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(71) Applicant: **BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 210 West 7th St., Austin, TX 78701 (US).

(72) Inventors: **ZHANG, Yu**; C/o The University Of Texas Md Anderson Cancer, Center, 1515 Holcombe Blvd., Houston, TX 77030 (US). **RIQUELME, Erick**; C/o The University Of Texas Md Anderson Cancer, Center, 1515 Holcombe Blvd., Houston, TX 77030 (US). **MCALLISTER, Floren-**

cia; C/o The University Of Texas Md Anderson Cancer, Center, 1515 Holcombe Blvd., Houston, TX 77030 (US).

(74) Agent: **SCHNEPP, Amanda, S.J.**; Parker Highlander PLLC, 1120 So. Capital Of Texas Highway, Bldg. One Suite 200, Austin, TX 78746 (US).

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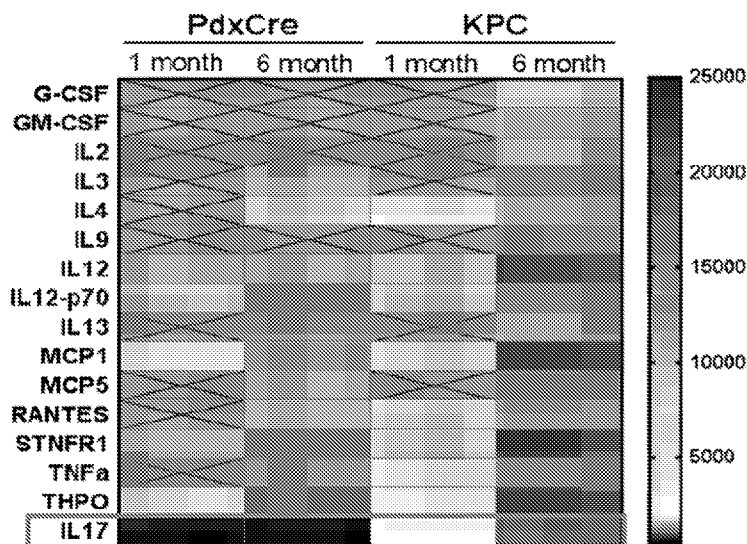


FIG. 1A

(57) Abstract: Provided herein are methods of treating the cancer patients, such as pancreatic cancer patients, with a combination of an IL-17 signaling inhibitor and an immune checkpoint blockade therapy, such as an anti-PD1 therapy and/or an anti-CTLA-4 therapy. Also provided herein are methods of determining if such therapy is efficacious based on level of lactate in the patient's cancer and/or serum.

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DESCRIPTION

COMPOSITIONS AND METHODS FOR TREATING CANCER USING IL-17

SIGNALING INHIBITORS AND IMMUNE CHECKPOINT INHIBITORS

REFERENCE TO RELATED APPLICATIONS

5 **[0001]** The present application claims the priority benefit of United States provisional application number 62/792,198, filed January 14, 2019, the entire contents of which is incorporated herein by reference.

BACKGROUND

1. Field

10 **[0002]** The present disclosure relates generally to the fields of cancer biology and immunotherapy. More particularly, it concerns methods for selecting patients for treatment with a combination of an IL-17 signaling inhibitor and an immune checkpoint inhibitor as well as treating patients so selected.

2. Description of Related Art

15 **[0003]** Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies (Noone et al., 2018) with limited treatment options and surgery being the only potentially curative modality (Siegel et al., 2018). The surrounding tumor microenvironment is very complex and constituted mostly by a dense fibro-inflammatory stroma infiltrated by immunosuppressive cells which have been implicated in the tumorigenesis process and as
20 contributors to the lack of responses to most therapies.

[0004] Several immunotherapy approaches have emerged in the past decade and immune checkpoint blockade has been approved for use in several cancer types (Le et al., 2015; Brahmer et al., 2015; Diaz et al., 2015; Wolchok et al., 2017; D'Angelo et al., 2017; Long et al., 2017). Unfortunately, immune checkpoint inhibitors have not proven efficacious
25 in treating pancreatic cancer (Royal et al., 2010; Brahmer et al., 2012; Herbst et al., 2014; Patnaik et al., 2015). Recently, considerable interest has been focused on combining checkpoint inhibitors with other immunotherapies, antibodies, or vaccines (Lutz et al., 2014; Highfill et al., 2014; Zhu et al., 2014; Winograd et al., 2015), which target or prime the tumor microenvironment, thought to be one of the main drivers of the immunosuppression that
30 prevails in pancreatic cancer.

[0005] Several cell types have been implicated in contributing to the immunosuppressive microenvironment that supports PDAC growth, including macrophages, myeloid derived-suppressor cells, fibroblasts, and T regulatory cells (Clark et al., 2007; Bayne et al., 2012). Strategies that target these cell types or specific molecules on the surface of these cells in combination with immune checkpoint inhibitors have proven to have synergistic anti-tumoral effect in preclinical models of pancreatic cancer as well as other cancer types (Highfill et al., 2014; Zhu et al., 2014; Zhang et al., 2017; Feig et al., 2013; Provenzano et al., 2012) and some of them are being tested in ongoing clinical trials.

[0006] However, the tumor microenvironment surrounding pancreatic cancer is very complex and several mechanisms contribute to initiation and maintaining immunosuppression. As such, there is an urgent need to develop efficient strategies to favor anti-tumoral immunity as well as rational synergistic combinatorial immunotherapies.

SUMMARY

[0007] In one embodiment, provided herein are methods for the treatment of a cancer in a patient, the methods comprising administering to the patient a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor. In some aspects, the patient has previously failed to respond to the administration of an immune checkpoint inhibitor. In some aspects, the methods are methods of overcoming resistance to immune checkpoint inhibitor therapy. In some aspects, a patient is selected for treatment because the patient's cancer expresses an increased level of Duoxa2, Duox2, and/or Duox1 relative to a Duoxa2, Duox2, and/or Duox1 expression level in a reference sample. In certain aspects, the reference sample is sourced from healthy or non-cancerous tissue from the patient. In certain aspects, the reference sample is sourced from a healthy subject. In some aspects, the methods further comprise reporting the Duoxa2, Duox2, and/or Duox1 expression level in the patient's cancer. In some aspects, the reporting comprises preparing a written or electronic report. In some aspects, the methods further comprise providing the report to the subject, a doctor, a hospital, or an insurance company.

[0008] In one embodiment, provided herein are methods of selecting a patient having a cancer for treatment with a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor, the method comprising (a) determining a level of Duoxa2, Duox2, and/or Duox1 expression in the patient's cancer, and (b) selecting the patient for

treatment if the patient's cancer has an increased level of Duoxa2, Duox2, and/or Duox1 relative to a Duoxa2, Duox2, and/or Duox1 expression level in a reference sample. In some aspects, the methods further comprise administering a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor to the selected patient. In some aspects, the methods further comprise selecting the patient for treatment if the patient has previously failed to respond to the administration of an immune checkpoint inhibitor. In some aspects, the reference sample is sourced from healthy or non-cancerous tissue from the patient. In some aspects, the reference sample is sourced from a healthy subject. In some aspects, the methods further comprise reporting the Duoxa2, Duox2, and/or Duox1 expression level in the patient's cancer. In some aspects, the reporting comprises preparing a written or electronic report. In some aspects, the methods further comprise providing the report to the subject, a doctor, a hospital, or an insurance company.

[0009] In one embodiment, provided herein are methods of assessing the efficacy of treatment of a cancer in a patient with a combined amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor, the method comprising (a) determining a level of lactate in the patient's cancer and/or serum, and (b) determining that the treatment is efficacious if the patient's cancer and/or serum has a decreased level of lactate relative to a lactate level in a reference sample. In some aspects, the methods further comprise continuing to administer a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor to the patient if the patient's cancer and/or serum has a decreased level of lactate relative to a lactate level in a reference sample. In some aspects, the reference sample is sourced from a patient cancer and/or serum sample taken prior to the patient being treated with the combined amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor. In some aspects, the methods comprise (i) determining a first level of lactate in the patient's cancer and/or serum; (ii) administering to the patient a combined amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor; (iii) determining a second level of lactate in the patient's cancer and/or serum; (iv) continuing to administer the combined amount of the IL-17 signaling inhibitor and the immune checkpoint inhibitor to the patient if the level at determined at step (iii) is lower than the level at step (i). In some aspects, the reference sample is sourced from a healthy subject. In some aspects, the lactate level in the patient's cancer is determined by PET or MRI-based hyperpolarization methods. In some aspects, the methods further comprise reporting the lactate level in the patient's cancer. In some aspects, the reporting comprises preparing a written or electronic report. In some

aspects, the methods further comprise providing the report to the subject, a doctor, a hospital, or an insurance company.

[0010] In one embodiment, provided herein are pharmaceutical formulations comprising an IL-17 signaling inhibitor and an immune checkpoint inhibitor.

5 [0011] In some aspects of any of the present embodiments, the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody, an IL-17R antagonist protein, and/or an IL-17R antagonist small molecule. In some aspects, the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody. In some aspects, the IL-17R antagonist protein comprises an IL-17R inhibitory antibody. In some aspects, the IL-17 signaling inhibitor comprises both an
10 IL-17 neutralizing antibody and an IL-17R inhibitory antibody.

[0012] In some aspects of any of the present embodiments, the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4 therapy. In some aspects, the anti-PD1 therapy comprises nivolumab, pembrolizumab, pidilizumab, AMP-223, AMP-514, cemiplimab, or PDR-001. In some
15 aspects, the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, BMS-036559, or CK-301. In some aspects, the anti-CTLA-4 therapy comprises ipilimumab or tremelimumab.

[0013] In some aspects of any of the present embodiments, the methods further comprise administering a further anti-cancer therapy to the patient. In some aspects, the
20 second anti-cancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy, or cytokine therapy. In some aspects, the further anti-cancer therapy comprises gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, or docetaxel.

[0014] In some aspects of any of the present embodiments, the cancer is a pancreatic
25 cancer. In some aspects, the patient has previously undergone at least one round of anti-cancer therapy. In some aspects, the patient is a human.

[0015] In one embodiment, provided herein is the use of a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor for the treatment of a cancer in a patient. In one embodiment, provided herein are pharmaceutical compositions
30 comprising a combined effective amount of an IL-17 signaling inhibitor and an immune

checkpoint inhibitor for use as a medicament for treating a cancer in a patient. In one embodiment, provided herein is the use of an IL-17 signaling inhibitor and an immune checkpoint inhibitor in the manufacture of a medicament for the treatment of cancer.

[0016] As used herein, “essentially free,” in terms of a specified component, is used
5 herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical
10 methods.

[0017] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one.

[0018] The use of the term “or” in the claims is used to mean “and/or” unless
15 explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0019] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to
20 determine the value, the variation that exists among the study subjects, or a value that is within 10% of a stated value.

[0020] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the
25 invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0022] **FIGS. 1A-M.** IL17-secreting cells are increased in murine and human pancreatic adenocarcinoma carcinoma, and IL17 signaling modulates the pancreatic tumor microenvironment. FIG. 1A: Heat map representing serum levels of cytokines from spontaneous pancreatic adenocarcinoma mouse model (KRAS^{G12D}; Trp53^{R172H}; Pdx1-Cre or KPC) and control mice (Pdx1-Cre or C) at 1 month and 6 months old. FIG. 1B: Serum IL17 levels measured by luminex in C and KPC mice at 1 month and 6 months old. Data is expressed as fold changes compared to levels in C mice and standard deviation (SD) is shown. Results show the mean $\pm$ SD (n=5) of fold changes from KPC over C (**P* < .05). FIG. 1C: Relative IL17 mRNA expression measured by quantitative RT-PCR in normal pancreatic tissue and tumor tissue formed from KPC cells orthotopically implanted into syngeneic mice. Results show the mean $\pm$ SD (n=5) of fold changes from KPC tumors over normal pancreas (**P* < .05). FIG. 1D: Kaplan-Meier survival curves comparing PDAC with low vs. high expression of IL17A. FIG. 1E: Top panel: Experimental Protocol for orthotopic implantation of KPC cells into syngeneic wild-type and mTmG C57 mice followed by treatment with anti-IL17 and anti-IL17R monoclonal antibodies (aIL17/aIL17R) vs control isotype IgG (Isotype IgG). Scheme of time points at which RNAseq (14 days) and Immunoprofiling were performed (28 days). Bottom panel: Ingenuity Pathway Analysis showing top five cellular functions predicted using genes significantly down-regulated in tumors from mice orthotopically implanted with KPC cells and treated with neutralizing aIL17/aIL17R monoclonal antibodies vs isotype. As indicated on X-axis, biological functions with *p*<0.05 are sorted based on Z scores. FIG. 1F: Quantification of neutrophils infiltrating human PDAC tissue vs normal adjacent tissue by CD15 staining performed by IHC. Results expressed as number of CD15+ cells/hpf. FIG. 1G: Quantification of tumor infiltrating Gr1+ cells from KPC orthotopically implanted mice treated with Isotype IgG or aIL17/aIL17RA antibodies measured by flow cytometry. Results are expressed as relative % of Gr1+ cells from total gated CD45+ cells. FIG. 1H: Representative pictures of multiplex immunofluorescence staining (Multiplex IF) showing CD8, Gr1, CK19, SMA and DAPI staining in tumor tissues

from KPC orthotopically implanted mice treated with Isotype IgG or aIL17/aIL17R antibodies. FIG. 1I: Quantification of tumor infiltrating cytotoxic T cells from KPC orthotopically implanted mice treated with Isotype IgG or aIL17/aIL17R antibodies. Flow cytometry results are expressed as relative % of CD8+ from total gated CD45+ cells. FIG. 1J: Quantification of tumor infiltrating CD8+T cells expressing GranzymeB (GzmB)/mm2, based on tissue immunofluorescence staining. FIG. 1K: Quantification of tumor infiltrating cytotoxic T cells from KPC orthotopically implanted mice treated with isotype IgG or aIL17/aIL17R antibodies. Results are expressed as relative % of CD8+ and CD8+/IFN γ + cells from total gated CD45+ cells. FIG. 1L: Gzm+/Gr1 ratio calculated based on individual cell types quantification from multiplex IF. FIG. 1M: Spatial quantification of CD8+GzmB+ cells surrounding CK19+ cells (within 40 μ m) in tumor tissues from KPC orthotopically implanted mice treated with isotype IgG or aIL17/aIL17R antibodies by multiplex IF staining.

[0023] FIGS. 2A-J. Suppression of IL17 signaling overcomes resistance to PD-1 inhibitors. FIG. 2A: Experimental procedure for subcutaneous implantation of KPC cells into syngeneic mice and subsequently treated with isotype IgG, anti-PD-1 (aPD-1 antibody), anti-IL17/IL17R (aIL17/aIL17R antibodies), or anti-PD-1/IL17/IL17R (aPD-1/aIL17/aIL17R antibodies). FIG. 2B: Tumor growth curves for tumors subcutaneously implanted KPC cells upon treatments delineated in (FIG. 2A) (n=10 mice/group). Quantification was done plotting tumor volume (measured in mm³) (**P* < .05). FIG. 2C: Quantitative RT-PCR analysis of PD-L1 gene relative expression in tumor tissues from KPC orthotopically implanted mice treated with isotype IgG or aIL17/aIL17R (n=10 mice/group) (**P* < .05). FIG. 2D: Experimental procedure for orthotopic implantation of KPC cells into syngeneic mice and subsequently treated with isotype IgG, aPD-1, aIL17/aIL17R or aIL17/aIL17R/aPD-1 antibodies. FIG. 2E: Quantification of tumor volume (mm³) from orthotopically implanted KPC cells into syngeneic mice treated with antibodies as described in (FIG. 2D) (n=10 mice/group) (**P* < .05). FIG. 2F: Kaplan-Meier curves for syngeneic mice orthotopically implanted with KPC cells and treated with antibodies described in (FIG. 2D) (n=10 mice/group) (**P* < .05). FIG. 2G: Quantification of tumor volume (mm³) from orthotopically implanted mT3 cells into syngeneic mice treated with antibodies as described in (FIG. 2D) (n=8 mice/group) (**P* < .05). FIG. 2H: Immunoblotting for IL17RA on CRISPR/Cas9 IL17RA knockout KPC cell clones. β -actin was used as a loading control. Parental KPC cells and scramble negative control were also included. FIG. 2I: Quantification of tumor volume (mm³) from

orthotopically implanted KPC cells (with IL17RA deleted by CRISPR/Cas9 vs. scramble control) into syngeneic mice in presence/absence of aPD-1 (n=5 mice/group) (**P* < .05). FIG. 2J: Quantification of tumor volume (mm³) from subcutaneous implantation of KPC cells into syngeneic mice treated with isotype IgG, anti-PD-1 (aPD-1), anti-IL17/PD-1, or anti-IL17R/PD-1. Y-axis is tumor volume.

[0024] **FIGS. 3A-E.** The anti-tumoral effect of combinatorial IL17 and PD-1 blockade is CD8+T cell dependent. FIG. 3A: Flow cytometry-based analysis of tumor infiltrating CD8+ and CD8-IFN γ + cells. Tumors obtained from syngeneic mice orthotopically implanted with KPC cells and treated with isotype IgG, aPD-1, aIL17/aIL17R, or aIL17/aIL17R/aPD-1 antibodies (n=10 mice/group). Results expressed in percent of total CD45+ gated viable cells (**P* < .05). The bars in each group represent, from left to right, IgG, aPD-1, aIL17/aIL17R, and aIL17/aIL17R/aPD-1. FIG. 3B: Immunohistochemistry-based quantification of tumor infiltrating cells expressing Granzyme-B (GzmB+) in same groups/treatments as FIG. 3A (**P* < .05). Results expressed as total number of GzmB+ cells/mm². The bars represent, from left to right, IgG, aPD-1, aIL17/aIL17R, and aIL17/aIL17R/aPD-1. FIG. 3C: Representative pictures of multiplex immunofluorescence staining (Multiplex IF) showing CD8, GzmB, CK19, SMA, and DAPI (top panels) staining in tumor tissues from KPC orthotopically implanted mice under treatment described in FIG. 2E. Bottom panels show only CD8/GzmB. FIG. 3D: Quantification of tumor volume (mm³) from orthotopically implanted KPC cells into wild-type syngeneic mice treated with isotype IgG, aIL17/aIL17R/aPD-1, anti-CD8 (aCD8), or aIL17/aIL17R/aPD-1 and aCD8 antibodies (n=10) (**P* < .05). FIG. 3E: Quantification of tumor volume (mm³) from orthotopically implanted KPC cells into CD8-deficient mice (CD8-/-) treated with isotype IgG or aIL17/aIL17R/aPD-1 antibodies (n=6-7 mice/group) (**P* < .05).

[0025] **FIGS. 4A-J.** Combinatorial PD-1 and IL17 inhibition induces metabolic changes, which may mediate immunosuppression and serve as activity biomarker. FIG. 4A: Venn diagram with IL17-regulated genes from 3 datasets: Genes down-regulated by *in vivo* IL17 blockade in orthotopic PDAC model detected by RNA-seq (A). Genes down-regulated upon *in vivo* IL17 blockade in oncogenic epithelium of KC^{iMist1} mice detected by Microarray (B). Genes directly upregulated on enteroids exposed to IL17 and detected by RNAseq (C). *DUOX2* and *DUOX2* genes are highlighted. FIG. 4B: Quantitative RT-PCR analysis of

NOX/DUOX family genes of KPC cells *in vitro* stimulated by IL17 recombinant protein 10 ng/ml for 3 days. In each pair of columns, the left column represents Ctrl and the right column represents IL17. FIG. 4C: Quantitative RT-PCR analysis for *DUOX2* and *DUOX2* in mTmG negative sorted cancer cells (KPC unlabeled cells were implanted orthotopically into mTmG recipient mice) from mice exposed *in vivo* to IL17 neutralizing antibodies vs. isotype. In each pair of columns, the left column represents Ctrl and the right column represents IL17. FIG. 4D: Flow cytometry analysis of reactive oxygen species levels detected by DCFDA staining in KPC cells *in vitro* stimulated by IL17 recombinant protein. FIG. 4E: Representative pictures of reactive oxygen species stained by dihydroethidium in KPC cells after *in vitro* stimulation with IL17 recombinant protein for 24 h. FIG. 4F: Medium H₂O₂ levels produced by KPC cells *in vitro* stimulated by IL17 recombinant protein. Representative experiment of two conducted is shown. In each pair of columns, the left column represents Ctrl and the right column represents IL17. FIG. 4G: Percentage inhibition of medium IFN γ released by splenocyte after co-culturing with culture medium from KPC cells *in vitro* stimulated by IL17 recombinant protein 10 ng/ml for 24 h, 48 h, and 72 h. In each pair of columns, the left column represents Ctrl and the right column represents IL17. FIG. 4H: Heat map representing normalized metabolites in tumor tissues from KPC cells orthotopically implanted mice treated with isotype IgG, anti-PD-1, and anti-IL17/IL17R or aIL17/aIL17R/aPD-1 antibodies for 4 weeks (n=10). FIG. 4I: Heat map representing normalized metabolites in tumor tissues from KPC cells orthotopically implanted mice treated with isotype IgG or aIL17/aIL17R/aPD-1 antibodies for 2 weeks (n=10). FIG. 4J: Lactate levels of lactate in serum from KPC cells orthotopically implanted mice treated with isotype IgG or aIL17/aIL17R/aPD-1 antibodies for 2 weeks (n=10).

[0026] FIG. 5. Graphic abstract showing the mechanism that IL17A modulates pancreatic cancer immunosuppression and its inhibition overcomes resistance to anti-PD-1.

[0027] FIGS. 6A-F. FIG. 6A-B: Flow cytometry analysis showing Tregs (CD4+FOXP3+) (FIG. 6A) and MDSCs (CD45+/Cd11b+/Cd11b+) (FIG. 6B) in tumor tissues from KPC orthotopically implanted mice treated with Isotype IgG or anti-IL17/IL17R antibodies. FIGS. 6C-E: Flow cytometry analysis showing Tregs (FIG. 6C), MDSCs (FIG. 6D) and neutrophils (CD45+/Gr1+/Cd11b-) (FIG. 6E) cells in spleens from KPC orthotopically implanted mice treated with Isotype IgG or anti-IL17/IL17R antibodies. FIG.

6F: Relative mRNA expression of cytokines and chemokines in KPC cells *in vitro* stimulated by IL17 recombinant protein 10 ng/ml for 24 h compared to KPC cells control by RNAseq.

[0028] **FIGS. 7A-D.** FIG. 7A: Exhaustion markers Eomes and CD44 on CD4+ and CD8+T cells detected by flow cytometry on tumors from mice treated with isotype vs. IL17 blockade. FIGS. 7B-C: Response evaluation criteria in solid tumors (RECIST) analysis on KPC subcutaneously (FIG. 7B) or orthotopically (FIG. 7C) implanted mice treated with isotype IgG, anti-PD1, and anti-IL17/IL17R or anti-PD1/IL17/IL17R antibodies (At least a 30% decrease in tumor volume taken as responder (R)). FIG. 7D: Representative pictures of H&E, and immunohistochemistry for Ki67 and Cleaved caspase-3 on tumor tissues from KPC cells orthotopically implanted mice treated with Isotype IgG or anti-IL17/IL17R antibodies.

[0029] **FIGS. 8A-D.** Correlation analysis between tumor volume and CD8+ T cell frequency in the four treatment arms. FIG. 8A: Isotype IgG. FIG. 8B: aPD-1. FIG. 8C: aIL17/aIL17R. FIG. 8D: aIL17/aIL17R/aPD-1.

[0030] **FIGS. 9A-E.** FIGS. 9A-C: Correlation of DUOX2/DUOXA2 (FIG. 9A), DUOX2/GZMB (FIG. 9B), and DUOXA2/GZMB (FIG. 9C) mRNA expression in human PDAC on RNA-seq data retrieved from TCGA. FIG. 9D: Normalized lactate levels in tumor tissues from KPC orthotopically implanted mice treated with isotype IgG, anti-PD1, and anti-IL17/IL17R or anti-PD1/IL17/IL17R antibodies for 4 weeks. FIG. 9E: Normalized lactate levels in tumor tissues from KPC orthotopically implanted mice treated with isotype IgG or anti-IL17/IL17R for 2 weeks.

[0031] **FIG. 10.** IL17 blockade sensitizes tumors to other checkpoints like CTLA4. Quantification of tumor volume (mm³) from orthotopically implanted KPC cells into syngeneic mice treated with isotype IgG, aCTLA-4, aIL17/aIL17R or aIL17/aIL17R/aCTLA-4 antibodies.

DETAILED DESCRIPTION

[0032] Interleukin-17 (IL17), a cytokine secreted from immune cells recruited to the pancreas in response to Kras and inflammation, is involved in the initiation and development of pancreatic precursor lesions of PDAC by interacting with IL17 receptor, which is overexpressed in epithelium upon Kras activation, promoting a stemness signature. IL17

inhibition limits the neutrophils recruitment into the tumors, increases CD8+ T cells activation, and induces a spatial reconfiguration situating this cytotoxic cell population in closer contact with tumors. Moreover, IL17 blockade turned resistant tumors into immune checkpoint inhibitor-sensitive tumors, in a CD8+ T cells dependent manner. Furthermore, Duox2/Duoxa2 are novel biomarkers for selecting patients for treatment with the IL17/PD-1 combination. Lactate levels are decreased in tumors and/or serum prior to anti-tumoral efficacy, which makes it a biomarker for the efficacy of the IL17/PD-1 combination therapy.

I. Aspects of the Present Invention

[0033] Knowing that IL17 has a pro-tumorigenic role in initiation and progression of pancreatic premalignant lesions, the role of IL17 in established pancreatic cancer was studied and the mechanisms by which IL17 supports the immunosuppressive microenvironment that surrounds pancreatic cancer was explored. Furthermore, the mechanisms by which anti-IL17 monoclonal antibodies and PD-1 inhibitors exhibited synergistic anti-tumoral efficacy in preclinical models of pancreatic cancer was explored.

[0034] In this study, RNA sequencing of tumors, flow cytometry, immunohistochemical and multiplex analysis of tumors were used to understand the spatial remodeling of the tumor microenvironment. In short, IL17 blockade inhibits neutrophil recruitment to the tumors, which are not only decreased in numbers at the tumor site but also are located further away from CD8+ T cells, which exhibit an activated phenotype. Despite this potent immunomodulation induced by IL17 neutralizing antibodies, they are not capable of inducing anti-tumoral efficacy as single agents.

[0035] This study has identified therapeutic synergism between IL17 and immune checkpoint inhibition using pharmacological and CRISPR-Cas9 based genetic approaches in different preclinical models of PDAC. These results are not restricted to anti-PD-1 since combination of IL17 and CTLA-4 inhibitors is also synergistically effective against PDAC. Furthermore, using pharmacological and genetic suppression of CD8+ T cells, the synergistic effect of combining IL17 and PD-1 inhibition was found to be dependent on CD8+ T cells activation. Moreover, molecular mechanisms were searched for and it was found that IL17 can direct the expression and function of oxidases in 2 ways, first, by recruiting neutrophils, important source of Duox2/Duoxa2, into the tumors and second it directly regulates their expression in the oncogenic epithelium. Duox2 and Duoxa2 are important players in

regulating metabolism and regulating growth. Their main function is the regulation of H₂O₂ production, which can inhibit T cell activation. When looking at changes induced by the combination at the mRNA level, we found several metabolic pathways were involved and therefore explored metabolic biomarkers by NMR spectroscopy and found lactate as an early
5 predictor of responses to combination immunotherapy (FIG. 5).

[0036] Based on these findings, global metabolic changes induced by IL17 and the IL17/PD-1 combination were studied and significant changes in the lactate, which started early in the treatment course before any antitumoral effect was evident, were found, suggesting that imaging methods capable of measuring lactate (e.g., PET, MRI-based
10 hyperpolarization methods) could be useful as early biomarkers predictors of the combination's activity. This data suggests that, considering the delayed tumor responses usually seen with immunotherapies, metabolic imaging methods which rely on lactate measurements (ex: PET, MRI-based hyperpolarization methods) can be used for early prediction of responses to anti-IL17/anti-PD-1 activity. This is specifically important for
15 immunotherapies which usually have delayed anti-tumoral efficacy by standard imaging methodologies (Borcoman et al., 2018).

II. Inhibition of IL-17 signaling

[0037] An "IL-17 signaling inhibitor" as used herein includes any molecule that interferes with the function or binding of IL-17, blocks, and/or neutralizes a relevant activity
20 of IL-17. Thus, an IL-17 signaling inhibitor includes an anti-IL-17 antibody, an anti-IL-17 receptor antibody, including an anti-IL-17RA antibody and anti-IL-17RC antibody, or a soluble IL-17 receptor, including a soluble IL-17RA and a soluble IL-17RC.

[0038] An "IL-17 antibody," "anti-IL-17 antibody," or "antibody that binds to IL-17" refers to an antibody, or an antigen-binding fragment thereof, that is capable of binding to IL-
25 17A homodimer, IL-17F homodimer, and/or IL-17AF heterodimer, or a portion thereof, with sufficient affinity such that the antibody is useful as a detection, analytical, diagnostic and/or therapeutic agent in targeting IL-17. The IL-17 antibody may further interfere with IL-17 activities. In addition, the IL-17 antibody may interfere with expression of other genes or proteins. In an embodiment, the IL-17 antibody is capable of binding to IL-17AA, IL-17FF,
30 and/or IL-17AF. In some embodiments, an anti-IL17 antibody is capable of binding IL-17A homodimer. In some embodiments, an anti-IL17 antibody is capable of binding IL-17A

homodimer and IL-17AF heterodimer. In certain embodiments, an anti-IL-17 antibody is capable of binding to IL-17A homodimer and not capable of binding to IL-17AF heterodimer. In certain embodiments, an anti-IL-17 antibody is capable of binding to IL-17F homodimer and not capable of binding to IL-17AF heterodimer. In some embodiments, an anti-IL-17 antibody is capable of binding IL-17A homodimer, IL-17F homodimer, and IL-17AF heterodimer. In some such embodiments, an anti-IL-17 antibody that is capable of binding IL-17A homodimer, IL-17F homodimer, and IL-17AF heterodimer can also be referred to as an IL-17A and F antibody or IL-17A and IL-17F cross-reactive antibody or IL-17A/F cross-reactive antibody. In certain such embodiments, the IL-17A and F cross-reactive antibody binds to identical or similar epitopes on IL-17A, IL-17F and/or IL-17AF heterodimer. In certain embodiments, the IL-17A and F cross-reactive antibody binds to identical or similar epitopes on IL-17A, IL-17F and/or IL-17AF heterodimer with sufficient affinity. In certain advantageous embodiments, the IL-17A and F cross-reactive antibody binds to IL-17A, IL-17F and IL-17AF with high affinity. The structures of IL-17A and IL-17F have been reported. See Hymowitz et al., 2001, *Embo J*, 20(19):5332-41, Ely et al., 2009, *Nature Immunology* 10(12):1245-1252, and Liu et al., 2013, *Nature Communications* DOI: 10.1038/ncomms2880. Similar or identical epitopes comprising amino acid residues present in the surface area of IL-17A and IL-17F can be deduced from the structures.

[0039] In a particular embodiment, the antibody is an IL-17 antibody or an IL-17 receptor antibody. In an embodiment, the antibody is at least one antibody selected from the group brodalumab (e.g., U.S. Pat. No. 7,833,527), secukinumab (e.g., U.S. Pat. No. 7,807,155), ixekizumab (e.g., U.S. Pat. No. 7,838,638), bimekizumab (e.g., U.S. Pat. No. 8,580,265), CNTO 6785 (e.g., U.S. Pat. No. 8,519,107), ALX-0761 (e.g., U.S. Pub. No. 20140314743), and afasevikumab (the anti-IL17 CDR sequences as shown in U.S. Pat. No. 8,715,669). In a further embodiment, the antibody is an IL-17 antibody and the IL-17 antibody binds to an IL-17A homodimer, IL-17F homodimer, and/or IL-17AF heterodimer. In an embodiment, the antibody is an IL-17 antibody that binds to IL-17A homodimer. In another embodiment, the IL-17 antibody binds to IL-17AA and IL-17AF. In yet another embodiment, the antibody is an IL-17 antibody that binds to IL-17F homodimer. In a further embodiment, the antibody is an IL-17 antibody that binds IL-17A/F heterodimer. In another embodiment, the antibody is a monoclonal antibody. In yet another embodiment, the antibody is a chimeric, humanized, or human antibody. In a yet further embodiment, the antibody is a bispecific, multi specific, or cross-reactive antibody.

[0040] Antibodies according to the present disclosure may be defined, in the first instance, by their binding specificity. Those of skill in the art, by assessing the binding specificity/affinity of a given antibody using techniques well known to those of skill in the art, can determine whether such antibodies fall within the scope of the instant claims. For example, the epitope to which a given antibody bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) amino acids located within the antigen molecule (*e.g.*, a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within the antigen molecule (*e.g.*, a conformational epitope).

[0041] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody “interacts with one or more amino acids” within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Cross-blocking can be measured in various binding assays such as ELISA, biolayer interferometry, or surface plasmon resonance. Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis, high-resolution electron microscopy techniques using single particle reconstruction, cryoEM, or tomography, crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. See,

e.g., Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A.

[0042] The term “epitope” refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Here, the preferred epitope is a conformational epitope that is present in homotrimeric type I collagen but absent in heterotrimeric type I collagen.

[0043] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the disclosure into groups of antibodies binding different epitopes.

[0044] The present disclosure includes antibodies that may bind to the same epitope, or a portion of the epitope. Likewise, the present disclosure also includes antibodies that compete for binding to a target or a fragment thereof with any of the specific exemplary antibodies described herein. One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference, the reference antibody is allowed to bind to target under saturating conditions. Next, the ability of a test antibody to bind to the target molecule is assessed. If the test antibody is able to bind to the target molecule following saturation binding with the reference antibody, it can be concluded that the test antibody binds to a different epitope than the

reference antibody. On the other hand, if the test antibody is not able to bind to the target molecule following saturation binding with the reference antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference antibody.

[0045] Two antibodies bind to the same or overlapping epitope if each competitively
5 inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially
10 all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0046] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test
15 antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. Structural studies with EM or crystallography also can demonstrate whether or not
20 two antibodies that compete for binding recognize the same epitope.

[0047] In another aspect, the antibodies may be defined by their variable sequence, which include additional “framework” regions. Furthermore, the antibodies sequences may vary from these sequences, optionally using methods discussed in greater detail below. For example, nucleic acid sequences may vary from those set out above in that (a) the variable
25 regions may be segregated away from the constant domains of the light and heavy chains, (b) the nucleic acids may vary from those set out above while not affecting the residues encoded thereby, (c) the nucleic acids may vary from those set out above by a given percentage, *e.g.*, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, (d) the nucleic acids may vary from those set out above by virtue of the ability to hybridize
30 under high stringency conditions, as exemplified by low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C, (e) the amino acids may vary from those set out above by a given

percentage, *e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, or (f) the amino acids may vary from those set out above by permitting conservative substitutions (discussed below).

[0048] When comparing polynucleotide and polypeptide sequences, two sequences
5 are said to be “identical” if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous
10 positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0049] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc.,
15 Madison, Wis.), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M. O. (1978) A model of evolutionary change in proteins--Matrices for detecting distant relationships. In Dayhoff, M. O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington D.C. Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and
20 Phylogeny pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, Calif.; Higgins, D. G. and Sharp, P. M. (1989) CABIOS 5:151-153; Myers, E. W. and Muller W. (1988) CABIOS 4:11-17; Robinson, E. D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) Mol. Biol. Evol. 4:406-425; Sneath, P. H. A. and Sokal, R. R. (1973) Numerical Taxonomy--the Principles and Practice of Numerical Taxonomy, Freeman Press, San
25 Francisco, Calif.; Wilbur, W. J. and Lipman, D. J. (1983) Proc. Natl. Acad., Sci. USA 80:726-730.

[0050] Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) Add. APL. Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol.
30 48:443, by the search for similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. USA 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT,

BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.), or by inspection.

[0051] One particular example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) Nucl. Acids Res. 25:3389-3402 and Altschul *et al.* (1990) J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the disclosure. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. The rearranged nature of an antibody sequence and the variable length of each gene requires multiple rounds of BLAST searches for a single antibody sequence. Also, manual assembly of different genes is difficult and error-prone. The sequence analysis tool IgBLAST (world-wide-web at ncbi.nlm.nih.gov/igblast/) identifies matches to the germline V, D and J genes, details at rearrangement junctions, the delineation of Ig V domain framework regions and complementarity determining regions. IgBLAST can analyze nucleotide or protein sequences and can process sequences in batches and allows searches against the germline gene databases and other sequence databases simultaneously to minimize the chance of missing possibly the best matching germline V gene.

[0052] In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

[0053] For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the

cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

[0054] In one approach, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0055] Yet another way of defining an antibody is as a “derivative” of any of the below-described antibodies and their antigen-binding fragments. The term “derivative” refers to an antibody or antigen-binding fragment thereof that immunospecifically binds to an antigen but which comprises, one, two, three, four, five or more amino acid substitutions, additions, deletions or modifications relative to a “parental” (or wild-type) molecule. Such amino acid substitutions or additions may introduce naturally occurring (*i.e.*, DNA-encoded) or non-naturally occurring amino acid residues. The term “derivative” encompasses, for example, as variants having altered CH1, hinge, CH2, CH3 or CH4 regions, so as to form, for example antibodies, *etc.*, having variant Fc regions that exhibit enhanced or impaired effector or binding characteristics. The term “derivative” additionally encompasses non-amino acid modifications, for example, amino acids that may be glycosylated (*e.g.*, have altered mannose, 2-N-acetylglucosamine, galactose, fucose, glucose, sialic acid, 5-N-acetylneuraminic acid, 5-glycolneuraminic acid, *etc.* content), acetylated, pegylated, phosphorylated, amidated, derivatized by known protecting/blocking groups, proteolytic cleavage, linked to a cellular ligand or other protein, *etc.* In some embodiments, the altered carbohydrate modifications modulate one or more of the following: solubilization of the antibody, facilitation of subcellular transport and secretion of the antibody, promotion of antibody assembly, conformational integrity, and antibody-mediated effector function. In a

specific embodiment the altered carbohydrate modifications enhance antibody mediated effector function relative to the antibody lacking the carbohydrate modification. Carbohydrate modifications that lead to altered antibody mediated effector function are well known in the art (for example, see Shields, R. L. et al. (2002) "Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcγ₃ And Antibody-Dependent Cellular Toxicity," J. Biol. Chem. 277(30): 26733-26740; Davies J. et al. (2001) "Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line: Expression Of Antibodies With Altered Glycoforms Leads To An Increase In ADCC Through Higher Affinity For FC γ₃ RIII," Biotechnology & Bioengineering 74(4): 288-294). Methods of altering carbohydrate contents are known to those skilled in the art, see, e.g., Wallick, S. C. et al. (1988) "Glycosylation Of A VH Residue Of A Monoclonal Antibody Against Alpha (1----6) Dextran Increases Its Affinity For Antigen," J. Exp. Med. 168(3): 1099-1109; Tao, M. H. et al. (1989) "Studies Of Aglycosylated Chimeric Mouse-Human IgG. Role Of Carbohydrate In The Structure And Effector Functions Mediated By The Human IgG Constant Region," J. Immunol. 143(8): 2595-2601; Routledge, E. G. et al. (1995) "The Effect Of Aglycosylation On The Immunogenicity Of A Humanized Therapeutic CD3 Monoclonal Antibody," Transplantation 60(8):847-53; Elliott, S. et al. (2003) "Enhancement Of Therapeutic Protein In Vivo Activities Through Glycoengineering," Nature Biotechnol. 21:414-21; Shields, R. L. et al. (2002) "Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcγ₃ RIII And Antibody-Dependent Cellular Toxicity," J. Biol. Chem. 277(30): 26733-26740).

[0056] A derivative antibody or antibody fragment can be generated with an engineered sequence or glycosylation state to confer preferred levels of activity in antibody dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), or antibody-dependent complement deposition (ADCD) functions as measured by bead-based or cell-based assays or *in vivo* studies in animal models.

[0057] A derivative antibody or antibody fragment may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, *etc.* In one embodiment, an antibody derivative will possess a similar or identical function as the parental antibody. In another embodiment, an antibody derivative will exhibit an altered

activity relative to the parental antibody. For example, a derivative antibody (or fragment thereof) can bind to its epitope more tightly or be more resistant to proteolysis than the parental antibody.

III. Immune Checkpoint Inhibitors

5 **[0058]** Immunomodulatory agents include immune checkpoint inhibitors, agonists of co-stimulatory molecules, and antagonists of immune inhibitory molecules. The immunomodulatory agents may be drugs, such as small molecules, recombinant forms of ligand or receptors, or antibodies, such as human antibodies (*e.g.*, International Patent Publication WO2015/016718; Pardoll, *Nat Rev Cancer*, 12(4): 252-264, 2012; both
10 incorporated herein by reference). Known inhibitors of immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized, or human forms of antibodies may be used. As the skilled person will know, alternative and/or equivalent names may be in use for certain antibodies mentioned in the present disclosure. Such alternative and/or equivalent names are interchangeable in the context of the present disclosure. For
15 example, it is known that lambrolizumab is also known under the alternative and equivalent names MK-3475 and pembrolizumab.

[0059] Co-stimulatory molecules are ligands that interact with receptors on the surface of the immune cells, *e.g.*, CD28, 4-1BB, OX40 (also known as CD134), ICOS, and GITR. As an example, the complete protein sequence of human OX40 has Genbank
20 accession number NP_003318. In some embodiments, the immunomodulatory agent is an anti-OX40 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-OX40 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively,
25 art recognized anti-OX40 antibodies can be used. An exemplary anti-OX40 antibody is PF-04518600 (see, *e.g.*, WO 2017/130076). ATOR-1015 is a bispecific antibody targeting CTLA4 and OX40 (see, *e.g.*, WO 2017/182672, WO 2018/091740, WO 2018/202649, WO 2018/002339).

[0060] Another co-stimulatory molecule that can be targeted in the methods provided
30 herein is ICOS, also known as CD278. The complete protein sequence of human ICOS has Genbank accession number NP_036224. In some embodiments, the immune checkpoint

inhibitor is an anti-ICOS antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-ICOS antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art.

5 Alternatively, art recognized anti-ICOS antibodies can be used. Exemplary anti-ICOS antibodies include JTX-2011 (see, *e.g.*, WO 2016/154177, WO 2018/187191) and GSK3359609 (see, *e.g.*, WO 2016/059602).

[0061] Yet another co-stimulatory molecule that can be targeted in the methods provided herein is glucocorticoid-induced tumour necrosis factor receptor-related protein

10 (GITR), also known as TNFRSF18 and AITR. The complete protein sequence of human GITR has Genbank accession number NP_004186. In some embodiments, the immunomodulatory agent is an anti-GITR antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-GITR antibodies (or VH and/or VL domains

15 derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-GITR antibodies can be used. An exemplary anti-GITR antibody is TRX518 (see, *e.g.*, WO 2006/105021).

[0062] Immune checkpoint proteins that may be targeted by immune checkpoint blockade include adenosine A2A receptor (A2AR), B7-H3 (also known as CD276), B and T

20 lymphocyte attenuator (BTLA), CCL5, CD27, CD38, CD8A, CMKLR1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), CXCL9, CXCR5, HLA-DRB1, HLA-DQA1, HLA-E, killer-cell immunoglobulin (KIR), lymphocyte activation gene-3 (LAG-3, also known as CD223), Mer tyrosine kinase (MerTK), NKG7, programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1, also known as CD274), PDCD1LG2,

25 PSMB10, STAT1, T cell immunoreceptor with Ig and ITIM domains (TIGIT), T-cell immunoglobulin domain and mucin domain 3 (TIM-3), and V-domain Ig suppressor of T cell activation (VISTA, also known as C10orf54). In particular, immune checkpoint inhibitors targeting the PD-1 axis and/or CTLA-4 have received FDA approval broadly across diverse cancer types.

30 [0063] In some embodiments, a PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding

antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or an oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. 8,735,553, 8,354,509, and 8,008,449, all of which are incorporated herein by reference. Other PD-1 axis antagonists for use in the methods provided herein are known in the art, such as described in U.S. Patent Application Publication Nos. 2014/0294898, 2014/022021, and 2011/0008369, all of which are incorporated herein by reference.

[0064] In some embodiments, a PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and CT-011. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence)). In some embodiments, the PD-1 binding antagonist is AMP- 224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0065] Another immune checkpoint protein that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an “off” switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CTLA-4 is similar 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 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA-4 is also found in regulatory T cells and may be important to their function. T cell activation through

the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[0066] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in US Patent No. 8,119,129; PCT Publn. Nos. WO 01/14424, WO 98/42752, WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab); U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc Natl Acad Sci USA*, 95(17): 10067-10071; Camacho *et al.* (2004) *J Clin Oncology*, 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res*, 58:5301-5304 can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No. WO2001/014424, WO2000/037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[0067] An exemplary anti-CTLA-4 antibody is ipilimumab (also known as 10D1, MDX- 010, MDX- 101, and Yervoy®) or antigen binding fragments and variants thereof (see, *e.g.*, WO 01/14424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises the CDR1, CDR2, and CDR3 domains of the VH region of ipilimumab, and the CDR1, CDR2, and CDR3 domains of the VL region of ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on CTLA-4 as the above-mentioned antibodies. In another embodiment, the antibody has an at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95%, or 99% variable region identity with ipilimumab). Other molecules for modulating CTLA-4 include CTLA-4 ligands and receptors such as described in U.S. Patent Nos. 5844905, 5885796 and International Patent Application Nos. WO1995001994 and WO1998042752; all incorporated herein by reference, and immunoadhesins such as described in U.S. Patent No. 8329867, incorporated herein by reference.

[0068] Another immune checkpoint protein that can be targeted in the methods provided herein is lymphocyte-activation gene 3 (LAG-3), also known as CD223. The complete protein sequence of human LAG-3 has the Genbank accession number NP-002277. LAG-3 is found on the surface of activated T cells, natural killer cells, B cells, and plasmacytoid dendritic cells. LAG-3 acts as an “off” switch when bound to MHC class II on the surface of antigen-presenting cells. Inhibition of LAG-3 both activates effector T cells and inhibitor regulatory T cells. In some embodiments, the immune checkpoint inhibitor is an anti-LAG-3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-LAG-3 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-LAG-3 antibodies can be used. An exemplary anti-LAG-3 antibody is relatlimab (also known as BMS-986016) or antigen binding fragments and variants thereof (see, *e.g.*, WO 2015/116539). Other exemplary anti-LAG-3 antibodies include TSR-033 (see, *e.g.*, WO 2018/201096), MK-4280, and REGN3767. MGD013 is an anti-LAG-3/PD-1 bispecific antibody described in WO 2017/019846. FS118 is an anti-LAG-3/PD-L1 bispecific antibody described in WO 2017/220569.

[0069] Another immune checkpoint protein that can be targeted in the methods provided herein is V-domain Ig suppressor of T cell activation (VISTA), also known as C10orf54. The complete protein sequence of human VISTA has the Genbank accession number NP_071436. VISTA is found on white blood cells and inhibits T cell effector function. In some embodiments, the immune checkpoint inhibitor is an anti-VISTA3 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-VISTA antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-VISTA antibodies can be used. An exemplary anti-VISTA antibody is JNJ-61610588 (also known as onvatilimab) (see, *e.g.*, WO 2015/097536, WO 2016/207717, WO 2017/137830, WO 2017/175058). VISTA can also be inhibited with the small molecule CA-170, which selectively targets both PD-L1 and VISTA (see, *e.g.*, WO 2015/033299, WO 2015/033301).

[0070] Another immune checkpoint protein that can be targeted in the methods provided herein is CD38. The complete protein sequence of human CD38 has Genbank accession number NP_001766. In some embodiments, the immune checkpoint inhibitor is an anti-CD38 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody),
5 an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-CD38 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CD38 antibodies can be used. An exemplary anti-CD38 antibody is daratumumab (see, *e.g.*, U.S. Pat. No. 7,829,673).

[0071] Another immune checkpoint protein that can be targeted in the methods provided herein is T cell immunoreceptor with Ig and ITIM domains (TIGIT). The complete protein sequence of human TIGIT has Genbank accession number NP_776160. In some
10 embodiments, the immune checkpoint inhibitor is an anti-TIGIT antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment
15 thereof, an immunoadhesin, a fusion protein, or oligopeptide. Anti-human-TIGIT antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-TIGIT antibodies can be used. An exemplary anti-TIGIT antibody is MK-7684 (see, *e.g.*, WO 2017/030823, WO 2016/028656).

[0072] Other immune inhibitory molecules that can be targeted for immunomodulation include STAT3 and indoleamine 2,3-dioxygenase (IDO). By way of example, the complete protein sequence of human IDO has Genbank accession number NP_002155. In some embodiments, the immunomodulatory agent is a small molecule IDO inhibitor. Exemplary small molecules include BMS-986205, epacadostat (INCB24360), and
20 navoximod (GDC-0919).
25

IV. Methods of Treatment

[0073] The present invention provides methods of treating a cancer patient with a combination of an IL-17 signaling inhibitor and an immune checkpoint inhibitor. Such treatment may also be in combination with another therapeutic regime, such as
30 chemotherapy. Certain aspects of the present invention can be used to select a cancer patient for treatment based on the presence of upregulated Duoxa2, Duox2, and/or Duox1 expression

in the patient's tumor. In various aspects, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the cells that comprise the cancer may harbor an increase in one or more of the listed markers. Thus, in some aspects, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 5 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the cells that comprise the cancer may comprise normal levels of one or more of the listed markers. In other aspects, various percentages of cells comprising the cancer may harbor an altered expression level of one or more the listed markers. Other aspects of the present invention provide for selecting a cancer patient for treatment based on the patient having previously failed to respond to the 10 administration of an immune checkpoint inhibitor. In some embodiments, methods are provided for assessing the efficacy of treatment by measuring, for example, lactate in the patient's tumor and/or serum, where a decrease in lactate levels is indicative of efficacy. In some aspects, lactate levels serve as an early marker of efficacy such that decreased lactate levels may be detection before any antitumoral effect is otherwise evident.

15 **[0074]** The term "subject" or "patient" as used herein refers to any individual to which the subject methods are performed. Generally the patient is human, although as will be appreciated by those in the art, the patient 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 20 (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of patient.

[0075] "Treatment" and "treating" refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For 25 example, a treatment may include administration chemotherapy, immunotherapy, radiotherapy, performance of surgery, or any combination thereof.

[0076] The methods described herein are useful in treating cancer. Generally, the terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. More specifically, cancers that are 30 treated in connection with the methods provided herein include, but are not limited to, solid tumors, metastatic cancers, or non-metastatic cancers. In certain embodiments, the cancer may originate in the lung, kidney, bladder, blood, bone, bone marrow, brain, breast, colon,

esophagus, duodenum, small intestine, large intestine, colon, rectum, anus, gum, head, liver, nasopharynx, neck, ovary, pancreas, prostate, skin, stomach, testis, tongue, or uterus.

[0077] The cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; non-small cell lung cancer; 5 renal cancer; renal cell carcinoma; clear cell renal cell carcinoma; lymphoma; blastoma; sarcoma; carcinoma, undifferentiated; meningioma; brain cancer; oropharyngeal cancer; nasopharyngeal cancer; biliary cancer; pheochromocytoma; pancreatic islet cell cancer; Li-Fraumeni tumor; thyroid cancer; parathyroid cancer; pituitary tumor; adrenal gland tumor; osteogenic sarcoma tumor; neuroendocrine tumor; breast cancer; lung cancer; head and neck 10 cancer; prostate cancer; esophageal cancer; tracheal cancer; liver cancer; bladder cancer; stomach cancer; pancreatic cancer; ovarian cancer; uterine cancer; cervical cancer; testicular cancer; colon cancer; rectal cancer; skin cancer; giant and spindle cell carcinoma; small cell carcinoma; small cell lung cancer; papillary carcinoma; oral cancer; oropharyngeal cancer; nasopharyngeal cancer; respiratory cancer; urogenital cancer; squamous cell carcinoma; 15 lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrointestinal cancer; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial 20 polyposis coli; solid carcinoma; carcinoid tumor, malignant; bronchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; 25 skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar 30 cell carcinoma; adenosquamous carcinoma; adenocarcinoma with squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary

paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; lentigo maligna melanoma; acral lentiginous melanoma; nodular melanoma; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; an endocrine or neuroendocrine cancer or hematopoietic cancer; pinealoma, malignant; chordoma; central or peripheral nervous system tissue cancer; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; B-cell lymphoma; malignant lymphoma; Hodgkin's disease; Hodgkin's; low grade/follicular non-Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; mantle cell lymphoma; Waldenstrom's macroglobulinemia; other specified non-hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and hairy cell leukemia.

[0078] The term “therapeutic benefit” or “therapeutically effective” as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

[0079] Likewise, an effective response of a patient or a patient’s “responsiveness” to treatment refers to the clinical or therapeutic benefit imparted to a patient at risk for, or suffering from, a disease or disorder. Such benefit may include cellular or biological responses, a complete response, a partial response, a stable disease (without progression or relapse), or a response with a later relapse. For example, an effective response can be reduced tumor size or progression-free survival in a patient diagnosed with cancer.

[0080] Regarding neoplastic condition treatment, depending on the stage of the neoplastic condition, neoplastic condition treatment involves one or a combination of the following therapies: surgery to remove the neoplastic tissue, radiation therapy, and chemotherapy. Other therapeutic regimens may be combined with the administration of the anticancer agents, e.g., therapeutic compositions and chemotherapeutic agents. For example, the patient to be treated with such anti-cancer agents may also receive radiation therapy and/or may undergo surgery.

[0081] In the case of non-small cell lung cancer, the patient may undergo surgery to remove cancerous tissue. The patient may undergo chemotherapy with one or more of gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, and docetaxel.

[0082] For the treatment of disease, the appropriate dosage of a therapeutic composition will depend on the type of disease to be treated, as defined above, the severity and course of the disease, previous therapy, the patient’s clinical history and response to the agent, and the discretion of the physician. The agent may be suitably administered to the patient at one time or over a series of treatments.

A. Combination Treatments

[0083] The methods and compositions, including combination therapies, enhance the therapeutic or protective effect, and/or increase the therapeutic effect of another anti-cancer or anti-hyperproliferative therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect, such as the killing of a cancer cell and/or the inhibition of cellular hyperproliferation. A tissue, tumor, or cell can be contacted with one or more compositions or pharmacological formulation(s) comprising one or more of the agents or by contacting the tissue, tumor, and/or cell with two or more distinct compositions or formulations. Also, it is contemplated that such a combination therapy can be used in conjunction with radiotherapy, surgical therapy, or immunotherapy.

[0084] Administration in combination can include simultaneous administration of two or more agents in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, the subject therapeutic composition and another therapeutic agent can be formulated together in the same dosage form and administered simultaneously. Alternatively, subject therapeutic composition and another therapeutic agent can be simultaneously administered, wherein both the agents are present in separate formulations. In another alternative, the therapeutic agent can be administered just followed by the other therapeutic agent or vice versa. In the separate administration protocol, the subject therapeutic composition and another therapeutic agent may be administered a few minutes apart, or a few hours apart, or a few days apart.

[0085] An anti-cancer first treatment may be administered before, during, after, or in various combinations relative to a second anti-cancer treatment. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the first treatment is provided to a patient separately from the second treatment, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the first therapy and the second therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[0086] In certain embodiments, a course of treatment will last 1-90 days or more (this such range includes intervening days). It is contemplated that one agent may be given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof, and another agent is given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment, it is contemplated that there is a period of time at which no anti-cancer treatment is administered. This time period may last 1-7 days, and/or 1-5 weeks, and/or 1-12 months or more (this such range includes intervening days), depending on the condition of the patient, such as their prognosis, strength, health, *etc.* It is expected that the treatment cycles would be repeated as necessary.

[0087] Various combinations may be employed. For the example below a combination of an IL-17 signaling inhibitor and an immune checkpoint inhibitor is “A” and another anti-cancer therapy is “B”:

[0088] A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

[0089] B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A

[0090] B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[0091] Administration of any compound or therapy of the present invention to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

1. Chemotherapy

[0092] A wide variety of chemotherapeutic agents may be used in accordance with the present invention. The term “chemotherapy” refers to the use of drugs to treat cancer. A “chemotherapeutic agent” is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA,

to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[0093] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclophosphamide; alkyl sulfonates, such as busulfan, improsulfan, and pposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics, such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammall and calicheamicin omegaI1); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone;

aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKpolysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatin, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

2. Radiotherapy

[0094] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

3. Immunotherapy

[0095] The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the invention. In the context

of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (Rituxan®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may
5 recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, *etc.*) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

10 **[0096]** In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor,
15 erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[0097] Examples of immunotherapies currently under investigation or in use
20 are immune adjuvants, *e.g.*, Mycobacterium bovis, Plasmodium falciparum, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, Infection Immun., 66(11):5329-5336, 1998; Christodoulides et al., Microbiology, 144(Pt 11):3027-3037, 1998); cytokine therapy, *e.g.*, interferons α , β , and γ , IL-1, GM-CSF, and TNF (Bukowski et al., Clinical Cancer Res., 4(10):2337-2347, 1998;
25 Davidson et al., J. Immunother., 21(5):389-398, 1998; Hellstrand et al., Acta Oncologica, 37(4):347-353, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin et al., Proc. Natl. Acad. Sci. USA, 95(24):14411-14416, 1998; Austin-Ward and Villaseca, Revista Medica de Chile, 126(7):838-845, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hanibuchi et al., Int. J.
30 Cancer, 78(4):480-485, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

[0098] In some embodiment, the immune therapy could be adoptive immunotherapy, which involves the transfer of autologous antigen- specific T cells generated *ex vivo*. The T cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific T cells or redirection of T cells through genetic engineering. Isolation and transfer of tumor specific T cells has been shown to be successful in treating melanoma. Novel specificities in T cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single fusion molecule. In general, the binding moiety of a CAR consists of an antigen-binding domain of a single-chain antibody (scFv), comprising the light and variable fragments of a monoclonal antibody joined by a flexible linker. Binding moieties based on receptor or ligand domains have also been used successfully. The signaling domains for first generation CARs are derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. CARs have successfully allowed T cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors.

[0099] In one embodiment, the present application provides for a combination therapy for the treatment of cancer wherein the combination therapy comprises adoptive T cell therapy and a checkpoint inhibitor. In one aspect, the adoptive T cell therapy comprises autologous and/or allogenic T-cells. In another aspect, the autologous and/or allogenic T-cells are targeted against tumor antigens.

4. Surgery

[00100] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[00101] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or
5 every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

5. Other Agents

[00102] It is contemplated that other agents may be used in combination with certain aspects of the present invention to improve the therapeutic efficacy of treatment.
10 These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring
15 hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents
20 that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present invention to improve the treatment efficacy.

V. Kits and Diagnostics

[00103] In various aspects of the invention, a kit is envisioned containing,
25 diagnostic agents, therapeutic agents and/or delivery agents. In some embodiments, the kit may comprise reagents for assessing a patient selection marker, such as Duoxa2, Duox2, and/or Duox1 expression levels, in a patient sample. In some embodiments, the kit may comprise reagents for assessing the efficacy of treatment by measuring, for example, lactate in the patient's tumor and/or serum. In some embodiments, the present invention
30 contemplates a kit for preparing and/or administering a therapy of the invention. The kit may comprise reagents capable of use in administering an active or effective agent(s) of the invention. Reagents of the kit may include one or more anti-cancer component of a

combination therapy, as well as reagents to prepare, formulate, and/or administer the components of the invention or perform one or more steps of the inventive methods.

[00104] In some embodiments, the kit may also comprise a suitable container means, which is a container that will not react with components of the kit, such as an eppendorf tube, an assay plate, a syringe, a bottle, or a tube. The container may be made from sterilizable materials such as plastic or glass. The kit may further include an instruction sheet that outlines the procedural steps of the methods, and will follow substantially the same procedures as described herein or are known to those of ordinary skill.

VI. Examples

[00105] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Materials and Methods

[00106] *Cell lines.* Murine pancreatic adenocarcinoma cells derived from a spontaneous tumor in a KRAS^{G12D}; Trp53^{R172H}; Pdx1-Cre (KPC) mouse were used and named “KPC” cells. Cells were cultivated in Dulbecco’s modified Eagle medium (DMEM) with 4.5 g/L glucose (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (Sigma Aldrich, St. Louis, MO) and 1% penicillin/streptomycin (HyClone, South Logan, UT) at 37°C and 5% CO₂ in a humidified atmosphere.

[00107] *IL17 in vitro stimulation.* Murine recombinant IL17A proteins (R&D Systems, Minneapolis, MN) were using to stimulate cells at a concentration of 10 ng/mL.

[00108] *Animal models.* All animal experiments were conducted in compliance with the National Institutes of Health guidelines for animal research and were approved by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson

Cancer Center (MDACC). C57BL/6 mice purchased from Taconic Biosciences (Hudson, NY) were used for most experiments. mTmG mice were used as recipient for some of the experiments. CD8 knockout mice were purchased from Jackson Laboratory (Bar Harbor, ME). For orthotopic PDAC mouse models, eight-week-old male mice were anesthetized by inhalation of 2% isoflurane in oxygen. An incision was made on the left side of mouse to exteriorize the pancreas. 1×10^5 KPC cells in 10 μ l PBS/ Matrigel (Corning, NY) (v:v=1:1) were injected into pancreas. The incision was closed with 6-0 polyglycolic acid sutures (CP Medical, Portland, OR). For subcutaneous models, 5×10^5 KPC cells in 100 μ l PBS/ Matrigel (v:v=1:1) were injected into mouse flank subcutaneously.

[00109] *Neutralizing antibody administration.* Neutralizing antibodies against mouse IL17, IL17R, IL17E (generously provided by Amgen, Thousand Oaks, CA), PD-1, CD8a, and Rat IgG (Bio X Cell, Lebanon, NH). Doses, frequencies, species in which antibodies were raised, reactivity and injection mode are tabulated in Table 1.

Table 1. Antibody administration.

Antibody	Vendor	Species	Reactivity	Dose	Inoculation mode
IL17	Amgen	Rat	mouse	200 μ g/ twice a week	Intraperitoneal
IL17R	Amgen	Rat	mouse	200 μ g/ twice a week	Intraperitoneal
IL17E	Amgen	Rat	mouse	200 μ g/ twice a week	Intraperitoneal
PD1	Bio X Cell	Rat	mouse	100 μ g/ twice a week	Intraperitoneal
CD8	Bio X Cell	Rat	mouse	200 μ g/ twice a week	Intraperitoneal
IgG	Bio X Cell	Rat	mouse	200 μ g/ twice a week	Intraperitoneal

[00110] *Immunohistochemistry.* Paraformaldehyde-fixed, paraffin-embedded tissue sections were deparaffinized, rehydrated, and then boiled in EZ-retriever system (BioGenex, Fremont, CA) with 0.01 mol/L citrate buffer, pH 6.0 (Sigma-Aldrich, St. Louis, MO) for antigen retrieval. Endogenous peroxidases were blocked with 0.3% H_2O_2 for 15 minutes. Nonspecific epitopes were blocked with 10% normal goat serum (Seracare Life Sciences, Milford, MA) for 30 minutes. The sections were incubated overnight at 4°C with antibodies against one of the following mouse proteins: cleaved Caspase-3, Ki-67, CD8a, Gzmb. Antibody details, including final concentrations, can be found in Table 2. This was

followed by using a SignalStain Boost IHC Detection Reagent and DAB Substrate Kit (Cell Signaling Technology, Danvers, MA) following the manufacturer's instructions. Slides were then counterstained with hematoxylin, mounted in Acrymount (StatLab, McKinney, TX), and visualized under a light microscope.

5 **[00111]** *Opal multiplex IF.* Staining was performed manually using the same primary antibodies used for IHC analysis against the immune markers at specific: Monoclonal Anti-Mouse CD8a, Gr1, SMA, Gzmb, CK19. Staining was performed consecutively by using the same steps used in IHC, and the detection for each marker was completed before application of the next antibody. Details on primary antibodies are found in
10 Table 2. The Opal Polymer HRP Ms + Rb detection reagent (PerkinElmer, Boston, MA) was used for the primary antibody detection and Opal 7-Color Manual IHC, with 6 reactive fluorophores Opal 520, Opal 540, Opal 570, Opal 620, Opal 650, Opal and 690 plus DAPI nuclear counterstain, according to the manufacturer's instructions (catalogue # NEL811001KT PerkinElmer, Waltham, MA). Uniplex IF and Negative control were staining
15 with the same protocols. Slides were imaged using the Vectra 3.0 spectral imaging system (PerkinElmer) according to previously published instructions.

[00112] *Flow cytometry.* To characterize different subpopulations of immune cell from mice orthotopic PDAC tumor. Tumor tissues were harvested and digested into single cell suspensions by Collagenase P. The obtained cells were stained with Rat Anti-
20 Mouse CD45, Rat Anti-Mouse CD4, Rat Anti-Mouse Foxp3, Rat Anti-Mouse Ly-6G, Rat Anti-Mouse IFN- γ , CD8a Monoclonal Antibody. Antibodies details including final concentrations can be found in Table 2. Sample acquisition was carried out on LSRFortessa X-20 Analyzer Flow Cytometer (BD Biosciences, Franklin Lakes, NJ). Analysis was performed with FlowJo version 10 (Tree Star Inc., Ashland, OR).

25 **Table 2.** Antibodies.

Antibody	Vendor/ clone	Species	Reactivity	Concentration	Use
Cleaved Caspase-3	Cell Signaling Technology, 5A1E	Rabbit	H M R Mk	1:200	IHC
Ki67	Cell Signaling Technology, D3B5	Rabbit	Mouse	1:200	IHC

CD8	Cell Signaling Technology, D4W2Z	Rabbit	Mouse	1/200	IHC/ Opal Multiplex IF
Gzmb	eBioscience, 16G6	Rat	Mouse	1/100	IHC/ Opal Multiplex IF
CK19	DSHB, Troma-III-S	Rat	Mouse	1/40	Opal Multiplex IF
SMA	Invitrogen, PA5-16697	Rabbit	Several species	1/200	Opal Multiplex IF
CD45	BD Pharmigen	Rat	Mouse	0.5 µg	Flow Cytometry
CD4	BD Pharmigen, PerCP-Cy5.5, 30-F11	Rat	Mouse	0.5 µg	Flow Cytometry
Foxp3	BD Horizon, PE-CF594, RM4-5	Rat	Mouse	0.5 µg	Flow Cytometry
Ly-6G	BD Pharmigen, RB6-8C5	Rat	Mouse	0.5 µg	Flow Cytometry
CD8	EBioscience, 53-6.7	Rat	Mouse	0.5 µg	Flow Cytometry
Ly-6G	BD Pharmigen	Rat	Mouse	0.5 µg	Flow Cytometry

[00113] *RNA isolation and quantitative reverse transcription polymerase chain reaction.* Total RNA was extracted with RNeasy RNA isolation kit (Qiagen, Valencia, CA) and reverse transcribed with a cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative reverse transcription polymerase chain reaction was performed with Fast SYBRTM Green Master Mix (Applied Biosystems, Foster City, CA) on a ViiA 7 Real-Time PCR System machine (Applied Biosystems, Foster City, CA). Sequences of all validated PCR primers were obtained from PrimerBank (available at pga.mgh.harvard.edu/primerbank/); and primers were synthesized by Sigma-Aldrich (St. Louis, MO). GAPDH was used for normalization. Assays were run in triplicate.

[00114] *Histopathology.* Tumor-bearing mice were humanely sacrificed, and the pancreas or implanted tumors were excised, fixed in freshly prepared 4% paraformaldehyde in phosphate buffered saline, pH 7.2. Tissues were embedded in paraffin, and 5-µm sections obtained and stained with hematoxylin (Dako, Santa Clara, CA) and eosin

(VWR, West Chester, PA) following standard protocols for visual examination. The stained slides were reviewed and screened for representative tumor regions by a pathologist.

[00115] *Human Pancreatic Tumor microarrays.* Human pancreatic adenocarcinoma and normal tissue microarrays were collected and made by The University of Texas MD Anderson Cancer Center. IRB approved protocols include their use for biomarkers discovery.

[00116] *RNA sequencing.* KPC orthotopic PDAC mouse models were treated with isotype IgG or anti-IL17/IL17R neutralizing antibodies for two weeks. Total RNA was extracted from tumor tissues. Each sample was assessed using Qubit 2.0 fluorometer and Agilent TapeStation 2200 for RNA quantity and quality. Then sequencing was performed on Illumina NextSeq 500s, as previously described. Sequencing analysis was done using mRNA-seq Analysis on Maverix Analytic Platform (Maverix Biomics, Inc, San Mateo, CA). RNA sequencing data is being deposited at Sequence Read Archive (SRA accession number pending). Data can be found in Table 3.

Table 3. RNA Seq on tumors from mice treated with Isotype IgG vs IL-17/IL17RA monoclonal antibodies.

Gene ID	Gene Symbol	IL17 blockade vs Isotype fold changes	IL17 blockade vs Isotype p value
15130	Hbb-b2	-5.87659	0.00715563
15122	Hba-a1	-5.31419	0.00715563
399549	H2-M10.6	-5.14882	0.00715563
15129	Hbb-b1	-4.99837	0.00715563
11656	Alas2	-4.5484	0.00715563
11571	Crispl	-3.67369	0.00715563
13038	Ctsk	-3.31613	0.00715563
380702	Shisa6	-2.91609	0.00715563
18491	Pappa	-2.72917	0.0175695
68891	Cd177	-2.59422	0.0373309
17395	Mmp9	-2.5142	0.00715563
15458	Hpx	-2.5113	0.00715563
17229	Tpsb2	-2.48802	0.0262219
20377	Sfrpl	-2.41314	0.00715563
20201	S100a8	-2.35077	0.00715563
20845	Star	-2.1626	0.0339914
20202	S100a9	-2.12003	0.00715563
214593	Duox2	-2.1115	0.00715563
12814	Col11a1	-2.10603	0.0125058

20564	Slit3	-2.07398	0.00715563
68875	Tmcc2	-2.04813	0.0443712
76459	Car12	-1.98707	0.0339914
73230	Bmper	-1.81749	0.00715563
27206	Nrk	-1.80067	0.0443712
74145	Fl3a1	-1.71402	0.0125058
13717	Eln	-1.66284	0.00715563
12955	Cryab	-1.51099	0.0474108
14778	Gpx3	-1.51077	0.0339914

[00117] *Sorting of epithelial cells from orthotopic tumors.* mTmG mice (REF) were used as recipient for some of the experiments in which unlabeled KPC cells were implanted orthotopically into the mouse pancreas and after treatment, tumors were removed and after pancreatic tissue digestion, Tomato negative cells were sorted for PCR assays.

[00118] *Serum mouse cytokines, chemokines, and growth factors detection.* Analysis of cytokines was done on the serum of 1-month and 6-month-old KPC and Cre mice. Mouse Th1/Th2/Th17 cytokine array kit (BD Biosciences; 560485 and 551287) was used on 50 μ L of serum. Capture of cytokines from serum was done according to manufacturer's instruction and captured cytokines were analyzed in BD FACS CantoII. Instrument parameters were set up according to the instructions provided in the manufacturer's manual. Data was analyzed using BD FCAP array software.

[00119] *Cellular reactive oxygen species detection.* To measure ROS by immunofluorescence (IF) in living cells, 1×10^4 cells were plated into a 4 chamber culture slides (Falcon, Big Flats, NY) and treated with IL17A cytokine 0 or 10 ng/mL for 3 days. Before harvesting cells, 2 μ L 5 mM Dihydroethidium (Thermo Fisher Scientific, Waltham, MA) was added into each chamber for 30 minutes. Then cells were fixed by 4% Paraformaldehyde and stained with Alexa Fluor™ 647 Phalloidin (Thermo Fisher Scientific, Waltham, MA) at 1:50 dilution for 20 minutes. Slides were mounted in ProLong Diamond Antifade Mountant (Invitrogen, Carlsbad, CA). Images were acquired using a Nikon (Tokyo, Japan) confocal imaging microscope.

[00120] To measure ROS by flow cytometry, a total of 5×10^5 cells were plated into 6-well plate and treated with IL17A cytokine 0 or 10 ng/mL for 3 days. Then cells were harvested and stained using DCFDA / H2DCFDA – Cellular Reactive Oxygen Species Detection Assay Kit (Abcam, Cambridge, MA) following manufacturer protocol. ROS levels

were detected through Gallios 561 Flow Cytometer (Beckman, Indianapolis, IN). The experiment was performed in triplicate wells and repeated three times.

[00121] *H₂O₂ measurement in cell culture medium.* A total of 5x10⁵ cells were plated into 6-well plate and treated with IL17A cytokine 0 or 10 ng/mL for 3 days. Then culture medium was collected and passed through a 0.22 µm filter. Amplex™ Red Hydrogen Peroxide/Peroxidase Assay Kit (Thermo Fisher Scientific, Waltham, MA) were used to measure the H₂O₂ level in the cell culture medium following the manuals. This experiment was performed in triplicate wells.

[00122] *Lactate measurements.* Mouse sera from PDAC models or cell culture medium were collected and lactate levels were measured using Lactate Colorimetric/Fluorometric Assay Kit (BioVision, Milpitas, CA). Assays were run in triplicates.

[00123] *Ex vivo ¹H NMR spectroscopy.* Each tumor sample was weighed, crushed, and immersed in 3 mL of methanol-to-water mixture (2:1) on top of 0.5 mL of polymer vortex beads inside a 15 mL test tube. A process of mechanical homogenization was performed by vortexing the tubes for 15 seconds, flash-freezing in liquid nitrogen for one minute, and allowing the mixture to thaw, repeated three times. The samples were then subjected to centrifugation for ten minutes to separate the water-soluble metabolites from proteins and other cellular constituents. The supernatant was extracted and subjected to rotary evaporation to remove the methanol. The samples were further desiccated by placing them on a lyophilizer overnight, leaving just the collection of metabolites. The metabolites were then dissolved in a solution of 600 µL of H₂O, 36 µL of PO₄ buffer, and 4 µL of 80 mM DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid). Phosphate buffer was added to stabilize any potential pH variations, and DSS served as the reference standard to which the spectral signal from each metabolite was normalized.

[00124] NMR Spectra were obtained using a Bruker AVANCE III HD® NMR scanner (Bruker Bio Spin Corporation, The Woodlands, TX) at a temperature of 298°K. The spectrometer operates at a ¹H resonance frequency of 500 MHz and is endowed with a triple resonance (¹H, ¹³C, ¹⁵N) cryogenic temperature probe with a Z-axis shielded gradient. A pre-saturation technique was implemented for water suppression. Spectra were obtained with a 90° pulse width, a scan delay *t_{rel}* of 6.0 s, a 1024 Hz spectral width, and an acquisition time

$t_{\max}$ of 1.09 s (16,000 complex points). A total of 256 scans were collected and averaged for each spectrum, which resulted in a total scan time of 32 minutes and 49 seconds. Here, $t_{\text{rel}} + t_{\max}$ was nearly 8 s so that it was greater than $3 \cdot T_1$ of the metabolites observed. The time domain signal was apodized using an exponential function. After the spectra were acquired, metabolic profiling was performed using Chenomx NMR Suite 8.1 software (Chenomx Inc., Edmonton, Canada). Quantification of the metabolites was then performed using MestReNova software (Mestrelab Research, A Coruña, Spain) by integrating a nonzero region centered about the chemical shift at which the metabolite is known to resonate. This integral value for each metabolite is then normalized by the value of the integral of the DSS reference peak.

[00125] *Statistical analysis.* Data were expressed as the mean $\pm$ standard deviation. Data were analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Experimental values reported are means $\pm$ SD. Statistical significance among the tumor groups were assessed using a two-sample t-test assuming unequal variances. When more than 2 value sets were compared, 1-way analysis of variance (ANOVA) was used followed by Dunnett test when the data involved 3 or more groups. $P < .05$ (*), $P < .01$ (**), or $P < .001$ (***) were considered statistically significant. Kaplan Meier curve in FIG. 1D was performed using TCGA RNA-seq data from km-plotter web-tool as previously reported (Lanczky et al., 2016). Statistical analysis performed using log rank and hazard ratio calculated.

Example 1 – IL17-secreting cells are increased in murine and human pancreatic adenocarcinoma carcinoma

[00126] Given the key role that IL17 exerts in pancreatic premalignant lesion initiation and progression (McAllister et al., 2014), the inventors first aimed to determine if IL17 levels were elevated in pancreatic adenocarcinoma. To this end, serum concentrations of a panel of cytokines were measured in the autochthonous K-ras^{LSL.G12D/+};p53^{R172H/+};PdxCre mice (KPC) pancreatic adenocarcinoma mouse model of at two time points (1 and 6 months). Serum levels of several cytokines were increased in KPC mice in an age-dependent manner (FIG. 1A). When the results were compared with age-matched control PdxCre mice, serum levels of IL17 drastically increased with PDAC progression (FIGS. 1A&1B). Then, IL17 was measured in an orthotopic PDAC mouse model, in which KPC cells were allografted into the pancreas of syngeneic animals, and IL17 mRNA expression in orthotopic pancreatic tumors was found to be significantly upregulated compared to normal pancreas (FIG. 1C). TH17 cell

were also detected in human PDAC tissue and patients with higher IL17A expression, based on TCGA, had significantly worse prognosis than those with low IL17 expression (HR=2.2, p:0.0021) (FIG. 1D).

[00127] To obtain a global understanding of the cellular functions modulated by IL17 in the pancreatic tumor microenvironment, IL17 signaling was blocked using anti-IL17A/anti-IL17R neutralizing antibodies or IgG isotype control as previously described (McAllister et al., 2014) in an orthotopic pancreatic cancer mouse model (FIG. 1E). RNA sequencing analysis was performed on whole tumors obtained after 2 weeks of IL17 neutralization, and ingenuity pathway analysis was used to find that chemotaxis of myeloid cells, leukocytes and neutrophils, along with cell movement and adhesion of neutrophils were predicted to be the most significant cellular functions represented by the genes regulated by IL17 (FIG. 1E), processes that may contribute to the generation and maintenance of the immunosuppressive microenvironment that characterizes pancreatic adenocarcinoma. Human PDAC infiltration by CD15+ neutrophils was assessed and found to be significantly increased versus normal tissue (FIG. 1F).

Example 2 – IL17 signaling modulates the pancreatic tumor microenvironment

[00128] To further characterize the cellular populations modulated by IL17 throughout the course of tumor growth, immune cell analysis was performed by FACS on established tumors after IL17 neutralization. First, a panel of the most common immunosuppressive cells, including myeloid derived suppressor cells (MDSC), neutrophils, and T regulatory cells, was analyzed. Tumor recruitment of neutrophils (Gr1+) was significantly decreased upon IL17 blockade compared with control treated with IgG isotype (FIGS. 1G&1H) while all other populations did not significantly change (FIGS. 6A-6B). Also, the three cellular populations were not altered in the spleen (FIGS. 6C-6E), suggesting a local effect at the tumor microenvironment and not a systemic level.

[00129] To test if IL17 stimulation exerts a direct action on tumor cells, a transcriptomic assay on KPC cells exposed to IL17A *in vitro* was analyzed, as previously described (McAllister et al., 2014). Several cytokines and chemokines capable of recruiting suppressive populations were significantly induced after IL17 treatment, including Cxcl5, Cxcl3, Csf3, Ccl20, Cxcl1, Cxcl2 (FIG. 6F). Previous reports have attributed immunosuppressive activity to CXCR2-dependent neutrophils recruitment to the PDAC

tumor microenvironment (Nywening et al., 2018; Chao et al., 2016). IL17 signaling is upstream to these chemotactic molecules associated with neutrophils chemotaxis, therefore it is expected that its inhibition would result in broader efficacy.

[00130] Tumor immune cells analysis by FACS also revealed that IL17 blockade significantly increased the total number of CD8⁺ T cells (FIG. 1J). Furthermore, while IL17 blockade mildly increased the total number of CD8⁺ T cells, it significantly increased the number of tumor infiltrating activated T-cells (CD8⁺/IFN- γ ⁺) (FIGS. 1H&1K). Consistently, a ratio between Gzm⁺/Gr1⁺ cells was calculated and found to be significantly increased in tumors exposed to IL17 blockade (FIGS. 1H&1L). Then, it was asked whether IL17 blockade may also alter the spatial distribution of immune cells within the tumors. To achieve this, opal-multiplex immunofluorescence was performed, which allowed simultaneous detection of immune cells in the tumor. The number of activated CD8⁺ T cells (measured by CD8⁺/Gzm⁺) was significantly increased in the tumors (FIG. 1J) and they were also found in closer proximity to the tumor cells (CK19⁺) upon IL17 blockade versus IgG isotype-treated animals (FIG. 1H&1M), indicating that IL17 neutralization not only increases the number of activated CD8⁺ effector T cells but also modulates their spatial distribution favoring their migration to the tumor's proximity. Taken together, IL17-IL17RA epithelial signaling induces secretion of myeloid cells recruiting factors resulting in neutrophils recruitment to the tumors favoring inactivation and tumor exclusion of CD8⁺ T cells.

Example 3 – Suppression of IL17 signaling overcomes resistance to PD-1 inhibitors

[00131] Since IL17 blockade modulates the microenvironment favoring CD8⁺ T cells activation, it was hypothesized that it may have anti-tumor efficacy against pancreatic cancer. To test this, a subcutaneous syngeneic mouse model was used in which KPC tumor bearing-mice were treated with IgG isotype control versus anti-IL17/IL17R monoclonal antibodies (rat anti-mouse, Amgen) (FIG. 2A). Despite the strong favorable microenvironment modulation induced by IL17 blockade, it did not affect established tumors growth compared with isotype treated mice (FIG. 2B). It was hypothesized that this resistance could be mediated by modulation of checkpoint molecules in the setting of persistent immune activation. The levels of several exhaustion markers and immune checkpoint molecules were measured and it was found that the expression of PD-L1 mRNA in whole tumors increases in response to IL17 signaling inhibition while the exhaustion

markers Eomes and CD44 remained unchanged (FIG. 2C, FIG. 7A). To test if IL17 is partially responsible for inducing this effect in a direct manner, PD-L1 on KPC cells *in vitro* exposed to IL17 was measured and it was found that its mRNA expression was significantly decreased.

5 **[00132]** Based on these results, it was hypothesized that blockade of both IL17 and PDL1/PD-1 signaling would achieve synergistic anti-tumoral efficacy against pancreatic adenocarcinoma. To this end, KPC allografts bearing-mice were randomized into 4 groups which received the following treatments: (a) IgG isotype control antibodies, (b) dual anti-IL17/IL17R monoclonal antibodies (rat anti-mouse, Amgen), (c) anti-PD-1 monoclonal
10 antibodies (rat anti-mouse, BioXcell), and (d) triple combination of anti-IL17/IL17R and anti-PD-1 antibodies. Even though anti-IL17/IL17R or anti-PD-1 antibodies did not have anti-tumoral efficacy as single agents, the triple combination of anti-IL17/IL17R/PD-1 antibodies had a significant synergistic effect in decreasing tumor growth compared with other groups (FIGS. 2B&2J). Adapting RECIST criteria to assess murine response to the
15 treatment, it was found that five of nine mice exhibited responses to triple combination treatment; while only one of ten mice showed response to anti-PD-1 treatment and none of the treated mice had responses to anti-IL17 or isotype control treatment (FIG. 7B). The individual contribution of each anti-IL17 and anti-IL17R antibodies when added to anti-PD1 was sought to be teased out. Anti-IL17R added to anti-PD-1 did not affect tumors growth
20 significantly. However, the addition of anti-IL17A to anti-PD1 resulted in tumors smaller than isotype although the effect was not as dramatic as in the anti-IL17A, anti-IL17R and anti-PD-1 triple combination group. Based on these results, the combination of the three antibodies for the rest of the study.

[00133] A second experiment was performed with the same treatment arms
25 using a murine PDAC orthotopic tumor model (FIG. 2D), considering the different microenvironment formed in subcutaneous vs orthotopic implanted tumors. Similarly, a significant reduction in tumor size was detected following the combination of IL17/IL17RA and PD-1 inhibition compared to IgG control or single treatment controls (FIG. 2E). The response rate for triple combination treatment was 50% while no responders were found in
30 control or single treatment arms (FIG. 7C). A survival experiment was performed with the same four arms on the murine orthotopic model and a significant extension in mean survival was observed in mice treated with the triple combination of IL17/IL17R and PD-1 blockade

(62 days) compared to mice treated with IgG control (45 days), single agent anti-PD-1 (47 days), or dual anti-IL17/IL17R antibodies (45 days) (FIG. 2F). Of note, 4 out of 15 mice from the triple combination group survived by day 75 when the experiment ended. The histopathology, index of proliferation (KI67), and apoptosis level (caspase) were compared
5 between the groups and no major differences were found (FIG. 7D). To check if the antitumoral effect observed was consistent with other PDAC cell lines, mT3 cells, which are derived from murine organoids generated from KC mice PDAC (Boj et al., 2015), were used and a similar synergistic effect upon orthotopic implantation and treatment with anti-PD-1/anti-IL17/IL17R was found (FIG. 2G).

10 **[00134]** We then hypothesized that IL17 is mostly signaling through the pancreatic cancer cells, which by secreting chemotactic factors production direct neutrophils recruitment and ultimately induce and maintain pancreatic tumor immunosuppression. The role of the epithelial IL17/IL17RA signaling in the maintenance of tumor microenvironment immunosuppression was validated using a genetic strategy. IL17R was knocked out from
15 KPC cells by CRISPR/Cas9 gene editing (FIG. 2H), and orthotopic implantation of these cells versus scramble-control treated cells with intact IL17R was performed in the presence and absence of PD-1 inhibition. Differently from the pharmacological IL17 neutralization, pancreatic tumors formed with IL17R KO KPC cells were significantly smaller than those formed with IL17R intact scramble treated cells. When tumors formed with IL17R KO KPC
20 cells were treated with anti-PD-1 antibodies, tumors exhibited complete remission (FIG. 2I).

[00135] Finally, to determine if IL17 blockade sensitizes tumors selectively to PD-1 or to other checkpoint inhibitors as well, mice were treated with a combination of IL17/IL17R inhibitors and anti-CTLA4 monoclonal antibodies. IL17/IL17R blockade also significantly increases sensitivity to CTLA4 inhibitors (FIG. 10), suggesting that IL17
25 inhibition sensitizes tumors to checkpoint blockade nonspecifically.

[00136] In conclusion, through a combination of pharmacological and genetic approaches the role of IL17A/IL17RA axis in controlling the tumor development and growing and sensitizing to checkpoint blockade in established pancreatic carcinoma preclinical models was confirmed. Combinatorial IL17 inhibition and checkpoint blockade
30 results in a synergistic anti-tumoral effect.

Example 4 – The anti-tumoral effect of combinatorial IL17/IL17R and PD-1 blockade is CD8+ T cells dependent

[00137] To further understand the mechanisms behind the anti-tumoral synergistic effects of IL17/IL17R and PD-1 inhibition, effector immune cells in orthotopic tumors were quantified. Combinatorial treatment with anti-IL17/IL17R and anti-PD-1 antibodies significantly affected recruitment of CD8+ T cells and also cytotoxic IFN γ -secreting CD8+ T cells compared to control treatment arm (FIG. 3A). Increased CD8+T cells activation was also detected by quantification of the tumor infiltrating cells expressing granzyme-B with combinatorial IL17/IL17R and PD-1 blockade (FIG. 3B). Correlation analysis between tumor volume and CD8+ T cells frequency in the four treatment arms was also performed. While no correlation was found between the two variables in the control and single antibodies treatment arms, an inverse correlation was found between tumor volume and CD8+ T cells in mice treated with combinatorial anti-IL17A/RA/anti-PD-1 ($r = -0.77$), suggesting that T cells are only functionally active in tumors from this treatment arm (FIGS. 8A-8D). Next, the spatial distribution of cells was assessed, and it was found that CD8+/granzyme B+ cells were significantly increased in tumors from mice treated with combinatorial IL17 and PD-1 blockade (FIG. 3C).

[00138] Based on these results, it was hypothesized that CD8+ T cells are the mediators of the anti-tumoral effect induced by IL17/IL17R and PD-1 antibodies. To test this hypothesis, neutralizing antibodies against CD8 were used on tumor-bearing mice treated with anti-IL17 and anti-PD-1 antibodies. Blockade of CD8 resulted in the loss of efficacy of the IL17//IL17R/PD-1 combination when compared with mice that received the combination plus control IgG isotype (FIG. 3D). As validation of these results, CD8-deficient mice were used as recipients of KPC cells, and it was found that the combination of antibodies against IL17/IL17R and PD-1 were not effective in reducing tumor size in the absence of CD8+ T cells (FIG. 3E). These findings strongly indicate that the antitumoral synergistic effect of IL17 blockade and anti-PD-1 agents is mediated by CD8+ T cells.

Example 5 – IL17 upregulates Duoax2 in pancreatic cancer cells which may contribute to immunosuppression

[00139] In an attempt to find IL17-induced epithelial molecular mechanisms that preclude CD8+ T cells activation in the tumor microenvironment, genes commonly

regulated by IL17 were looked at in three independent experimental settings: A. RNA sequencing performed on PDAC orthotopic tumors from mice *in vivo* exposed to IL17 neutralizing antibodies vs isotype (Table 3); B. Microarray done on GFP+ oncogenic epithelial pancreatic cells sorted from Mist1Cre;LSLKras mice exposed to cerulean (KCiMist1;C) and *in vivo* treated with IL17 neutralizing antibodies vs isotype (McAllister et al., 2014); and C. RNA sequencing performed from enteroids *in vitro* exposed to IL17 (Kumar et al., 2016). *DUOX2* was the gene commonly upregulated by IL17 in the three datasets (FIG. 4A), which encodes for the enzyme dual oxidase 2 (Duox2) found in the thyroid gland and digestive tract and whose main function is the production of Reactive Oxygen Species (ROS). The gene *DUOXA2*, commonly regulated in B and C datasets, encodes for Duoxa2 (Dual oxidase maturation factor 2) and its function resides in favoring the maturation and transport from the endoplasmic reticulum to the cell membranes of functional Duox2 (Rada & Leto, 2008).

[00140] To validate the transcriptomic results and confirm if the IL-17-mediated induction on *DUOX2/DUOXA2* expression was direct, KPC cells were stimulated with IL17 *in vitro* and all *NOX/DUOX* family genes encoding for NADPH oxidases were measured. While a mild induction was evident on some NOX genes, the strongest induction occurred on DUOX family members, including Duox1 and Duox2, as well as the oxidase maturation factor Duoxa2 (FIG. 4B), confirming the direct role of IL17 in regulating Duox2/Duoxa2 system in pancreatic cancer cells. To specifically confirm if Duox2/Duoxa2 are regulated by IL17 *in vivo* in epithelial cancer cells and that their downregulation in tumors is not merely reflecting a decrease in tumor infiltrating neutrophil granulocytes, main cellular system capable of producing Duox2 (Donko et al., 2005), Duox2 mRNA expression was measured in epithelial cancer cells (Tomato negative fraction) sorted from the tumors that were formed by implantation of non-labelled KPC cells, to avoid immune activation, into mTmG recipient mice. A significant downregulation of Duox2 mRNA expression was found in epithelial cancer cells sorted from tumors formed in a microenvironment with IL17 blockade vs control (FIG. 4C).

[00141] Considering the main function of Duox2/Duoxa2, ROS on pancreatic cancer cells was measured *in vitro* and a significant increase upon IL17 direct stimulation was found (FIG. 4D). Immunofluorescence captured the increase in cytoplasmic ROS from the same cancer cells upon IL17 stimulation (FIG. 4E). Then, the levels of H₂O₂, the main

ROS regulated by Duox2/Duoxa2, secreted to the media by cancer cells stimulated by IL17 was measured, and a significant increase was found in a time-dependent manner (FIG. 4F). To test if the epithelial production of ROS could result in immune cells suppression, splenocytes were treated with media from IL17 treated and untreated cells and a significant reduction in IFN- γ production in splenocytes treated with media from KPC cells exposed to IL17 vs media was found (FIG. 4G). Last, DUOX2, DUOXA2, and GZMB mRNA expression levels were looked at in TCGA, and a significant co-expression of DUOX2 and DUOXA2 was found as expected (FIG. 9A) but a significant lack of correlation between DUOX2, DUOXA2, and GZMB was found (FIGS. 9B&9C).

Example 6 – Combinatorial PD-1 and IL17/IL17R inhibition induces metabolic changes in lactate which can serve as an activity biomarker

[00142] Considering the effect of IL17 on Duox2/Duoxa2, important players in regulation of metabolism and growth (Rada & Leto, 2008; Donko et al., 2005), it was hypothesized that global metabolic changes may be induced by the combinatorial IL17/PD-1 blockade. To test for this, *ex vivo* ^1H -NMR spectroscopy-based metabolomics was performed on pancreatic tumors from the different treatment arms. After detailed analysis of 16 metabolites, it was found that the levels of lactate were significantly decreased in the tumors from mice treated with IL17 or IL17/PD-1 blockade while acetate was increased in tumors from the combination arm (FIG. 4H, FIG. 9D). Previous reports have described the production of lactate by neutrophils (Rodriguez-Espinosa et al., 2015).

[00143] The decrease in tumors lactate could be reflecting the decreased neutrophils infiltration due to IL17 inhibition. Since lactate is mainly reflecting the proliferation rate, it was assessed whether lactate levels were already affected as early as 10 days post-treatment initiation, when the tumors from all treatment arms were still similar in size, and it was found that lactate levels in the tumors are significantly lower in tumors treated with the combination of antibodies (FIG. 4I, FIG. 9E). To determine if tumor changes in lactate can be detected systemically, lactate levels in serum of mice treated with combinatorial IL17/IL17R/PD-1 inhibitors were measured and a significant decrease in lactate in the combination arm was found (FIG. 4J).

[00144] These finding suggest that the changes in the tumor microenvironment induced by IL17 and PD-1 neutralization cause early metabolic changes that favor immune

activation. These findings provide an early metabolic biomarker of efficacy for this novel combinatorial immunotherapy.

* * *

[00145] All of the methods disclosed and claimed herein can be made and executed
5 without undue experimentation in light of the present disclosure. While the compositions and
methods of this invention have been described in terms of preferred embodiments, it will be
apparent to those of skill in the art that variations may be applied to the methods and in the
steps or in the sequence of steps of the method described herein without departing from the
concept, spirit and scope of the invention. More specifically, it will be apparent that certain
10 agents which are both chemically and physiologically related may be substituted for the
agents described herein while the same or similar results would be achieved. All such similar
substitutes and modifications apparent to those skilled in the art are deemed to be within the
spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A method for the treatment of a cancer in a patient, the method comprising administering to the patient a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor.
- 5 2. The method of claim 1, wherein the patient has previously failed to respond to the administration of an immune checkpoint inhibitor.
3. The method of claim 2, wherein the method is a method of overcoming resistance to immune checkpoint inhibitor therapy.
4. The method of any one of claims 1-3, wherein the IL-17 signaling inhibitor comprises
10 an IL-17 neutralizing antibody, an IL-17R antagonist protein, and/or an IL-17R antagonist small molecule.
5. The method of claim 4, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody.
6. The method of claim 4, wherein the IL-17R antagonist protein comprises an IL-17R
15 inhibitory antibody.
7. The method of any one of claims 1-3, wherein wherein the IL-17 signaling inhibitor comprises both an IL-17 neutralizing antibody and an IL-17R inhibitory antibody.
8. The method of any one of claims 1-7, wherein the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4
20 therapy.
9. The method of claim 8, wherein the anti-PD1 therapy comprises nivolumab, pembrolizumab, pidilizumab, AMP-223, AMP-514, cemiplimab, or PDR-001.
10. The method of claim 8, wherein the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, BMS-036559, or CK-301.
- 25 11. The method of claim 8, wherein the anti-CTLA-4 therapy comprises ipilimumab or tremelimumab.

12. The method of any one of claims 1-11, further comprising administering a further anti-cancer therapy to the patient.
13. The method of claim 12, wherein the further anti-cancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy,
5 immunotherapy, or cytokine therapy.
14. The method of claim 12, wherein the further anti-cancer therapy comprises gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, or docetaxel.
15. The method of any one of claims 1-14, wherein the cancer is a pancreatic cancer.
- 10 16. The method of any one of claims 1-15, wherein the patient has previously undergone at least one round of anti-cancer therapy.
17. The method of any one of claims 1-16, wherein the patient is a human.
18. The method of any one of claims 1-17, wherein the patient's cancer expresses an increased level of Duoxa2, Duox2, and/or Duox1 relative to a Duoxa2, Duox2, and/or Duox1
15 expression level in a reference sample.
19. The method of claim 18, wherein the reference sample is sourced from healthy or non-cancerous tissue from the patient.
20. The method of claim 18, wherein the reference sample is sourced from a healthy subject.
- 20 21. The method of any one of claims 18-20, further comprising reporting the Duoxa2, Duox2, and/or Duox1 expression level in the patient's cancer.
22. The method of claim 21, wherein the reporting comprises preparing a written or electronic report.
23. The method of claim 22, further comprising providing the report to the subject, a
25 doctor, a hospital, or an insurance company.
24. A method of assessing the efficacy of treatment of a cancer in a patient with a combined amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor, the

method comprising (a) determining a level of lactate in the patient's cancer and/or serum, and (b) determining that the treatment is efficacious if the patient's cancer and/or serum has a decreased level of lactate relative to a lactate level in a reference sample.

25. The method of claim 24, further comprising continuing to administer a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor to the patient if the patient's cancer and/or serum has a decreased level of lactate relative to a lactate level in a reference sample.

26. The method of claim 24 or 25, wherein the reference sample is sourced from a patient cancer and/or serum sample taken prior to the patient being treated with the combined amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor.

27. The method of claim 26, comprising:
(i) determining a first level of lactate in the patient's cancer and/or serum;
(ii) administering to the patient a combined amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor;
(iii) determining a second level of lactate in the patient's cancer and/or serum;
(iv) continuing to administer the combined amount of the IL-17 signaling inhibitor and the immune checkpoint inhibitor to the patient if the level at determined at step (iii) is lower than the level at step (i).

28. The method of any one of claims 24-27, wherein the reference sample is sourced from a healthy subject.

29. The method of any one of claims 24-27, wherein the lactate level in the patient's cancer is determined by PET or MRI-based hyperpolarization methods.

30. The method of any one of claims 24-29, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody, an IL-17R antagonist protein, or an IL-17R antagonist small molecule.

31. The method of claim 30, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody.

32. The method of claim 30, wherein the IL-17R antagonist protein comprises an IL-17R inhibitory antibody.

33. The method of any one of claims 24-29, wherein the IL-17 signaling inhibitor comprises both a IL-17 neutralizing antibody and an IL-17R inhibitory antibody.
34. The method of any one of claims 24-33, wherein the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4
5 therapy.
35. The method of claim 34, wherein the anti-PD1 therapy comprises nivolumab, pembrolizumab, pidilizumab, AMP-223, AMP-514, cemiplimab, or PDR-001.
36. The method of claim 34, wherein the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, BMS-036559, or CK-301.
- 10 37. The method of claim 34, wherein the anti-CTLA-4 therapy comprises ipilimumab or tremelimumab.
38. The method of any one of claims 24-37, further comprising administering a further anti-cancer therapy to the patient.
39. The method of claim 38, wherein the second anti-cancer therapy is a surgical therapy,
15 chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy, or cytokine therapy.
40. The method of claim 38, wherein the further anti-cancer therapy comprises gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, or docetaxel.
- 20 41. The method of any one of claims 24-40, wherein the cancer is a pancreatic cancer.
42. The method of any one of claims 24-41, wherein the patient has previously undergone at least one round of anti-cancer therapy.
43. The method of any one of claims 24-42, wherein the patient is a human.
44. The method of any one of claims 24-43, further comprising reporting the lactate level
25 in the patient's cancer.
45. The method of claim 44, wherein the reporting comprises preparing a written or electronic report.

46. The method of claim 45, further comprising providing the report to the subject, a doctor, a hospital, or an insurance company.

47. A method of selecting a patient having a cancer for treatment with a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor, the method comprising (a) determining a level of Duoxa2, Duox2, and/or Duox1 expression in the patient's cancer, and (b) selecting the patient for treatment if the patient's cancer has an increased level of Duoxa2, Duox2, and/or Duox1 relative to a Duoxa2, Duox2, and/or Duox1 expression level in a reference sample.

48. The method of claim 47, further comprising administering a combined effective amount of an IL-17 signaling inhibitor and an immune checkpoint inhibitor to the selected patient.

49. The method of claim 47, further comprising selecting the patient for treatment if the patient has previously failed to respond to the administration of an immune checkpoint inhibitor.

50. The method of claim 47, wherein the reference sample is sourced from healthy or non-cancerous tissue from the patient.

51. The method of claim 47, wherein the reference sample is sourced from a healthy subject.

52. The method of any one of claims 47-51, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody, an IL-17R antagonist protein, or an IL-17R antagonist small molecule.

53. The method of claim 52, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody.

54. The method of claim 52, wherein the IL-17R antagonist protein comprises an IL-17R inhibitory antibody.

55. The method of any one of claims 47-51, wherein the IL-17 signaling inhibitor comprises both a IL-17 neutralizing antibody and an IL-17R inhibitory antibody.

56. The method of any one of claims 47-55, wherein the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4 therapy.
57. The method of claim 56, wherein the anti-PD1 therapy comprises nivolumab,
5 pembrolizumab, pidilizumab, AMP-223, AMP-514, cemiplimab, or PDR-001.
58. The method of claim 56, wherein the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, BMS-036559, or CK-301.
59. The method of claim 56, wherein the anti-CTLA-4 therapy comprises ipilimumab or tremelimumab.
- 10 60. The method of any one of claims 47-59, further comprising administering a further anti-cancer therapy to the patient.
61. The method of claim 60, wherein the further anti-cancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy, or cytokine therapy.
- 15 62. The method of claim 60, wherein the further anti-cancer therapy comprises gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, or docetaxel.
63. The method of any one of claims 47-62, wherein the cancer is a pancreatic cancer.
64. The method of any one of claims 47-63, wherein the patient has previously undergone
20 at least one round of anti-cancer therapy.
65. The method of any one of claims 47-64, wherein the patient is a human.
66. The method of any one of claims 47-65, further comprising reporting the Duoxa2, Duox2, and/or Duox1 expression level in the patient's cancer.
67. The method of claim 66, wherein the reporting comprises preparing a written or
25 electronic report.
68. The method of claim 67, further comprising providing the report to the subject, a doctor, a hospital, or an insurance company.

69. A pharmaceutical formulation comprising an IL-17 signaling inhibitor and an immune checkpoint inhibitor.

70. The pharmaceutical formulation of claim 69, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody, an IL-17R antagonist protein, and/or an IL-17R antagonist small molecule.

71. The pharmaceutical formulation of claim 70, wherein the IL-17 signaling inhibitor comprises an IL-17 neutralizing antibody.

72. The pharmaceutical formulation of claim 70, wherein the IL-17R antagonist protein comprises an IL-17R inhibitory antibody.

73. The pharmaceutical formulation of claim 69, wherein the IL-17 signaling inhibitor comprises both a IL-17 neutralizing antibody and an IL-17R inhibitory antibody.

74. The pharmaceutical formulation of any one of claims 69-73, wherein the immune checkpoint inhibitor comprises one or more of an anti-PD1 therapy, an anti-PD-L1 therapy, and an anti-CTLA-4 therapy.

75. The pharmaceutical formulation of claim 74, wherein the anti-PD1 therapy comprises nivolumab, pembrolizumab, pidilizumab, AMP-223, AMP-514, cemiplimab, or PDR-001.

76. The pharmaceutical formulation of claim 74, wherein the anti-PD-L1 therapy comprises atezolizumab, avelumab, durvalumab, BMS-036559, or CK-301.

77. The pharmaceutical formulation of claim 74, wherein the anti-CTLA-4 therapy comprises ipilimumab or tremelimumab.

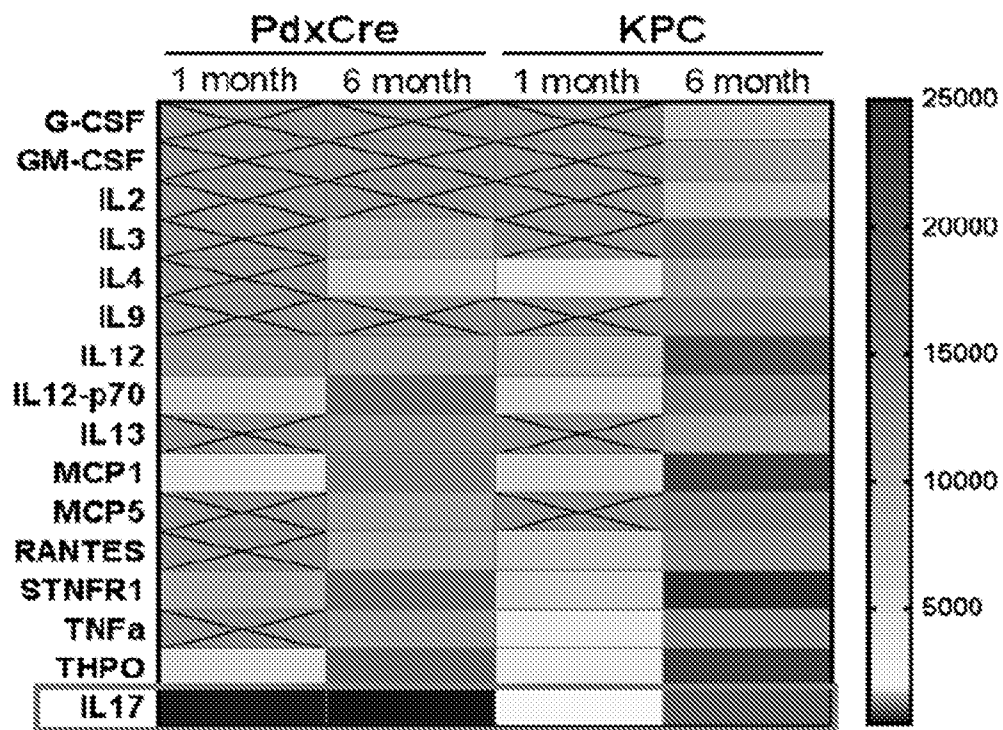


FIG. 1A

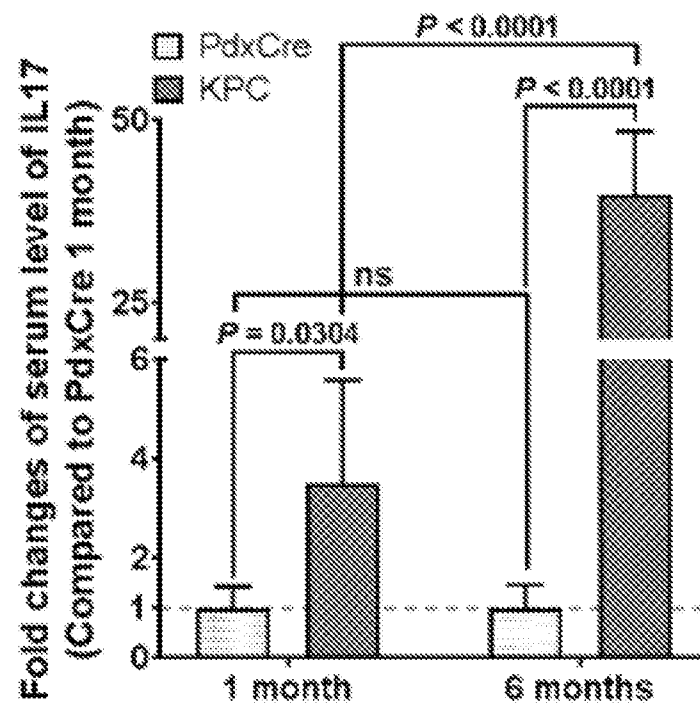


FIG. 1B

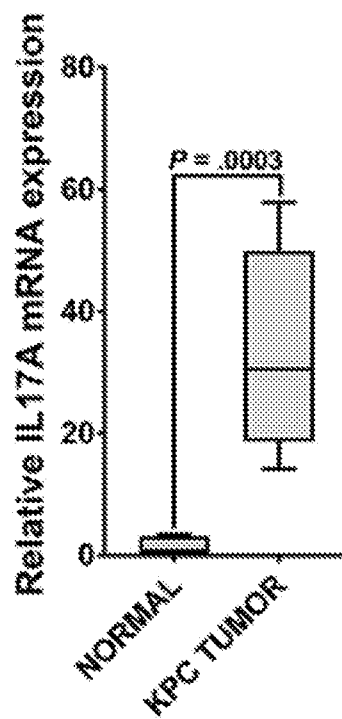


FIG. 1C

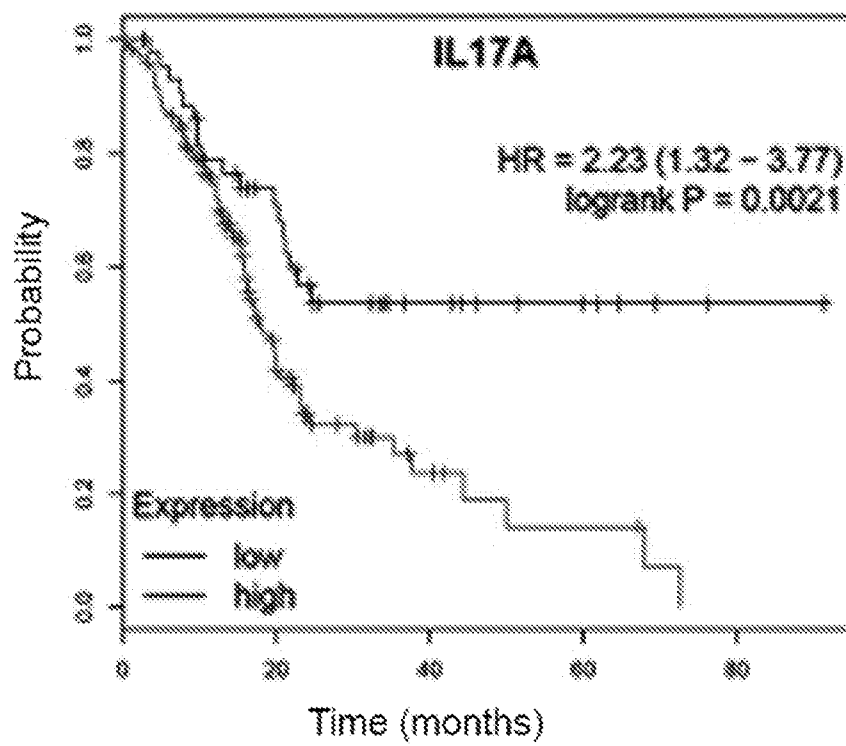


FIG. 1D

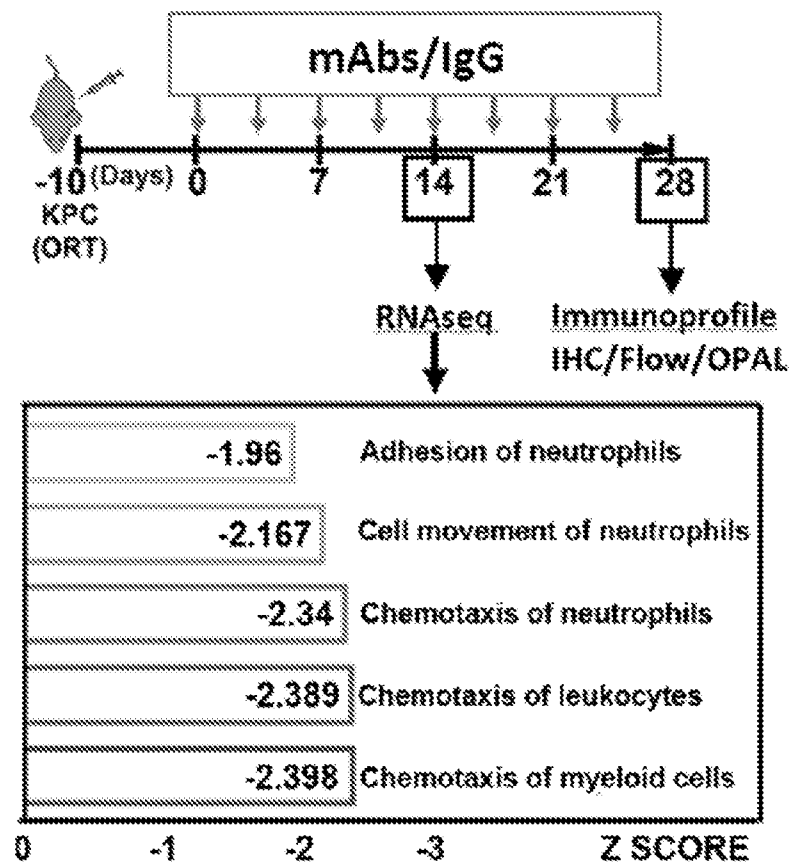


FIG. 1E

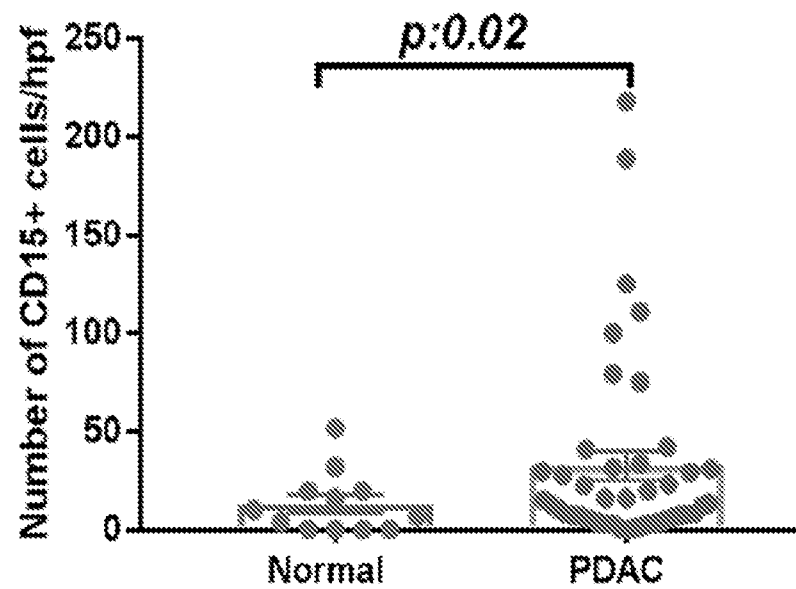


FIG. 1F

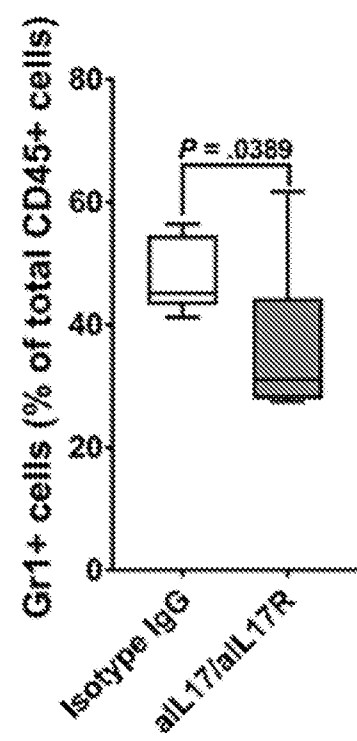


FIG. 1G

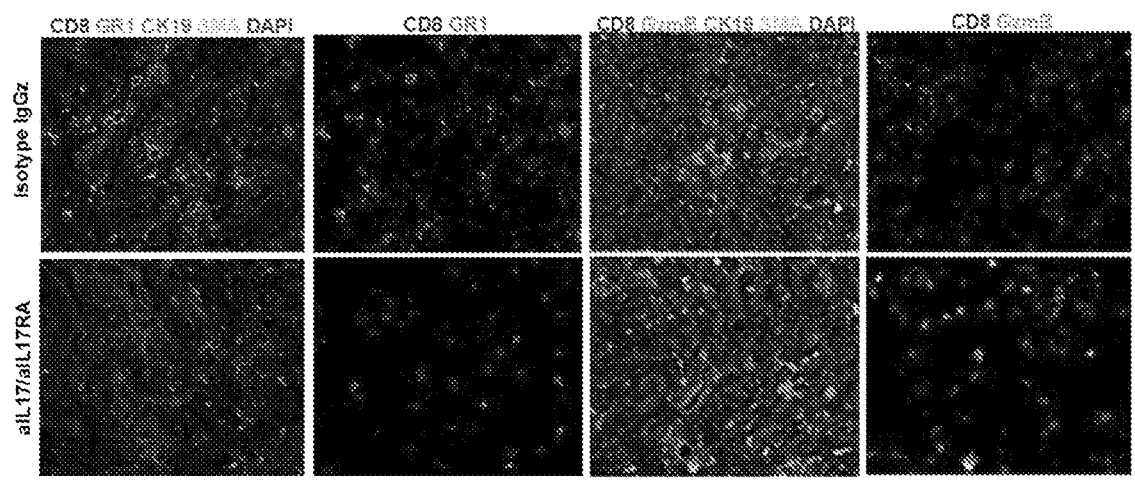


FIG. 1H

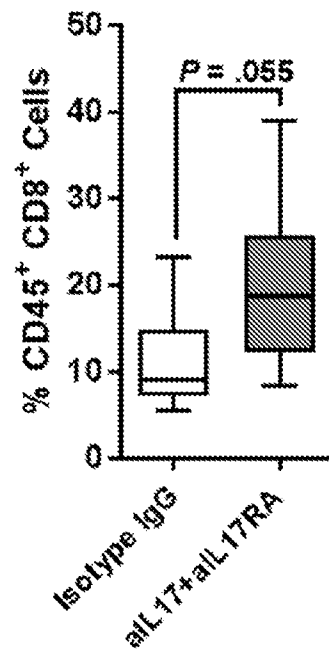


FIG. 1I

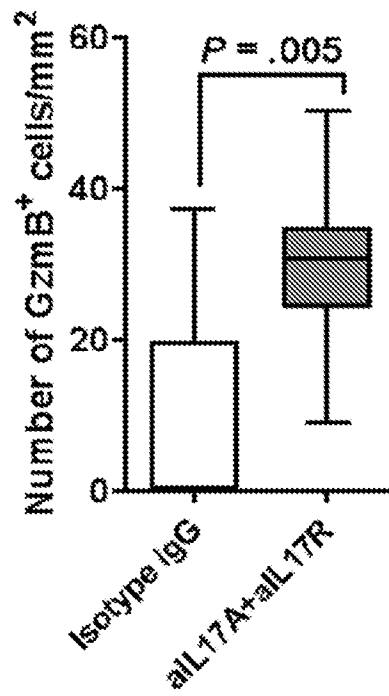


FIG. 1J

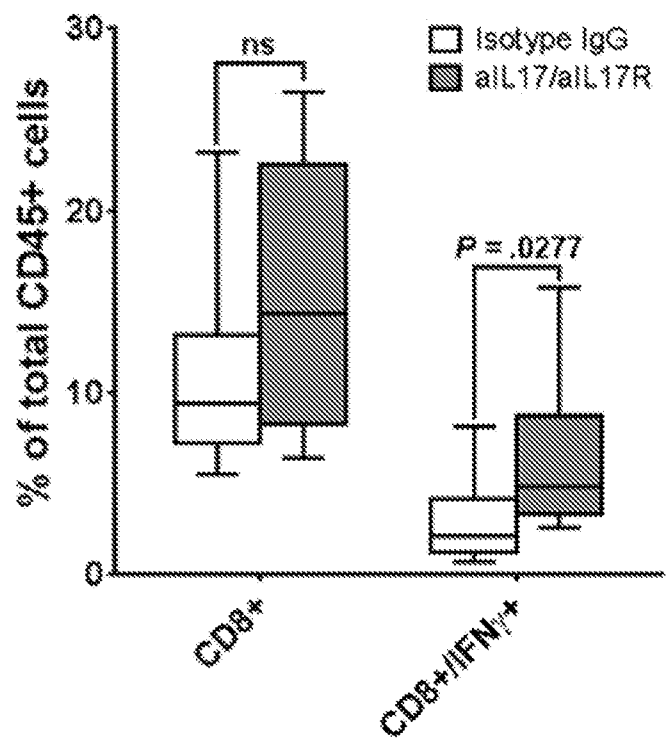


FIG. 1K

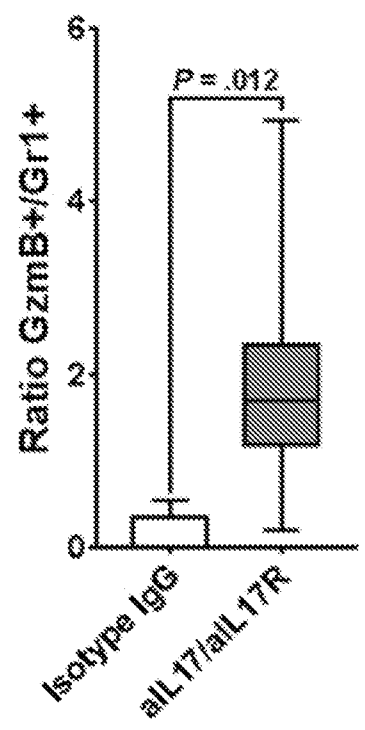


FIG. 1L

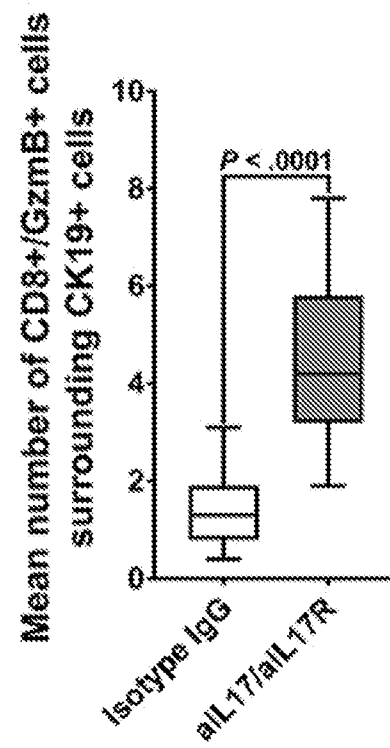


FIG. 1M



FIG. 2A

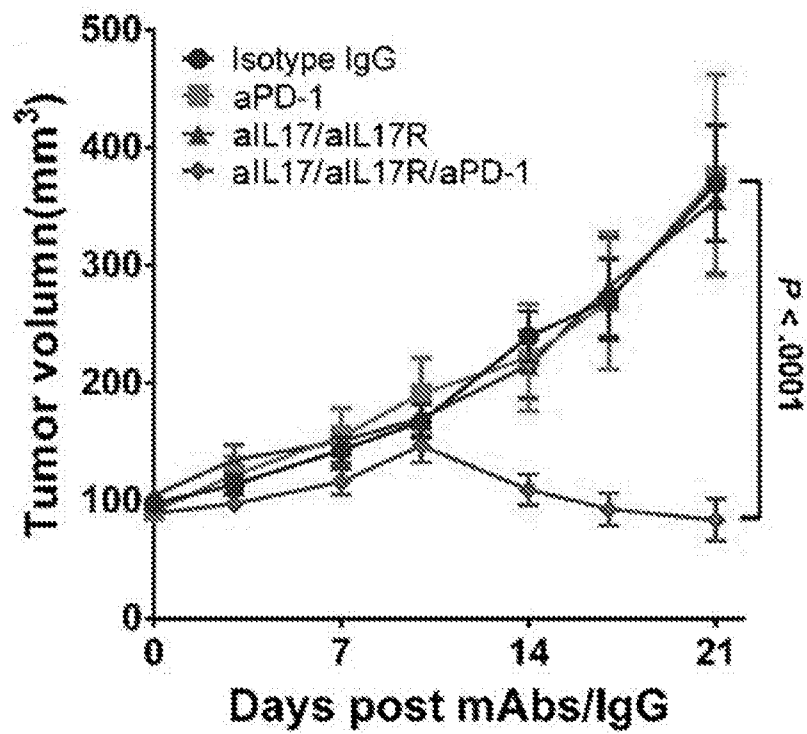


FIG. 2B

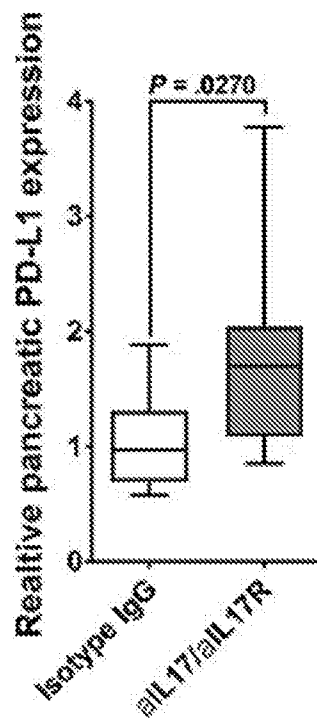


FIG. 2C

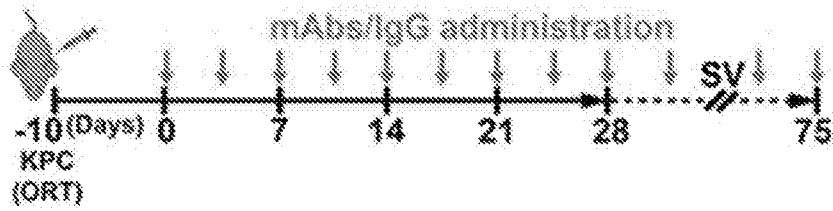


FIG. 2D

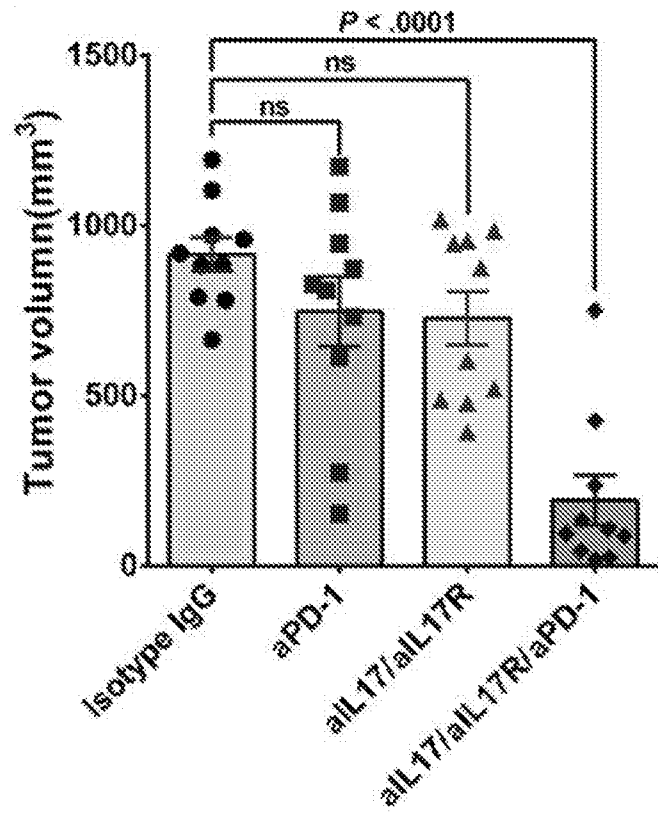


FIG. 2E

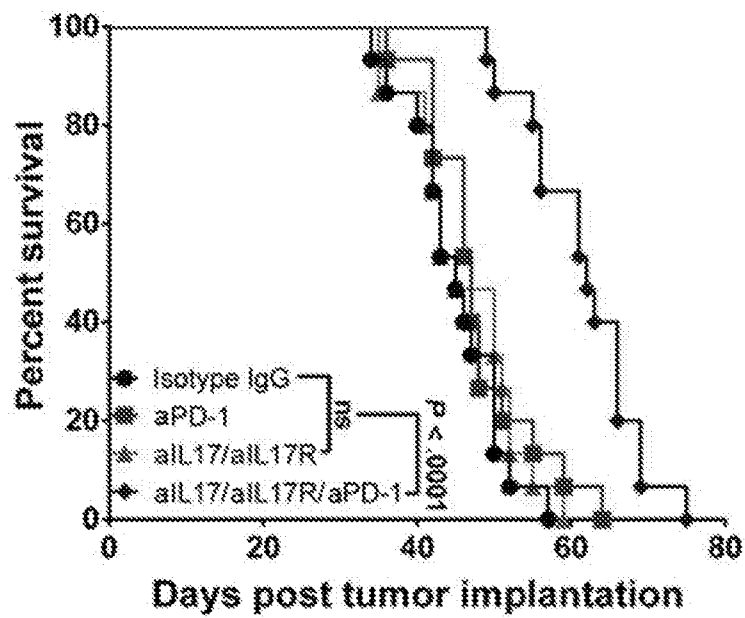


FIG. 2F

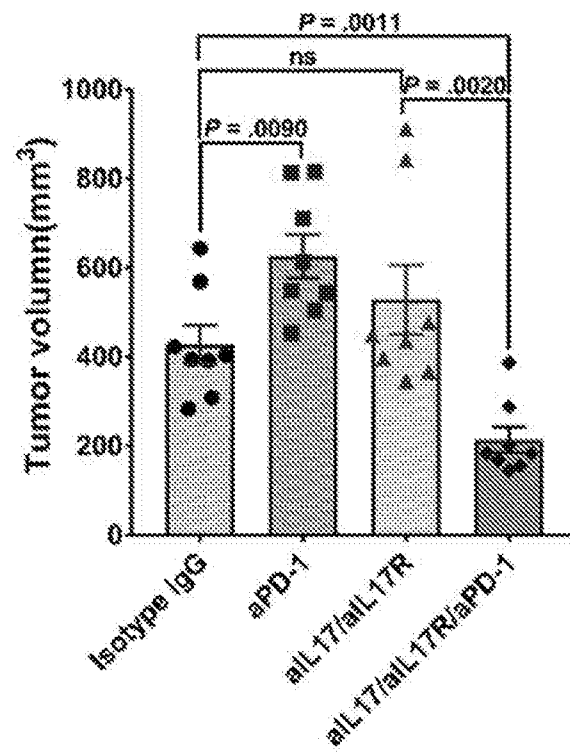


FIG. 2G

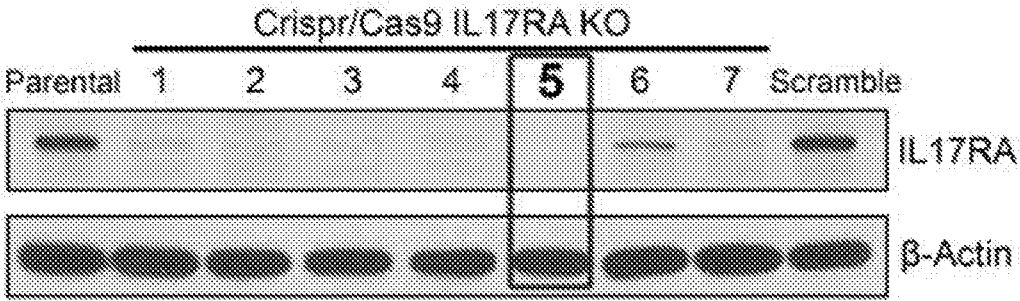


FIG. 2H

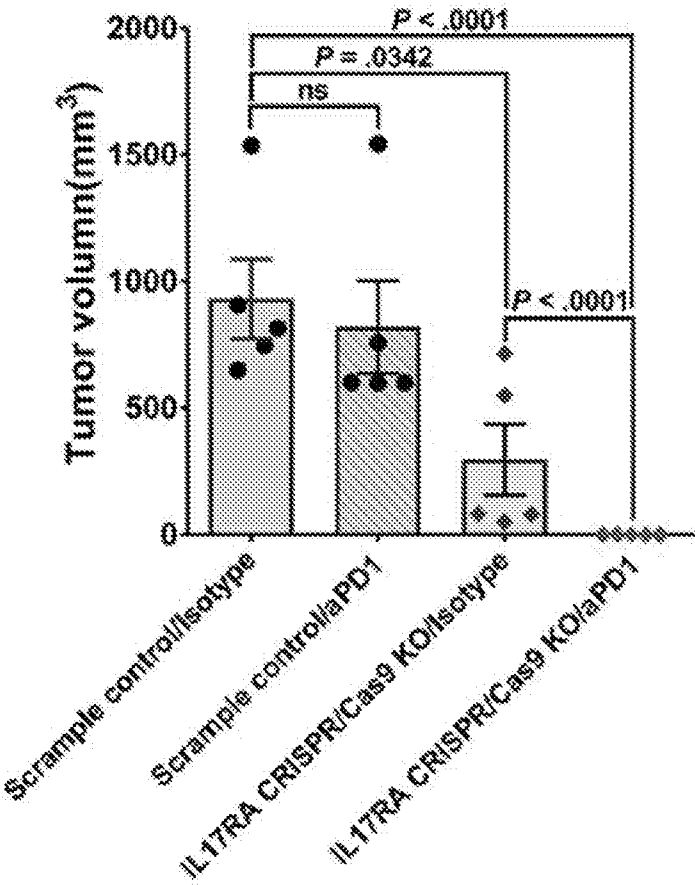


FIG. 2I

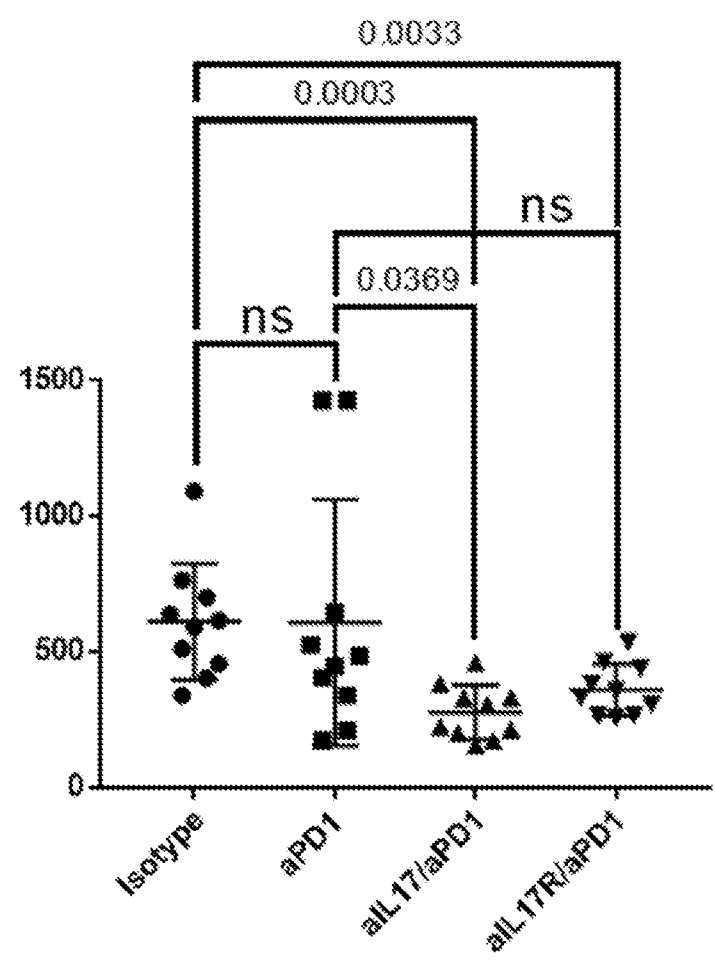


FIG. 2J

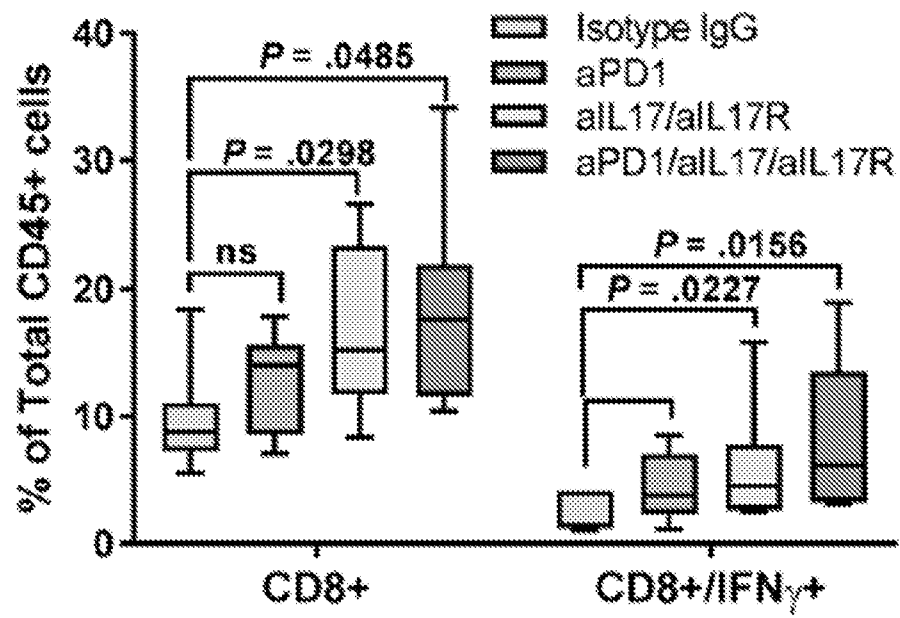


FIG. 3A

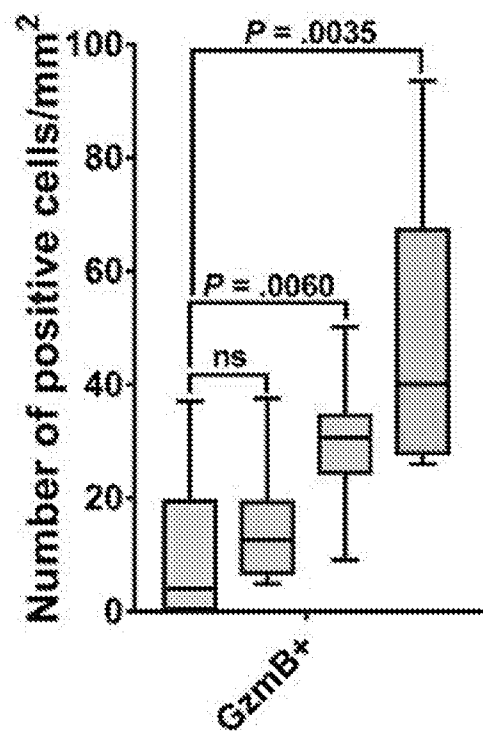


FIG. 3B

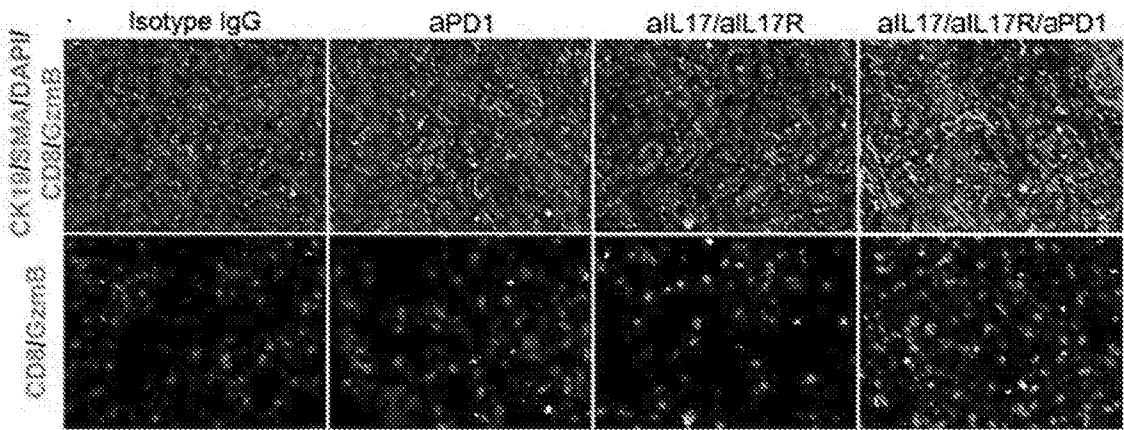


FIG. 3C

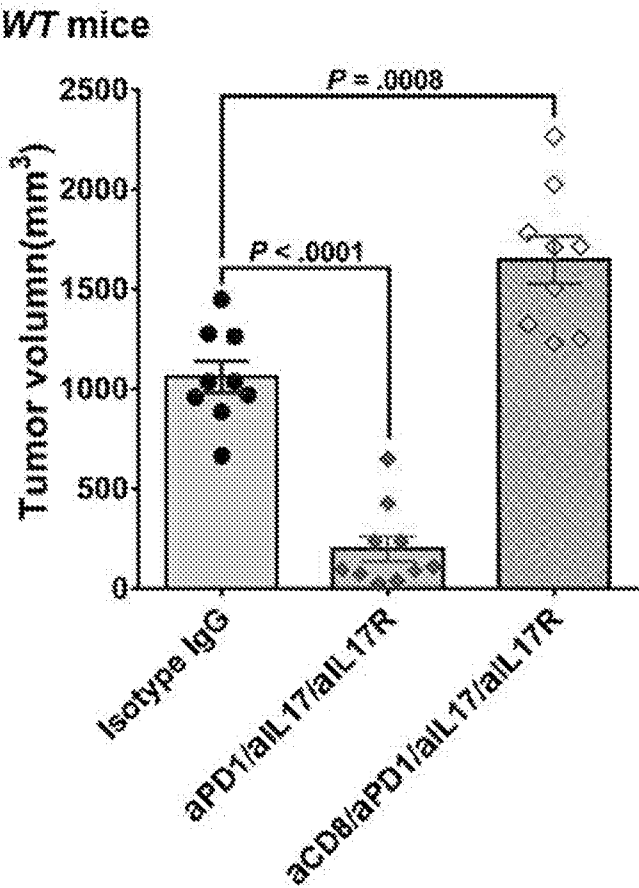


FIG. 3D

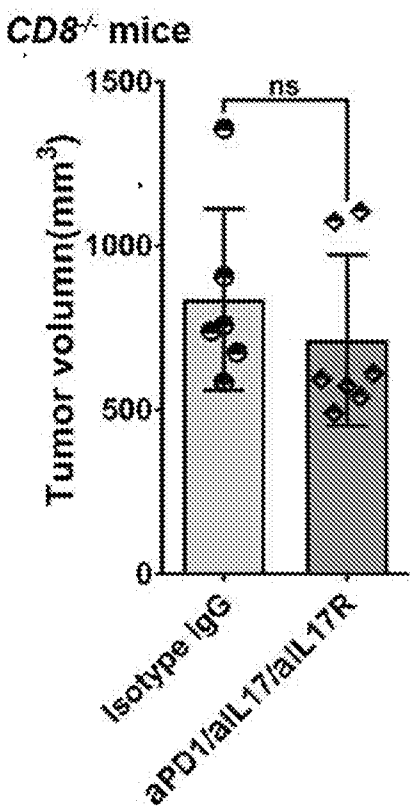
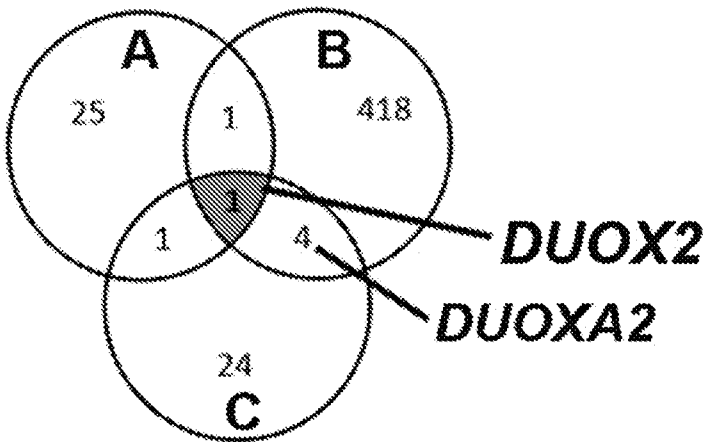


FIG. 3E



- A. IL-17-reg. genes in orthotopic PDAC model (RNAseq)
- B. IL-17-reg. genes in oncogenic epithelium of KCiMist1
- C. IL-17-reg. genes in vitro in enteroids (RNAseq)

FIG. 4A

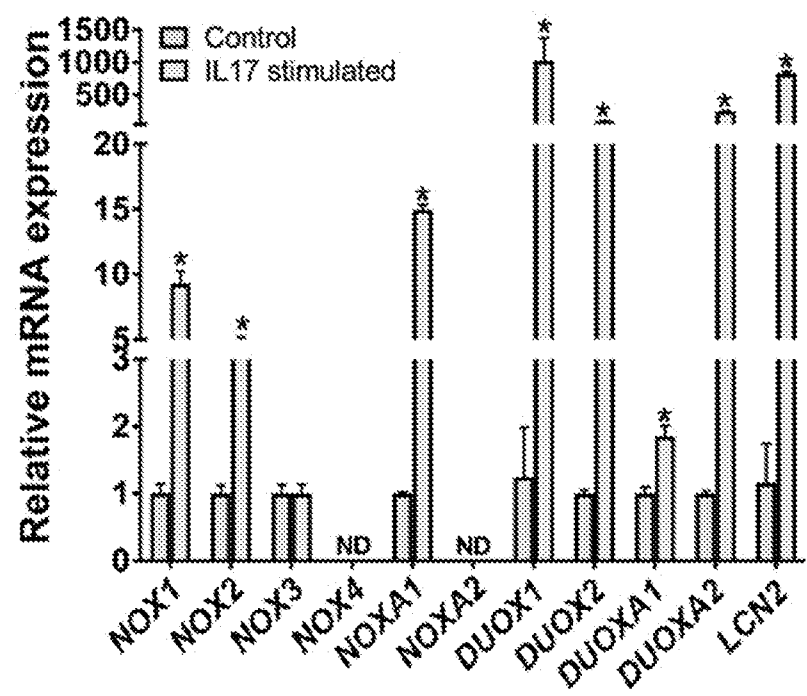


FIG. 4B

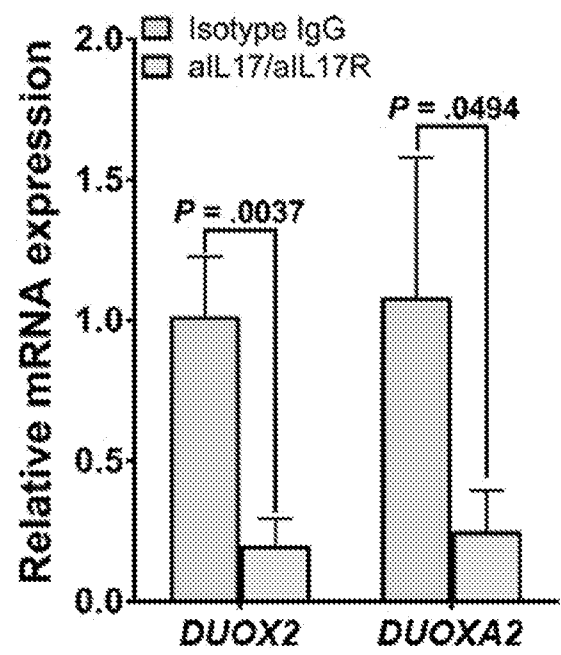


FIG. 4C

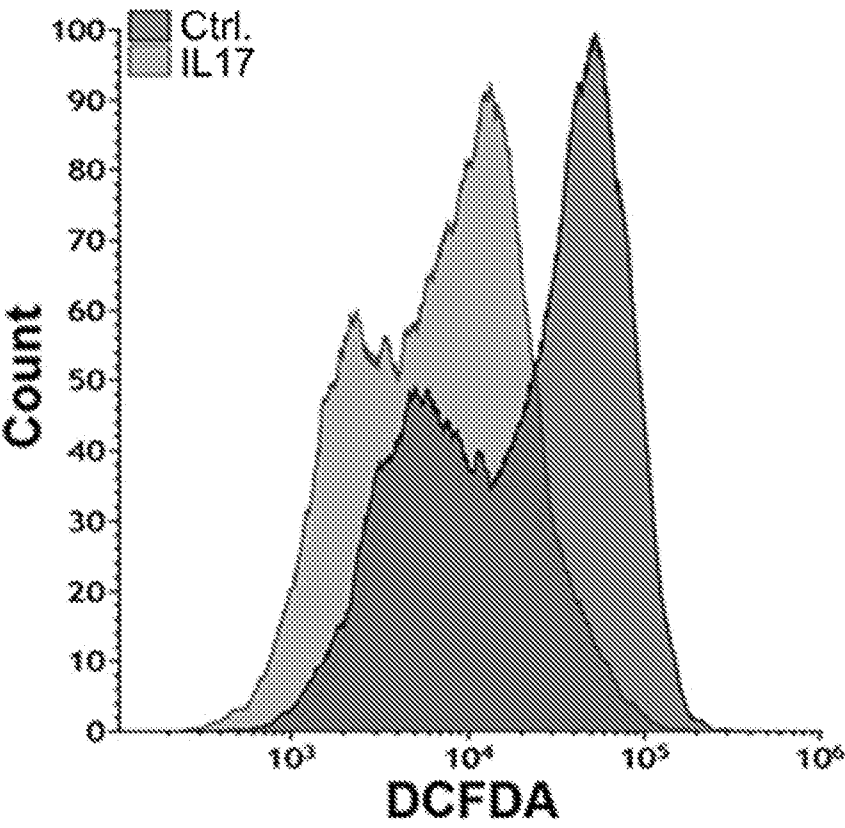


FIG. 4D

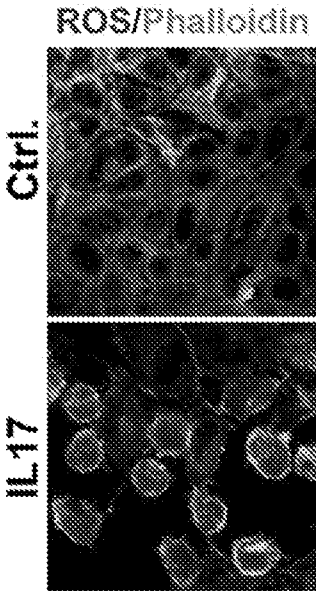


FIG. 4E

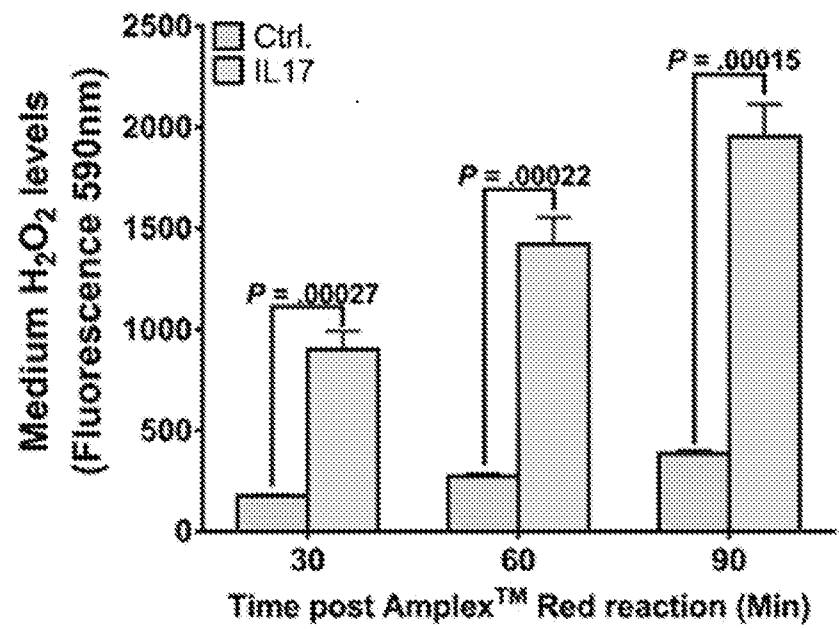


FIG. 4F

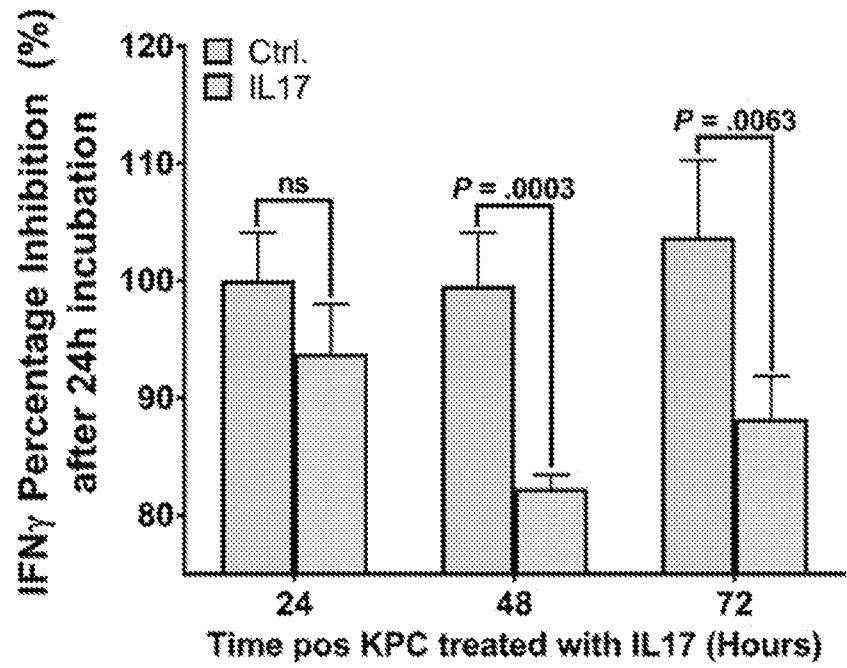


FIG. 4G

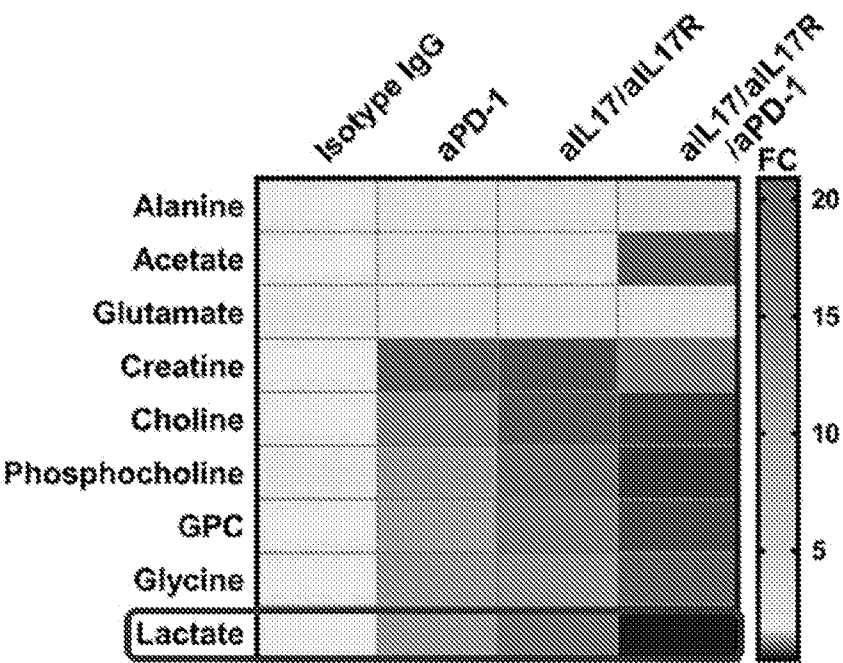


FIG. 4H

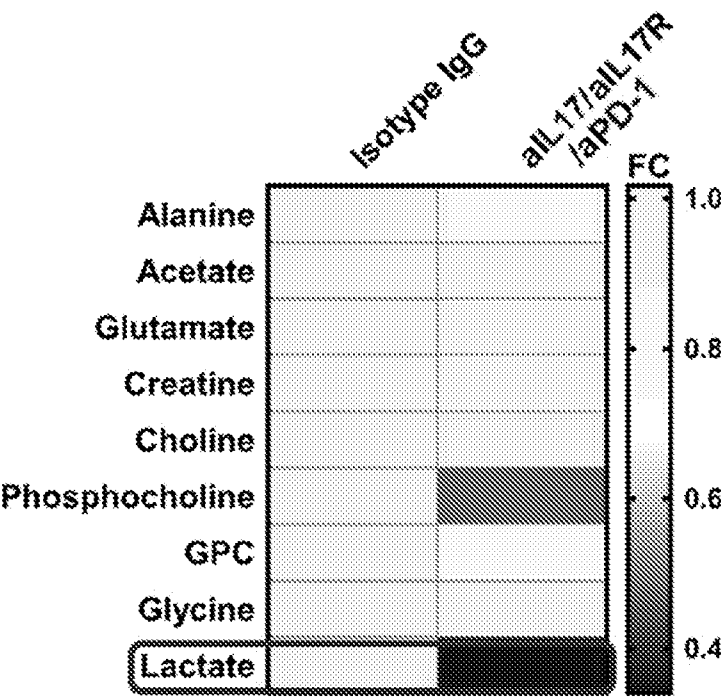


FIG. 4I

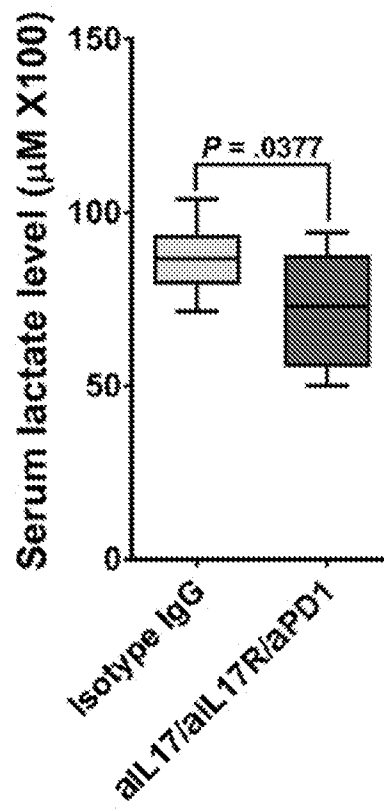


FIG. 4J

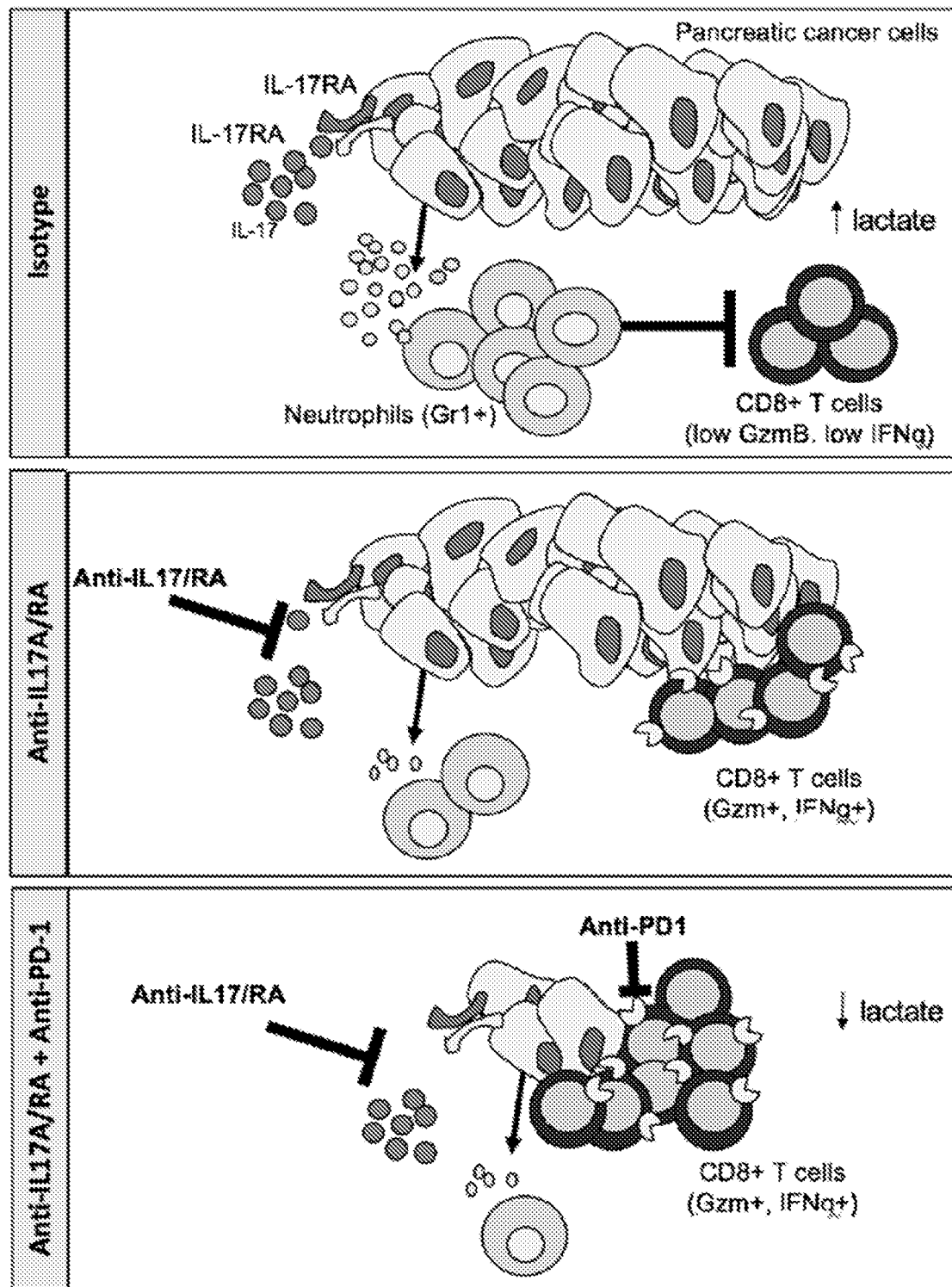


FIG. 5

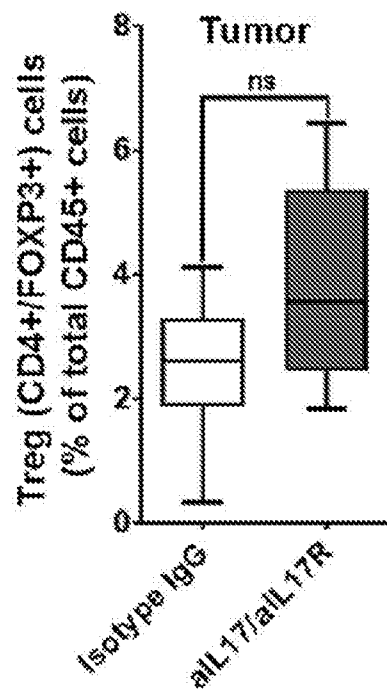


FIG. 6A

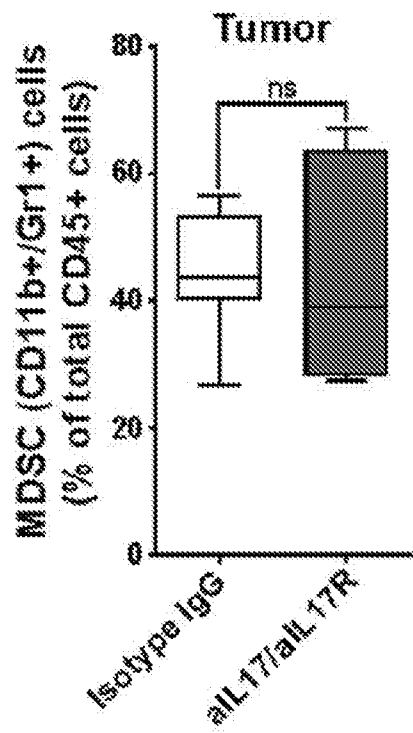


FIG. 6B

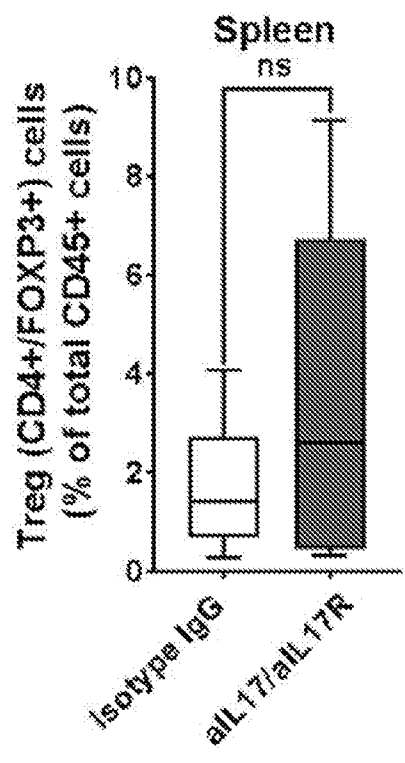


FIG. 6C

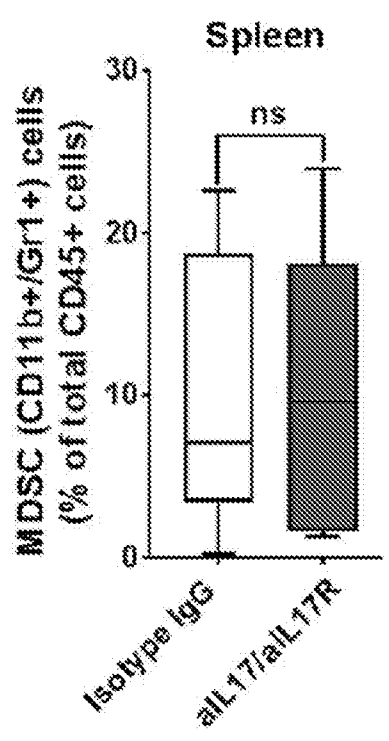


FIG. 6D

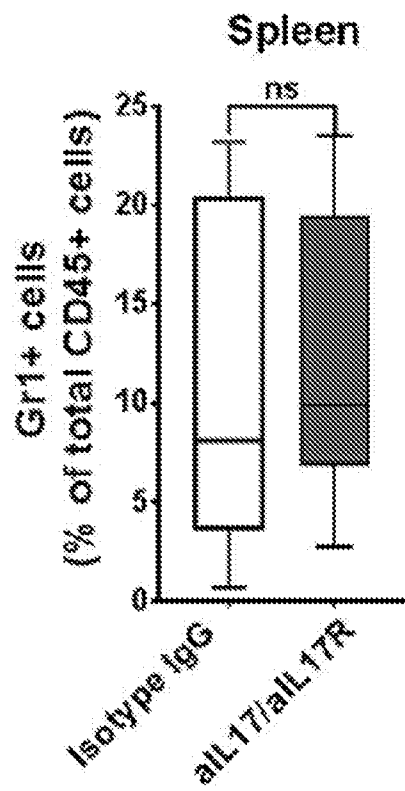


FIG. 6E

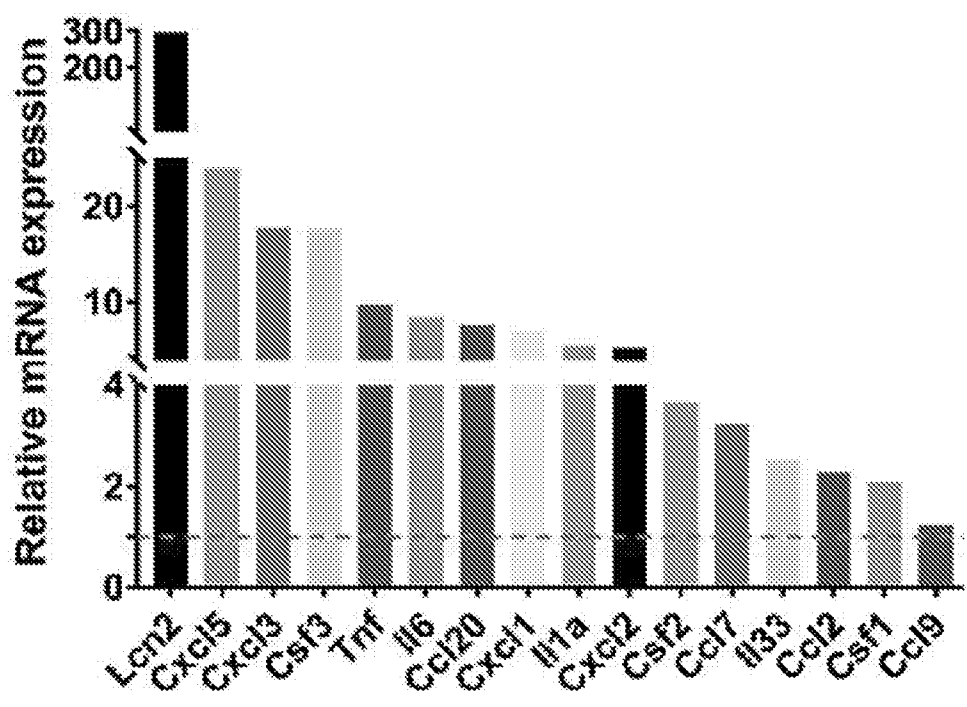


FIG. 6F

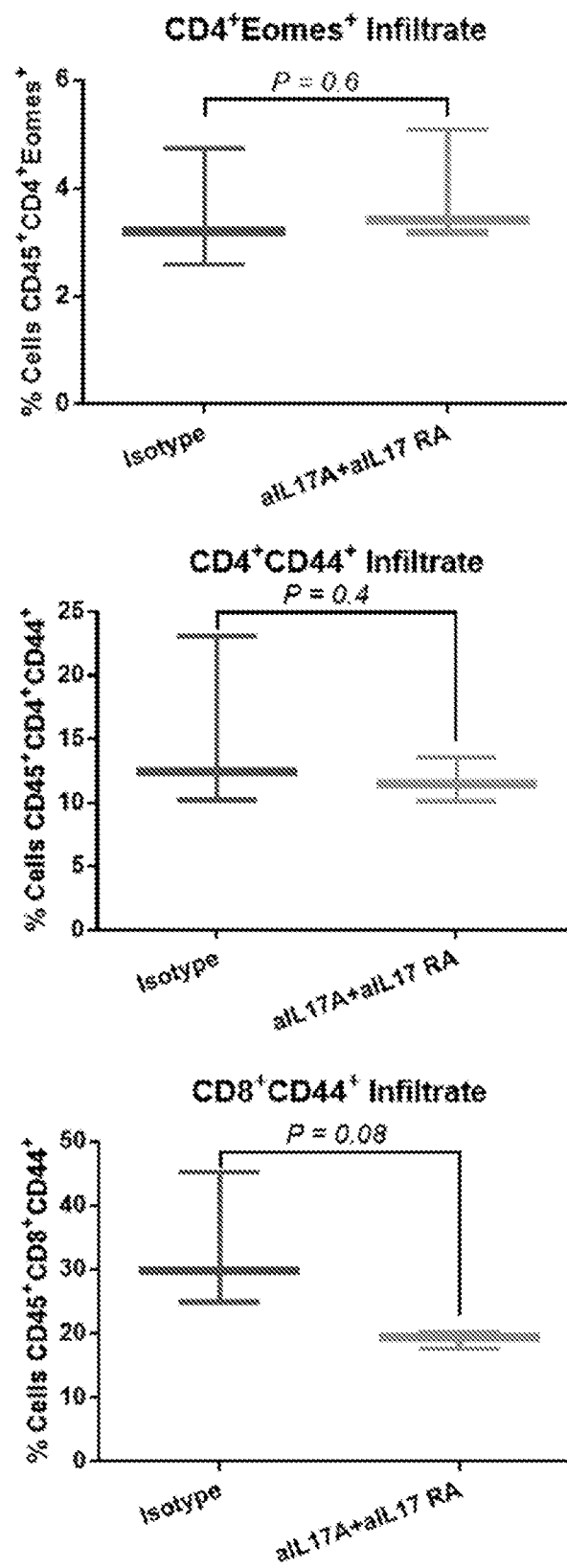


FIG. 7A

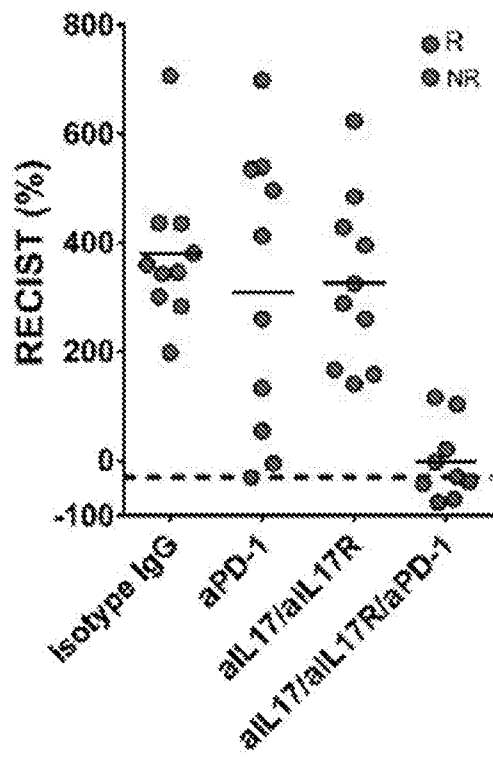


FIG. 7B

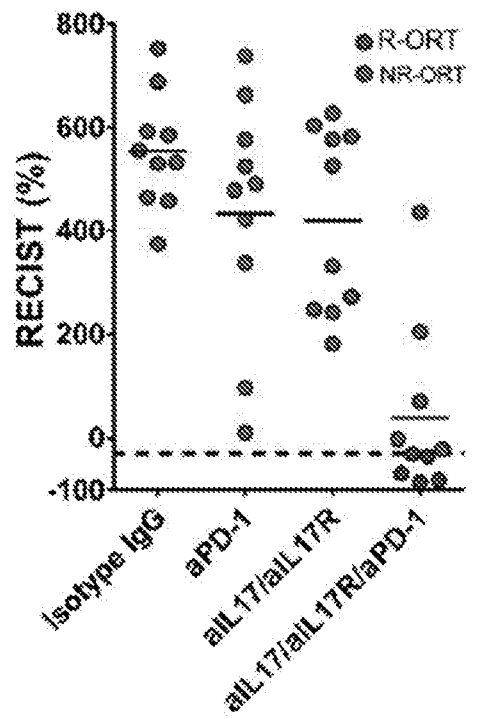


FIG. 7C

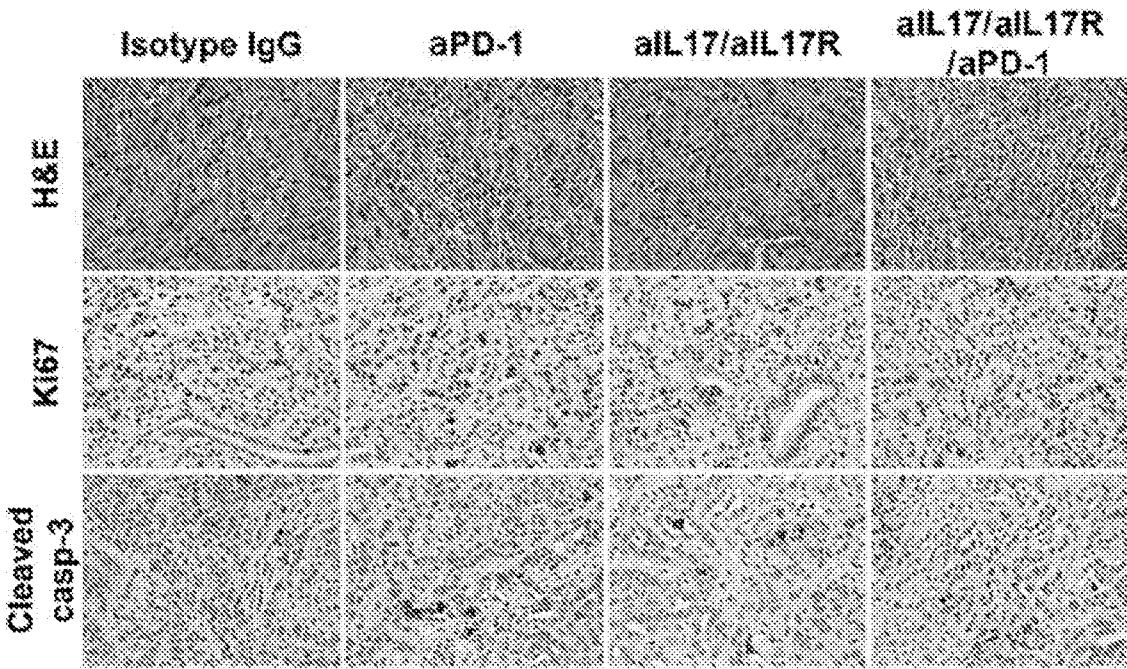
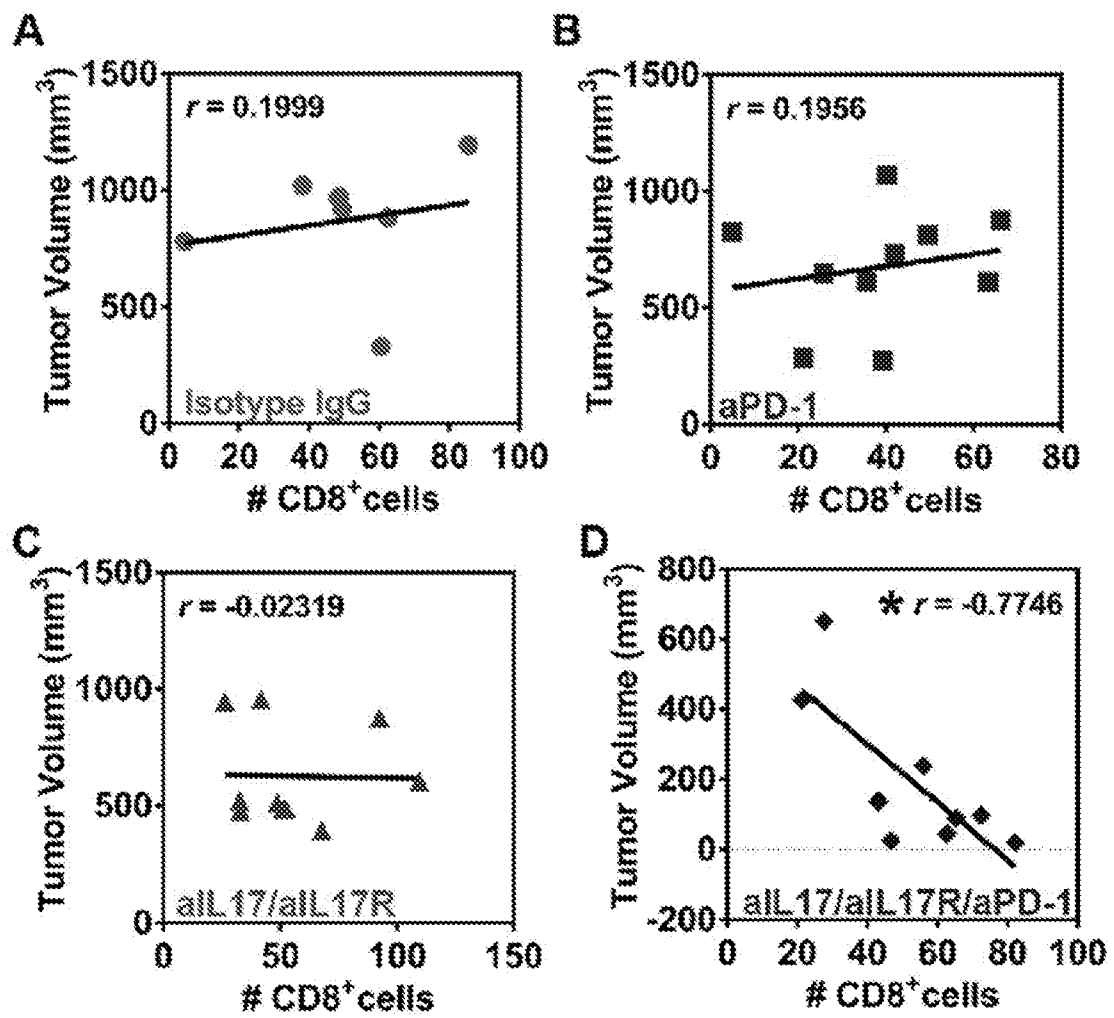


FIG. 7D



FIGS. 8A-D

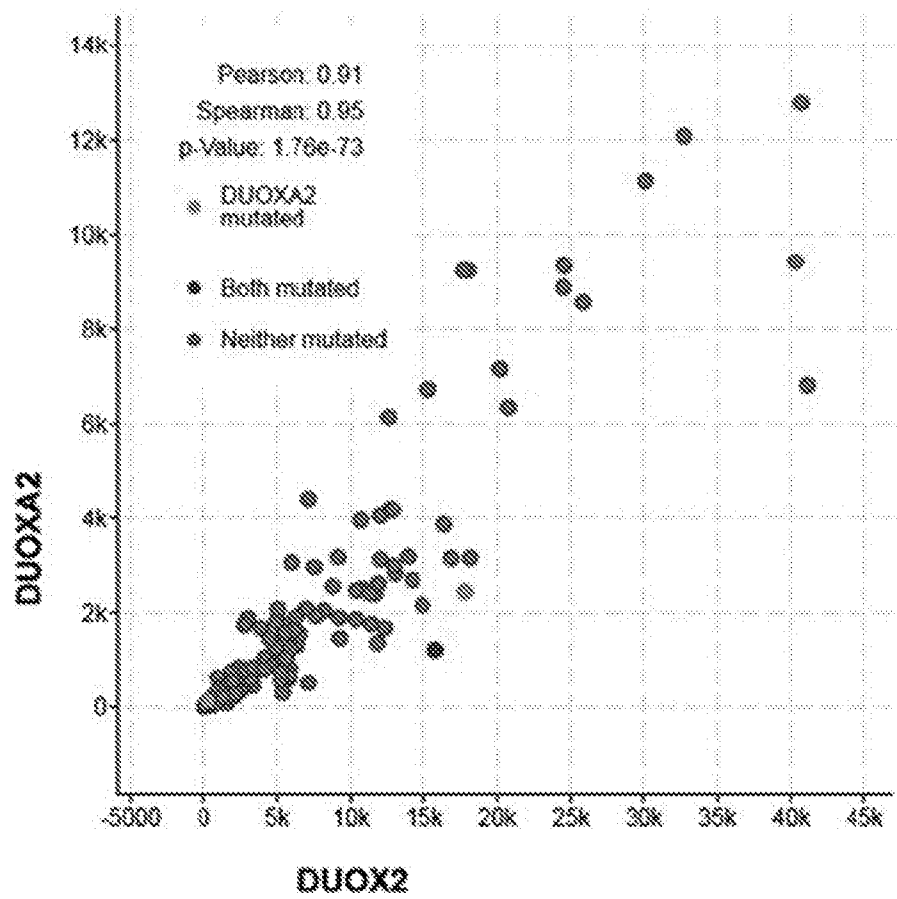


FIG. 9A

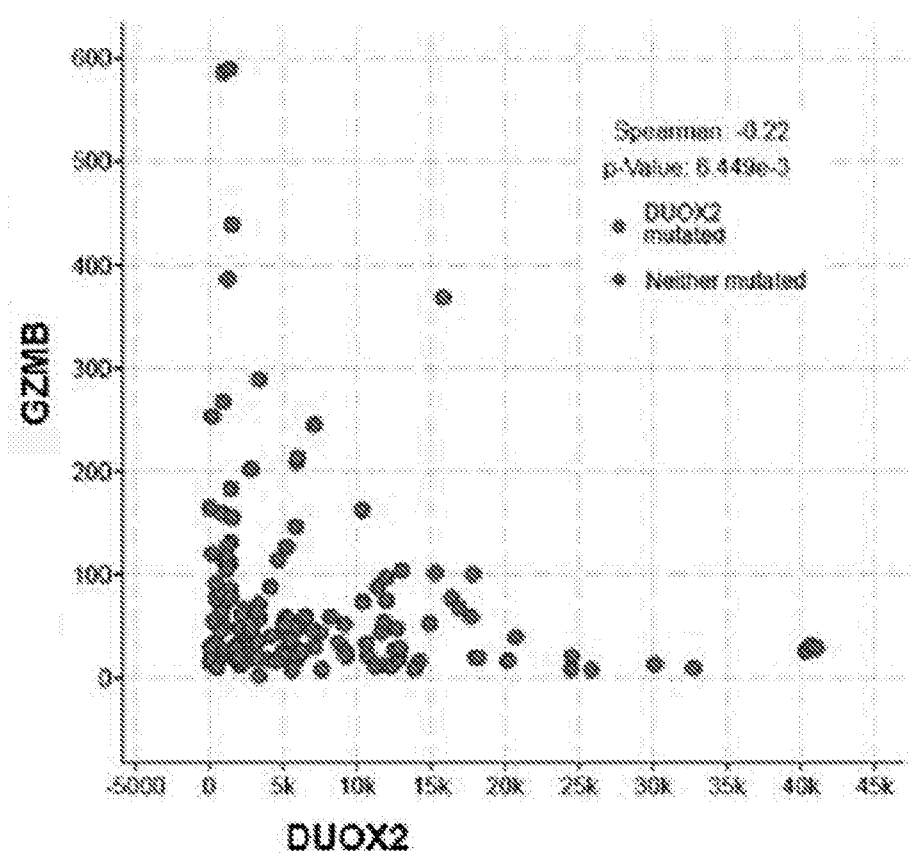


FIG. 9B

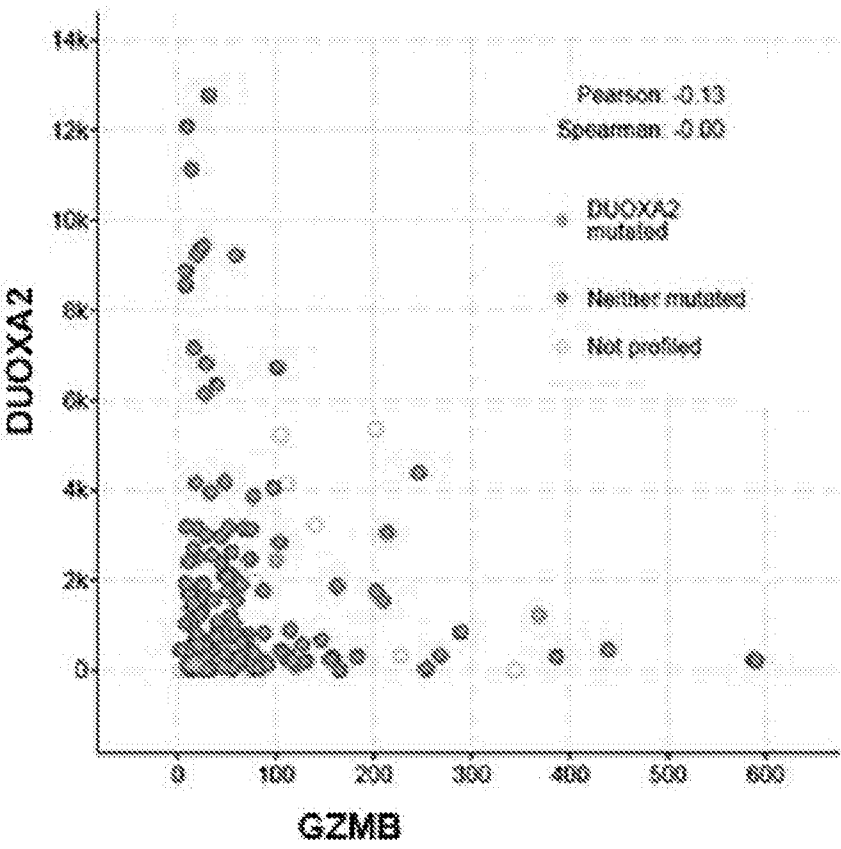


FIG. 9C

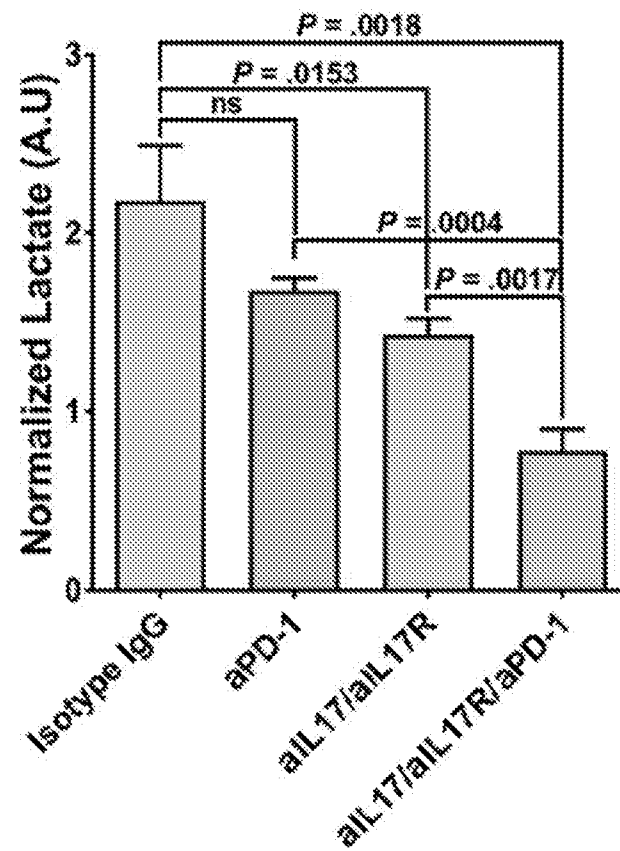


FIG. 9D

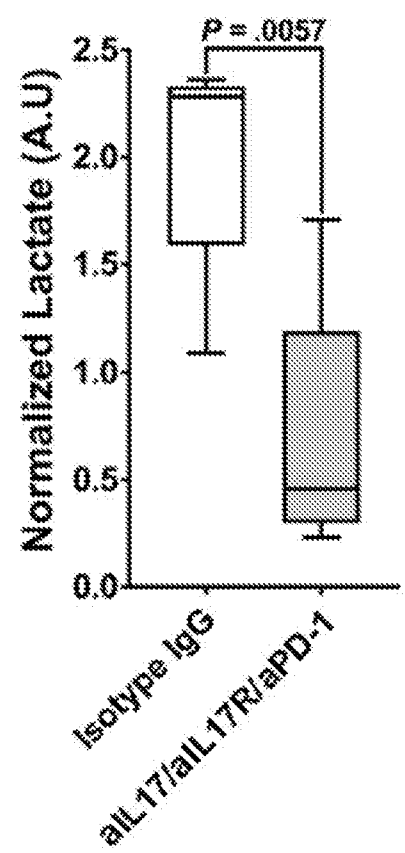


FIG. 9E

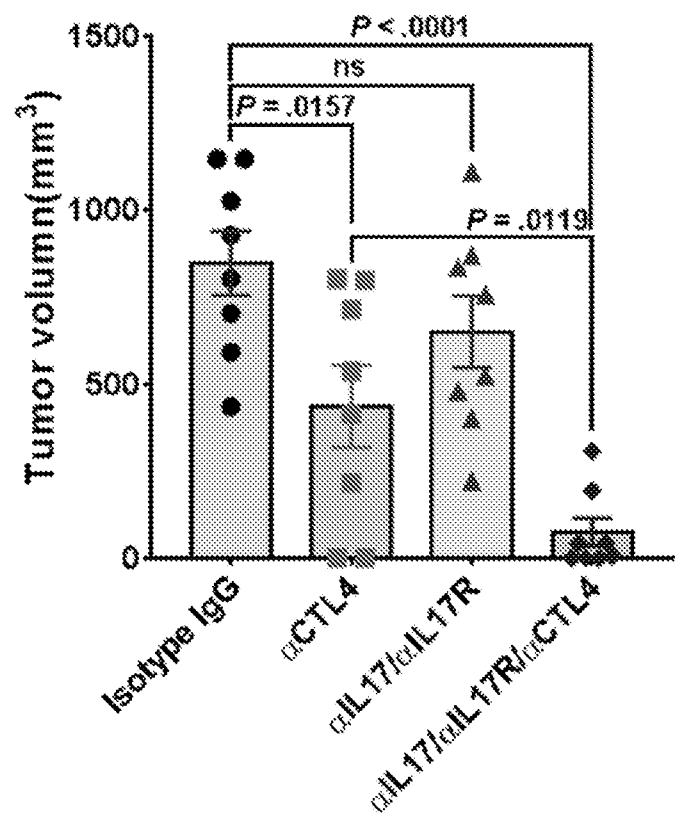


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/013467

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/20; C07K 16/24; C07K 16/28 (2020.01)

CPC - A61K 39/39558; A61K 2039/507; C07K 16/244; C07K 16/2818; C07K 16/2827; C07K 2317/73; C07K 2317/76 (2020.02)

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

USPC - 424/155.1; 424/174.1; 435/335; 435/344; 435/333.2; 530/388.23; 530/388.15 (keyword delimited)

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	WO 2016/044189 A1 (GENENTECH, INC. et al) 24 March 2016 (24.03.2016) entire document	1-7, 69-73
Y		24-27, 47-55, 74-77
Y	WALENTA et al. "High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers," Cancer Res, 15 February 2000 (15.02.2000), Vol. 60, No. 4, Pgs. 916-921. entire document	24-27
Y	KANG et al. "DUOX2-mediated production of reactive oxygen species induces epithelial mesenchymal transition in 5-fluorouracil resistant human colon cancer cells," Redox Biol, 23 April 2018 (23.04.2018), Vol. 17, Pgs. 224-235. entire document	47-55
Y	WO 2016/196377 A1 (MERRIMACK PHARMACEUTICALS, INC. et al) 08 December 2016 (08.12.2016) entire document	74-77
P, X	WO 2019/152344 A1 (UNIVERSITY OF LOUISVILLE RESEARCH FOUNDATION, INC.) 08 August 2019 (08.08.2019) entire document	1-7, 24-27, 47-55, 69-77
A	AKBAY et al. "Interleukin-17A Promotes Lung Tumor Progression through Neutrophil Attraction to Tumor Sites and Mediating Resistance to PD-1 Blockade," J Thorac Oncol, 06 May 2017 (06.05.2017), Vol. 12, No. 8, Pgs. 1268-1279. entire document	1-7, 24-27, 47-55, 69-77
A	MA et al. "Targeting of interleukin (IL)-17A inhibits PDL1 expression in tumor cells and induces anticancer immunity in an estrogen receptor-negative murine model of breast cancer," Oncotarget, 31 January 2017 (31.01.2017), Vol. 8, No. 5, Pgs. 7614-7624. entire document	1-7, 24-27, 47-55, 69-77



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

"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

03 March 2020

Date of mailing of the international search report

06 APR 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/013467

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LU et al. "NADPH oxidase DUOX1 and DUOX2 but not NOX4 are independent predictors in hepatocellular carcinoma after hepatectomy," Tumour Biol, 14 September 2011 (14.09.2011), Vol. 32, No. 6, Pgs. 1173-1182. entire document	1-7, 24-27, 47-55, 69-77
A	SHIME et al. "Tumor-secreted lactic acid promotes IL-23/IL-17 proinflammatory pathway," J Immunol, 01 June 2008 (01.06.2008), Vol. 180, No. 11, Pgs. 7175-7183. entire document	1-7, 24-27, 47-55, 69-77

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/013467

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8-23, 28-46, 56-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.