alone. In contrast, OX40-L+CIITA-expressing EL4 induced weaker antibody response (Figure 4C and 4D). Antibodies induced by EL4-expressing 4-1BB-L+CIITA were specific to EL4 as evidenced by control activated murine T cells, B16F10 melanoma, and MC38 colon cancer which were not stained by the serum (Figure 4E). Antibodies induced by EL4-expressing 4-1BB-L+CIITA induced complement dependent cytotoxicity against EL4 (Figure 4F).

[0045] The therapeutic potential of engineered cancer cells was analyzed using a therapeutic vaccine model. In this model, CIITA overexpressing EL4 cells that express both MHC class I and MHC-II or MHC-loss EL4 were inoculated in C57BL/6 mice, and mice were vaccinated by irradiated engineered EL4 (Figure 5A). Therapeutic vaccination with 4-1BB-L+CIITA-expressing EL4 induced significant antitumor effect including complete elimination in 2/5 mice (Figure 5B). In addition, the same vaccination eliminated MHC-loss EL4 in 2/7 mice and prolonged survival of remaining mice (Figure 5C and 5D).

[0046] The effect of engineered cancer cells was tested in other tumor models. In both MC38 colon cancer and B16F10 melanoma models, 4-1BB-L+CIITA expressing cancer cells were spontaneously rejected in all mice (Figure 6A), which was associated with higher circulating antibodies specific against respective cancers (Figure 6B). Using murine ovarian cancer cell line, ID8, vaccination of mice with irradiated 4-1BB-L+CIITA-expressing ID8, and to a lesser extent OX40-L+CIITA-expressing ID8, induced antibodies that bound on the parental ID8 (Figure 6C).

[0047] The following representative murine sequences were used to demonstrate embodiments of this disclosure. Those skilled in the art will recognize, given the benefit of this disclosure that the human sequences provide below the murine sequences, can be adapted

for use in human cancer vaccines, and other therapeutic approaches based on the present disclosure.

[0048] **Mouse**

[0049] **In DNA sequences, bold codons indicate the Start or Stop codon.**

<CIITA>

>Mus musculus class II major histocompatibility complex transactivator (CIITA) (also known as "aka" C2ta; Gm9475; Mhc2ta; EG669998)

>DNA sequence (NCBI Reference Sequence: NM_001302618.1)

ATGAACCACTTCCAGGCCATCCTGGCCCAAGTACAGACACTGCTCTCCAGCCAG
AAGCCCAGGCAGGTGCGGGCCCTCCTGGATGGCCTGCTGGAAGAAGAGCTGCTC
TCACGGGAATACCACTGTGCCTTGCTGCATGAGCCTGATGGTGATGCCCTGGCCC
GGAAGATTTCCCTGACCCTGCTGGAGAAAGGGGACTTAGACTTGACTTTCTTGAG
CTGGGTCTGCAACAGTCTGCAGGCTCCCACGGTAGAGAGGGGCACCAGCTACAG
GGACCATGGAGACCATAGTCTGTGTGCCACCATGGATCTGGGATCTCCAGAGGG
CAGCTACCTGGAACTCCTTAACAGTGATGCCGACCCCCTACATCTCTACCACCTC
TATGACCAGATGGACCTGGCTGGGGAGGAGGAGATCGAACTCAGCTCAGAGCCA
GACACAGATACCATCAACTGCGACCAGTTCAGCAAGCTGTTGCAGGACATGGAA
CTGGATGAAGAGACCCGGGAGGCCTATGCCAACATTGCGGAACTGGATCAGTAC
GTGTTCCAGGATACCCAGCTCGAGGGCCTGAGCAAGGACCTCTTCATAGAGCAC
ATTGGAGCAGAGGAAGGCTTTGGTGAGAACATAGAGATCCCTGTAGAAGCAGGA
CAGAAGCCTCAGAAGAGACGCTTCCCGGAAGAGCATGCTATGGACTCAAAGCAC
AGGAAGCTAGTGCCCACCTCTAGGACCTCACTGAACTATTTGGATCTCCCCACTG
GGCACATCCAGATCTTCACCACTCTGCCCCAGGGACTCTGGCAAATCTCAGGGGC
TGGCACAGGTCTCTCCAGTGTCCTAATCTACCACGGTGAGATGCCCCAGGTCAAC
CAAGTGCTCCCTTCAAGCAGCCTCAGTATCCCCAGTCTCCCCGAGTCCCCAGACC
GGCCTGGCTCCACCAGCCCCTTCACACCATCTGCAGCTGACCTGCCCAGCATGCC
CGAACCTGCGCTGACCTCCCGTGTAAATGAGACAGAGGACACATCTCCCTCCCCA
TGCCAAGAGGGTCCCGAGTCTTCCATCAAGCTTCCAAAATGGCCAGAGGCTGTG
GAGCGATTCCAGCACTCCCTACAGGACAAATACAAGGCATTGCCCCAGAGCCCA
AGGGGTCCTCTGGTGGCCGTGGAGCTGGTACGGGCCAGGCTGGAAAGAGGCAGC
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AGCCCACGGTGGTCTGGCAGAGGTACTTCAGGTTGTCAGTGACTGCAGGCGACC
AGGAGAGACACAGGTGGTCGCTGTGCTGGGCAAGGCTGGCCAGGGAAAGAGCC
ACTGGGCCAGGACAGTGAGTCACACCTGGGCATGTGGCCAGTTGCTACAATATG
ACTTTGTCTTCTATGTCCCCTGTCATTGCTTGGATCGTCCCGGGGACACCTACCAC
CTGCGGGATCTGCTCTGTCCCCCGAGCCTGCAGCCACTGGCCATGGATGACGAGG
TCCTTGATTATATCGTGAGGCAGCCAGACCGTGTTCTGCTCATCCTAGATGCTTTC
GAGGAGCTAGAGGCCCAAGATGGCCTCCTGCACGGACCCTGTGGATCTCTGTCC
CCAGAGCCCTGCTCCCTCCGAGGACTGCTGGCTGGGATCTTCCAGCGGAAGCTAC
TGCGAGGCTGCACACTGCTCCTCACAGCCCGGCCCCGGGGCCGCCTGGCTCAGA
GCCTGAGCAAGGCAGATGCCATCTTTGAGGTGCCCAGCTTCTCTACCAAGCAGGC
CAAGACTTACATGAGGCACTACTTTGAGAACTCAGGGACAGCGGGGAACCAAGA
CAAGGCCCTGGGCCTCCTGGAGGGCCAGCCTCTTCTCTGCAGCTATAGTCACAGC
CCTGTTGTGTGCAGGGCTGTGTGCCAGCTCTCCAAGGCCCTGCTAGAACAGGGCA
CAGAGGCCCAGCTACCTTGTACACTTACAGGACTCTATGTCAGCCTGCTAGGTCC
TGCAGCTCAGAACAGTCCTCCCGGAGCCTTAGTCGAGCTGGCCAAGCTGGCCTG

GGAGCTGGGACGAAGACACCAAAGCACCTTGCAAGAAACCCGGTTTTCATCCGT
GGAGGTGAAAACCTGGGCAGTGACCCAAGGCTTGATGCAGCAGACCCTGGAGAC
CACGGAGGCTCAACTGGCCTTCTCCAGTTTTCTGCTACAGTGTTTCCTGGGTGCTG
TGTGGCTGGCACAGTGCAATGAAATCAAAGACAAGGAGCTGCCACAGTACCTGG
CCTTGACTCCGAGGAAGAAGAGACCCTATGACAACTGGCTGGAGGGTGTACCAC
GCTTTCTGGCTGGATTAGTTTTCCAGCCTCGAGCCCACTGCCTGGGAGCTCTGGT
GGAGCCTGCAGTGGCTGCAGTGGCGGATAGGAAACAGAAGGTTCTTACCAGGTA
CCTGAAGCGCCTGAAGCTGGGGACACTCCGGGCAGGGAGGCTGCTGGAGCTGCT
CCACTGTGCCCACGAGACACAGCAACCTGGGATATGGGAGCATGTTGCACACCA
GCTCCCTGGCCACCTCTCCTTCCTGGGCACCCGGCTCACACCCCCAGATGTGTAT
GTGCTGGGCAGGGCCTTGGAGACAGCCAGCCAGGACTTCTCCTTGGACCTTCGTC
AGACTGGCGTTGAGCCTTCTGGACTGGGAAACCTCGTGGGACTCAGCTGTGTCAC
CAGTTTCAGGGCCTCCTTGAGTGATACAATGGCATTATGGGAGTCCCTTCAGCAG
CAGGGAGAAGCCCAGCTACTCCAGGCGGCAGAGGAGAAGTTCACCATTGAGCCA
TTTAAAGCCAAATCCCCAAAGGATGTGGAAGACCTGGATCGTCTCGTGCAGACC
CAGAGGCTGAGAAACCCCTCAGAAGATGCAGCCAAGGATCTTCCTGCCATCCGG
GACCTTAAGAAGCTAGAGTTTGCGTTGGGCCCCATCTTGGGCCCCCAGGCTTTCC
CCACACTGGCAAAGATCCTTCCAGCCTTCTCTTCTCTGCAACACCTGGACCTGGA
CTCACTTAGTGAGAACAAGATCGGAGACAAGGGTGTGTCGAAGCTCTCAGCCAC
CTTCCCTCAGCTGAAGGCCCTGGAGACGCTCAACTTGTCCCAAAACAACATCACT
GATGTGGGTGCCTGCAAGCTTGCAGAAGCTCTGCCAGCCCTAGCCAAGTCCCTCC
TAAGGCTGAGCTTGTACAATAACTGCATCTGTGACAAAGGAGCCAAGAGCCTGG
CACAAGTACTTCCGGACATGGTGTCCCTGCGTGTGATGGATGTCCAGTTCAACAA
GTTCACGGCTGCCGGTGCCCAGCAACTGGCCTCCAGCCTTCAGAAGTGCCCTCAG
GTGGAAACACTGGCAATGTGGACACCCACTATCCCCTTTGGGGTTCAGGAACACC
TGCAGCAGCTGGATGCCAGGATCAGTCTGAGA**TGA** (SEQ ID NO:1)

CIITA Protein sequence (NCBI Reference Sequence: NP_001289547.1)

MNHFQAILAQVQTLLSSQKPRQVRALLDGLLEEELLSREYHCALLHEPDGDALARKI
SLTLLEKGDLDLTFLSWVCNSLQAPTVERGTSYRDHGDHSLCATMDLGSPEGSYLEL
LNSDADPLHLYHLYDQMDLAGEEEIELSSEPDTDTINCDQFSKLLQDMELDEETREA
YANIAELDQYVFQDTQLEGLSKDLFIEHIGAEEGFGENIEIPVEAGQKPQKRRFPEEHA
MDSKHRKLVPTSRTSLNYLDLPTGHIQIFTTLPQGLWQISGAGTGLSSVLIYHGEMPQ
VNQVLPSSSLSIPSLPESPDRPGSTSPFTPSAADLPSMPEPALTSRVNETEDTSPSPCQE
GPESSIKLPKWPEAVERFQHSLQDKYKALPQSPRGPLVAVELVRARLERGSNKSQER
ELATPDWTERQLAHGGLAEVLQVVSDCRRPGETQVVAVLGKAGQGKSHWARTVSH
TWACGQLLQYDFVFYVPCHCLDRPGDTYHLRDLLCPPSLQPLAMDDEVLDYIVRQP
DRVLLILDAFEELEAQDGLLHGPCGSLSPEPCSLRGLLAGIFQRKLLRGCTLLLTARPR
GRLAQSLSKADAIFEVPSFSTKQAKTYMRHYFENSGTAGNQDKALGLLEGQPLLCSY
SHSPVVCRAVCQLSKALLEQGTEAQLPCTLTGLYVSLLGPAAQNSPPGALVELAKLA
WELGRRHQSTLQETRFSSVEVKTWAVTQGLMQQTLETTEAQLAFSSFLLQCFLGAV
WLAQCNEIKDKELPQYLALTPRKKRPYDNWLEGVPRFLAGLVFQPRAHCLGALVEP
AVAAVADRKQKVLTRYLKRLKLGTLRAGRLLELLHCAHETQQPGIWEHVAHQLPG
HLSFLGTRLTPPDVYVLGRALETASQDFSLDLRQTGVEPSGLGNLVGLSCVTSFRASL
SDTMALWESLQQQGEAQLLQAAEEKFTIEPFKAKSPKDVEDLDRLVQTQRLRNPSED
AAKDLPAIRDLKKLEFALGPILGPQAFPTLAKILPAFSSLQHLDLDSLSENKIGDKGVS
KLSATFPQLKALETLNLSQNNITDVGACKLAEALPALAKSLLRLSLYNNCICDKGAK
SLAQVLPDMVSLRVMDVQFNKFTAAGAQQLASSLQKCPQVETLAMWTPTIPFGVQE
HLQQLDARISLR (SEQ ID NO:2)

<4-1BB-L>

>TNFSF9: TNF superfamily member 9 (aka Ly63l; 4-1BBL; Cd137l; 4-1BB-L; AI848817)

>DNA sequence (NCBI Reference Sequence: NM_009404.3)

ATGGACCAGCACACACTTGATGTGGAGGATACCGCGGATGCCAGACATCCAGCA
GGTACTTCGTGCCCCTCGGATGCGGCGCTCCTCAGAGATACCGGGCTCCTCGCGG
ACGCTGCGCTCCTCTCAGATACTGTGCGCCCCACAAATGCCGCGCTCCCCACGGA
TGCTGCCTACCCTGCGGTTAATGTTCGGGATCGCGAGGCCGCGTGGCCGCCTGCA
CTGAACTTCTGTTCCCGCCACCCAAAGCTCTATGGCCTAGTCGCTTTGGTTTTGCT
GCTTCTGATCGCCGCCTGTGTTCCTATCTTCACCCGCACCGAGCCTCGGCCAGCG
CTCACAATCACCACCTCGCCCAACCTGGGTACCCGAGAGAATAATGCAGACCAG
GTCACCCCTGTTTCCCACATTGGCTGCCCCAACACTACACAACAGGGCTCTCCTG
TGTTCGCCAAGCTACTGGCTAAAAACCAAGCATCGTTGTGCAATACAACTCTGAA
CTGGCACAGCCAAGATGGAGCTGGGAGCTCATACCTATCTCAAGGTCTGAGGTA
CGAAGAAGACAAAAAGGAGTTGGTGGTAGACAGTCCCGGGCTCTACTACGTATT
TTTGGAACTGAAGCTCAGTCCAACATTCACAAACACAGGCCACAAGGTGCAGGG
CTGGGTCTCTCTTGTTTTGCAAGCAAAGCCTCAGGTAGATGACTTTGACAACTTG
GCCCTGACAGTGGAACTGTTCCCTTGCTCCATGGAGAACAAGTTAGTGGACCGTT
CCTGGAGTCAACTGTTGCTCCTGAAGGCTGGCCACCGCCTCAGTGTGGGTCTGAG
GGCTTATCTGCATGGAGCCCAGGATGCATACAGAGACTGGGAGCTGTCTTATCCC
AACACCACCAGCTTTGGACTCTTTCTTGTGAAACCCGACAACCCATGGGAA**TGA**
(SEQ ID NO:3)

4-1BB-L Protein sequence (NCBI Reference Sequence: NP_033430.1)

MDQHTLDVEDTADARHPAGTSCPSDAALLRDTGLLADAALLSDTVRPTNAALPTDA
AYPAVNVRDREAAWPPALNFCSRHPKLYGLVALVLLLLIAACVPIFTRTEPRPALTIT
TSPNLGTRENNADQVTPVSHIGCPNTTQQGSPVFAKLLAKNQASLCNTTLNWHSQD
GAGSSYLSQGLRYEEDKKELVVDSPGLYYVFLELKLSPTFTNTGHKVQGWVSLVLQ
AKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRAYLHGAQ
DAYRDWELSYPNTTSFGLFLVKPDNPWE (SEQ ID NO:4)

<OX40-L>

>TNFSF4: TNF superfamily member 4 (aka Ath1; gp34; Ath-1; Ox40l; TXGP1; CD134L; OX-40L; Tnlg2b; Txgp1l)

>DNA sequence (NCBI Reference Sequence: NM_009452.2)

ATGGAAGGGGAAGGGGTTCAACCCCTGGATGAGAATCTGGAAAACGGATCAAG
GCCAAGATTCAAGTGGAAGAAGACGCTAAGGCTGGTGGTCTCTGGGATCAAGGG
AGCAGGGATGCTTCTGTGCTTCATCTATGTCTGCCTGCAACTCTCTTCCTCTCCGG
CAAAGGACCCTCCAATCCAAAGACTCAGAGGAGCAGTTACCAGATGTGAGGATG
GGCAACTATTCATCAGCTCATACAAGAATGAGTATCAAACTATGGAGGTGCAGA
ACAATTCGGTTGTCATCAAGTGCGATGGGCTTTATATCATCTACCTGAAGGGCTC
CTTTTTCCAGGAGGTCAAGATTGACCTTCATTTCCGGGAGGATCATAATCCCATC
TCTATTCCAATGCTGAACGATGGTCGAAGGATTGTCTTCACTGTGGTGGCCTCTTT
GGCTTTCAAAGATAAAGTTTACCTGACTGTAAATGCTCCTGATACTCTCTGCGAA
CACCTCCAGATAAATGATGGGGAGCTGATTGTTGTCCAGCTAACGCCTGGATACT
GTGCTCCTGAAGGATCTTACCACAGCACTGTGAACCAAGTACCACTG**TGA** (SEQ ID NO:5)

> **OX40-L** Protein sequence (NCBI Reference Sequence: NP_033478.1)

MEGEGVQPLDENLENGSRPRFKWKKTLRLVVSGIKGAGMLLCFIYVCLQLSSSPAKD
PPIQRLRGAVTRCEDGQLFISSYKNEYQTMEVQNNSVVIKCDGLYIIYLKGSFFQEVKI
DLHFREDHNPISIPMLNDGRRIVFTVVASLAFKDKVYLTVNAPDTLCEHLQINDGELI
VVQLTPGYCAPEGSYHSTVNQVPL (SEQ ID NO:6)

<**GITR-L**>

>TNFSF18 TNF superfamily member 18 (aka Gitrl; Tnlg2a)

>DNA sequence (NCBI Reference Sequence: NM_183391.3)

ATGGAGGAAATGCCTTTGAGAGAATCAAGTCCTCAAAGGGCAGAGAGGTGCAA
GAAGTCATGGCTCTTGTGCATAGTGGCTCTGTTACTGATGTTGCTCTGTTCTTTGG
GTACACTGATCTATACTTCACTCAAGCCAACTGCCATCGAGTCCTGCATGGTTAA
GTTTGAACTATCATCCTCAAAATGGCACATGACATCTCCCAAACCTCACTGTGTG
AATACGACATCTGATGGGAAGCTGAAGATACTGCAGAGTGGCACATATTTAATC
TACGGCCAAGTGATTCCTGTGGATAAGAAATACATAAAAGACAATGCCCCCTTC
GTAGTACAGATATATAAAAAGAATGATGTCCTACAAACTCTAATGAATGATTTTC
AAATCTTGCCTATAGGAGGGGTTTATGAACTGCATGCTGGAGATAACATATATCT
GAAGTTCAACTCTAAAGACCATATTCAGAAAACTAACACATACTGGGGGATCAT
CTTAATGCCTGATCTACCATTCATCTCT**TAG** (SEQ ID NO:7)

> TNFSF18 Protein sequence (NCBI Reference Sequence: NP_899247.3)

MEEMPLRESSPQRAERCKKSWLLCIVALLLMLLCSLGTLIYTSLKPTAIESCMVKFEL
SSSKWHMTSPKPHCVNTTSDGKLKILQSGTYLIYGQVIPVDKKYIKDNAPFVVQIYK
KNDVLQTLMNDFQILPIGGVYELHAGDNIYLKFNSKDHIQKTNTYWGIILMPDLPFIS
(SEQ ID NO:8)

<**CD80**>

>CD80 (aka B71; Ly53; TSA1; Cd28l; Ly-53; MIC17)

>DNA sequence (NCBI Reference Sequence: NM_001359898.1)

ATGGCTTGCAATTGTCAGTTGATGCAGGATACACCACTCCTCAAGTTTCCATGTC
CAAGGCTCATTCTTCTCTTTGTGCTGCTGATTCGTCTTTCACAAGTGTCTTCAGAT
GTTGATGAACAACTGTCCAAGTCAGTGAAAGATAAGGTATTGCTGCCTTGCCGTT
ACAACTCTCCTCATGAAGATGAGTCTGAAGACCGAATCTACTGGCAAAAACATG
ACAAAGTGGTGCTGTCTGTCATTGCTGGGAAACTAAAAGTGTGGCCCGAGTATA
AGAACCGGACTTTATATGACAACACTACCTACTCTCTTATCATCCTGGGCCTGGT
CCTTTCAGACCGGGGCACATACAGCTGTGTCGTTCAAAAGAAGGAAAGAGGAAC
GTATGAAGTTAAACACTTGGCTTTAGTAAAGTTGTCCATCAAAGCTGACTTCTCT
ACCCCCAACATAACTGAGTCTGGAAACCCATCTGCAGACACTAAAAGGATTACC
TGCTTTGCTTCCGGGGGTTTCCCAAAGCCTCGCTTCTCTTGGTTGGAAAATGGAA
GAGAATTACCTGGCATCAATACGACAATTTCCCAGGATCCTGAATCTGAATTGTA
CACCATTAGTAGCCAACTAGATTTCAATACGACTCGCAACCACACCATTAAGTGT
CTCATTAAATATGGAGATGCTCACGTGTCAGAGGACTTCACCTGGGAAAAACCCC
CAGAAGACCCTCCTGATAGCAAGAACACACTTGTGCTCTTTGGGGCAGGATTCGG

CGCAGTAATAACAGTCGTCGTCATCGTTGTCATCATCAAATGCTTCTGTAAGCAC
AGAAGCTGTTTCAGAAGAAATGAGGCAAGCAGAGAAACAAACAACAGCCTTACC
TTCGGGCCTGAAGAAGCATTAGCTGAACAGACCGTCTTCCTT**TAG** (SEQ ID NO:9)

> CD80 Protein Sequence (NCBI Reference Sequence: NP_001346827.1)

MACNCQLMQDTPLLKFPCPRLILLFVLLIRLSQVSSDVDEQLSKSVKDKVLLPCRYNS
PHEDESEDRIYWQKHDKVVLSVIAGKLKVWPEYKNRTLYDNTTYSLIILGLVLSDRG
TYSCVVQKKERGTYEVKHLALVKLSIKADFSTPNITESGNPSADTKRITCFASGGFPK
PRFSWLENGRELPGINTTISQDPESELYTISSQLDFNTTRNHTIKCLIKYGDAHVSEDFT
WEKPPEDPPDSKNTLVLFGAGFGAVITVVVIVVIIKCFCKHRSCFRRNEASRETNNSL
TFGPEEALAEQTVFL (SEQ ID NO:10)

<**GM-CSF**>

> CSF2: colony stimulating factor 2 (aka CSF; Csfgm; GMCSF; Gm-CSf; MGI-IGM)

>DNA sequence (NCBI Reference Sequence: NM_009969.4)

ATGTGGCTGCAGAATTTACTTTTCCTGGGCATTGTGGTCTACAGCCTCTCAGCAC
CCACCCGCTCACCCATCACTGTCACCCGGCCTTGGAAGCATGTAGAGGCCATCAA
AGAAGCCCTGAACCTCCTGGATGACATGCCTGTCACGTTGAATGAAGAGGTAGA
AGTCGTCTCTAACGAGTTCTCCTTCAAGAAGCTAACATGTGTGCAGACCCGCCTG
AAGATATTCGAGCAGGGTCTACGGGGCAATTTCACCAAACTCAAGGGCGCCTTG
AACATGACAGCCAGCTACTACCAGACATACTGCCCCCCAACTCCGGAAACGGAC
TGTGAAACACAAGTTACCACCTATGCGGATTTCATAGACAGCCTTAAAACCTTTC
TGACTGATATCCCCTTTGAATGCAAAAAACCAGGCCAAAAA**TGA** (SEQ ID NO:11)

> GM-CSF Protein sequence (NCBI Reference Sequence: NP_034099.2)

MWLQNLLFLGIVVYSLSAPTRSPITVTRPWKHVEAIKEALNLLDDMPVTLNEEVEVV
SNEFSFKKLTCVQTRLKIFEQGLRGNFTKLKGALNMTASYYQTYCPPTPETDCETQV
TTYADFIDSLKTFLTDIPFECKKPGQK (SEQ ID NO:12)

[0050] Human

[0051] In the following DNA sequences, bold codons indicate the Start or Stop codon.

<**CIITA**>

>Homo sapiens class II major histocompatibility complex transactivator (CIITA) (also known in the art as C2TA; NLRA; MHC2TA; CIITAIV)

>DNA sequence (NCBI Reference Sequence: NM_001286402.1)

ATGCGTTGCCTGGCTCCACGCCCTGCTGGGTCCTACCTGTCAGAGCCCCAAGGCA
GCTCACAGTGTGCCACCATGGAGTTGGGGCCCCTAGAAGGTGGCTACCTGGAGC
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CCTGGCTGGAGAAGAAGAGATTGAGCTCTACTCAGAACCCGACACAGACACCAT
CAACTGCGACCAGTTCAGCAGGCTGTTGTGTGACATGGAAGGTGATGAAGAGAC
CAGGGAGGCTTATGCCAATATCGCGGAACTGGACCAGTATGTCTTCCAGGACTCC

CAGCTGGAGGGCCTGAGCAAGGACATTTTCATAGAGCACATAGGACCAGATGAA
GTGATCGGTGAGAGTATGGAGATGCCAGCAGAAGTTGGGCAGAAAAGTCAGAA
AAGACCCTTCCCAGAGGAGCTTCCGGCAGACCTGAAGCACTGGAAGCCAGCTGA
GCCCCCCACTGTGGTGACTGGCAGTCTCCTAGTGGGACCAGTGAGCGACTGCTCC
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AGATGCGCCTGGAGAAAACCGACCAGATTCCCATGCCTTTCTCCAGTTCCTCGTT
GAGCTGCCTGAATCTCCCTGAGGGACCCATCCAGTTTGTCCCCACCATCTCCACT
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TCATCTACCATGGTGAGGTGCCCCAGGCCAGCCAAGTACCCCCTCCCAGTGGATT
CACTGTCCACGGCCTCCCAACATCTCCAGACCGGCCAGGCTCCACCAGCCCCTTC
GCTCCATCAGCCACTGACCTGCCCAGCATGCCTGAACCTGCCCTGACCTCCCGAG
CAAACATGACAGAGCACAAGACGTCCCCCACCCAATGCCCGGCAGCTGGAGAGG
TCTCCAACAAGCTTCCAAAATGGCCTGAGCCGGTGGAGCAGTTCTACCGCTCACT
GCAGGACACGTATGGTGCCGAGCCCGCAGGCCCGGATGGCATCCTAGTGGAGGT
GGATCTGGTGCAGGCCAGGCTGGAGAGGAGCAGCAGCAAGAGCCTGGAGCGGG
AACTGGCCACCCCGGACTGGGCAGAACGGCAGCTGGCCCAAGGAGGCCTGGCTG
AGGTGCTGTTGGCTGCCAAGGAGCACCGGCGGCCGCGTGAGACACGAGTGATTG
CTGTGCTGGGCAAAGCTGGTCAGGGCAAGAGCTATTGGGCTGGGGCAGTGAGCC
GGGCCTGGGCTTGTGGCCGGCTTCCCCAGTACGACTTTGTCTTCTCTGTCCCCTGC
CATTGCTTGAACCGTCCGGGGGATGCCTATGGCCTGCAGGATCTGCTCTTCTCCC
TGGGCCCACAGCCACTCGTGGCGGCCGATGAGGTTTTCAGCCACATCTTGAAGAG
ACCTGACCGCGTTCTGCTCATCCTAGACGGCTTCGAGGAGCTGGAAGCGCAAGAT
GGCTTCCTGCACAGCACGTGCGGACCGGCACCGGCGGAGCCCTGCTCCCTCCGG
GGGCTGCTGGCCGGCCTTTTCCAGAAGAAGCTGCTCCGAGGTTGCACCCTCCTCC
TCACAGCCCGGCCCCGGGGCCGCCTGGTCCAGAGCCTGAGCAAGGCCGACGCCC
TATTTGAGCTGTCCGGCTTCTCCATGGAGCAGGCCCAGGCATACGTGATGCGCTA
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GGACCGGCCACTTCTTCTCAGTCACAGCCACAGCCCTACTTTGTGCCGGGCAGTG
TGCCAGCTCTCAGAGGCCCTGCTGGAGCTTGGGGAGGACGCCAAGCTGCCCTCC
ACGCTCACGGGACTCTATGTCGGCCTGCTGGGCCGTGCAGCCCTCGACAGCCCCC
CCGGGGCCCTGGCAGAGCTGGCCAAGCTGGCCTGGGAGCTGGGCCGCAGACATC
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TGGCCAAAGGCTTAGTCCAACACCCACCGCGGGCCGCAGAGTCCGAGCTGGCCT
TCCCCAGCTTCCTCCTGCAATGCTTCCTGGGGGCCCTGTGGCTGGCTCTGAGTGG
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GAGGCCCTATGACAACTGGCTGGAGGGCGTGCCACGCTTTCTGGCTGGGCTGATC
TTCCAGCCTCCCGCCCGCTGCCTGGGAGCCCTACTCGGGCCATCGGCGGCTGCCT
CGGTGGACAGGAAGCAGAAGGTGCTTGCGAGGTACCTGAAGCGGCTGCAGCCGG
GGACACTGCGGGCGCGGCAGCTGCTGGAGCTGCTGCACTGCGCCCACGAGGCCG
AGGAGGCTGGAATTTGGCAGCACGTGGTACAGGAGCTCCCCGGCCGCCTCTCTTT
TCTGGGCACCCGCCTCACGCCTCCTGATGCACATGTACTGGGCAAGGCCTTGGAG
GCGGCGGGCCAAGACTTCTCCCTGGACCTCCGCAGCACTGGCATTTGCCCCTCTG
GATTGGGGAGCCTCGTGGGACTCAGCTGTGTCACCCGTTTCAGGGCTGCCTTGAG
CGACACGGTGGCGCTGTGGGAGTCCCTGCAGCAGCATGGGGAGACCAAGCTACT
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GGATGTGGAAGACCTGGGAAAGCTTGTGCAGACTCAGAGGACGAGAAGTTCCTC
GGAAGACACAGCTGGGGAGCTCCCTGCTGTTCGGGACCTAAAGAAACTGGAGTT
TGCGCTGGGCCCTGTCTCAGGCCCCCAGGCTTTCCCCAAACTGGTGCGGATCCTC
ACGGCCTTTTCCTCCCTGCAGCATCTGGACCTGGATGCGCTGAGTGAGAACAAGA
TCGGGGACGAGGGTGTCTCGCAGCTCTCAGCCACCTTCCCCCAGCTGAAGTCCTT

GGAAACCCTCAATCTGTCCCAGAACAACATCACTGACCTGGGTGCCTACAAACTC
GCCGAGGCCCTGCCTTCGCTCGCTGCATCCCTGCTCAGGCTAAGCTTGTACAATA
ACTGCATCTGCGACGTGGGAGCCGAGAGCTTGGCTCGTGTGCTTCCGGACATGGT
GTCCCTCCGGGTGATGGACGTCCAGTACAACAAGTTCACGGCTGCCGGGGCCCA
GCAGCTCGCTGCCAGCCTTCGGAGGTGTCCTCATGTGGAGACGCTGGCGATGTGG
ACGCCCACCATCCCATTCAGTGTCCAGGAACACCTGCAACAACAGGATTCACGG
ATCAGCCTGAGA**TGA** (SEQ ID NO:13)

> Human CIITA Protein sequence (NCBI Reference Sequence: NP_001273331.1)

MRCLAPRPAGSYLSEPQGSSQCATMELGPLEGGYLELLNSDADPLCLYHFYDQMDL
AGEEEIELYSEPDTDTINCDQFSRLLCDMEGDEETREAYANIAELDQYVFQDSQLEGL
SKDIFIEHIGPDEVIGESMEMPAEVGQKSQKRPFPEELPADLKHWKPAEPPTVVTGSL
LVGPVSDCSTLPCLPLPALFNQEPASGQMRLEKTDQIPMPFSSSSLSCLNLPEGPIQFV
PTISTLPHGLWQISEAGTGVSSIFIYHGEVPQASQVPPPSGFTVHGLPTSPDRPGSTSPF
APSATDLPSMPEPALTSRANMTEHKTSPTQCPAAGEVSNKLPKWPEPVEQFYRSLQD
TYGAEPAGPDGILVEVDLVQARLERSSSKSLERELATPDWAERQLAQGGLAEVLLAA
KEHRRPRETRVIAVLGKAGQGKSYWAGAVSRAWACGRLPQYDFVFSVPCHCLNRP
GDAYGLQDLLFSLGPQPLVAADEVFSHILKRPDRVLLILDGFEELEAQDGFLHSTCGP
APAEPCSLRGLLAGLFQKKLLRGCTLLLTARPRGRLVQSLSKADALFELSGFSMEQA
QAYVMRYFESSGMTEHQDRALTLLRDRPLLLSHSHSPTLCRAVCQLSEALLELGEDA
KLPSTLTGLYVGLLGRAALDSPPGALAELAKLAWELGRRHQSTLQEDQFPSADVRT
WAMAKGLVQHPPRAAESELAFPSFLLQCFLGALWLALSGEIKDKELPQYLALTPRKK
RPYDNWLEGVPRFLAGLIFQPPARCLGALLGPSAAASVDRKQKVLARYLKRLQPGT
LRARQLLELLHCAHEAEEAGIWQHVVQELPGRLSFLGTRLTPPDAHVLGKALEAAG
QDFSLDLRSTGICPSGLGSLVGLSCVTRFRAALSDTVALWESLQQHGETKLLQAAEE
KFTIEPFKAKSLKDVEDLGKLVQTQRTRSSSEDTAGELPAVRDLKKLEFALGPVSGPQ
AFPKLVRILTAFSSLQHLDLDALSENKIGDEGVSQLSATFPQLKSLETLNLSQNNITDL
GAYKLAEALPSLAASLLRLSLYNNCICDVGAESLARVLPDMVSLRVMDVQYNKFTA
AGAQQLAASLRRCPHVETLAMWTPTIPFSVQEHLQQQDSRISLR (SEQ ID NO:14)

<4-1BB-L>

>Human TNFSF9: TNF superfamily member 9 (aka CD137L; TNLG5A; 4-1BB-L)

>DNA sequence (NCBI Reference Sequence: NM_003811.3)

ATGGAATACGCCTCTGACGCTTCACTGGACCCCGAAGCCCCGTGGCCTCCCGCGC
CCCGCGCTCGCGCCTGCCGCGTACTGCCTTGGGCCCTGGTCGCGGGGCTGCTGCT
GCTGCTGCTGCTCGCTGCCGCCTGCGCCGTCTTCCTCGCCTGCCCCTGGGCCGTGT
CCGGGGCTCGCGCCTCGCCCGGCTCCGCGGCCAGCCCGAGACTCCGCGAGGGTC
CCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTT
TGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTAC
AGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAG
GACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAAC
TAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCT
GCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTG
GACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCC
GCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGC
CAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTC

CGGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA**TAA** (SEQ ID NO:15)

>Human 4-1BB-L protein sequence (NCBI Reference Sequence: NP_003802.1)

MEYASDASLDPEAPWPPAPRARACRVLPWALVAGLLLLLLLAAACAVFLACPWAV SGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSD PGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQ PLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARH AWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO:16)

<**OX40-L**>

>TNFSF4: TNF superfamily member 4 (aka GP34; CD252; OX4OL; TXGP1; CD134L; OX-40L; TNLG2B)

>DNA sequence (NCBI Reference Sequence: NM_003326.4)

ATGGAAAGGGTCCAACCCCTGGAAGAGAATGTGGGAAATGCAGCCAGGCCAAG ATTCGAGAGGAACAAGCTATTGCTGGTGGCCTCTGTAATTCAGGGACTGGGGCTG CTCCTGTGCTTCACCTACATCTGCCTGCACTTCTCTGCTCTTCAGGTATCACATCG GTATCCTCGAATTCAAAGTATCAAAGTACAATTTACCGAATATAAGAAGGAGAA AGGTTTCATCCTCACTTCCCAAAAGGAGGATGAAATCATGAAGGTGCAGAACAA CTCAGTCATCATCAACTGTGATGGGTTTTATCTCATCTCCCTGAAGGGCTACTTCT CCCAGGAAGTCAACATTAGCCTTCATTACCAGAAGGATGAGGAGCCCCTCTTCCA ACTGAAGAAGGTCAGGTCTGTCAACTCCTTGATGGTGGCCTCTCTGACTTACAAA GACAAAGTCTACTTGAATGTGACCACTGACAATACCTCCCTGGATGACTTCCATG TGAATGGCGGAGAACTGATTCTTATCCATCAAAATCCTGGTGAATTCTGTGTCCT T**TGA** (SEQ ID NO:17)

>Human OX40-L Protein sequence (NCBI Reference Sequence: NP_003317.1)

MERVQPLEENVGNAARPRFERNKLLLVASVIQGLGLLLCFTYICLHFSALQVSHRYP RIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINCDGFYLISLKGYFSQEVNISL HYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVTTDNTSLDDFHVNGGELILI HQNPGEFCVL (SEQ ID NO:18)

<**GITR-L**>

>TNFSF18 TNF superfamily member 18 (aka TL6; AITRL; GITRL; TNLG2A; hGITRL)

>DNA sequence (NCBI Reference Sequence: NM_005092.3)

ATGACATTGCATCCTTCACCCATCACTTGTGAATTTTTGTTTTCCACAGCTCTCAT TTCTCCAAAAATGTGTTTGAGCCACTTGGAAAATATGCCTTTAAGCCATTCAAGA ACTCAAGGAGCTCAGAGATCATCCTGGAAGCTGTGGCTCTTTTGCTCAATAGTTA TGTTGCTATTTCTTTGCTCCTTCAGTTGGCTAATCTTTATTTTTCTCCAATTAGAGA CTGCTAAGGAGCCCTGTATGGCTAAGTTTGGACCATTACCCTCAAAATGGCAAAT GGCATCTTCTGAACCTCCTTGCGTGAATAAGGTGTCTGACTGGAAGCTGGAGATA CTTCAGAATGGCTTATATTTAATTTATGGCCAAGTGGCTCCCAATGCAAACTACA ATGATGTAGCTCCTTTTGAGGTGCGGCTGTATAAAAACAAAGACATGATACAAA

CTCTAACAAACAAATCTAAAATCCAAAATGTAGGAGGGACTTATGAATTGCATG
TTGGGGACACCATAGACTTGATATTCAACTCTGAGCATCAGGTTCTAAAAAATAA
TACATACTGGGGTATCATTTTACTAGCAAATCCCCAATTCATCTCC**TAG** (SEQ ID NO:19)

>Human GITR-L Protein sequence (NCBI Reference Sequence: NP_005083.2)

MTLHPSPITCEFLFSTALISPKMCLSHLENMPLSHSRTQGAQRSSWKLWLFCSIVMLL
FLCSFSWLIFIFLQLETAKEPCMAKFGPLPSKWQMASSEPPCVNKVSDWKLEILQNGL
YLIYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSKIQNVGGTYELHVGDTIDLI
FNSEHQVLKNNTYWGIILLANPQFIS (SEQ ID NO:20)

<**CD86**>

>CD86 (aka B70; B7-2; B7.2; LAB72; CD28LG2)

>DNA sequences (NCBI Reference Sequence: NM_175862.4)

ATGGATCCCCAGTGCACTATGGGACTGAGTAACATTCTCTTTGTGATGGCCTTCC
TGCTCTCTGGTGCTGCTCCTCTGAAGATTCAAGCTTATTTCAATGAGACTGCAGA
CCTGCCATGCCAATTTGCAAACTCTCAAAACCAAAGCCTGAGTGAGCTAGTAGTA
TTTTGGCAGGACCAGGAAAACTTGGTTCTGAATGAGGTATACTTAGGCAAAGAG
AAATTTGACAGTGTTCATTCCAAGTATATGGGCCGCACAAGTTTTGATTCGGACA
GTTGGACCCTGAGACTTCACAATCTTCAGATCAAGGACAAGGGCTTGTATCAATG
TATCATCCATCACAAAAAGCCCACAGGAATGATTCGCATCCACCAGATGAATTCT
GAACTGTCAGTGCTTGCTAACTTCAGTCAACCTGAAATAGTACCAATTTCTAATA
TAACAGAAAATGTGTACATAAATTTGACCTGCTCATCTATACACGGTTACCCAGA
ACCTAAGAAGATGAGTGTTTTGCTAAGAACCAAGAATTCAACTATCGAGTATGAT
GGTATTATGCAGAAATCTCAAGATAATGTCACAGAACTGTACGACGTTTCCATCA
GCTTGTCTGTTTCATTCCCTGATGTTACGAGCAATATGACCATCTTCTGTATTCTG
GAAACTGACAAGACGCGGCTTTTATCTTCACCTTTCTCTATAGAGCTTGAGGACC
CTCAGCCTCCCCCAGACCACATTCCTTGGATTACAGCTGTACTTCCAACAGTTATT
ATATGTGTGATGGTTTTCTGTCTAATTCTATGGAAATGGAAGAAGAAGAAGCGGC
CTCGCAACTCTTATAAATGTGGAACCAACACAATGGAGAGGGAAGAGAGTGAAC
AGACCAAGAAAAGAGAAAAAATCCATATACCTGAAAGATCTGATGAAGCCCAGC
GTGTTTTTAAAAGTTCGAAGACATCTTCATGCGACAAAAGTGATACATGTTTTT**TA**
A (SEQ ID NO:21)

>Human CD86 Protein sequence (NCBI Reference Sequence: NP_787058.4)

MDPQCTMGLSNILFVMAFLLSGAAPLKIQAYFNETADLPCQFANSQNQSLSELVVFW
QDQENLVLNEVYLGKEKFDSVHSKYMGRTSFDSDSWTLRLHNLQIKDKGLYQCIIH
HKKPTGMIRIHQMNSELSVLANFSQPEIVPISNITENVYINLTCSSIHGYPEPKKMSVLL
RTKNSTIEYDGIMQKSQDNVTELYDVSISLSVSFPDVTSNMTIFCILETDKTRLLSSPFS
IELEDPQPPPDHIPWITAVLPTVIICVMVFCLILWKWKKKRPRNSYKCGTNTMEREE
SEQTKKREKIHIPERSDEAQRVFKSSKTSSCDKSDTCF (SEQ ID NO:22)

<**GM-CSF**>

> CSF2: colony stimulating factor 2 (aka CSF; GMCSF)

>DNA sequence (NCBI Reference Sequence: NM_000758.3)

ATGTGGCTGCAGAGCCTGCTGCTCTTGGGCACTGTGGCCTGCAGCATCTCTGCAC
CCGCCCGCTCGCCCAGCCCCAGCACGCAGCCCTGGGAGCATGTGAATGCCATCC
AGGAGGCCCGGCGTCTCCTGAACCTGAGTAGAGACACTGCTGCTGAGATGAATG
AAACAGTAGAAGTCATCTCAGAAATGTTTGACCTCCAGGAGCCGACCTGCCTAC
AGACCCGCCTGGAGCTGTACAAGCAGGGCCTGCGGGGCAGCCTCACCAAGCTCA
AGGGCCCCTTGACCATGATGGCCAGCCACTACAAGCAGCACTGCCCTCCAACCC
CGGAAACTTCCTGTGCAACCCAGATTATCACCTTTGAAAGTTTCAAAGAGAACCT
GAAGGACTTTCTGCTTGTCATCCCCTTTGACTGCTGGGAGCCAGTCCAGGAG**TGA**
(SEQ ID NO:23)

>Human GM-CSF protein sequence (NCBI Reference Sequence: NP_000749.2)

MWLQSLLLLGTVACSISAPARSPSPSTQPWEHVNAIQEARRLLNLSRDTAAEMNETV
EVISEMFDLQEPTCLQTRLELYKQGLRGSLTKLKGPLTMMASHYKQHCPPTPETSCA
TQIITFESFKENLKDFLLVIPFDCWEPVQE (SEQ ID NO:24)

[0052] The foregoing description of specific embodiments is for the purpose of illustration and is not to be construed as restrictive. From the teachings of the present invention, those skilled in the art will recognize that various modifications and changes may be made without departing from the spirit of the invention.

What is claimed is:

1. Modified cancer cells that are modified to co-express class II trans-activator (CIITA), and an immuno-stimulatory molecule.

2. The modified cancer cells of claim 1, wherein the immuno-stimulatory molecule is selected from OX-40-ligand and 4-1BB-Ligand.

3. The modified cancer cells of claim 2, wherein the immuno-stimulatory molecule is the 4-1BB-Ligand.

4. A pharmaceutical composition comprising the modified cancer cells of any one of claims 1-3.

5. A cell line comprising the modified cancer cells of any one of claims 1-3.

6. A method of making modified cancer cells for use in a cancer vaccine, the method comprising introducing into the cancer cells one or more polynucleotides that result in expression of class II trans-activator (CIITA), and an immuno-stimulatory molecule.

7. The method of claim 6, wherein the immuno-stimulatory molecule is selected from OX-40-ligand and 4-1BB-Ligand.

8. The method of claim 7, wherein the immuno-stimulatory molecule is the 4-1BB-Ligand.

9. A method for stimulating an immune response in an individual against one or more cancer antigens, the method comprising;

i) introducing into the individual modified cancer cells of any one of claims 1-3 such that the immune response against the one or more antigens expressed by the cancer cells is stimulated; or

ii) introducing into cancer cells in the individual one or more polynucleotides encoding class II trans-activator (CIITA) and an immuno-stimulatory molecule to produce modified cancer cells in the individual, wherein the modified cancer cells express the CITTA

and the immune-stimulatory molecule from the one or more polynucleotides, and wherein the immune response is stimulated to one or more antigens expressed by the modified cancer cells.

10. The method of claim 9, wherein the modified cancer cells express an immuno-stimulatory molecule that is selected from OX-40-ligand and 4-1BB-Ligand, and/or wherein one of the polynucleotides express OX-40-ligand or 4-1BB-Ligand

11. The method of claim 10, wherein the modified cancer cells express the 4-1BB-Ligand.

12. The method of claim 9, wherein the stimulated immune response comprises one or a combination of: a durable memory antitumor CD8+ T-cell response that is specific for the same cancer type as the modified cancer cells, or an antitumor antibody response against the same cancer type as the modified cancer cells, or an inhibition of growth of a tumor comprising cancer cells that are the same cancer type as the modified cancer cells, or eradication of one or more existing tumors that comprise cancer cells that are the same cancer type as the modified cancer cells.

13. The method of claim 12, wherein the modified cancer cells express the 4-1BB-Ligand.

14. The method of claim 9, wherein the modified cancer cells of i) are introduced into the individual.

15. The method of claim 9, wherein the one or more polynucleotides of ii) are introduced into the individual.

16. An isolated expression vector or combination of isolated expression vectors encoding class II trans-activator (CIITA) and an immuno-stimulatory molecule.

17. The expression vector or combination of expression vectors of claim 16, wherein the immuno-stimulatory molecule is OX-40-ligand or 4-1BB-Ligand.

18. The expression vector or combination of expression vectors of claim 17, wherein the immuno-stimulatory is the 4-1BB-Ligand.

19. One or more modified cancer cells that is/are breast cancer cell(s) selected from the group consisting, prostate cancer cell(s), pancreatic cancer cell(s), lung cancer cell(s), liver cancer cell(s), ovarian cancer cell(s), cervical cancer cell(s), colon cancer cell(s)r, esophageal cancer cell(s), stomach cancer cell(s), bladder cancer cell(s), brain cancer cell(s), testicular cancer cell(s), head and neck cancer cell(s), melanoma cell(s), skin cancer cell(s), any sarcoma cell(s), leukemia cell(s), lymphoma cell(s), myeloma cell(s), and combinations thereof, wherein the one or more modified cancer cells express class II trans-activator (CIITA) and an immuno-stimulatory molecule from one or more recombinant polynucleotides.

20. The one or more modified cancer cells of claim 19, wherein the immuno-stimulatory molecule comprises OX-40-ligand or 4-1BB-Ligand.

21 The one or more modified cancer cells of claim 20, wherein the immuno-stimulatory molecule comprises the 4-1BB-Ligand.

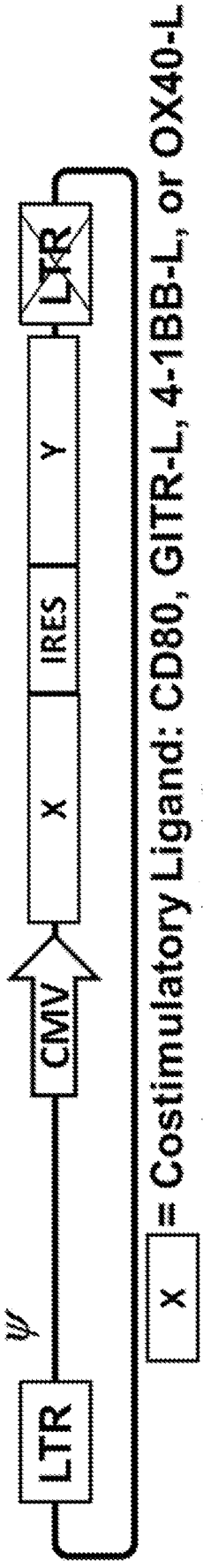

LTR
ψ
CMV
X
IRES
Y
LTR
X = Costimulatory Ligand: CD80, GITR-L, 4-1BB-L, or OX40-L
or Cytokine: GM-CSF or Empty
Y = CIITA or GFP

Figure 1

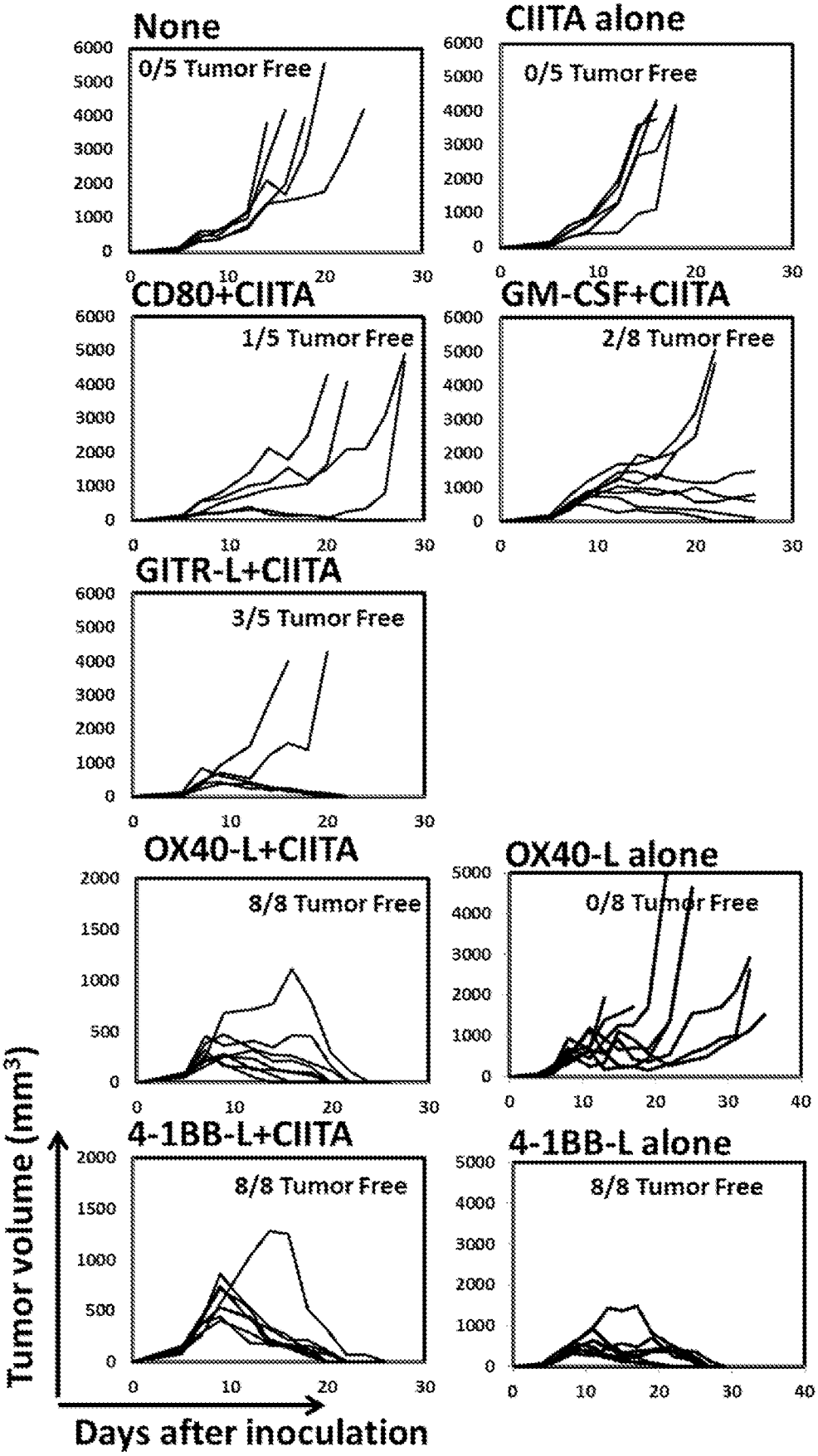
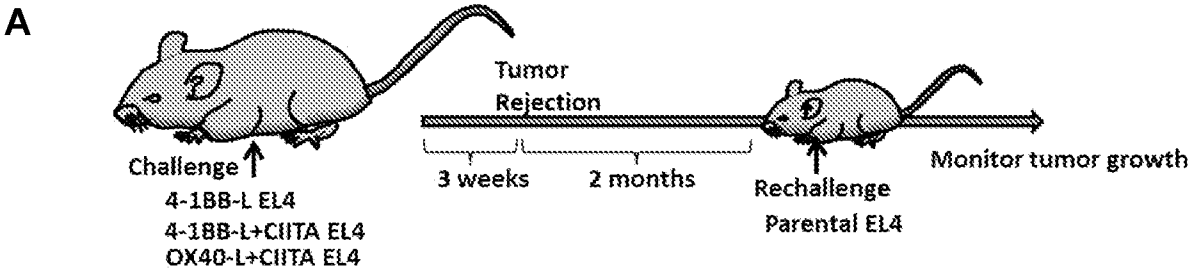
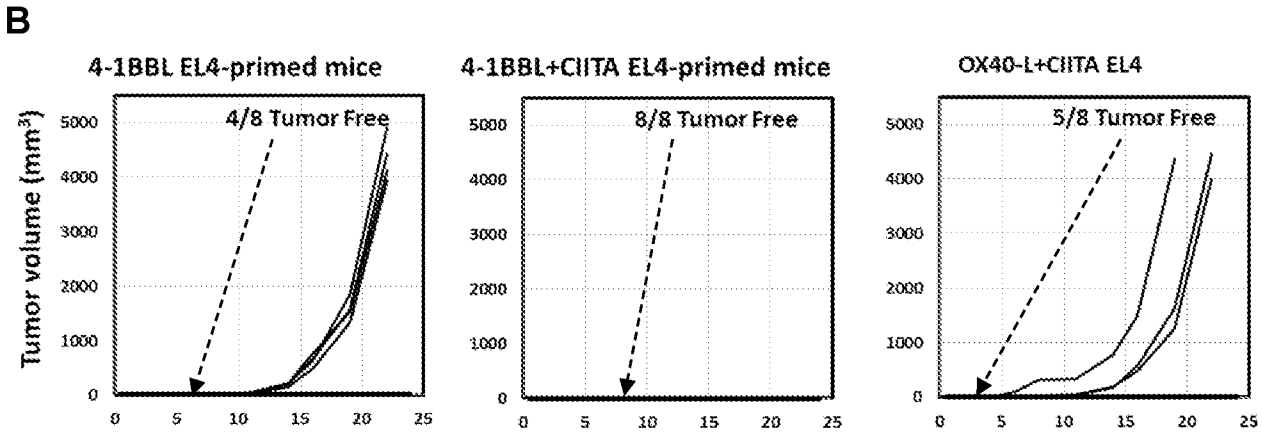
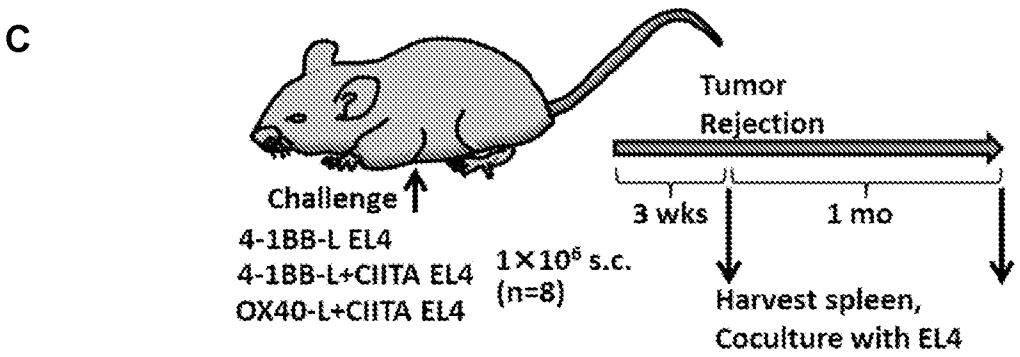

None
0/5 Tumor Free
CIITA alone
0/5 Tumor Free
CD80+CIITA
1/5 Tumor Free
GM-CSF+CIITA
2/8 Tumor Free
GITR-L+CIITA
3/5 Tumor Free
OX40-L+CIITA
8/8 Tumor Free
OX40-L alone
0/8 Tumor Free
4-1BB-L+CIITA
8/8 Tumor Free
4-1BB-L alone
8/8 Tumor Free
Tumor volume (mm3)
Days after inoculation

Figure 2


A
Challenge
4-1BB-L EL4
4-1BB-L+CIITA EL4
OX40-L+CIITA EL4
Tumor
Rejection
3 weeks
2 months
Rechallenge
Parental EL4
Monitor tumor growth


B
4-1BBL EL4-primed mice
4-1BBL+CIITA EL4-primed mice
OX40-L+CIITA EL4
Tumor volume (mm3)
4/8 Tumor Free
8/8 Tumor Free
5/8 Tumor Free


C
Challenge
4-1BB-L EL4
4-1BB-L+CIITA EL4
OX40-L+CIITA EL4
1×10^6 s.c.
(n=8)
Tumor
Rejection
3 wks
1 mo
Harvest spleen,
Coculture with EL4

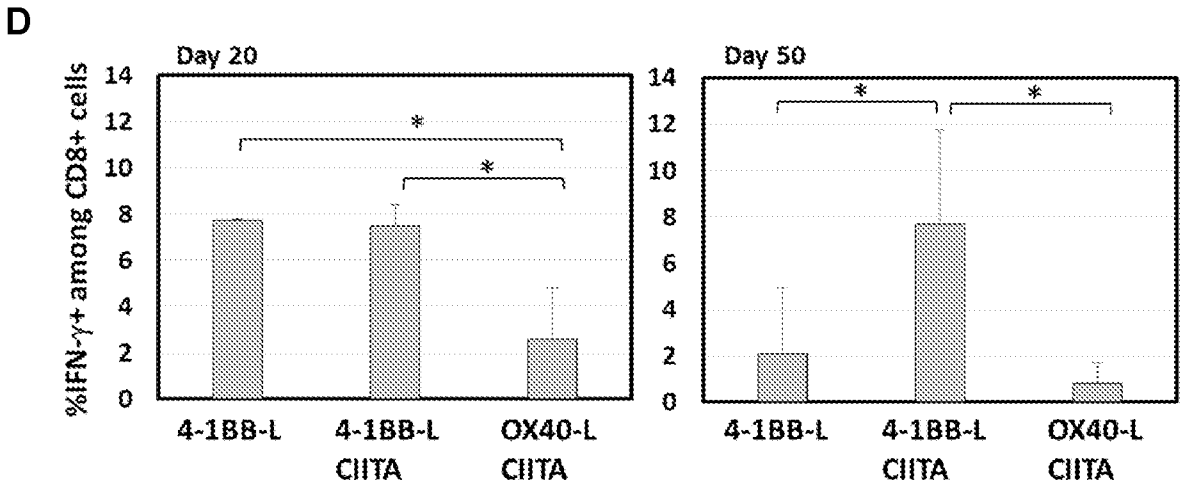

D
Day 20
Day 50
%IFN-γ+ among CD8+ cells
4-1BB-L
4-1BB-L CIITA
OX40-L CIITA

Figure 3

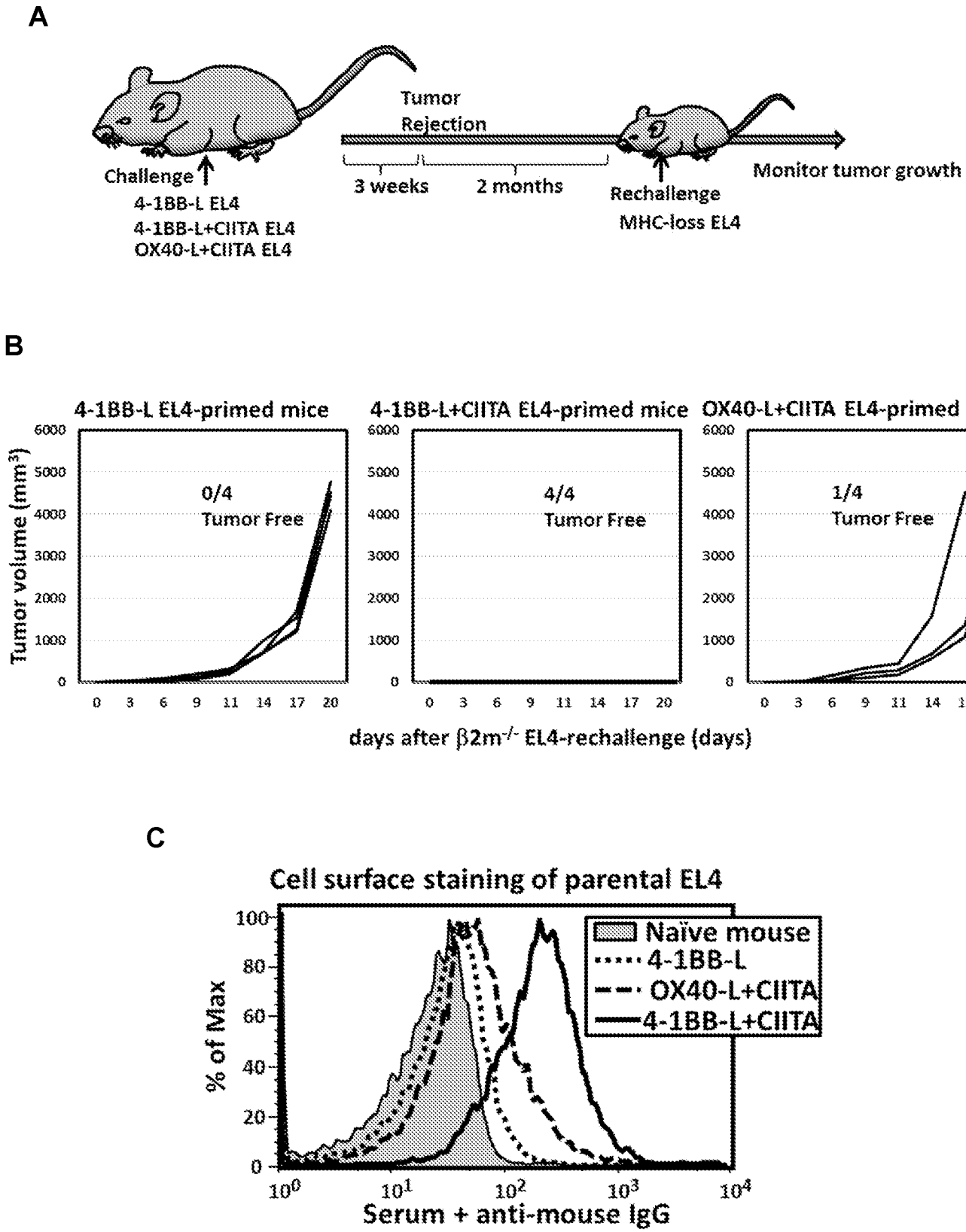

A
Tumor
Rejection
Challenge
4-1BB-L EL4
4-1BB-L+CIITA EL4
OX40-L+CIITA EL4
3 weeks
2 months
Rechallenge
MHC-loss EL4
Monitor tumor growth
B
4-1BB-L EL4-primed mice
4-1BB-L+CIITA EL4-primed mice
OX40-L+CIITA EL4-primed mice
Tumor volume (mm³)
0/4
Tumor Free
4/4
Tumor Free
1/4
Tumor Free
days after β2m-/- EL4-rechallenge (days)
C
Cell surface staining of parental EL4
% of Max
Naïve mouse
4-1BB-L
OX40-L+CIITA
4-1BB-L+CIITA
Serum + anti-mouse IgG

Figure 4

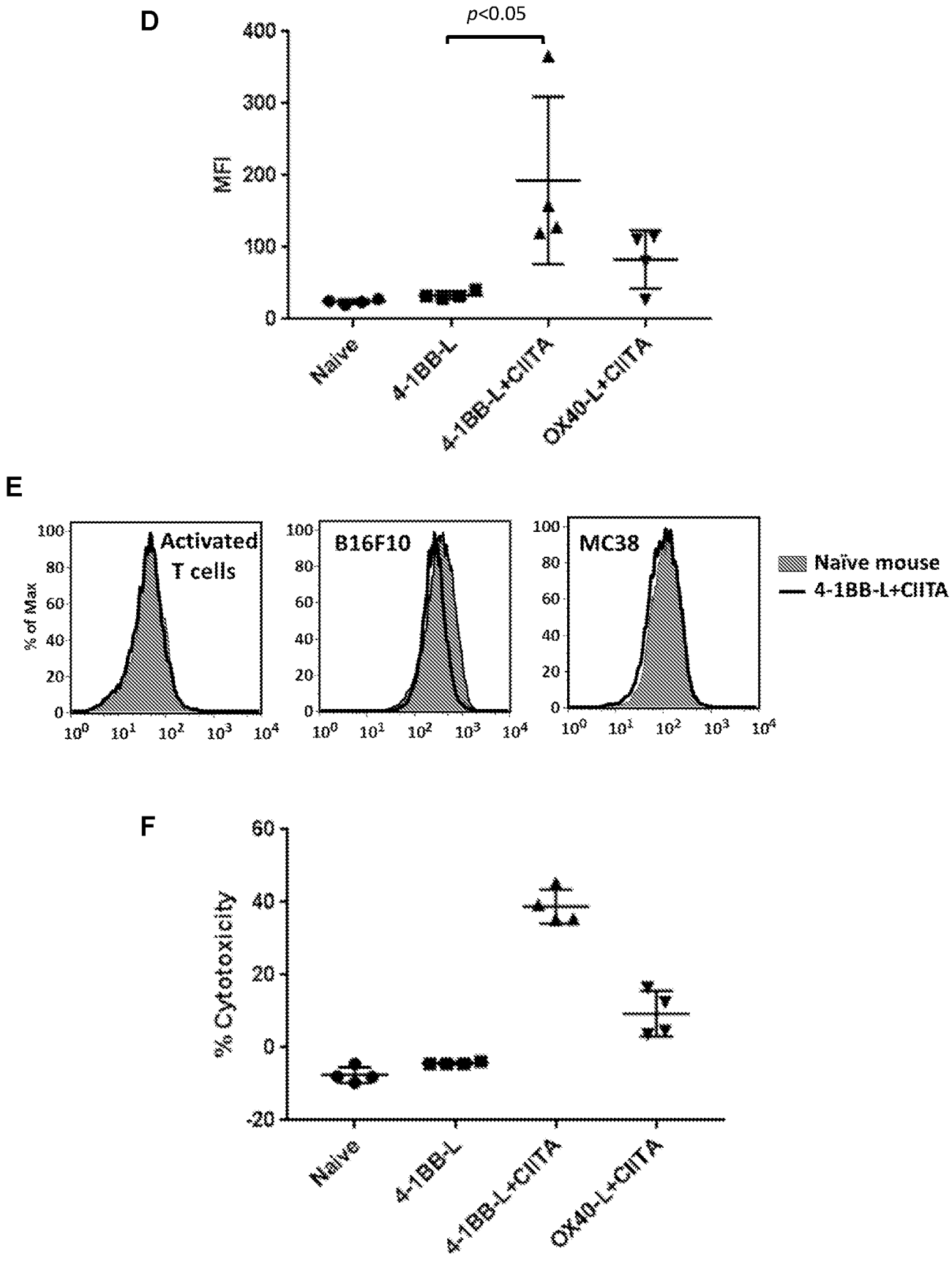
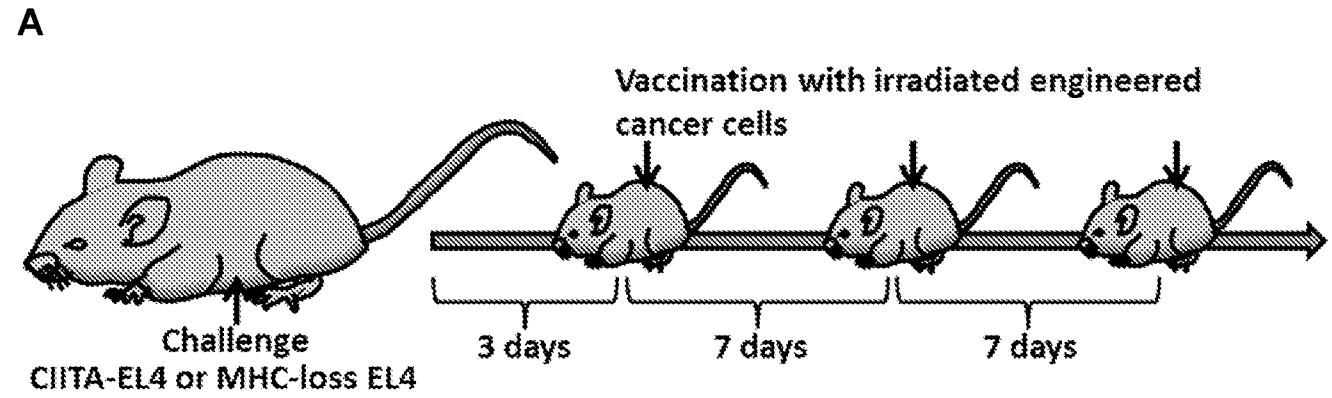


Figure 4, continued


A
Vaccination with irradiated engineered cancer cells
Challenge
CIITA-EL4 or MHC-loss EL4
3 days
7 days
7 days

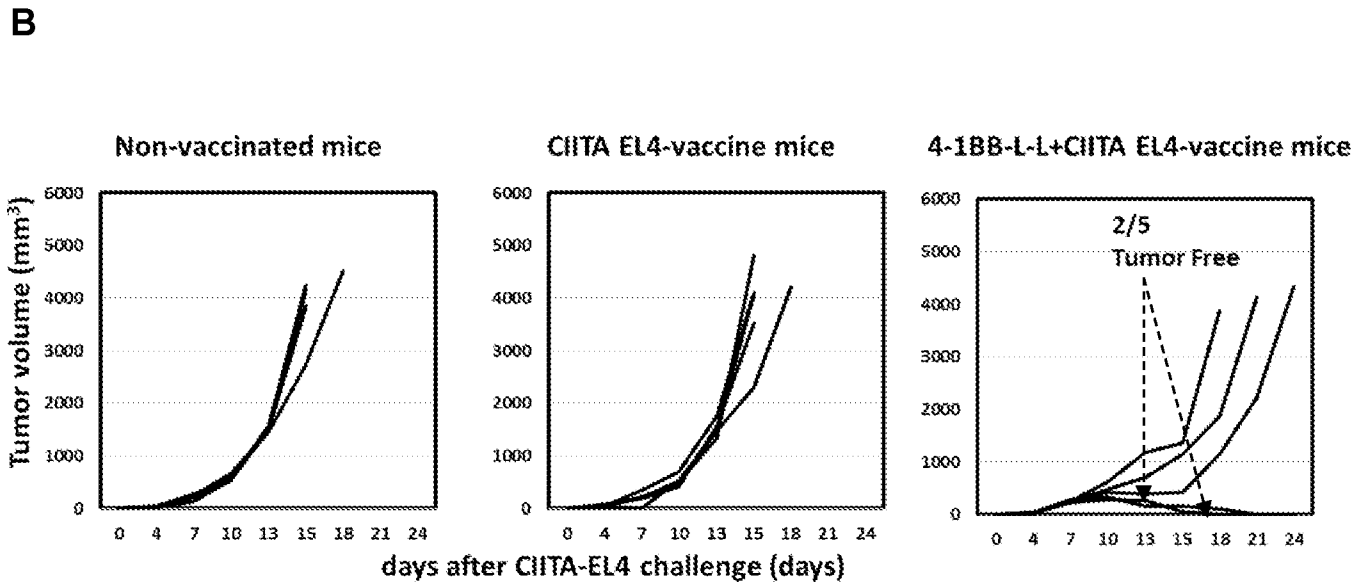
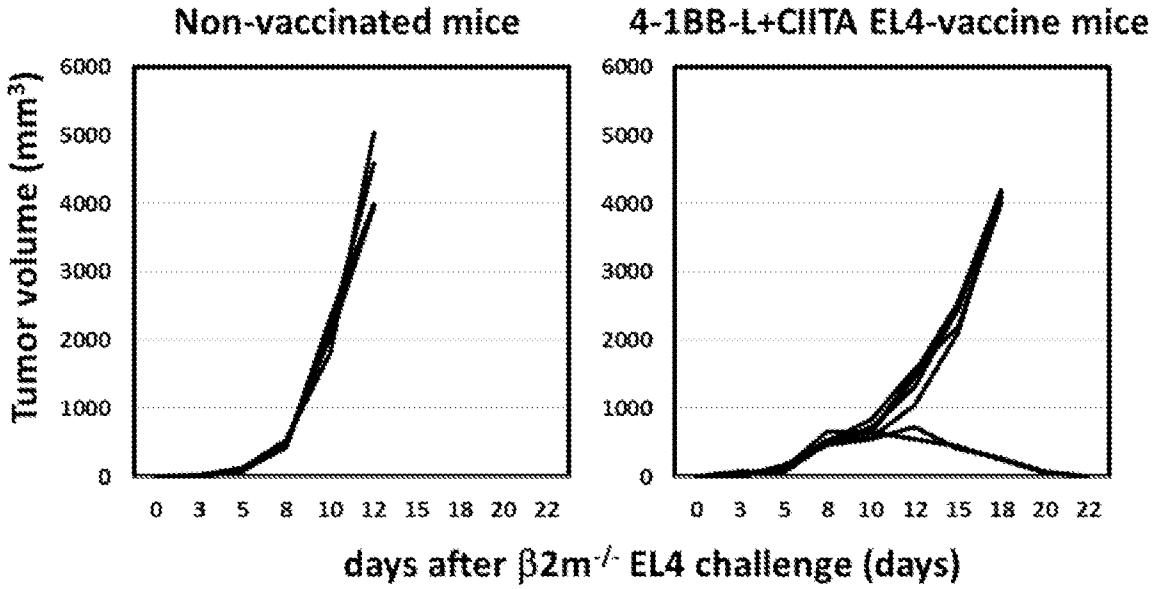

B
Non-vaccinated mice
CIITA EL4-vaccine mice
4-1BB-L-L+CIITA EL4-vaccine mice
Tumor volume (mm3)
2/5
Tumor Free
days after CIITA-EL4 challenge (days)

Figure 5

C



D

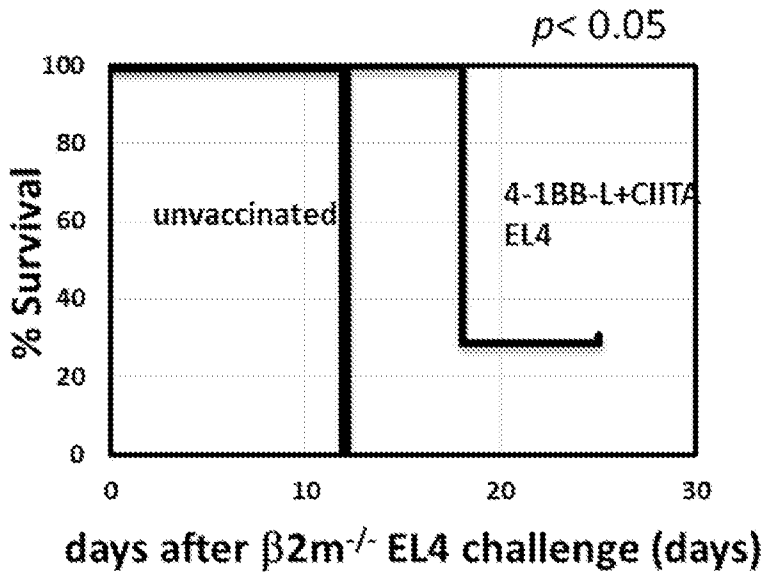
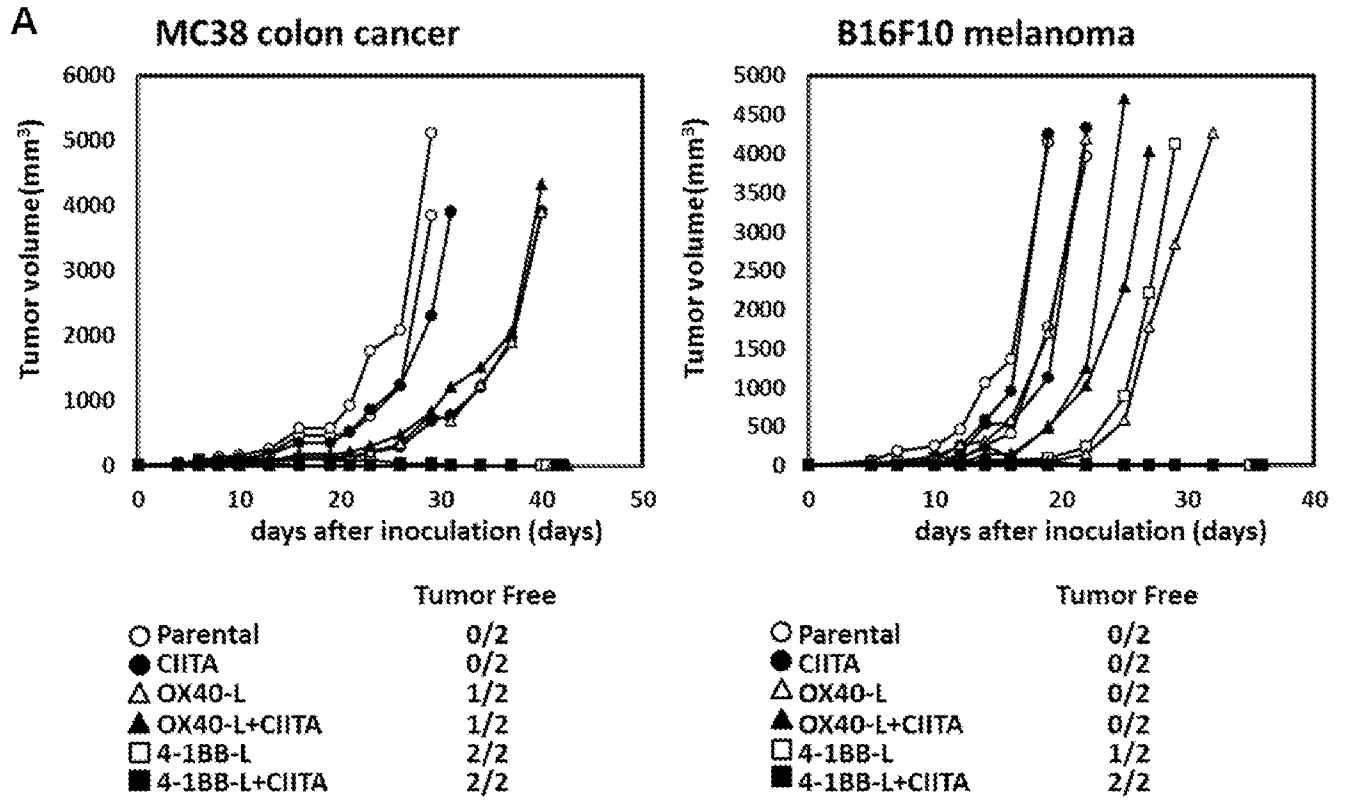
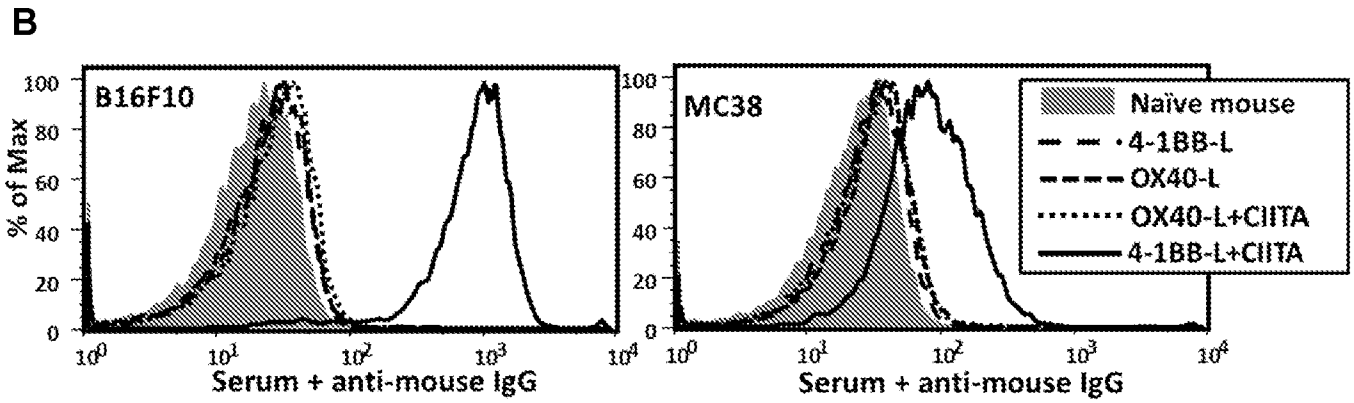


Figure 5, continued


A
MC38 colon cancer
Tumor volume(mm3)
days after inoculation (days)
Tumor Free
Parental 0/2
CIITA 0/2
OX40-L 1/2
OX40-L+CIITA 1/2
4-1BB-L 2/2
4-1BB-L+CIITA 2/2
B16F10 melanoma
Tumor volume(mm3)
days after inoculation (days)
Tumor Free
Parental 0/2
CIITA 0/2
OX40-L 0/2
OX40-L+CIITA 0/2
4-1BB-L 1/2
4-1BB-L+CIITA 2/2


B
B16F10
MC38
% of Max
Serum + anti-mouse IgG
Naïve mouse
4-1BB-L
OX40-L
OX40-L+CIITA
4-1BB-L+CIITA

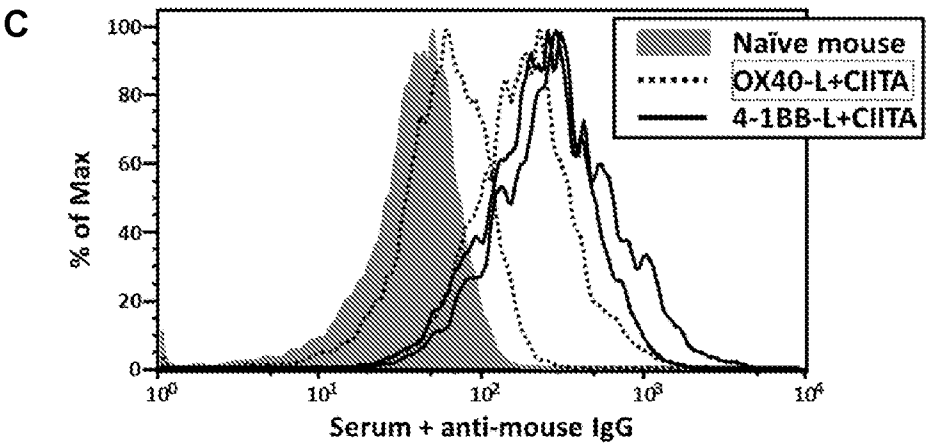

C
% of Max
Serum + anti-mouse IgG
Naïve mouse
OX40-L+CIITA
4-1BB-L+CIITA

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/42764

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/68; C12N 5/07, 5/071, 5/09, 5/16, 15/79, 15/85, 15/86; A61K 38/16, 38/19 (2019.01)

CPC - C12Q 1/68, 1/6883, 1/6886; C12N 15/79, 15/8201, 15/85, 15/86, 5/06, 5/0602, 5/0693, 5/16; A61K 38/16, 38/191

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	(JING, W et al.) Induction of Immunity to Neuroblastoma Early after Syngeneic Hematopoietic Stem Cell Transplantation Using a Novel Mouse Tumor Vaccine. Biology of Blood and Marrow Transplantation. March 2007, Vol. 13, No. 3; pages 277-292; abstract; page 277, 2nd column, 2nd paragraph; page 278, 1st column, 1st-2nd paragraphs and 2nd column, 2nd paragraph; page 279, 1st column, 2nd-3rd paragraphs; page 282, 2nd column, 3rd paragraph; DOI: 10.1016/j.bbmt.2006.11.018	1-3, 4/1-3, 5/1-3, 6-8, 9/1-3, 10/9/1-3, 11/10/9/1-3, 12/9/1-3, 13/12/9/1-3, 14/9/1-3, 15/9/1-3, 16-21
A	WO 99/42585 A1 (SISTERS OF PROVIDENCE IN OREGON) 26 August 1999; entire document	1-3, 4/1-3, 5/1-3, 6-8, 9/1-3, 10/9/1-3, 11/10/9/1-3, 12/9/1-3, 13/12/9/1-3, 14/9/1-3, 15/9/1-3, 16-21
A	(CHOU, SD et al.) Histone acetylation regulates the cell type specific CIITA promoters, MHC class II expression and antigen presentation in tumor cells. International Immunology. November 2005, Epub 6 October 2005, Vol. 17, No. 11; pages 1483-1494; DOI: 10.1093/intimm/dxh326	1-3, 4/1-3, 5/1-3, 6-8, 9/1-3, 10/9/1-3, 11/10/9/1-3, 12/9/1-3, 13/12/9/1-3, 14/9/1-3, 15/9/1-3, 16-21
A	(LU, ZY et al.) B7-1 and 4-1BB ligand expression on a myeloma cell line makes it possible to expand autologous tumor-specific cytotoxic T cells in vitro. Experimental Hematology. 21 August 2007, Vol. 35, No. 3, pages 443-453; DOI: 10.1016/j.exphem.2006.11.002	1-3, 4/1-3, 5/1-3, 6-8, 9/1-3, 10/9/1-3, 11/10/9/1-3, 12/9/1-3, 13/12/9/1-3, 14/9/1-3, 15/9/1-3, 16-21

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
23 September 2019 (23.09.2019)	02 OCT 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/42764

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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

3. Additional comments: