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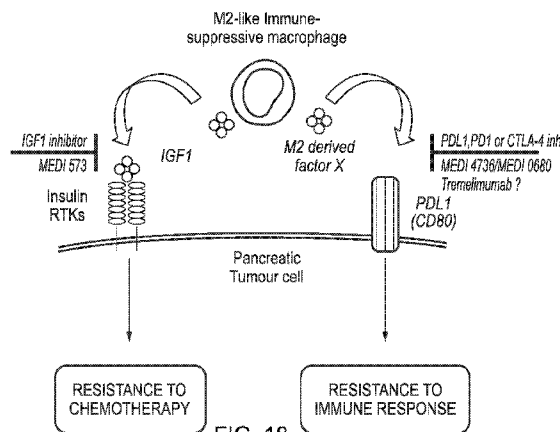


FIG. 18

(57) Abstract: The present invention relates to combinations and pharmaceutical compositions comprising an IGF inhibitor and a chemotherapeutic agent, and their use in treating a proliferative disorder such as cancer (for example a solid cancer such as pancreatic cancer). The invention also provides an IGF inhibitor for use in treating such proliferative disorders in combination with a chemotherapeutic agent, and a chemotherapeutic agent for use in treating such proliferative disorders in combination with an IGF inhibitor and a biomarker for identifying proliferative disorders which have increased responsiveness to the combined treatment.



INSULIN-LIKE GROWTH FACTOR INHIBITOR AND CHEMOTHERAPEUTIC AGENT FOR
USE IN CANCER THERAPY

The present invention relates to combinations and pharmaceutical compositions comprising an IGF inhibitor and a chemotherapeutic agent, and their use in treating a proliferative disorder such as cancer (for example a solid cancer such as pancreatic cancer). The invention also provides an IGF inhibitor for use in treating such proliferative disorders in combination with a chemotherapeutic agent, and a chemotherapeutic agent for use in treating such proliferative disorders in combination with an IGF inhibitor and a biomarker for identifying proliferative disorders which have increased responsiveness to the combined treatment.

BACKGROUND

Proliferative disorders such as cancer pose a significant health problem worldwide. Current options for treating such disorders include surgical resection, external beam radiation therapy and/or systemic chemotherapy. Such treatments are partially successful for some forms of cancer but are less successful for others. Drug resistance is one of the biggest challenges in cancer therapeutics and is the cause of relapse in the majority of cancer patients, including pancreatic cancer patients (Zahreddine and Borden, 2013).

By way of example, gemcitabine is the standard chemotherapeutic treatment for pancreatic cancer (also known as pancreatic ductal adenocarcinoma (PDA)) and yet 95% of patients diagnosed with pancreatic cancer and treated with gemcitabine die within 5 years of commencing treatment. The low efficacy of gemcitabine in the treatment of PDA is in part due to the ability of pancreatic cancer cells to become resistant to gemcitabine.

Understanding the molecular mechanisms underlying the development of cancer cell resistance to chemotherapy is critical to the development of durable treatment strategies for such proliferative disorders.

Although multiple factors can contribute to the resistance of cancers such as PDA to chemotherapeutic therapies, one dominant player is the presence of a rich pro-tumoral microenvironment (Gore and Korc, 2014; Lunardi et al., 2014; McMillin et al., 2013; Mielgo and Schmid, 2013; Noy and Pollard, 2014). Tumour associated macrophages (TAMs) are key drivers of this pro-tumoral microenvironment and can promote tumour cell proliferation, drug resistance and metastasis in many cancers including PDA (Noy and Pollard, 2014). Thus, high numbers of TAMs often correlate with resistance to chemotherapy and

metastasis leading to poor survival in pancreatic cancer patients (Ino et al., 2013; Kurahara et al., 2011). Previous studies have shown that inhibition of myeloid cell infiltration into the tumour restrains cancer progression (Schmid et al., 2011a; Schmid et al., 2013). However, TAMs can be polarised into M1 inflammatory/anti-tumoral
5 macrophages that will activate an immune response against the tumour or M2 immunosuppressive/pro-tumoral macrophages that promote tumour immunity (Kurahara et al., 2011; Mielgo and Schmid, 2013; Ruffell et al., 2012).

TAMs are present in the tumour microenvironment of many cancers, especially solid
10 cancers such as pancreatic cancer, lung cancer, breast cancer, melanoma, colorectal cancer, ovarian cancer, gastric cancer, thyroid cancer, liver cancer and prostate cancer. Therapeutics that specifically inhibit the pro-tumoral functions of TAMs, while sparing their anti-tumoral capacity, hold great promise in the goal of restraining cancer progression, metastasis and relapse.

15 There is a clear need for new therapeutic agents and methods for the treatment of proliferative disorders such as cancer.

BRIEF SUMMARY OF THE DISCLOSURE

20 The inventors have investigated the mechanisms by which pro-tumoral TAMs impact PDA and breast cancer resistance to chemotherapy and tumour progression.

The invention is based on the surprising finding that M2-like macrophages express high
25 levels of IGFs, such as IGF-1 and IGF-2 and are an important source of these growth factors in the tumour microenvironment. Surprisingly, the inventors have identified that secretion of IGF-1 and IGF-2 by M2-like macrophages leads to the activation of Insulin Receptor signaling and subsequent cancer cell resistance to chemotherapy.

The inventors have also found that within the tumour microenvironment, M2-like
30 macrophages and α SMA+ stromal cells are the main sources of IGFs.

Advantageously, the inventors have found that directly blocking IGF binding, as opposed to blocking the IGF receptors, counteracts this resistance to chemotherapy and restores sensitivity to chemotherapeutic agents, resulting in reduced tumour growth in pancreatic
35 cancer mice treated with a combination of chemotherapeutic agent (gemcitabine) and an IGF inhibitor (an IGF-blocking antibody). Furthermore, the inventors have shown that directly blocking IGF binding also counteracts pancreatic cancer cell resistance to

paclitaxel and 5'FU (also known as fluorouracil herein), two additional chemotherapeutic agents commonly used in the treatment of pancreatic cancer.

These novel findings indicate that combinations and pharmaceutical compositions

5 comprising an IGF inhibitor (e.g. an IGF blocking antibody) and a chemotherapeutic agent (e.g. gemcitabine and/or 5'FU and/or nab-paclitaxel) are useful in the treatment of a proliferative disorder such as cancer (for example a solid cancer such as pancreatic cancer or breast cancer).

10 The inventors have also identified that murine pancreatic cancer cells exposed to M2-like macrophages upregulate expression of PD-L1 and CD80. Consistent with this, the inventors have found that human PDA tissue samples show an increase in PD-L1 expression compared to controls. Binding of PD-L1 or CD80 with its receptor PD-1 and CTLA-4 respectively leads to functional exhaustion of T cells (reviewed by Pardoll D,
15 nature reviews cancer, 12, 252-264 (April 2012); the blockade of immune checkpoints in cancer immunotherapy). Accordingly, the observed increase in PD-L1 and/or CD80 expression in cancer cells of a subject may attenuate, e.g. suppress and/or inhibit the subject from eliciting an effective immune response.

20 Novel combinations and pharmaceutical compositions comprising an IGF inhibitor, a chemotherapeutic agent and optionally a PD-L1 inhibitor and/or a CD80 inhibitor, and their use in treating a proliferative disorder such as cancer in a subject are therefore provided. Such combinations and pharmaceutical compositions advantageously counteract the development of resistance to chemotherapy (and optionally resistance to immune
25 response) in such subjects.

In one aspect, the invention provides a pharmaceutical composition comprising a first composition comprising an IGF inhibitor and a pharmaceutically acceptable excipient, adjuvant, diluent or carrier, and a second composition comprising a chemotherapeutic
30 agent and a pharmaceutically-acceptable excipient, adjuvant, diluent or carrier, wherein the IGF inhibitor directly inhibits the biological activity of IGF.

Optionally, the first and second compositions are provided in a form which is suitable for sequential, separate and/or simultaneous administration.

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Optionally, the pharmaceutical composition further comprises a PD-L1 and/or CD80 inhibitor.

In another aspect, the invention provides a pharmaceutical composition according to the invention for use in treating a proliferative disorder in a subject.

- 5 In another aspect, the invention provides the use of the pharmaceutical composition of the invention in the treatment of a proliferative disorder in a subject.

In another aspect, the invention provides a combination comprising an IGF inhibitor and a chemotherapeutic agent for use in treating a proliferative disorder in a subject, wherein the
10 IGF inhibitor directly inhibits the biological activity of IGF.

Optionally, the combination further comprises a PD-L1 inhibitor and/or a CD80 inhibitor.

In another aspect, the invention provides an IGF inhibitor for use in treating a proliferative
15 disorder in a subject in combination with a chemotherapeutic agent, wherein the IGF inhibitor directly inhibits the biological activity of IGF.

Optionally, the IGF inhibitor is for use in combination with (i) the chemotherapeutic agent and (ii) a PD-L1 inhibitor and/or a CD80 inhibitor.
20

In another aspect, the invention provides a chemotherapeutic agent for use in treating a proliferative disorder in a subject in combination with an IGF inhibitor, wherein the IGF inhibitor directly inhibits the biological activity of IGF.

- 25 Optionally, the chemotherapeutic agent is for use in combination with (i) the IGF inhibitor and (ii) a PD-L1 inhibitor and/or a CD80 inhibitor.

Optionally, in accordance with all aspects of the invention, the proliferative disorder is cancer.
30

Optionally, in accordance with all aspects of the invention, the cancer is selected from pancreatic cancer, lung cancer, breast cancer, melanoma, colorectal cancer, ovarian cancer, gastric cancer, thyroid cancer, liver cancer, and prostate cancer.

- 35 Optionally, in accordance with all aspects of the invention, the proliferative disorder is pancreatic cancer and/or breast cancer.

Optionally, in accordance with all aspects of the invention, a tumor sample isolated from a patient has increased levels of M2-like macrophages (preferably M2-like CD163+ macrophages) compared to a control sample (e.g. normal tissue) or compared to the predetermined reference level.

5

Optionally, in accordance with all aspects of the invention, said subject is susceptible to developing IGF-induced resistance to said chemotherapeutic agent.

Optionally, in accordance with all aspects of the invention, the IGF inhibitor inhibits at least one IGF selected from IGF-1 and IGF-2.

Optionally, in accordance with all aspects of the invention, the IGF inhibitor inhibits binding of at least one IGF to the insulin receptor, IGFR or hybrid receptors. Further optionally, the IGF inhibitor inhibits binding of IGF-1 to the insulin receptor, IGFR or hybrid receptors and/or inhibits binding of IGF-2 to the insulin receptor, IGFR or hybrid receptors.

Optionally, in accordance with all aspects of the invention, the IGF inhibitor is an anti-IGF antibody or an antigen binding fragment thereof.

Optionally, in accordance with all aspects of the invention, the chemotherapeutic agent is selected from the group consisting of a nucleoside analogue, a topoisomerase inhibitor, a platinum complex, an anti-mitotic agent and combinations thereof. Further optionally, the nucleoside analogue is gemcitabine or fluorouracil and the anti-mitotic agent is paclitaxel

Optionally, in accordance with all aspects of the invention, the IGF inhibitor is an anti-IGF antibody or antigen binding fragment thereof, and wherein the chemotherapeutic agent is gemcitabine, fluorouracil or paclitaxel

Optionally, in accordance with all aspects of the invention, the PD-L1 inhibitor inhibits binding of PDL1 to a PD-1 receptor.

Optionally, in accordance with all aspects of the invention, the PD-L1 inhibitor is an anti-PD-L1 antibody or an antigen binding fragment thereof.

Optionally, in accordance with all aspects of the invention, the CD80 inhibitor inhibits binding of CD80 to a CTLA-4 receptor.

Optionally, in accordance with all aspects of the invention, the CD80 inhibitor is an anti-CD80 antibody or an antigen binding fragment thereof.

In another aspect, the invention provides the use of M2-like macrophages and/or Insulin/IGF receptor activation (measured as expression of phospho-Insulin/IGF receptors) as biomarkers to select a patient population responsive to or sensitive cancer treatment with an IGF inhibitor and a chemotherapeutic agent.

In another aspect, the invention provides the use of a M2-like macrophages and/or phosphor-Insulin/IGFR as biomarkers to predict a patient population at risk of chemoresistance to a chemotherapeutic agent.

In another aspect, the invention provides a method of increasing the sensitivity rate (efficacy rate) of a combination of an IGF inhibitor and a chemotherapeutic agent to treat cancer in a patient population said method comprising selecting a sub population expressing M2-like macrophage and/or phospho-Insulin/IGFR biomarkers.

In another aspect, the invention provides a method of identifying a subject having increased likelihood of responsiveness or sensitivity to a combination of an IGF inhibitor and a chemotherapeutic agent comprising:

- a. Determining the level of M2-like macrophages in a tumor sample isolated from the subject; and
 - b. comparing the level of M2-like macrophages in the patient sample with the level of M2-like macrophages in a control sample (e.g. normal non cancerous tissue) or with a predetermined reference level for M2-like macrophages
- wherein an increased level of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level is prognostic of an increased likelihood of responsiveness or sensitivity to an IGF inhibitor and a chemotherapeutic agent in the subject.

In another aspect, the invention provides a method of identifying a subject having responsiveness or sensitivity to an IGF inhibitor comprising:

- a. Determining the level of M2-like macrophages in a tumor sample isolated from the subject; and
- b. comparing the level of M2-like macrophages in the patient sample with the level of M2-like macrophages in a control sample or with a predetermined reference level for M2-like macrophages

wherein an increased level of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level is prognostic of an increased likelihood of responsiveness or sensitivity to an IGF inhibitor.

5 In another aspect, the invention provides a method of treating a subject having cancer comprising:

a. Determining the level of M2-like macrophages in a tumor sample isolated from the subject; and

b. comparing the level of M2-like macrophages in the patient sample with the level of
10 M2-like macrophages in a control sample or with a predetermined reference level for M2-like macrophages

c. administering a therapeutically effective amount of a chemotherapeutic agent and an IGF inhibitor when there is an increased level of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level.

15

Optionally, in accordance with all aspects of the invention, the M2-like macrophage is CD163+.

Optionally, in accordance with all aspects of the invention, the predetermined reference
20 level of M2-like macrophages is at least 20 M2-like macrophages in the tumour core sample.

Optionally, in accordance with all aspects of the invention, the cancer may be pancreatic cancer or breast cancer.

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Optionally, in accordance with all aspects of the invention, the chemotherapeutic agent may be gemcitabine, fluorouracil or paclitaxel.

In another aspect, the invention provides a kit for identifying a patient population
30 responsive to a combined treatment with an IGF inhibitor and a chemotherapeutic agent comprising:

a. a detectably labelled agent that specifically binds to a M2-like CD163+ macrophage; and

b. reagents for the assay.

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In another aspect, the invention provides an assay device comprising a compound or agent capable of detecting a M2-like macrophage.

In another aspect, the invention provides a pharmaceutical composition as hereinbefore described with reference to the accompanying drawings.

- 5 In another aspect, the invention provides a pharmaceutical composition for use in treating a proliferative disorder in a subject as herein before described with reference to the accompanying drawings.

- 10 In another aspect, the invention provides the use of the pharmaceutical composition in the treatment of a proliferative disorder in a subject as herein before described with reference to the accompanying drawings.

- 15 In another aspect, the invention provides a combination comprising an IGF inhibitor and a chemotherapeutic agent for use in treating a proliferative disorder in a subject as herein before described with reference to the accompanying drawings.

- 20 In another aspect, the invention provides an IGF inhibitor for use in treating a proliferative disorder in a subject in combination with a chemotherapeutic agent, as herein before described with reference to the accompanying drawings.

- In another aspect, the invention provides a chemotherapeutic agent for use in treating a proliferative disorder in a subject in combination with an IGF inhibitor as herein before described with reference to the accompanying drawings.

25 BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention are further described hereinafter with reference to the accompanying drawings:

- 30 Figure 1 illustrates increased macrophage infiltration in biopsies from PDA patients.

Figure 2 illustrates increased M2 macrophage infiltration in biopsies from PDA patients.

- 35 Figure 3 illustrates that human macrophages stimulated with human pancreatic cancer cells behave like M2 tumour promoting (immunosuppressive) macrophages.

Figure 4 illustrates that macrophages promote resistance of pancreatic cancer cells to gemcitabine.

5 Figure 5 illustrates that macrophage derived factors promote chemoresistance of pancreatic cancer cells.

Figure 6 illustrates that macrophages promote chemoresistance of pancreatic cancer cells.

10 Figure 7 illustrates that macrophage (M2) derived factors specifically activate Insulin receptor in pancreatic cancer cells.

Figure 8 illustrates expression of Insulin receptor ligands in human M2-like macrophages.

15 Figure 9 illustrates expression levels of IGF-1 in human primary macrophages unstimulated or stimulated with human pancreatic cancer cells in two independent experiments.

20 Figure 10 illustrates that IGF-1 expression is increased in murine primary macrophages stimulated with murine KPC tumour cells.

Figure 11 illustrates that blocking of IGF counteracts macrophage-induced chemoresistance of mouse pancreatic KPC cancer cells and restores their sensitivity to gemcitabine.

25 Figure 12 illustrates that blocking of IGF counteracts macrophage-induced chemoresistance of mouse KPC pancreatic cancer cells and restores their sensitivity to gemcitabine.

30 Figure 13 illustrates that blocking of IGF restores KPC cells sensitivity to gemcitabine and that recombinant IGF-1 is sufficient to mediate resistance of pancreatic cancer cells to gemcitabine.

35 Figure 14 illustrates that blocking of IGF restores tumour cells sensitivity to gemcitabine and that recombinant IGF-1 is sufficient to mediate resistance of pancreatic cancer cells to gemcitabine.

Figure 15 illustrates that in vivo blockade of IGF in combination with gemcitabine reduces pancreatic tumour growth in mice with pancreatic cancer.

Figure 16 illustrates that KPC tumour cells exposed to M2 like macrophages up-regulate PDL1 and CD80 expression. Murine pancreatic cancer cells isolated from the genetic KPC (Kras^{G12D/+}; p53^{R172H/+}; Pdx1-Cre) mouse model were cultured in the absence or presence of primary murine macrophage conditioned media for 24 hours. Cells were harvested and stained with anti-PDL1-PE/Cy7, anti-CD80-PE/Cy7 and anti-CD86-PE antibodies from Biolegend. Cells were analysed by flow cytometry for the percentage of cells expressing PDL1, CD80 and CD86. Expression of PDL1 and CD80 was found to be significantly increased in KPC cells when cultured with macrophage conditioned media. However, KPC cells did not seem to express any CD86 in the absence or presence of macrophage conditioned media.

Figure 17 illustrates that human epithelial cells from malignant pancreatic ducts express high levels of PDL1. Tissue samples from normal pancreas and PDA were stained for PDL1 (using the anti-PDL1 antibody from abcam - ab58810). It was found that PDL1 is expressed in the epithelial malignant pancreatic cancer cells of biopsies from PDA patients.

Figure 18 illustrates the rationale for combining IGF inhibitors, checkpoint inhibitors (e.g. a PDL-1 inhibitor and/or a CD80 inhibitor) and chemotherapy in the treatment of pancreatic cancer.

Figure 19 illustrates that IGF blockade partially restores sensitivity of pancreatic cancer cells to 5-FU (fluorouracil).

Figure 20 illustrates macrophage secreted factors induce chemoresistance in A: human pancreatic Suit-2 cancer cells, B: primary mouse pancreatic cells and C: primary mouse breast cancer cells following culture in the presence or absence of human primary macrophages or macrophage conditioned media (MCM) from human or mouse primary macrophages.

Figure 21 illustrates increased presence of 1: phospho-Insulin receptor, 2: phospho-AXL receptor and 3: phospho- Ephrin receptor in human pancreatic cancer Suit-2 cells which were serum starved for 24 hours and exposed for 2 hours to human MCM compared to those left unexposed using a phospho-receptor tyrosine kinase array.

Figure 22 illustrates an immunoblot analysis of phospho-Insulin receptor, Insulin receptor, phospho- IGF receptor 1, IGFR1 and tubulin in Suit-2 cells serum starved or exposed to MCM for 30 min or 3 hours.

5

Figure 23 illustrates an immunoblot analysis of pan-phospho tyrosine, Insulin receptor and IGFR1 in Insulin and IGF receptor immunoprecipitates of Suit-2 cells treated with human MCM for 3 hours or left untreated.

10 Figure 24 illustrates quantification of *IGF-1* and *IGF-2* mRNA expression levels in human primary macrophages unexposed or exposed to tumor conditioned media (TCM) from human pancreatic cancer Suit-2 cells.

15 Figure 25 illustrates quantification of *IGF-1*, *IGF-2* and *Insulin* mRNA expression levels in mouse primary macrophages.

Figure 26 illustrates quantification of *IGF-1* and *IGF-2* mRNA expression levels in: primary murine macrophages cultured under standard conditions with M-CSF1, M1 polarized macrophages, M2 polarized macrophages, macrophages cultured in the presence of TCM from murine primary Py230 breast cancer cells and from murine primary KPC pancreatic cancer cells.

25 Figure 27 illustrates Immunoblot analysis of phospho-Insulin/IGF receptor, phospho-Insulin receptor, Insulin receptor, phospho- IGF receptor 1, IGFR1 and tubulin, in human pancreatic cancer Suit-2 cells, murine primary KPC pancreatic cancer and Py230 breast cancer derived cells, serum starved, exposed to MCM or exposed to recombinant IGF for 3 hours.

30 Figure 28 illustrates that blockade of IGF impairs macrophage-mediated activation of Insulin/IGF receptor and downstream signalling molecules IRS1, IRS2 and AKT in cancer cells by Immunoblotting analysis of Suit-2 cells untreated or treated with MCM or MCM+IGF blocking antibody for 3 hours.

35 Figure 29 illustrates quantification of cell death in Suit-2 cells treated with gemcitabine, MCM and IGF blocking antibody for 24 hours. Error bars represent s.d. (n=3); ** two tailed p value ≤ 0.01 , * two tailed p value ≤ 0.05 using a student's t- test.

Figure 30 illustrates primary mouse KPC-derived pancreatic cancer cells were untreated or treated with gemcitabine, MCM, IGF blocking antibody or recombinant IGF for 24 hours and percentage of cell death was quantified by flow cytometry. Error bars represent s.d. (n=3); ** two tailed p value ≤ 0.01 using a student's t- test.

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Figure 31 illustrates representative flow cytometry dot blots of KPC-derived cells exposed to gemcitabine, MCM, IGF blocking antibody and recombinant IGF.

Figure 32 illustrates KPC-derived cells which were cultured in the presence or absence of MCM or recombinant IGF and treated with 100 or 1000 nM nab-paclitaxel for 24 hours. Percentage of cell death was quantified by flow cytometry. Error bars represent s.d. (n=3); * two tailed p value ≤ 0.05 using a student's t- test.

Figure 33 illustrates human breast MDA-MB-231 cancer cells cultured in the presence or absence of MCM or recombinant IGF and treated with 500 nM nab-paclitaxel for 48 hours. Percentage of cell death was quantified by flow cytometry. Error bars represent s.d. (n=3); * two tailed p value ≤ 0.05 , ** two tailed p value ≤ 0.01 using a student's t- test.

Figure 34 illustrates that phospho-insulin/IGF receptors confocal microscopy images of frozen human PDA tissues immunofluorescently co- stained for the tumor epithelial marker CK11 (in green), phospho-Insulin/IGF receptor (in red), and nuclei (in blue) and shows that phospho-insulin/IGF receptors are activated on cancer cells in biopsies from PDA patients. Scale bar, 100 μm .

Figure 35 illustrates immunohistochemical staining of phospho- Insulin/IGF receptor in normal human pancreas and biopsies from PDA patients. Scale bars, 100 μm and 50 μm .

Figure 36 illustrates a pie diagram representing the percentage of phospho-Insulin/IGF receptor positive (red) and negative (green) tumors assessed in tissue microarrays (TMA) containing biopsies from 54 different PDA patients.

Figure 37 illustrates confocal microscopy images of frozen human PDA tissues immunofluorescently co-stained for CD68 (in green), CK19 (in red) and nuclei (in blue). Scale bar, 100 μm .

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Figure 38 illustrates immunohistochemical staining of CD163 in normal human pancreas and biopsies from PDA patients. Scale bars, 100 μm and 50 μm .

Figure 39 illustrates serial sections of biopsies from PDA patients immunohistochemically stained for phospho- Insulin/IGF receptor and CD163. Scale bar, 100 μ m.

- 5 Figure 40 illustrates a contingency table and results from statistical analysis showing a positive correlation between phospho-Insulin/IGFR expression in tumors and increased M2-like macrophage infiltration. Chi-square = 9.272; $p=0.002$.

- Figure 41 illustrates a contingency table and results from statistical analysis
10 showing a positive correlation between tumors that are both phospho-Insulin/IGFR positive and highly infiltrated by M2-like macrophages and advanced tumor stage. Fisher Exact Test; $p=0.018$.

- Figure 42 illustrates serial sections of biopsies from: (a) non-malignant breast tissue
15 immunohistochemically stained for phospho-Insulin/IGF receptor and CD163. Scale bars, 100 μ m and 50 μ m. (b) and (c) from breast cancer patients immunohistochemically stained for phospho-Insulin/IGF receptor and CD163. Scale bars, 100 μ m and 50 μ m.

- Figure 43 illustrates a histogram depicting the quantification of CD68 and CD163 positive
20 macrophages in normal breast and breast cancer tissue samples. $n = 6-8$ fields counted. Error bars represent s.d. ($n=3$); * two tailed p value ≤ 0.05 , *** two tailed p value ≤ 0.005 using a student's t - test.

- Figure 44 illustrates a pie diagram representing the percentage of phospho-Insulin/IGF
25 receptor positive (red) and negative (green) tumors assessed in a tissue microarray containing biopsies from 75 different breast cancer patients.

- Figure 45 illustrates a positive correlation between phospho-Insulin/IGFR expression in
30 breast cancer tumors and increased M2-like macrophage infiltration.

- Figure 46 illustrates that intra-tumoral macrophages and α -SMA+ stromal cells are the
main sources of IGF in pancreatic tumors *in vivo* **(a)** KPC-derived tumor cells were
orthotopically implanted into the pancreas of syngeneic recipient mice, mimicking PDA.
(b) Hematoxylin and Eosin (H&E) and immunohistochemical staining of Ki-67, α SMA,
35 phospho-Insulin/IGF receptor, CD68 and CD206 staining of naïve mouse pancreas and
murine PDA tissue samples harvested at day 30 after tumor implantation. **(c)** KPC^{luc/zsGreen}
(zsGreen) -derived tumor cells were orthotopically implanted into the pancreas of syngeneic

recipient mice, mimicking PDA. Tumors were harvested at day 23 after implantation digested and tumor cells, non-immune stromal cells and M1-like and M2-like macrophages were sorted by flow cytometry. **(d)** Quantification of IGF-1, mRNA expression levels in CD45+/F4/80+/CD206- M1-like macrophages, CD45+/F4/80+/CD206+ M2-like macrophages, CD45-/GFP- non-immune stromal cells and CD45-/GFP+ tumor cells isolated from murine pancreatic tumors. Error bars represent s.d. (n=3). **(e)** Quantification of IGF-2, mRNA expression levels in CD45+/F4/80+/CD206- M1-like macrophages, CD45+/F4/80+/CD206+ M2-like macrophages, CD45-/GFP- non-immune stromal cells and CD45-/GFP+ tumor cells isolated from murine pancreatic tumors. Error bars represent s.d. (n=3). Error bars represent s.d. (n=3).

Figure 47 illustrates a combination with gemcitabine with IGF blockade inhibites tumor growth in the syngeneic orthotopic pancreatic cancer model: **(a)** KPC^{luc/zsGreen} (zsGreen) - derived pancreatic tumor cells were orthotopically implanted into the pancreas of syngeneic recipient mice, and mice were treated, starting at day 7 after tumor implantation, twice a week i.p., with either control IgG antibody, gemcitabine (100mg/Kg), IGF blocking antibody BI 836845 (100mg/Kg) or a combination of gemcitabine with IGF blocking antibody. **(b)** Representative images of tumors and tumor weights are shown (n= 6 mice per group). **(c)** Pancreatic tumors were digested and percentage of intra- tumoral F4/80+ macrophages, Ly6C+/Ly6G- inflammatory monocytes, Gr1+/CD11b+ neutrophils and myeloid derived suppressor cells (MDSCs) and CD8+ cytotoxic T cells (CTCs), among CD45+ immune cells, were quantified by flow cytometry (n=3). **(d)** Percentage of intra-tumoral CD206- M1-like macrophages and CD206+ M2-like macrophages, among F4/80+ macrophages, were quantified by flow cytometry (n=3). **(e)** Immunohistochemical staining of phospho-Insulin/IGFR and cleaved caspase-3 in murine PDA tumors treated with IgG (control), gemcitabine, IGF blocking antibody BI 836845 or gemcitabine + BI 836845. **(f)** Quantification of cleaved caspase-3 positive apoptotic cells in PDA tumors treated with IgG (control), gemcitabine, IGF blocking antibody BI 836845 or gemcitabine + BI 836845 (n=6 fields counted).

Figure 48 shows schematics depicting the role of M2-like macrophage and myofibroblasts-derived IGFs in activation of the Insulin/IGFR signaling survival pathway and in mediating chemoresistance of cancer cells.

Figure 49 illustrates human pancreatic cancer Suit-2 cells cultured in the presence or absence of MCM and treated with 0, 100, 200 and 500 nM gemcitabine for 24 hours.

Percentage of cell death was quantified by flow cytometry. Error bars represent s.d. (n=3);
* two tailed p value ≤ 0.05 , ** two tailed p value ≤ 0.01 using a student's t- test.

5 Figure 50 illustrates CD206 and IL-12 RNA expression levels quantified in primary human macrophages that were cultured under standard conditions in the presence of macrophage colony-stimulating factor 1 (M-CSF1), polarized into M1 macrophages or polarized into M2 macrophages.

10 Figure 51 illustrates CD206 and IL-12 RNA expression levels quantified in primary murine macrophages that were cultured under standard conditions in the presence of macrophage colony-stimulating factor 1 (M-CSF1), polarized into M1 macrophages or polarized into M2 macrophages.

15 Figure 52 illustrates quantification of cell death in Suit-2 cells exposed to 200 nM gemcitabine, MCM and/or IGF blocking antibody (10 μ g/ml). Error bars represent s.d. (n=3); ** two tailed p value ≤ 0.01 using a student's t- test.

20 Figure 53 illustrates cell cycle analysis of primary mouse KPC derived pancreatic cancer cells exposed to MCM, IGF blocking antibody (10 μ g/ml) or recombinant IGF (100 ng/ml).

Figure 54 illustrates confocal microscopy images of frozen human PDA biopsies immunofluorescently co- stained for CK11 (green), phospho-Insulin/IGF receptor (red) and DNA (blue). Scale bar, 50 μ m.

25 Figure 55 illustrates immunohistochemical staining of phospho-Insulin/IGF receptor, CD68 and CD163 in human normal pancreas and serial sections from biopsies of PDA patients.

30 Figure 56 illustrates quantification of CD68 and CD163+ macrophages in human normal pancreas and PDA samples (n= 6-8 fields).

Figure 57 illustrates clinical information from the 54 PDA samples analysed by immunohistochemistry for phospho-Insulin/IGFR expression on cancer cells and CD163+ macrophage infiltration.

35 Figure 58 illustrates clinical information from the 75 breast cancer samples analysed by immunohistochemistry for phospho-Insulin/IGFR expression on cancer cells and CD163+ macrophage infiltration.

Figure 59 illustrates immunofluorescent staining of EpCAM (green), α SMA (red) and nuclei (blue) in frozen tissues from naïve mouse pancreas and mouse pancreatic tumors from orthotopic syngeneic model. Scale bar, 100 μ m.

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Figure 60 illustrates immunohistochemical staining of α SMA and CD68 in paraffin embedded tissues from naïve mouse pancreas and mouse pancreatic tumors from orthotopic syngeneic model. Scale bar, 50 μ m.

10 Figure 61 illustrates immunohistochemical staining of phospho-Insulin/IGFR, CD206 and α SMA, in serial sections of pancreatic tumor tissues from the genetically engineered KPC mouse model and in adjacent normal pancreas. Scale bar, 50 μ m.

Figure 62 illustrates: a) KPC-derived tumor cells orthotopically implanted into the pancreas of syngeneic recipient mice, mimicking PDA. Normal pancreas from naïve mice and pancreatic tumors were harvested and digested on day 29 after implantation. Percentage of intra-tumoral F4/80+ macrophages, Gr1+/CD11b+ neutrophils and myeloid derived suppressor cells (MDSCs), CD4+ and CD8+ T cells, among CD45+ immune cells, were quantified by flow cytometry (n= 2-4 mice). (b) Quantification of IGF-1, mRNA expression levels in F4/80+ and F4/80- cells isolated from murine pancreatic tumors. Error bars represent s.d. (n=3) (c) Quantification of IGF-2, mRNA expression levels in F4/80+ and F4/80- cells isolated from murine pancreatic tumors. Error bars represent s.d. (n=3).

Figure 63 illustrates the percentage of intra-tumoral immune cells (among CD45+ cells) in Naïve and Tumor samples.

Figure 64 illustrates the gating strategy used to sort CD45-/GFP+ KPC-derived tumor cells, CD45-/GFP- non immune stromal cells, CD45+F4/80+/CD206- M1-like macrophages and CD45+/F4/80+/CD206+ M2-like macrophages from pancreatic tumors.

30

Figure 65 illustrates the quantification of α SMA mRNA expression levels in the CD45-/GFP- non immune stromal cell population isolated from pancreatic tumors using flow cytometry.

35 Figure 66 illustrates a bright field microscopy image of primary pancreatic myofibroblasts isolated from naïve murine pancreas (top). Immunoblotting analysis of α SMA and GAPDH expression in primary pancreatic myofibroblasts (bottom).

Figure 67 illustrates quantification of IGF-1 mRNA expression levels in primary murine pancreatic myofibroblasts untreated and treated with KPC-derived tumor conditioned media.

5

Figure 68 illustrates quantification of IGF-2 mRNA expression levels in primary murine pancreatic myofibroblasts untreated and treated with KPC-derived tumor conditioned media.

10 Figure 69 illustrates: (a) Primary mouse KPC-derived pancreatic cancer cells implanted orthotopically in the pancreas of syngeneic recipient mice. Mice were administered i.p., twice a week with IgG antibody, gemcitabine alone or gemcitabine with IGF blocking antibody (ab9572). Tumors were harvested at day 30 and representative images are shown. (b) Tumor weights are shown (n=9-12 mice per group).

15

Figure 70 illustrates Immunohistochemical staining of cleaved caspase-3, phospho-Insulin/IGFR, CD206 and CD68 in naïve pancreas, and murine PDA tumors treated with IgG, gemcitabine or gemcitabine+ IGF blocking antibody. Right, Bargraph showing the percentage of apoptotic cancer cells (cleaved caspase-3 positive) quantified in tumor tissues harvested from mice treated with IgG control antibody, gemcitabine or gemcitabine+IGF blocking antibody.

20

DETAILED DESCRIPTION

25 The invention is based on the surprising finding that pancreatic TAMs (but not naïve macrophages) express high levels of Insulin growth factor 1 (IGF-1) and Insulin growth factor 2 (IGF-2) and that pancreatic TAMs are an important source of these growth factors in the tumour microenvironment.

30 Surprisingly, the inventors have also found that secretion of IGF-1 and IGF-2 by TAMs leads to the activation of Insulin Receptor signaling and the development of cancer cell resistance to chemotherapy (specifically, resistance of pancreatic cancer cells to gemcitabine and/or 5-FU).

35 Importantly, blockade of IGF activity e.g. IGF-1 and IGF-2 activity (but not IGFR activity) using an IGF blocking antibody restored sensitivity of pancreatic cancer cells to chemotherapy (specifically gemcitabine and/or 5FU) *in vitro* and restrained tumour

progression and reduced the immuno-suppressive microenvironment in a pre-clinical model of pancreatic cancer.

5 The findings presented herein suggest that: i) in PDA, IGF ligands rather signal through either Insulin receptor or hybrid Insulin/IGF-R1 receptors but not through IGF-R1 homodimers, which might explain why inhibition of IGFR has failed in pancreatic cancer clinical trials (Basu et al., 2011; Guha, 2013; Pollak, 2012), ii) inhibition of IGF ligands can sensitize pancreatic cancer cells to chemotherapy. Combinatorial treatments using means that directly block IGF ligand activity and chemotherapy therefore provide exciting new
10 therapeutic opportunities to treat proliferative disorders such as PDA.

As TAMs are present in the tumour microenvironment of many cancers, therapeutics that can specifically inhibit the pro-tumoral functions of TAMs, while sparing their anti-tumoral capacity, hold great promise in the goal of restraining cancer progression, metastasis and
15 relapse in more general terms.

The invention therefore provides a new approach to treating a proliferative disorder such as cancer using a combination of compounds, specifically an IGF inhibitor and a chemotherapeutic agent.
20

The inventors have also identified that murine pancreatic cancer cells exposed to M2-like macrophages upregulate expression of PDL-1 (also known as PDL-1, B7-H1 or CD274) and CD80 (also known as B7-1) and that human PDA tissue samples also show an increase in PDL-1 expression compared to controls. Binding of PDL-1 or CD80 with its
25 receptor PD-1 (also known as CD279) and CTLA-4 (also known as CD152) respectively leads to functional exhaustion of T cells (reviewed by Pardoll D, nature reviews cancer, 12, 252-264 (April 2012); the blockade of immune checkpoints in cancer immunotherapy). Accordingly, the observed increase in PDL-1 and/or CD80 expression in cancer cells of a subject may attenuate, e.g. suppress and/or inhibit the subject from eliciting an effective
30 immune response.

The invention therefore provides a new approach to treating a proliferative disorder such as cancer using a combination of compounds, specifically an IGF inhibitor, a chemotherapeutic agent and (i) a PDL-1 inhibitor and/or (ii) a CD80 inhibitor.

35 The inventors have also surprisingly found that M2-like macrophages and myofibroblasts are the main sources of Insulin-like growth factors in the tumour microenvironment but do not express insulin and that insulin-IGF receptor activation positively correlates with

increased M2-like CD163+ macrophage infiltration in tumours and with tumour progression. Thus, the present invention has identified M2-like macrophages and phospho-Insulin/IGFR as biomarkers and their use as a stratification tool.

5 **Compounds**

The invention provides one or more compounds that are suitable for use in treating a proliferative disorder in a subject.

As used herein, the term “compound” is intended to include one or more compounds and
10 refers to a substance that is suitable for use in treating a proliferative disorder in a subject. By way of example, the compound may be an antibody or at least an antigen binding fragment thereof, an antisense molecule, a polypeptide, a nucleic acid, a carbohydrate, a lipid, a small molecular weight compound, an oligonucleotide, an oligopeptide, siRNA, a recombinant protein, a peptibody, or conjugates or fusion proteins thereof.

15 Preferably, the compound is an IGF inhibitor, a chemotherapeutic agent, a PDL-1 inhibitor and/or a CD80 inhibitor.

In one particular example, the compound of the invention (e.g. the compound capable of
20 inhibiting IGF, e.g. the IGF inhibitor) is an antibody or at least an antigen binding fragment thereof.

Accordingly, an antibody may be provided, or at least an antigen binding fragment thereof, wherein the antibody or antigen binding fragment is capable of inhibiting IGF (e.g. is an
25 IGF inhibitor). In such an example, the antibody or antigen binding fragment thereof may bind to IGF directly (e.g. specifically).

It is noted that any of the compounds of the invention may be an antibody or at least an antigen binding fragment thereof (i.e. it is not limited to compounds capable of inhibiting
30 IGF). By way of example, but not by way of limitation, a compound that is capable of inhibiting PDL-1 (e.g. a PDL-1 inhibitor) may be an antibody or at least an antigen binding fragment thereof in the context provided herein. By way of a further example, but not by way of limitation, a compound that is capable of inhibiting CD80 (e.g. a CD80 inhibitor) may be an antibody or at least an antigen binding fragment thereof in the context provided
35 herein. Accordingly, the term “IGF” as used below when describing antibodies may equally be replaced with the terms “CD80” or “PDL-1” where the context allows.

The term "antibody" is used herein in its broadest sense and refers to immunoglobulin molecules and immunologically active portions thereof, i.e., molecules that contain an antigen binding site which specifically binds an antigen, such as IGF (e.g. IGF-1 and/or IGF-2), preferably human IGF (e.g. human IGF-1 and/or IGF-2). A molecule which
5 specifically binds to IGF is a molecule which binds IGF (e.g. IGF-1 and/or IGF-2), but does not substantially bind other molecules in a sample, e.g., a biological sample, which naturally contains IGF (e.g. IGF-1 and/or IGF-2).

The antigen may be such as CD80 or PDL-1, preferably CD80 or PDL-1. A molecule
10 which specifically binds to CD80 or PDL-1 is a molecule which binds CD80 or PDL-1, but does not substantially bind other molecules in a sample, e.g., a biological sample, which naturally contains CD80 or PDL-1.

Immunoglobulins (Ig) are a class of structurally related proteins consisting of two pairs of
15 polypeptide chains, one pair of light (L) (low molecular weight) chain (κ or λ), and one pair of heavy (H) chains (γ , α , μ , δ and ϵ), all four linked together by disulphide bonds. Both H and L chains have regions that contribute to the binding of antigen and that are highly variable from one Ig molecule to another. In addition, H and L chains contain regions that are non-variable or constant. The carboxy-terminal domain is essentially identical among
20 L chains of a given type and is referred to as the "constant" (C) region. The amino terminal domain varies from L chain to L chain and contributes to the binding site of the antibody. Because of its variability, it is referred to as the "variable" (V) region.

The H chains of Ig molecules are of several classes: α , μ , σ , ϵ , and γ (of which there are
25 several sub-classes). An assembled Ig molecule consisting of one or more units of two identical H and L chains, derives its name from the H chain that it possesses. Thus, there are five Ig isotypes: IgA, IgM, IgD, IgE and IgG (with four sub-classes based on the differences in the H chains, i.e., IgG1, IgG2, IgG3 and IgG4). Further detail regarding antibody structure and their various functions can be found in, Using Antibodies: A
30 laboratory manual, Cold Spring Harbour Laboratory Press.

The antibody may be a polyclonal or a monoclonal antibody that binds IGF, CD80 or PDL-1. As used herein, the term "monoclonal antibody" refers to a population of antibody molecules that contain only one species of an antigen binding site capable of
35 immunoreacting with a particular epitope of IGF (e.g. IGF-1 and/or IGF-2), CD80 or PDL-1. A monoclonal antibody composition thus typically displays a single binding affinity for a particular protein with which it immunoreacts. This particular protein may be a class of

proteins (such as IGF; which includes IGF-1 and IGF-2) or may be a specific singular protein (such as IGF-1, IGF-2, CD80 or PDL-1).

5 The antibody may be humanised. A humanised monoclonal antibody to an IGF, CD80 or PDL-1 polypeptide may be produced as a fusion polypeptide in an expression vector suitably adapted for transfection or transformation of prokaryotic or eukaryotic cells. Said antibody may be humanised by recombinant methods to combine the complementarity determining regions of said antibody with both the constant (C) regions and the framework regions from the variable (V) regions of a human antibody.

10 Optionally, said antibody is provided with a marker including a conventional label or tag, for example a radioactive and/or fluorescent and/or epitope label or tag.

Alternatively, said antibody is a chimeric antibody. Chimeric antibodies are recombinant antibodies in which all of the V-regions of a mouse or rat antibody are combined with
15 human antibody C-regions. Humanised antibodies are recombinant hybrid antibodies which fuse the complementarity determining regions from a rodent antibody V-region with the framework regions from the human antibody V-regions. The C-regions from the human antibody are also used. The complementarity determining regions (CDRs) are the regions within the N-terminal domain of both the heavy and light chain of the antibody to where the
20 majority of the variation of the V-region is restricted. These regions form loops at the surface of the antibody molecule. These loops provide the binding surface between the antibody and antigen.

Antibodies from non-human animals provoke an immune response to the foreign antibody
25 and its removal from the circulation. Both chimeric and humanised antibodies have reduced antigenicity when injected to a human subject because there is a reduced amount of rodent (i.e. foreign) antibody within the recombinant hybrid antibody, while the human antibody regions do not illicit an immune response. This results in a weaker immune response and a decrease in the clearance of the antibody. This is desirable when using
30 therapeutic antibodies in the treatment of diseases. Humanised antibodies are designed to have less "foreign" antibody regions and are therefore thought to be less immunogenic than chimeric antibodies.

The terms "antibody", "antigen binding fragment" and "at least an antigen binding
35 fragment" are used interchangeably herein unless the context dictates otherwise.

As used herein, an “at least an antigen binding fragment” of an antibody may be an antibody fragment. The antibody fragment may be, but is not limited to, a fab fragment, a F(ab')₂ fragment, or a fragment produced by a fab expression library.

- 5 The antibody or antibody fragment may be a monoclonal antibody, a humanised antibody, a chimeric antibody or a single chain antibody, or an epitope binding fragment thereof.

The antibody, or at least an antigen binding fragment thereof (e.g. an antibody fragment), may specifically bind to an IGF polypeptide (e.g. IGF-1 and/or IGF-2), CD80 or PDL-1,
10 preferably a human IGF, CD80 or PDL-1 polypeptide. The molecular structure of IGF-1 and IGF-2, CD80 or PDL-1 is discussed herein in more detail elsewhere.

The inventors have surprisingly found that a compound that is capable of inhibiting IGF, CD80 or PDL-1 (e.g. an IGF, CD80 or PDL-1 inhibitor) is useful in the treatment of a
15 proliferative disorder in a subject in combination with a chemotherapeutic agent because it is capable of inhibiting, reducing and/or preventing resistance to chemotherapeutic agent(s) in the subject.

Compounds that are capable of inhibiting IGF, CD80 or PDL-1

20 As used herein, the phrase “capable of inhibiting IGF, CD80 or PDL-1” refers to (but is not limited to) compounds that have the ability to reduce, inhibit and/or prevent the biological activity of IGF, CD80 or PDL-1. As used herein, the phrases “IGF, CD80 or PDL-1 activity”, “IGF, CD80 or PDL-1 biological activity” and “the biological activity of IGF, CD80 or PDL-1” are used interchangeably to describe the overall mechanism by which IGF, CD80 or PDL-1
25 carries out their function within a cell (e.g. a cell having or at risk of having a proliferative disorder). A compound that is capable of inhibiting IGF, CD80 or PDL-1 activity may inhibit IGF, CD80 or PDL-1 activity directly (e.g. by interaction with the IGF, CD80 or PDL-1 polypeptide or by interaction with a nucleic acid encoding the IGF, CD80 or PDL-1 polypeptide).

30

Preferably, a compound that is capable of inhibiting IGF, CD80 or PDL-1 is an IGF, CD80 or PDL-1 inhibitor.

As used herein, an “inhibitor” may be an antibody, a polypeptide, a nucleic acid, a
35 carbohydrate, a lipid, a small molecular weight compound, an oligonucleotide, an oligopeptide, siRNA, antisense, a recombinant protein, a peptibody, or conjugates or fusion proteins thereof.

As used herein, the phrase “IGF, CD80 or PDL-1 inhibitor” refers to an inhibitor that directly inhibits the biological activity of IGF, CD80 or PDL-1 (e.g. by binding to and/or interacting with IGF, CD80 or PDL-1 so as to inhibit the biological activity of IGF, CD80 or PDL-1; by sequestering circulating IGF, CD80 or PDL-1 in the cell; and/or by inhibiting upstream pathways that are required for IGF activity such as IGF, CD80 or PDL-1 expression and/or IGF, CD80 or PDL-1 post-translational modification). For example, the IGF, CD80 or PDL-1 inhibitor may inhibit binding of IGF, CD80 or PDL-1 to its receptor (e.g. the insulin receptor, the IGFR and Insulin/IGFR hybrids, the CD80 receptor or the PDL-1 receptor) through direct interaction with IGF, CD80 or PDL-1.

By way of example only, the IGF, CD80 or PDL-1 inhibitor may be an antibody (or at least an antigen binding fragment thereof) that, when bound to IGF, CD80 or PDL-1, inhibits IGF, CD80 or PDL-1 from binding to its receptor (e.g. the insulin receptor, the IGFR and Insulin/IGFR hybrids the CD80 receptor or the PDL-1 receptor).

As used herein, the terms “inhibit”, “down-regulate”, “prevent” and “reduce” refer to an alteration of the level of IGF, CD80 or PDL-1 biological activity such that the aforementioned level of activity is less than that observed in the absence of the inhibiting substance or compound (e.g. inhibitor). Inhibition may be reversible or irreversible.

A compound that is capable of inhibiting IGF (e.g. the IGF inhibitor) may be capable of inhibiting one or more IGFs (e.g. IGF-1 and/or IGF-2 i.e. it may be an IGF-1 and/or IGF-2 inhibitor).

As used herein, the term “IGF” refers to one or more insulin-like growth factors, which are proteins with high sequence similarity to insulin. The term “IGF” encompasses the two native ligands, IGF-1 and IGF-2, and also natural IGF variants such as brain IGF, otherwise known as des(1-3)IGF-1. The IGF referred to herein may be from any species, including human, murine, bovine, ovine, porcine, and equine.

IGF-1 (insulin-like growth factor 1; also known as somatomedin C) is encoded by the *IGF1* gene in humans. IGF-1 undergoes alternative splicing. There are six different isoforms of IGF-1 which share the same mature peptide. Accordingly, the invention is considered equally applicable to all IGF-1 isoforms. The Genbank details for human IGF-1 are shown below:

IGF1**Nucleotide (coding mRNA)**

LOCUS NM_001111283 7370 bp mRNA linear PRI 25-MAY-2014
 DEFINITION Homo sapiens insulin-like growth factor 1 (somatomedin C) (IGF1),
 transcript variant 1, mRNA.
 ACCESSION NM_001111283
 VERSION NM_001111283.1 GI:163659898

Peptide (Amino Acid)

LOCUS CAG46659 153 aa linear PRI 16-OCT-2008
 DEFINITION IGF1 [Homo sapiens].
 ACCESSION CAG46659
 VERSION CAG46659.1 GI:49456677

- IGF-1 is an endocrine hormone with a similar molecular structure to insulin. It is mainly secreted by the liver as a result of growth hormone (GH) stimulation. It is required for achieving maximal growth. IGF-I has also been shown to have an involvement in regulating neural development including neurogenesis, myelination, synaptogenesis, and dendritic branching and neuroprotection after neuronal damage. Its primary action is mediated by binding to its specific receptor, the insulin-like growth factor 1 receptor (IGF1R), which is present on many cell types in many tissues. Mutagenesis of IGF-1 has shown Ala8, Asp12, Phe23 and Tyr24 in the B domain; Tyr31, Arg36, Arg37 in the C peptide and Met59, Tyr60 and Ala62 in the A domain to be important for high affinity binding to IGF1R (Meyts and Whittaker, 2002). The C peptide plays a major role in IGF binding to IGF1R.

- IGF-1 also binds to the insulin receptor (albeit at significantly lower affinity than it binds to IGF1R). IGF-1 has been shown to bind and interact with all the IGF binding proteins (IGFBPs), of which there are seven: IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, and IGFBP7. Some IGFBPs are inhibitory. For example, both IGFBP-2 and IGFBP-5 bind IGF-1 at a higher affinity than IGF-1 binds the IGF1R. Therefore, increases in serum levels of these two IGFBPs result in a decrease in IGF-1 activity.

- IGF-2 (insulin-like growth factor 2) is encoded by the *IGF2* gene in humans. IGF-2 undergoes alternative splicing. There are three different isoforms of IGF-2, one of which is known as the canonical isoform and the other two are highly similar. Accordingly, the invention is considered equally applicable to all IGF-2 isoforms. The Genbank details for human IGF-2 are shown below:

IGF2**Nucleotide (coding mRNA)**

LOCUS: HUMGFIII 1045 bp mRNA linear PRI 08-NOV-1994
 DEFINITION: Human insulin-like growth factor II mRNA, complete cds.
 ACCESSION: M29645

VERSION: M29645.1 GI:183121

Peptide (Amino Acid)

5 insulin-like growth factor II precursor [Homo sapiens]
GenBank: AAA52544.1

LOCUS AAA52544 180 aa linear PRI 08-NOV-1994
DEFINITION insulin-like growth factor II precursor [Homo sapiens].
10 ACCESSION AAA52544
VERSION AAA52544.1 GI:183122

IGF-2 is also a hormone with similar molecular structure to insulin. It is thought to be a primary growth factor necessary in early development, and is also required for
15 development and function of organs such as the brain, liver and kidney. IGF-2 exerts its effects by binding to the IGF-1 receptor. IGF-2 also binds to the insulin receptor and to the IGF-2 receptor (also called the cation-independent mannose 6-phosphate receptor), which functions as an IGF-2 sequestering agent and thus prevents IGF-2 signaling.

20 As used herein, an IGF inhibitor that inhibits binding of IGF to its receptor may inhibit binding of IGF (e.g. IGF-1 and/or IGF-2) to one or more receptors selected from IGF1R, IGF2R, insulin receptor and hybrid receptors e.g. hybrid insulin/IGFR1 receptors. Preferably, the one or more receptors comprises the insulin receptor. This is because the inventors have surprisingly found that secretion of IGF-1 (and IGF-2) by TAMs leads to
25 activation of insulin receptor signalling and the development of cancer cell resistance to chemotherapy (specifically, resistance of pancreatic cancer cells to gemcitabine and/or 5'FU), and importantly, that blockade of IGF-1 and IGF-2 activity (but not IGFR activity) using an IGF blocking antibody restored sensitivity of pancreatic cancer cells to gemcitabine and/or 5'FU.

30 The findings presented herein also indicate that especially high levels of IGF-1 are expressed by pancreatic TAMs and that the presence of recombinant IGF-1 alone is sufficient to induce resistance to gemcitabine in pancreatic cancer cells. Accordingly, a compound capable of inhibiting IGF-1 specifically, without inhibiting IGF-2 and/or other
35 IGFs (e.g. an IGF-1 specific inhibitor such as an anti-IGF-1 antibody or antigen binding fragment thereof) is also considered to be a compound for use in accordance with the invention.

Compounds that are chemotherapeutic agents

40 As used herein, the phrase "chemotherapeutic agent" refers to (but is not limited to) compounds that are used in chemotherapy for the treatment of proliferative disorders such as cancer.

Several chemotherapeutic agents are known, some of which are clinically approved or awaiting approval as cancer therapies. Suitable examples include nucleoside analogues, topoisomerase inhibitors, platinum complexes, and combinations thereof. A specific
5 example of a nucleoside analogue is gemcitabine, although many others are well known. Another specific example of a nucleoside analogue is fluorouracil (also known as 5-FU). A specific example of a topoisomerase inhibitor is irinotecan, although many others are well known. A specific example of a platinum complex is oxaliplatin, although many others are well known.

10

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN(TM) cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine,
15 triethylenephosphoramide, triethylenethiophosphoramide 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
20 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the
25 enediyne antibiotics (e.g., calicheamicin, especially calicheamicin (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomorphores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin,
30 chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN(TM) doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin,
35 streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-

- mercaptapurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane;
- 5 folic acid replenisher such as froinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amisacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet;
- 10 pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKO polysaccharide complex (JHS Natural Products, Eugene, Oreg.); 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;
- 15 arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL(R) paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.) and TAXOTERE(R) doxetaxel (Rhône-Poulenc Rorer, Antony, France); chlorambucil; GEMZAR(TM) gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine;
- 20 NAVELBINE(TM) vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.
- 25 Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumours such as anti-oestrogens and selective oestrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX(TM) tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON(TM) toremifene; aromatase inhibitors that inhibit the enzyme
- 30 aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGAS(TM) megestrol acetate, AROMASIN(TM) exemestane, formestane, fadrozole, RIVISOR(TM) vorozole, FEMARA(TM) letrozole, and ARIMIDEX(TM) anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a
- 35 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, and H-Ras; ribozymes such as a

VEGF expression inhibitor (e.g., ANGIOZYME(R) ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, ALLOVECTIN(TM) vaccine, LEUVECTIN(TM) vaccine, and VAXID(TM) vaccine; PROLEUKIN(TM) rIL-2; LURTOTECAN(TM) topoisomerase I inhibitor; ABARELIX(TM) rGnRH; and
5 pharmaceutically acceptable salts, acids or derivatives of any of the above.

The selection of suitable chemotherapeutic agents may depend on the specific proliferative disorder being treated. By way of example, but not by way of limitation, suitable chemotherapeutic agents for use in the treatment of pancreatic cancer include
10 gemcitabine, fluorouracil, FOLFIRINOX (= Leucovorin also known as folinic acid, fluorouracil, Irinotecan and Oxaliplatin) and nab-paclitaxel.

The inventors have identified that a compound that is capable of inhibiting IGF (e.g. an IGF inhibitor) is useful in the treatment of a proliferative disorder in a subject in combination
15 with a chemotherapeutic agent and (i) a compound that is capable of inhibiting PDL-1 (e.g. a PDL-1 inhibitor) and/or (ii) a compound that is capable of inhibiting CD80 (e.g. a CD80 inhibitor). Advantageously, this combination is capable of inhibiting, reducing and/or preventing resistance to chemotherapeutic agent(s) and is capable of inhibiting, reducing and/or preventing suppression of an immune response in the subject.

20

Compounds that are capable of inhibiting PDL-1

As used herein, the phrase "capable of inhibiting PDL-1" refers to (but is not limited to) compounds that have the ability to reduce, inhibit and/or prevent the biological activity of PDL-1. As used herein, the phrases "PDL-1 activity", "PDL-1 biological activity" and "the
25 biological activity of PDL-1" are used interchangeably to describe the overall mechanism by which PDL-1 carries out its function within a cell (e.g. a cell having or at risk of having a proliferative disorder). A compound that is capable of inhibiting PDL-1 activity may inhibit PDL-1 activity directly (e.g. by interaction with the PDL-1 polypeptide or by interaction with a nucleic acid encoding the PDL-1 polypeptide), or may interfere with PDL-1 binding to its
30 receptor (PD-1), either by directly blocking the interaction between PDL-1 and its receptor or by affecting PD-1 activity such that it inhibits PD-1- mediated PDL-1 activity.

Preferably, a compound that is capable of inhibiting PDL-1 is a PDL-1 inhibitor.

35 As stated above, an "inhibitor" may be an antibody, a polypeptide, a nucleic acid, a carbohydrate, a lipid, a small molecular weight compound, an oligonucleotide, an

oligopeptide, siRNA, antisense, a recombinant protein, a peptibody, or conjugates or fusion proteins thereof.

As used herein, the phrase "PDL-1 inhibitor" refers to an inhibitor that directly inhibits the biological activity of PDL-1 (e.g. by binding to and/or interacting with PDL-1 so as to inhibit the biological activity of PDL-1; by sequestering circulating PDL-1 in the cell; and/or by inhibiting upstream or downstream pathways that are required for PDL-1 activity such as PDL-1 expression, PDL-1 post-translational modification and/or PDL-1 signalling via the PD-1 receptor). For example, the PDL-1 inhibitor may inhibit binding of PDL-1 to its receptor (e.g. the PD-1 receptor) through direct interaction with PDL-1 or through direct interaction with the PD-1 receptor.

By way of example only, the PDL-1 inhibitor may be an antibody (or at least an antigen binding fragment thereof) that, when bound to PDL-1, inhibits PDL-1 from binding to its receptor (e.g. the PD-1 receptor). An example of such an inhibitor is the anti-PDL-1 antibody MEDI 4736.

By way of an alternative example, the PDL-1 inhibitor may be an antibody (or at least an antigen binding fragment thereof) that, when bound to a PD-1 receptor, inhibits PDL-1 from binding to the PD-1 receptor. An example of such an inhibitor is the anti-PD-1 antibody MEDI 0680.

Further examples of agents that target immune-checkpoint pathways are provided in table 1 below, which is reproduced from Pardoll D, Nature Reviews cancer, 12, 252-264 (April 2012):

Target	Biological function	Antibody or Ig fusion protein	State of clinical development*
CTLA4	Inhibitory receptor	Ipilimumab	FDA approved for melanoma, Phase II and Phase III trials ongoing for multiple cancers
		Tremelimumab	Previously tested in a Phase III trial of patients with melanoma; not currently active
PD1	Inhibitory receptor	MDX-1106 (also known as BMS-936558)	Phase I/II trials in patients with melanoma and renal and lung cancers
		MK3475	Phase I trial in multiple cancers
		CT-011†	Phase I trial in multiple cancers
		AMP-224§	Phase I trial in multiple cancers
PDL1	Ligand for PD1	MDX-1105	Phase I trial in multiple cancers
		Multiple mAbs	Phase I trials planned for 2012
LAG3	Inhibitory receptor	IMP321	Phase III trial in breast cancer
		Multiple mAbs	Preclinical development
B7-H3	Inhibitory ligand	MGA271	Phase I trial in multiple cancers

Target	Biological function	Antibody or Ig fusion protein	State of clinical development [†]
B7-H4	Inhibitory ligand		Preclinical development
TIM3	Inhibitory receptor		Preclinical development

CTLA4, cytotoxic T-lymphocyte-associated antigen 4; FDA, US Food and Drug Administration; Ig, immunoglobulin; LAG3, lymphocyte activation gene 3; mAbs, monoclonal antibodies; PD1, programmed cell death protein 1; PDL, PD1 ligand; TIM3, T cell membrane protein 3. ^{*}As of January 2012. [†]PD1 specificity not validated in any published material. [§]PDL2-Ig fusion protein. ^{||}LAG3-Ig fusion protein.

As used herein, the terms “inhibit”, “down-regulate”, “prevent” and “reduce” refer to an alteration of the level of PDL-1 biological activity such that the aforementioned level of activity is less than that observed in the absence of the inhibiting substance or compound
5 (e.g. inhibitor). Inhibition may be reversible or irreversible.

As used herein, the term “PDL-1” refers to programmed death ligand-1 (PDL-1), also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1). In humans, PDL-1 is encoded by the *CD274* gene. The PDL-1 referred to herein may be from any
10 species, including human, murine, bovine, ovine, porcine, and equine.

PDL-1 is a 40kDa type 1 transmembrane protein that is thought to play an important role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. PDL-1
15 binds to its receptor, the PD-1 receptor, which is found on activated T cells, B cells, and myeloid cells, to modulate activation or inhibition of these immune cells. By way of example, binding of PDL-1 or CD80 with its receptor PD-1 and CTLA-4 respectively leads to functional exhaustion of T cells (reviewed by Pardoll D, Nature Reviews Cancer 12, 252-264 (April 2012; The blockade of immune checkpoints in cancer immunotherapy).
20 Accordingly, the PDL-1 expression *in vivo* can act to suppress an immune response.

The Genbank details for human PDL-1 are shown below:

PD-L1 = CD274 molecule [Homo sapiens]

25 GenBank: AAH69381.1
LOCUS AAH69381 290 aa
DEFINITION CD274 molecule [Homo sapiens].
ACCESSION AAH69381
VERSION AAH69381.1 GI:46854604

30

The Genbank details for human PD-1 are shown below:

PD-1 = CD279 molecule [Homo sapiens]

GenBank: AAH74740.1

LOCUS AAH74740 288 aa linear PRI 15-JUL-2006

5 DEFINITION Programmed cell death 1 [Homo sapiens].

ACCESSION AAH74740

VERSION AAH74740.1 GI:49902307

Compounds that are capable of inhibiting CD80

10 As used herein, the phrase “capable of inhibiting CD80” refers to (but is not limited to) compounds that have the ability to reduce, inhibit and/or prevent the biological activity of CD80. As used herein, the phrases “CD80 activity”, “CD80 biological activity” and “the biological activity of CD80” are used interchangeably to describe the overall mechanism by which CD80 carries out its function within a cell (e.g. a cell having or at risk of having a
15 proliferative disorder). A compound that is capable of inhibiting CD80 activity may inhibit CD80 activity directly (e.g. by interaction with the CD80 polypeptide or by interaction with a nucleic acid encoding the CD80 polypeptide), or may interfere with CD80 binding to its receptor (CTLA-4), either by directly blocking the interaction between CD80 and its receptor or by affecting CD80 activity such that it inhibits CTLA-4- mediated CD80 activity.

20

Preferably, a compound that is capable of inhibiting CD80 is a CD80 inhibitor.

As stated above, an “inhibitor” may be an antibody, a polypeptide, a nucleic acid, a carbohydrate, a lipid, a small molecular weight compound, an oligonucleotide, an
25 oligopeptide, siRNA, antisense, a recombinant protein, a peptibody, or conjugates or fusion proteins thereof.

As used herein, the phrase “CD80 inhibitor” refers to an inhibitor that directly inhibits the biological activity of CD80 (e.g. by binding to and/or interacting with CD80 so as to inhibit
30 the biological activity of CD80; by sequestering circulating CD80 in the cell; and/or by inhibiting upstream or downstream pathways that are required for CD80 activity such as CD80 expression, CD80 post-translational modification and/or CD80 signalling via the CTLA-4 receptor). For example, the CD80 inhibitor may inhibit binding of CD80 to its receptor (e.g. the CTLA-4 receptor) through direct interaction with CD80 or through direct
35 interaction with the CTLA-4 receptor.

By way of example only, the CD80 inhibitor may be an antibody (or at least an antigen binding fragment thereof) that, when bound to CD80, inhibits CD80 from binding to its receptor (e.g. the CTLA-4 receptor). An example of such an inhibitor is the anti-CD80 antibody [1G10] (ab25208) (Abcam).

5

The CD80 inhibitor may be an antibody (or at least an antigen binding fragment thereof) that acts as a CTLA-4 blocking antibody, e.g. when bound it inhibits CD80 from binding to the CTLA-4 receptor. An example of such an inhibitor is the anti-CTLA-4 antibody tremelimumab or Ipilimumab.

10

As used herein, the terms “inhibit”, “down-regulate”, “prevent” and “reduce” refer to an alteration of the level of CD80 biological activity such that the aforementioned level of activity is less than that observed in the absence of the inhibiting substance or compound (e.g. inhibitor). Inhibition may be reversible or irreversible.

15

As used herein, the term “CD80” refers to cluster of differentiation 80 (CD80), also known as B7-1. The CD80 referred to herein may be from any species, including human, murine, bovine, ovine, porcine, and equine. CD80 is found on activated B cells and monocytes and provides the costimulatory signal that is necessary for T cell activation and survival. It is the ligand for two different receptors found on T cells, namely CD28 and CTLA-4.

20

The Genbank details for human CD80 are shown below:

CD80 molecule [Homo sapiens]

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GenBank: AAH42665.1

LOCUS AAH42665 288 aa linear PRI 08-SEP-2006

DEFINITION CD80 molecule [Homo sapiens].

ACCESSION AAH42665

VERSION AAH42665.1 GI:27503576

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The Genbank details for human CTLA-4 are shown below:

CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4) [Homo sapiens]

GenBank: AAH69566.1

35

LOCUS AAH69566 223 aa linear PRI 15-JUL-2006

DEFINITION Cytotoxic T-lymphocyte-associated protein 4 [Homo sapiens].

ACCESSION AAH69566

VERSION AAH69566.1 GI:46854814

M2-like Macrophages

5 The present inventors have surprisingly shown that M2 but not M1-like macrophages directly support chemoresistance of cancer cells by secreting Insulin-like growth factors (IGFs), which activate Insulin/IGF receptors on cancer cells. Immunohistochemical analysis of biopsies from pancreatic and breast cancer patients revealed that 57% and 75% of the patients respectively express activated Insulin/IGF receptors, and this 10 positively correlates with increased M2-like macrophage infiltration and tumor progression. The present inventors surprising found that, in vivo, both M2-like macrophages and α SMA+ stromal cells are the main sources of IGF production.

Accordingly, the present invention is predicated on the surprising finding that M2-like 15 macrophages and phospho-Insulin/IGFR can be used as biomarkers to identify patient populations which may be sensitive to a combination of an IGF inhibitor and a chemotherapeutic agent.

In another aspect, the invention provides the use of a M2-like macrophage and phospho- 20 Insulin/IGFR as biomarkers to select a patient population responsive to or sensitive cancer treatment with an IGF inhibitor and a chemotherapeutic agent.

In another aspect, the invention provides the use of a M2-like macrophage and phospho- Insulin/IGFR as biomarkers to predict select a patient population at risk of 25 chemoresistance to a chemotherapeutic agent.

In another aspect, the invention provides a method of increasing the sensitivity rate (efficacy rate) of a combination of an IGF inhibitor and a chemotherapeutic agent to treat cancer in a patient population said method comprising selecting a sub population having a 30 M2-like macrophage and/or phospho-Insulin/IGFR biomarker.

In another aspect, the invention provides a method of identifying a subject having increased likelihood of responsiveness or sensitivity to a combination of an IGF inhibitor and a chemotherapeutic agent comprising:

35 a. Determining the level of M2-like macrophages and/or phospho-Insulin/IGFR in a tumor sample isolated from the subject; and

- b. comparing the level of M2-like macrophages and/or phospho-Insulin/IGFR in the patient sample with the level of M2-like macrophages and/or phospho-Insulin/IGFR in a control sample or with a predetermined reference level for M2-like macrophages wherein an increased level of M2-like macrophages and/or phospho-Insulin/IGFR in the tumor sample compared to the control sample or compared to the predetermined reference level is prognostic of an increased likelihood of responsiveness or sensitivity to an IGF inhibitor and a chemotherapeutic agent in the subject.

In another aspect, the invention provides a method of identifying a subject having responsiveness or sensitivity to an IGF inhibitor comprising:

- a. Determining the level of M2-like macrophages and/or phospho-Insulin/IGFR in a tumor sample isolated from the subject; and
- b. comparing the level of M2-like macrophages and/or phospho-Insulin/IGFR in the patient sample with the level of M2-like macrophages in a control sample or with a predetermined reference level for M2-like macrophages wherein an increased level of M2-like macrophages and/or phospho-Insulin/IGFR in the tumor sample compared to the control sample or compared to the predetermined reference level is prognostic of an increased likelihood of responsiveness or sensitivity to an IGF inhibitor.

In another aspect, the invention provides a method of treating a subject having cancer comprising:

- a. Determining the level of M2-like macrophages and/or phospho-Insulin/IGFR in a tumor sample isolated from the subject; and
- b. comparing the level of M2-like macrophages and/or phospho-Insulin/IGFR in the patient sample with the level of M2-like macrophages and/or phospho-Insulin/IGFR in a control sample or with a predetermined reference level for M2-like macrophages
- c. administering a therapeutically effective amount of a chemotherapeutic agent and an IGF inhibitor when there is an increased level of M2-like macrophages and/or phospho-Insulin/IGFR in the tumor sample compared to the control sample or compared to the predetermined reference level.

In another aspect, the invention provides a kit for identifying a patient population which would benefit from a combined treatment with an IGF inhibitor and a chemotherapeutic agent comprising:

- a. a detectably labelled agent that specifically binds to a M2-like CD163+ macrophage and to phospho-Insulin/IGFR; and

b. reagents for the assay.

In another aspect, the invention provides an assay device comprising a compound or agent capable of detecting a M2-like macrophage and phospho-Insulin/IGFR.

5

Optionally, in accordance with all aspects of the invention, the M2-like macrophage is CD163+.

Optionally, in accordance with all aspects of the invention, the predetermined reference level of M2-like macrophages is at least 20 or at least 30 or at least 40 or at least 50 M2-like macrophages in the tumour core sample.

10

Optionally, in accordance with all aspects of the invention a scoring may be allocated to the levels of M2-like macrophages in a tumour sample isolated from a patient. The scorings may be as follows None = 0 macrophages/core, Low = up to about 30 macrophages/core, Medium= 31-50 macrophages/core, High= 51 and above macrophages/core. Optionally, in accordance with all aspects of the invention the predetermined reference level may differentiate no or low macrophage levels from medium/high levels.

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Optionally, in accordance with all aspects of the invention a core sample may be approximately 1.5mm.

25

Optionally, in accordance with all aspects of the invention, the chemotherapeutic agent is gemcitabine, fluorouracil, paclitaxel or nab-paclitaxel.

As used herein "patient" refers to an individual, e.g., a human, having a proliferative disorder such as cancer.

30

As used herein the term "labeled", refers to direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance.

35

The term "prognosis" is used herein to refer to the prediction of the likelihood of cancer-attributable death or progression, including, for example, recurrence, metastatic spread, and drug resistance, of a neoplastic disease, such as cancer.

The term "prediction" or (and variations such as predicting) is used herein to refer to the likelihood that a patient will respond either favorably or unfavorably to a drug or set of drugs. In one embodiment, the prediction relates to the extent of those responses. In another embodiment, the prediction relates to whether and/or the probability that a patient will survive following treatment, for example treatment with a particular therapeutic agent and/or surgical removal of the primary tumor, and/or chemotherapy for a certain period of time without cancer recurrence. The predictive methods of the invention can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular patient. The predictive methods of the present invention are valuable tools in predicting if a patient is likely to respond favorably to a treatment regimen, such as a given therapeutic regimen, including for example, administration of a given therapeutic agent (e.g. a chemotherapeutic agent) or combination (e.g. an IGF inhibitor in combination with a chemotherapeutic agent), chemotherapy, etc. or whether long-term survival of the patient, following a therapeutic regimen is likely.

The term "increased resistance" to a particular therapeutic agent or treatment option, when used in accordance with the invention, means decreased response to a standard dose of the drug or to a standard treatment protocol.

The term "decreased sensitivity" to a particular therapeutic agent or treatment option, when used in accordance with the invention, means decreased response to a standard dose of the agent or to a standard treatment protocol, where decreased response can be compensated for (at least partially) by increasing the dose of agent, or the intensity of treatment.

"Responsive to cancer treatment" and "sensitive to cancer treatment" can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of tumor growth, including slowing down or complete growth arrest; (2) reduction in the number of tumor cells; (3) reduction in tumor size; (4) inhibition (e.g., reduction, slowing down or complete stopping) of tumor cell infiltration into adjacent peripheral organs and/or tissues; (5) inhibition (e.g., reduction, slowing down or complete stopping) of metastasis; (6) enhancement of antitumor immune response, which may, but does not have to, result in the regression or rejection of the tumor; (7) relief, to some extent, of one or more symptoms associated with the tumor; (8) increase in the length of survival following treatment; and/or (9) decreased mortality at a given point of time following treatment.

The term "prediction" or (and variations such as predicting) is used herein to refer to the likelihood that a patient will respond either favorably or unfavorably to a drug or set of drugs (e.g. the combination of an IGF inhibitor and a chemotherapeutic agent). In one embodiment, the prediction relates to the extent of those responses. In another

5 embodiment, the prediction relates to whether and/or the probability that a patient will survive following treatment, for example treatment with a particular therapeutic agent and/or surgical removal of the primary tumor, and/or chemotherapy for a certain period of time without cancer recurrence. The predictive methods of the invention can be used clinically to make treatment decisions by choosing the most appropriate treatment
10 modalities for any particular patient. The predictive methods of the present invention are valuable tools in predicting if a patient is likely to respond favorably to a treatment regimen, such as a given therapeutic regimen, including for example, administration of a given therapeutic agent or combination, chemotherapy, etc. , or whether long-term survival of the patient, following a therapeutic regimen is likely.

15

The level of M2-like macrophage in a sample may be determined by techniques known in the art, such as enzyme linked immunosorbent assays (ELISAs), immunoprecipitation, immunofluorescence, Immunohistochemistry, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. For in vivo detection of an M2-like
20 macrophages a labeled anti-M2-like (e.g. CD163+) macrophage antibody may be introduced into a patient. Such an antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

25

Alternatively, M2-like macrophages could be used as a non-invasive biomarker. For example, M2-like macrophages could be detected in a biological sample (e.g.blood) and analysis could be undertaken to determine if such M2-like macrophages express IGF.

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The level of M2-like macrophage in a sample may also be determined by determining the level of M2-like macrophage activity in a sample.

35

Methods of the invention further comprise comparing the level or activity of M2-like macrophage in the patient (e.g. core) sample with the level or activity of M2-like macrophage in a control sample or with a predetermined reference level for M2-like macrophage.

In one embodiment, methods of the invention include contacting a control sample with a compound or agent capable of detecting an M2-like macrophage nucleic acid molecule, such as mRNA, or genomic DNA, and comparing the level of the M2-like macrophage nucleic acid molecule in the control sample with the level of M2-like macrophage molecule in the patient sample.

In another embodiment, the methods of the invention further include contacting the control sample with a compound or agent capable of detecting an M2-like macrophage, and comparing the level of M2-like macrophage in the control sample with the presence of M2-like macrophage in the test sample.

As used herein "reference level" or "control", refers to a sample having a normal level of M2-like macrophage expression, for example a sample from a healthy subject not having or suspected of having a proliferative disorder such as cancer. Alternatively, the reference level may be comprised of an M2-like macrophage level from a reference database, which may be used to generate a pre-determined cut off value, i.e. a diagnostic score that is statistically predictive of a symptom (e.g. chemoresistance) or disease or lack thereof or may be a pre-determined reference level based on a standard population sample.

Alternatively, predictions may be based on the normalized expression level of M2-like macrophage. Expression levels are normalized by correcting the absolute expression level of M2-like macrophage in a sample by comparing its expression to the expression of a reference nucleic acid that is not a marker, e.g., an mRNA, such as an mRNA that is constitutively expressed. This normalization allows the comparison of the expression level in one sample to another sample, or between samples from different sources. This normalized expression can then optionally be compared to a reference level or control.

In one embodiment the diagnostic, predictive or stratification methods involve determining the level of M2-like macrophage in a sample and determining the level of at least one further biomarker, for example a biomarker predictive or indicative associated with chemoresistance. Preferably, the at least one further biomarker may be selected from Insulin/IGFR. Preferably, the level of the at least one further biomarker is determined using any one of the above mentioned methods.

The level of at least one further biomarker may be determined in the same biological sample or a different biological sample to the level of M2-like macrophage.

Alternatively, the level of M2-like macrophage in a sample can be detected and quantified using mass spectrometry.

5 In one aspect the invention includes an assay device, for example a solid support such as an array or a chip, that has attached to a surface thereof a compound or agent capable of detecting an M2-like macrophage. Preferably, compound or agent capable of detecting an M2-like macrophage is an anti- M2-like macrophage antibody, more preferably an M2-like macrophage capture antibody. In one embodiment the assay device further comprises at least one additional compound or agent for detecting a further biomarker,
10 preferably Insulin/IGFR.

The methods of the invention allow a skilled person to make informed treatment decisions on the basis of M2-like macrophage. For example, the methods of diagnosis and prognosis described herein may further comprise a step of treating a patient or organ, on
15 the basis of the diagnosis or prognosis. The step of treating a patient or organ may, by way of example only, be administration of a therapeutically effective amount of an IGF inhibitor and a chemotherapeutic agent. The combination may be administered simultaneously or sequentially.

20 Kits

The invention also includes kits for detecting the presence of M2-like macrophage in a biological sample. For example, the kit can include a compound or agent capable of detecting an M2-like macrophage in a biological sample. The compound or agent can be
25 packaged in a suitable container. The kit can further comprise instructions for using the kit to detect M2-like macrophage.

In one aspect the invention provides a kit for diagnosing determining the treatment strategy for a patient with cancer comprising: a detectably labelled agent that specifically binds to
30 M2-like macrophage; and
ii) reagents for performing a diagnostic assay.

The agent may be an antibody or a nucleic acid molecule. Suitably, the labelled agent may be an anti-CD163 agent that binds to macrophages.

35

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which specifically binds to an M2-like macrophage corresponding to a marker of

the invention; and, optionally, (2) a second, different antibody which binds to either the M2-like macrophage or the first M2-like macrophage antibody and is conjugated to a detectable agent.

- 5 For oligonucleotide-based kits, the kit can include: (1) a nucleotide probe, e.g., a detectably labeled primer, which hybridizes to an M2-like macrophage or (2) a pair of primers for amplifying a M2-like macrophage.

10 In one aspect the invention provides a kit for diagnosing ischaemia-reperfusion injury or

The kits can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kits can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Suitably, the kits and assay devices of the present invention may be used to determining
15 tumour stage, to predict tumour prognosis and/or to predict responsiveness to treatment.

Compound combinations and compositions

A compound of the invention can be for use or administration alone or in combination with at least one or more other compounds. Administration "in combination with" at least one or
20 more other compounds includes separate, simultaneous (concurrent) and sequential (consecutive) administration in any order. "Combined use" and "combination" in the context of the invention also includes a product comprising both the compound and at least one or more other compounds, as discrete separate dosage forms, in separate containers or e. g. in blisters containing both types of drugs in discrete solid dosage units, e.g. in a
25 form in which the dosage units which have to be taken together or which have to be taken within one day are grouped together in a manner which is convenient for the patient. Said product itself or as a part of a kit may contain instructions for the simultaneous, sequential or separate administration of the discrete separate dosage units, to a subject. Accordingly, the product may comprise at least two compounds (e.g. an IGF inhibitor and a
30 chemotherapeutic agent; and optionally a PDL-1 inhibitor and/or a CD80 inhibitor) as discrete separate dosage forms, in a form which is suitable for sequential, separate and/or simultaneous administration.

The separate formulations of e.g. an IGF inhibitor and a chemotherapeutic agent (and
35 optionally a PDL-1 inhibitor and/or a CD80 inhibitor) may therefore be administered sequentially, separately and/or simultaneously. For example, the separate formulations of an IGF inhibitor and a chemotherapeutic agent (and optionally a PDL-1 inhibitor and/or a

CD80 inhibitor) of the combination product may be administered simultaneously (optionally repeatedly). For example, the separate formulations of an IGF inhibitor and a chemotherapeutic agent (and optionally a PDL-1 inhibitor and/or a CD80 inhibitor) of the combination product may be administered sequentially (optionally repeatedly). For
5 example, the separate formulations of an IGF inhibitor and a chemotherapeutic agent (and optionally a PDL-1 inhibitor and/or a CD80 inhibitor) of the combination product may be administered separately (optionally repeatedly). Where the administration of the separate formulations of an IGF inhibitor and a chemotherapeutic agent (and optionally a PDL-1 inhibitor and/or a CD80 inhibitor) of the combination product is sequential or separate, the
10 delay in administering the second formulation (and optionally third formulation) should not be such as to lose the beneficial effect of the combination therapy.

By way of example, a combination is provided comprising an IGF inhibitor and a chemotherapeutic agent (and optionally a PDL-1 inhibitor and/or a CD80 inhibitor). The
15 combination is useful for the treatment of a proliferative disorder.

By way of an alternative example, a pharmaceutical composition is provided comprising a first composition comprising an IGF inhibitor and a pharmaceutically acceptable excipient, adjuvant, diluent and/or carrier, and a second composition comprising a chemotherapeutic
20 agent and a pharmaceutically acceptable excipient, adjuvant, diluent and/or carrier. The first and second compositions may be provided in a form which is suitable for sequential, separate and/or simultaneous administration.

In one example, a pharmaceutical composition is provided comprising a first composition
25 comprising an IGF inhibitor and a pharmaceutically acceptable excipient, adjuvant, diluent and/or carrier, a second composition comprising a chemotherapeutic agent and a pharmaceutically acceptable excipient, adjuvant, diluent and/or carrier, and a third composition comprising a PDL-1 inhibitor and/or a CD80 inhibitor and a pharmaceutically acceptable excipient, adjuvant, diluent and/or carrier. The first, second and third
30 compositions may be provided in a form which is suitable for sequential, separate and/or simultaneous administration.

A compound of the invention may thus be part of a composition (e.g. a pharmaceutical composition) that comprises the compound and one or more other components. A
35 composition may be a pharmaceutical composition that comprises a compound of the invention and a pharmaceutically acceptable excipient, adjuvant, diluent and/or carrier. Pharmaceutical compositions may routinely contain pharmaceutically acceptable

concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents or compounds.

- 5 As used herein, "pharmaceutically acceptable" refers to a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

10

- Excipients are natural or synthetic substances formulated alongside an active ingredient (e.g. a compound of the invention), included for the purpose of bulking-up the formulation or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating drug absorption or solubility. Excipients can also be useful in the manufacturing process, to aid in the handling of the active substance concerned such as by facilitating powder flowability or non-stick properties, in addition to aiding *in vitro* stability such as prevention of denaturation over the expected shelf life. Pharmaceutically acceptable excipients are well known in the art. A suitable excipient is therefore easily identifiable by one of ordinary skill in the art. By way of example, suitable pharmaceutically acceptable excipients include water, saline, aqueous dextrose, glycerol, ethanol, and the like.

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- Adjuvants are pharmacological and/or immunological agents that modify the effect of other agents in a formulation. Pharmaceutically acceptable adjuvants are well known in the art. A suitable adjuvant is therefore easily identifiable by one of ordinary skill in the art.

25

Diluents are diluting agents. Pharmaceutically acceptable diluents are well known in the art. A suitable diluent is therefore easily identifiable by one of ordinary skill in the art.

- 30 Carriers are non-toxic to recipients at the dosages and concentrations employed and are compatible with other ingredients of the formulation. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. Pharmaceutically acceptable carriers are well known in the art. A suitable carrier is therefore easily identifiable by one of ordinary skill in the art.

35

The compound combinations and/or compositions of the present invention may be used as a sole therapy or may involve additional surgery or radiotherapy or an additional

chemotherapeutic agent or a therapeutic antibody in addition. Such chemotherapeutic agents may include one or more of the following categories of anti-tumour agents:

- (i) other antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as alkylating agents (for example cis-platin, oxaliplatin, carboplatin, cyclophosphamide, nitrogen mustard, melphalan, chlorambucil, busulphan, temozolamide and nitrosoureas); antimetabolites (for example gemcitabine and antifolates such as fluoropyrimidines like 5-fluorouracil and tegafur, raltitrexed, methotrexate, cytosine arabinoside, and hydroxyurea); antitumour antibiotics (for example anthracyclines like adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin and mithramycin); antimitotic agents (for example vinca alkaloids like vincristine, vinblastine, vindesine and vinorelbine and taxoids like taxol and taxotere and polokinese inhibitors); and topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan and camptothecin);
- (ii) cytostatic agents such as antioestrogens (for example tamoxifen, fulvestrant, toremifene, raloxifene, droloxifene and idoxifyfene), antiandrogens (for example bicalutamide, flutamide, nilutamide and cyproterone acetate), LHRH antagonists or LHRH agonists (for example goserelin, leuprorelin and buserelin), progestogens (for example megestrol acetate), aromatase inhibitors (for example as anastrozole, letrozole, vorazole and exemestane) and inhibitors of 5 α -reductase such as finasteride;
- (iii) anti-invasion agents (for example c-Src kinase family inhibitors like 4-(6-chloro-2,3-methylenedioxyanilino)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-tetrahydropyran-4-yloxyquinazoline (AZD0530; International Patent Application WO 01/94341) and *N*-(2-chloro-6-methylphenyl)-2-{6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-ylamino}thiazole-5-carboxamide (dasatinib, BMS-354825; J. Med. Chem., 2004, 47, 6658-6661), and metalloproteinase inhibitors like marimastat, inhibitors of urokinase plasminogen activator receptor function or antibodies to Heparanase);
- (iv) inhibitors of growth factor function: for example such inhibitors include growth factor antibodies and growth factor receptor antibodies (for example the anti-erbB2 antibody trastuzumab [HerceptinTM], the anti-EGFR antibody panitumumab, the anti-erbB1 antibody cetuximab [Erbix, C225] and any growth factor or growth factor receptor antibodies disclosed by Stern *et al.* Critical reviews in oncology/haematology, 2005, Vol. 54, pp11-29); such inhibitors also include tyrosine kinase inhibitors, for example inhibitors of the epidermal growth factor family (for example EGFR family tyrosine kinase inhibitors such as *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine (gefitinib, ZD1839), *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (erlotinib, OSI-774) and 6-acrylamido-*N*-(3-chloro-4-fluorophenyl)-7-(3-morpholinopropoxy)-quinazolin-4-amine (CI 1033), erbB2 tyrosine kinase inhibitors such

as lapatinib, inhibitors of the hepatocyte growth factor family, inhibitors of the platelet-derived growth factor family such as imatinib, inhibitors of serine/threonine kinases (for example Ras signalling inhibitors such as farnesyl transferase inhibitors, for example sorafenib (BAY 43-9006)), inhibitors of cell signalling through AKT kinases, inhibitors of the hepatocyte growth factor family, c-kit inhibitors, abl kinase inhibitors, IGF receptor (insulin-like growth factor) kinase inhibitors; aurora kinase inhibitors (for example AZD1152, PH739358, VX-680, MLN8054, R763, MP235, MP529, VX-528 AND AX39459) and cyclin dependent kinase inhibitors such as CDK2 and/or CDK4 inhibitors;

(v) antiangiogenic agents such as those which inhibit the effects of vascular endothelial growth factor, [for example the anti-vascular endothelial cell growth factor antibody bevacizumab (Avastin™) and VEGF receptor tyrosine kinase inhibitors such as 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474; Example 2 within WO 01/32651), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171; Example 240 within WO 00/47212), vatalanib (PTK787; WO 98/35985) and SU11248 (sunitinib; WO 01/60814), compounds such as those disclosed in International Patent Applications WO97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 and compounds that work by other mechanisms (for example linomide, inhibitors of integrin $\alpha v \beta 3$ function and angiostatin)];

(vi) vascular damaging agents such as Combretastatin A4 and compounds disclosed in International Patent Applications WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 and WO 02/08213;

(vii) antisense therapies, for example those which are directed to the targets listed above, such as ISIS 2503, an anti-ras antisense;

(viii) gene therapy approaches, including for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene-directed enzyme pro-drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi-drug resistance gene therapy; and

(ix) immunotherapy approaches, including for example ex-vivo and in-vivo approaches to increase the immunogenicity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies.

Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment.

Treatment of a subject

The inventors have surprisingly found that a compound that is capable of inhibiting IGF (e.g. an IGF inhibitor) is useful in the treatment of a proliferative disorder in a subject in combination with a chemotherapeutic agent because it is capable of reducing and/or preventing resistance to chemotherapeutic agent(s) in the subject.

Furthermore, the inventors have identified that the above combination may be supplemented with a compound capable of inhibiting PDL-1 (e.g. a PDL-1 inhibitor) and/or a compound capable of inhibiting CD80 (e.g. a CD80 inhibitor) because such compounds are capable of reducing and/or preventing suppression of the immune response in the subject.

As used herein, the terms "treat", "treating" and "treatment" are taken to include an intervention performed with the intention of preventing the development or altering the pathology of a disorder or symptom. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathological disorder or symptom.

As used here in the term "subject" refers to an individual, e.g., a human, pig, horse, mouse, cow, rat etc having or at risk of having a proliferative disorder e.g. cancer. Optionally, the subject is a subject having or at risk of having cancer, e.g. a solid cancer such as pancreatic cancer. The subject may be a patient i.e. a subject in need of treatment in accordance with the invention. The subject may have received treatment for the disorder. Alternatively, the subject has not been treated prior to treatment in accordance with the present invention.

As used herein, the terms "disease" and "disorder" are used interchangeably.

As used herein, the phrase "a proliferative disorder" is intended to include cancer, for example cancer of the lung, head and neck, brain, colon, rectum, oesophagus, stomach, liver, biliary tract, thyroid, kidney, cervix, ovary, uterus, skin, breast, bladder, prostate, pancreas and including haematological malignancies such as leukaemia, multiple myeloma and lymphoma. In particular, "a proliferative disorder" is intended to encompass solid tumours (cancers) (e.g. primary and recurrent solid tumours) such as pancreatic cancer, lung cancer, breast cancer, melanoma, colorectal cancer, ovarian cancer, gastric

cancer, thyroid cancer, liver cancer and prostate cancer. A proliferative disorder of particular interest in the context of the invention is pancreatic cancer.

The compounds, combinations and/or compositions described herein can be administered to the subject by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be topical, oral, parenteral, intravenous, intraperitoneal, intramuscular, intravascular, intracavity, intranasal, intracerebral, intratracheal, intralesional, intraperitoneal, intratumoural, rectal, subcutaneous, transdermal, epidural, percutaneous, or by infusion.

The compounds, combinations and/or compositions described herein may be in a form suitable for the above modes of administration. For example, suitable forms for oral administration include a tablet or capsule; suitable forms for nasal administration or administration by inhalation include a powder or solution; suitable forms for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) include a sterile solution, suspension or emulsion; suitable forms for topical administration include an ointment or cream; and suitable forms for rectal administration include a suppository. Alternatively, the route of administration may be by direct injection into, for example, the tumour, or by regional delivery or by local delivery.

The compounds, combinations and/or compositions described herein are for administration in an effective amount. An "effective amount" (or "therapeutically effective amount") is an amount that alone, or together with further doses, produces the desired (therapeutic) response. The (therapeutically) effective amount to be used will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. For example, the dosage of the IGF inhibitor and/or the chemotherapeutic agent for a given patient will be determined by the attending physician, taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. The dosages and schedules may be varied according to the particular disease state and the overall condition of the patient. For example, it may be necessary or desirable to reduce the above-mentioned doses of the components of the combination treatment in order to reduce toxicity. Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods.

The compositions of the present invention are advantageously presented in unit dosage form.

The compounds, combinations and/or compositions described herein are therefore administered to a subject in an effective amount to produce the desired response.

Examples of such responses include, but are not limited to, anti-tumour effects, the

5 response rate, the time to disease progression and the survival rate. Anti-tumour effects associated with treatment as described herein include but are not limited to, inhibition of tumour growth, tumour growth delay, regression of tumour, shrinkage of tumour, increased time to regrowth of tumour on cessation of treatment, slowing of disease progression. It is expected that when a compound, combination and/or composition described herein is
10 administered to a subject in need of treatment for cancer, said treatment will produce an effect, as measured by, for example, one or more of: the extent of the anti-tumour effect, the response rate, the time to disease progression and the survival rate. Anti-cancer effects include prophylactic treatment as well as treatment of existing disease.

15 The invention therefore also provides a method of treating a proliferative disorder such as cancer, which comprises administration of a compound, combination and/or composition according to the invention to a subject having or suspected of having said proliferative disorder. Optionally, the cancer is selected from pancreatic cancer, lung cancer, breast cancer, melanoma, colorectal cancer, ovarian cancer, gastric cancer, thyroid cancer, liver
20 cancer and prostate cancer.

Accordingly, *in vivo* methods of treatment are provided, which may be prophylactic and/or therapeutic.

25 Preferably, the combination(s) and/or composition(s) described herein will provide a beneficial or synergistic effect on the treatment or prophylaxis of a proliferative disorder such as cancer. A combination treatment is defined as affording a "synergistic effect" or a "synergistic treatment" if the effect is therapeutically superior, as measured by, for example, the extent of the response, the response rate, the time to disease progression or
30 the survival period, to that achievable on dosing one or other of the components of the combination treatment at its conventional dose. For example, the effect of the combination treatment is synergistic if the effect is therapeutically superior to the effect achievable with the IGF inhibitor alone or chemotherapeutic agent alone. Further, the effect of the combination is synergistic if a beneficial effect is obtained in a group of subjects that does
35 not respond (or responds poorly) to the IGF inhibitor or the chemotherapeutic agent alone. In addition, the effect of the combination treatment is defined as affording a synergistic effect if one of the components is dosed at its conventional dose and the other component

is dosed at a reduced dose and the therapeutic effect, as measured by, for example, the extent of the response, the response rate, the time to disease progression or the survival period, is equivalent to or better than that achievable on dosing conventional amounts of either one of the components of the combination treatment. In particular, synergy is deemed to be present if the conventional dose of the IGF inhibitor or the chemotherapeutic agent may be reduced without detriment to one or more of the extent of the response, the response rate, the time to disease progression and survival data, in particular without detriment to the duration of the response, but with fewer and/or less troublesome side-effects than those that occur when conventional doses of each component are used.

Predictive methods

The data provided herein indicate that PDA patients with high levels of IGF will not respond well to gemcitabine and/or 5'FU (i.e. such patients display IGF-induced resistance to chemotherapy). Accordingly, patient IGF levels may be used as a predictive biomarker for response to gemcitabine and/or 5'FU. Use of IGF as a predictive marker would advantageously allow PDA patients with high levels of IGF to be selected for combinatorial treatment in accordance with the invention (i.e. treatment with a combination of chemotherapy and IGF inhibitor, with an optional addition of (i) a PDL-1 inhibitor and/or (ii) a CD80 inhibitor).

The invention therefore provides a method for determining the resistance of a patient with a proliferative disorder to treatment with a chemotherapeutic agent, said method comprising:

- (i) measuring IGF protein levels present within a microtumour environment within the patient; and
- (ii) comparing the measurement of (i) to control IGF protein levels (e.g. derived from a non-microtumour environment within said patient)

wherein an increase in IGF protein levels in (i) compared to control IGF protein levels is predictive of resistance to treatment with a chemotherapeutic agent.

The invention also provides a method for determining whether a patient with a proliferative disorder will benefit from combinatorial treatment with a chemotherapeutic agent and an IGF inhibitor, said method comprising:

- (i) measuring IGF protein levels; IGFR levels; and/or M2-like macrophage levels present within a microtumour environment within the patient; and

- (ii) comparing the measurement of (i) to control IGF protein levels; IGFR levels; and/or M2-like macrophage levels (e.g. derived from a non-microtumour environment within said patient)

wherein an increase in IGF protein levels; IGFR levels; and/or M2-like macrophage levels in (i) compared to control IGF protein levels; IGFR levels; and/or M2-like macrophage levels predicts that said patient will benefit from combinatorial treatment with a chemotherapeutic agent and an IGF inhibitor.

EXAMPLE 1

Introduction

Pancreatic ductal adenocarcinoma (PDA) is an extremely lethal disease of the exocrine pancreas for which novel therapeutic strategies are urgently needed. Drug resistance is one of the biggest challenges in cancer therapeutics and the cause of relapse in the majority of cancer patient, including pancreatic cancer patients. Therefore, understanding the molecular mechanisms of drug resistance is critical to the development of durable treatment strategies.

Although multiple factors can contribute to the resistance of PDA to therapies, one dominant player is the presence of a rich pro-tumoral microenvironment. Tumour associated macrophages (TAMs) are key drivers of this pro-tumoral microenvironment and promote PDA progression and resistance to chemotherapy. However, the molecular basis of how tumour associated macrophages promote resistance of pancreatic cancer cells to chemotherapy remains elusive. In these studies, the inventors found that tumour associated macrophages support resistance to pancreatic cancer cells to chemotherapy by secreting IGF. Mechanistically, our data suggest that macrophage derived IGF leads to Insulin receptor activation on pancreatic cancer cells, which consequently leads to resistance to chemotherapy. Importantly, blockade of IGF restored response of tumours to gemcitabine in pre-clinical models of pancreatic cancer and decreased PDA progression. Accordingly, biopsies from PDA patients show a strong correlation between phospho-Insulin levels, increased numbers of TAMs and poor response to gemcitabine. These findings suggest that specific inhibition of IGF in combination with gemcitabine holds great promise in the treatment of PDA.

Materials and Methods

Cell culture, generation of primary pancreatic cancer cells, macrophages and conditioned media

5 KPC cells were isolated in our laboratory from PDA tumour tissues obtained from $Kras^{G12D/+}$; $p53^{R172H/+}$; Pdx1-Cre mice as described in (Torres et al., PLOS ONE, 2013). Human pancreatic Suit-2 cells and murine KPC cells were cultured in DMEM supplemented with 10% FBS. Murine and human primary isolated monocytes were differentiated into macrophages in M-CSF media and polarised to M1, M2 or cancer associated macrophages with IFN γ /LPS, IL-2 or tumour conditioned media respectively. 10 To generated conditioned media, macrophages and pancreatic cancer cells were cultured in serum free DMEM for 24 h, supernatant was harvested, filtered with 0.45 μ m filter and stored at -20°C.

15 Purification of Myeloid Cells

Murine and human myeloid cells were purified from murine bone marrow or human buffy coats obtained from the University of Liverpool Blood Bank (healthy volunteers) using anti-CD11b magnetic bead affinity chromatography according to manufacturer's directions (Miltenyi Biotec). To assess the purity of the CD11b $^{+}$ cell population, allophycocyanin- 20 labelled anti-CD11b antibodies was added to cells, and flow cytometry was performed. Cell were >95% CD11b $^{+}$.

Treatment with Gemcitabine and IGF-1 blocking antibody

Suit-2 and KPC cells were cultured in serum free or DMEM with 2% FBS respectively, 25 pretreated for 3 h with human or mouse macrophage conditioned media respectively, and IGF-1 IgG blocking antibody (abcam 9572) at 10 μ g/ml or IgG antibody as control, followed by Gemcitabine at 200 nM. Cells were harvested after 24 hours, and subjected to flow cytometry. Cells were stained with annexinV conjugated to FITC and PI (eBioscience) following the manufacturer's protocol. Apoptosis was evaluated by flow cytometry (FACS 30 Calibur, Becton Dickinson).

RTK arrays and immunoblotting

Cells were serum starved or treated with macrophage conditioned media for 30 min, 2 h or 3h, harvested and lysed in RIPA buffer (150 mM NaCl, 10 mM Tris-HCl pH 7.2, 0.1% SDS, 35 1% Triton X-100, 5 mM EDTA) supplemented with a complete protease inhibitor mixture (SIGMA), a phosphatase inhibitor cocktail (Invitrogen), 1 mM PMSF and 0.2 mM Na_3VO_4 . Cell lysates (300 μ g) were analyzed with the Human Phospho-RTK Array Kit (R&D

Systems). Protein samples were resuspended in 4× Laemmli sample buffer, and immunoblotting was performed on nitrocellulose membranes blocked in 5% BSA in Tris-buffered saline Tween-20. Membranes were incubated with antibodies in 5% milk or 5% bovine serum albumin overnight at 4°C: anti-pInsulin/pIGFR1 (R&D), anti-pInsulin (LSBioScience), anti-tubulin (Sigma), anti-Insulin (abcam), anti-Insulin (R&D) anti-pIGF1R, anti-IGF1R diluted to 1:1000.

Orthotopic pancreas cancer syngeneic model

KPC primary pancreatic carcinoma cells (1×10^6 tumour cells in 30 µl of matrigel) were implanted into the pancreas of six- to eight-week-old female isogenic PC recipient mice obtained from Cambridge, as previously described (Qiu et al., *Methods Mol Biol.* 2013ref). Tumours were established for 2 weeks before beginning treatment. Mice were administered i.p with Gemcitabine (125 mg/kg), IGF-1 blocking antibody (abcam 9572) 25 µg/mouse or IgG isotype control antibody every 2 -3 days for 15 days before harvest. At endpoint, tumours were harvested, photographed, weighed, cryopreserved in OCT, fixed in formalin, solubilized for RNA purification, or collagenase-digested for flow cytometric analysis of immune cell populations (CD11b, Gr1, CD206, CD8, Treg expression. Phospho-Insulin levels, immune cell infiltration and apoptosis was measured by phospho-insulin (R&D and LSBioscience), CD68 (DAKO), CD206 (Abcam), CD8 Abcam) and cleaved caspase 3 (Cell signalling) immunofluorescent and immunohistochemical staining, composition of immune cell populations was assessed on digested tumours by flow cytometry and CD206, collagen and IGF-1 expression levels were determined by qPCR. All in vivo experiments were performed with n=8-10 mice.

Gene expression

Total RNA was isolated from cells and tissues using ISOGEN (Nippon Gene). cDNA was prepared from 1 µg RNA/sample, and qPCR was performed using gene specific QuantiTect Primer Assay primers from Qiagen. Relative expression levels were normalized to *gapdh* expression according to the formula $2^{-(Ct_{gene\ of\ interest} - Ct_{gapdh})}$. Fold increase in expression levels were calculated by comparative Ct method $2^{-(ddCt)}$.

Quantification of Immune Cells in Tissues by Flow Cytometry

To quantify the different immune cell populations in tissues, tumours were excised, minced and digested to single cell suspensions for 1 h at 37°C in 5 ml of Hanks Balanced Salt Solution (HBSS, GIBCO) containing 1 mg/ml Collagenase type IV, 10 µg/ml Hyaluronidase type V and 20 units/ml DNase type IV (Sigma). Red blood cells were solubilized with RBC Lysis Buffer (eBioscience) and then cells were incubated in FC-blocking reagent (BD

Bioscience), followed by anti-CD45-PE/Cy7 (Biolegend), anti-CD11b-FITC (Biolegend), anti-Gr1-APC/Cy7 (Biolegend), anti-F4/80-APC (Biolegend), anti-CD206-PerCP/Cy5.5 (Biolegend), anti-CD8-PerCP/Cy5.5 (Biolegend), anti-CD3-FITC (Biolegend), anti-CD4-APC (Biolegend). To exclude dead cells, 0.5 µg/ml propidium iodide was added.

5

Immunohistochemical analysis

Deparaffinization and antigen retrieval was performed using an automated DAKO PT-link. Paraffin-embedded human and mouse PDA tumours were immunostained using the DAKO envision+ system-HRP. Tissue sections were incubated overnight at 4°C with primary antibodies against phospho-Insulin (R&D) (1:50), phospho-Insulin (LSBios) (1:100), CD68 (DAKO) (1:1000), CD163 (abcam) (pre-diluted), F4/80 (affymetrix) (1:100), αSMA (abcam) (1:100), CD8 (abcam) (1:100). Staining was developed using diaminobenzidine and counterstained with hematoxylin.

10

Immunofluorescence analysis

Human and mouse PDA frozen tissue sections were fixed with cold acetone, permeabilized in 0.1% Triton, blocked in 8% goat serum and incubated overnight at 4°C with anti-phospho Insulin (R&D) (1:200), anti-phospho-Insulin (LSBio) (1:100), CD68 (DAKO, clone KP1) 1:200, CK19 (abcam) (1:200), CD8 (Biolegend) (1:100), F4/80 (Biolegend) (1:100), CD206 (abcam) (1:100), α SMA (abcam) (1:100), EpCAM (BD Pharmingen) (1:100) and cleaved caspase 3 (Cell signaling) (1:300). The appropriate fluorescently labelled secondary antibodies were co-incubated with the DNA dye TOPRO-3 (Invitrogen).

20

Statistical Methods

All error bars indicate standard error of the mean (SEM) of 3-5 replicates (in vitro studies) or 8-10 replicates (animal studies). Statistical significance was assessed with the Student *t* test for in vitro studies and ANOVA coupled with posthoc tests for in vivo studies using SigmaXL 6.02. A value of $P < 0.05$ was considered significant and signified by an asterisk.

30

Institutional approvals

All studies involving human tissues were approved by the University of Liverpool and were considered exempt according to national guidelines. All experiments on live animals were performed in accordance with institutional and national guidelines and regulations, under approval by the University of Liverpool BSU.

35

Results and discussion

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal diseases worldwide. Current therapies are unfortunately in most cases ineffective. Drug resistance is one of the biggest challenges in cancer therapeutics and the cause of relapse in the majority of cancer patient, including pancreatic cancer patients (Zahreddine and Borden, 2013). Therefore, understanding the molecular mechanisms of resistance is critical to the development of durable treatment strategies.

Despite extensive efforts invested in the clinical development of therapies against PDA, current standard treatments only include chemotherapeutic agents highly toxic and of modest efficacy (Li et al., 2004). Thus, the development of more effective and less toxic therapies to treat PDA is an urgent need. Although multiple factors can contribute to the resistance of PDA to therapies, one dominant player is the presence of a rich pro-tumoral microenvironment (Gore and Korc, 2014; Lunardi et al., 2014; McMillin et al., 2013; Mielgo and Schmid, 2013; Neesse et al., 2013; Noy and Pollard, 2014; Weizman et al., 2014). Tumour associated macrophages (TAMs) are key drivers of this pro-tumoral microenvironment and can promote tumour cell proliferation, drug resistance and metastasis in many carcinomas including PDA (Noy and Pollard, 2014; Ruffell et al., 2014). Thus, high numbers of TAMs often correlate with resistance to chemotherapy and metastasis leading to poor survival in pancreatic cancer patients (Ino et al., 2013; Kurahara et al., 2011). Previous studies from our group and others have shown that inhibition of myeloid cell infiltration into the tumour restrains cancer progression (DeNardo et al., 2011; Schmid et al., 2011a; Schmid et al., 2011b; Schmid et al., 2013; Zhu et al., 2014) However, TAMs can be polarised into M1 inflammatory/anti-tumoral macrophages that will activate an immune response against the tumour or M2 immunosuppressive/pro-tumoral macrophages that promote tumour immunity (Kurahara et al., 2011; Mielgo and Schmid, 2013; Ruffell et al., 2012). Thus, therapeutics that can specifically inhibit the pro-tumoral functions of TAMs, while sparing their anti-tumoral capacity, hold great promise in the goal of restraining PDA progression, metastasis and relapse.

The inventors have investigated the mechanisms by which pro-tumoral TAMs impact PDA resistance to chemotherapy and tumour progression.

The inventors have demonstrated that pancreatic TAMs (but not naïve macrophages) express high levels of IGF-1 (and are the main source of these growth factors in the tumour microenvironment). Secretion of IGF by TAMs consequently lead to the activation of Insulin Receptor signaling and cancer cells resistance to chemotherapy. The inventors

also found that phospho-Insulin receptor is highly expressed in epithelial malignant pancreatic ductal cells in biopsies from PDA patients and that increased phosphorylation of Insulin Receptor on cancer cells correlates with PDA progression and response to gemcitabine. Importantly, blockade of IGF ligands (but not IGFR) restored sensitivity of pancreatic cancer cells to chemotherapy *in vitro* and restrained tumour progression and reduced the immuno-suppressive microenvironment in a pre-clinical model of pancreatic cancer. These findings suggest that: i) in PDA, IGF ligands rather signal through either Insulin receptor or hybrid Insulin/IGF-R1 receptors but not through IGF-R1 homodimers, which might explain why inhibition of IGFR has failed in pancreatic cancer clinical trials (Basu et al., 2011; Guha, 2013; Pollak, 2012), ii) inhibition of IGF ligands can sensitize pancreatic cancer cells to chemotherapy. Thus, these studies strongly suggest that combinatorial treatments using IGF ligand blockade with chemotherapy may open new therapeutic opportunities to treat this devastating disease.

15 **Summary statements**

TAMs promote resistance of pancreatic cancer cells to chemotherapy.

A phospho-screen revealed that TAMs specifically trigger the activation of Insulin Receptor (but not IGF-1 Receptor) in pancreatic cancer cells.

20 TAMs express high levels of IGF.

Phospho-Insulin is highly expressed in pancreatic tumour cells in biopsies from PDA patients.

25 In vitro blockade of IGF (ligands) inhibits macrophage-induced chemoresistance of pancreatic cancer cells.

30 *In vivo* blockade of IGF (ligand) in combination with Gemcitabine decreases tumour growth in a preclinical model of pancreatic cancer.

PD-L1 is highly expressed in pancreatic tumour cells in biopsies from PDA patients.

35 PD-L1 and CTLA-4 ligand (CD80) are upregulated in pancreatic cancer cells when exposed to TAMs.

These findings suggest that combinatorial treatment using:

1. IGF blocking agents with chemotherapy or
 2. IGF blocking agents + Checkpoint inhibitors (i.e. PD-L1/PD-1, CTLA-4 inhibitors)
- with chemotherapy could hold great promise in the treatment of PDA.

5 EXAMPLE 2

Introduction

In these studies, we found that M2 but not M1-like macrophages directly support chemoresistance of cancer cells by secreting Insulin-like growth factors (IGFs), which activate Insulin/IGF receptors on cancer cells. Immunohistochemical analysis of biopsies from pancreatic and breast cancer patients revealed that 57% and 75% of the patients respectively express activated Insulin/IGF receptors, and this positively correlates with increased M2-like macrophage infiltration and tumor progression. In vivo, we found that both M2-like macrophages but also α SMA+ stromal cells are the main sources of IGF production. Importantly, IGF blockade sensitized pancreatic tumors to gemcitabine treatment. These findings suggest that inhibition of IGFs in combination with chemotherapy could benefit cancer patients.

Materials and Methods

20

Cell culture, generation of primary pancreatic and breast cancer cells, macrophages and conditioned media

KPC cells were isolated in our laboratory from PDA tumor tissues obtained from KrasG12D/+; p53R172H/+; Pdx1-Cre mice as described previously (Olive et al., Science, 2009). Briefly, a 3mm³ fragment of PDA was excised, washed in 10 ml PBS, and then finely diced with sterile razors. Cells were incubated in 10 ml collagenase type V solution (1mg/ml in DMEM/F12) at 37C for 30-45 minutes with mixing. Cells were centrifuged (300 x g) and resuspended in 0.05% Trypsin/EDTA for 5 min at 37C. Digest was quenched by adding DMEM+ 10% fetal bovine serum and 96 μ M CaCl₂. Cells were washed 3 times with DMEM/F12 medium and plated in Biocoat dishes (Collagen I) in the ductal cell medium described previously (Schreiber et al., Gastroenterology, 2004). Cells were maintained on collagen coated plates at a minimal passage number (< p8) to allow initial expansion prior to use for in vitro and in vivo experiments.

35 Py230 cells were obtained from spontaneously arising tumors in MMTV-PyMT C57Bl/6 female mice by serial trypsinization and limiting dilution (Gibby et al., 2012). The mouse model used for obtaining these tumors has been described in detail previously (Guy et al.,

1992) and (Davie et al., 2007). Cells were maintained in Ham's F12K nutrient culture media (Life Technologies) with 5% HyClone fetal clone II (GE Life Sciences) and supplemented with MITO serum extender (BD Biosciences). Py230 cells were cultured until confluent and then passaged. Once confluent, they formed well-differentiated colonies
5 (Biswas et al., 2014; Gibby et al., 2012)

Human pancreatic cancer Suit-2 cells and breast cancer MDA-MB-231 cells were cultured in DMEM supplemented with 10% FBS. Human and murine primary isolated monocytes were differentiated into macrophages in DMEM + 10% FBS + 10 ng/ml recombinant
10 murine M-CSF media or 50 ng/ml recombinant human M-CSF, respectively, followed by polarizing to M1 (20 or 50 ng/ml INF γ and 100 or 10 ng/ml LPS), M2 (20 or 50 ng/ml IL-4 ng/ml) respectively, or tumor associated macrophages (using tumour conditioned media).

To generate conditioned media from macrophages and pancreatic cancer cells, also
15 referred to in these studies as macrophage and tumor derived factors, cells were cultured in serum free DMEM for 24h, supernatant was harvested, filtered with 0.45 μ m filter and stored at -20°C.

Generation of primary macrophages

20 Primary murine macrophages were generated by flushing the bone marrow from the femur and tibia of C57BL/6 or mixed 129/SvJae/C57Bl/6 (PC) mice followed by incubation for 5 days in DMEM containing 10% FBS and 10 ng/mL murine M-CSF (Peprotech). For some experiments, differentiated macrophages were polarized into an M1, M2, or tumor educated phenotype using INF γ (20 ng/ml, Peprotech) and LPS (100 ng/ml, Sigma
25 Aldrich); IL-4(20 ng/ml, Peprotech); or tumor conditioned media, respectively. Primary human macrophages were generated by purifying CD14 $^{+}$ monocytes from blood samples obtained from control healthy subjects using magnetic bead affinity chromatography according to manufacturer's directions (Miltenyi Biotec) followed by incubation for 5 days in DMEM containing 10% FBS and 50 ng/mL recombinant human M-CSF (Peprotech).
30 Studies were approved by the National Research Ethics (Research Integrity and Governance Ethics committee- Reference: RETH000807). All individuals provided informed consent for blood donation on approved institutional protocols.

Treatment with chemotherapy, IGF blocking antibody and recombinant IGF

35 Suit-2, KPC, cells were cultured in serum free or DMEM with 2% FBS respectively, pretreated for 3 hours with human or isogenic mouse macrophage derived factors (MDF) or recombinant IGF (Peprotech 100-11) at 100ng/ml, and IGF-1/2 IgG blocking

antibody (abcam 9572) at 10 µg/ml or irrelevant IgG antibody as control, followed by Gemcitabine at 200nM or 10-100nM of nab-paclitaxel. Cells were harvested after 24 hours, and subjected to flow cytometry. Cells were stained with annexinV conjugated to FITC and PI (eBioscience) following the manufacturer's protocol. Cell death was evaluated by flow
 5 cytometry (FACS Calibur, Becton Dickinson).

MDA-MB-231 cells were pretreated for 3 hours with human macrophage derived factors (MDF) or recombinant IGF (100ng/ml) followed by 500nM of nab-paclitaxel.

10 Py230 cells were pretreated for 3 hours with isogenic mouse macrophage derived factors (MDF) from M1 or M2 polarized macrophages followed by 100nM of paclitaxel.

Cell cycle analysis

KPC derived pancreatic cancer cells were treated with isogenic mouse MDFs, IGF1/2
 15 blocking antibody (abcam 9572) at 10µg/ml or recombinant IGF (Peprotech 100-11) at 100ng/ml. Cells were harvested, fixed with methanol, treated for 45 min with 10 µg/ml of RNase, resuspended in PBS containing 10 µg/ml of propidium iodide and subjected to flow cytometry.

20 RTK arrays and immunoblotting

Cells were serum starved or treated with macrophage conditioned media for 30 min, 2 h or 3h, harvested and lysed in RIPA buffer (150 mM NaCl, 10 mM Tris-HCl pH 7.2, 0.1% SDS, 1% Triton X-100, 5 mM EDTA) supplemented with a complete protease inhibitor mixture (SIGMA), a phosphatase inhibitor cocktail (Invitrogen), 1 mM PMSF and 0.2 mM Na₃VO₄.
 25 Cell lysates (300 µg) were analyzed with the Human Phospho-RTK Array Kit (R&D Systems). Protein samples were resuspended in 4×Laemmli sample buffer, and immunoblotting was performed on nitrocellulose membranes blocked in 5% BSA or milk (depending on the antibody) in Tris-buffered saline Tween-20 (TBST). Membranes were incubated with primary antibodies overnight at 4°C: anti-pInsulin/pIGFR1 (R&D, AF2507
 30 used 1:400 in 5% Milk-TBST), anti-pInsulin (LSBioScience, LS-C177981 used 1:1000 and repared in 2.5% BSA-TBST), anti-tubulin (Sigma, T6199 used 1:5000 in 2.5% BSA-TBST), anti-Insulin (abcam, 137747 used 1:1000 in 2.5% BSA-TBST), anti-Insulin (R&D, AF1544 used 1:2000 in 2.5% BSA-TBST), anti-pIGF1R (Biorbyt, orb97626 used 1:1000 in 2.5% BSA-TBST), anti-IGF1R (R&D, AF305-NA used 1:1000 in 2.5% BSA-TBST).

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Densitometry analysis of immunoblots was performed using ImageJ software and adjusted using the loading control.

Syngeneic Orthotopic pancreatic cancer model.

All animal experiments were performed in accordance with current UK legislation under an approved project licence (reference number: 403725). Mice were housed under specific pathogen-free conditions at the Biomedical Science Unit at the University of Liverpool.

Orthotopic pancreatic tumors were initiated by implanting 1×10^6 primary KPC cells into the pancreas of immune-competent syngeneic mice. Syngeneic recipient mice were six to eight-week-old female mice descended from mice used to generate the KPC PDAC cell lines but lacking oncogeneic KrasG12D expression (PC mice). Tumors were established for 2 week before beginning treatment. Mice were administered i.p with Gemcitabine (100 mg/kg), IGF-1/2 blocking antibody (abcam 9572) 25 µg/mouse or IgG isotype control antibody every 2 -3 days for 15 days before harvest. At endpoint, tumors were harvested, photographed, weighed, cryopreserved in OCT, fixed in formalin, solubilized for RNA purification, or collagenase-digested for flow cytometry analysis of immune cells and macrophages (CD45+ and F4/80+ cells respectively). Tumor tissues were analyzed by immunohistochemical and immunofluorescent staining for phospho-Insulin/IGFR expression, EpCAM expression, macrophage infiltration, fibroblast activation and apoptosis using the following antibodies: phospho-insulin/IGF receptor antibody (R&D), EpCAM (BD Pharmingen), CD68 antibody (DAKO), CD206 (abcam), αSMA (abcam) and cleaved caspase 3 (Cell signaling). Expression of IGF-1, IGF-2, Arginase, IL-10, IFNγ and IL-12 in intra-tumoral isolated macrophages were determined by qPCR. In vivo experiments was performed with n=9-12 mice.

Gene expression

Total RNA was isolated from purified cells as described for Qiagen Rneasy protocol. Total RNA from entire pancreatic tumor tissues was extracted using a high salt lysis buffer (Guadidine thiocynate 5 M, sodium citrate 2.5 uM, lauryl sarcosine 0.5% in H₂O) to improve RNA quality followed by purification using Qiagen Rneasy protocol. cDNA was prepared from 1µg RNA/sample, and qPCR was performed using gene specific QuantiTect Primer Assay primers from Qiagen. Relative expression levels were normalized to gapdh expression according to the formula $2^{-\Delta C_t}$ (Ct gene of interest – Ct gapdh) ((Schmittgen and Livak, 2008). In some cases values were multiplied by 100 for presentation purposes.

Quantification of immune cells in tissues by flow cytometry

Single cell suspensions from murine livers were prepared by mechanical and enzymatic disruption in Hanks Balanced Salt Solution (HBSS) with 1 mg/mL Collagenase P (Roche). Cells suspension were centrifuged for 5 min. at 1200 rpm, resuspended in HBSS and

filtered through a 500 µm polypropylene mesh (Spectrum Laboratories). Cell suspension was resuspended in 1mL 0.05% Trypsin and incubated at a 37°C for 5 minutes. Cells were filtered through a 70 µm cell strainer and resuspended in PBS + 5% BSA. Cells were blocked for 10 minutes on ice with FC Block (BD Pharmingen, Clone 2.4G2) and then stained with Sytox viability marker (Life Technologies) and conjugated antibodies against anti-CD45-PE/Cy7 (Biolegend, clone 30-F11), anti-F4/80-APC (Biolegend, clone BM8). Flow Cytometry was performed on a FACSCanto II (BD Biosciences). Part of the single cell suspension was used to isolated tumor associated macrophages using anti-F4/80 magnetic microbeads (Miltenyi Biotechnology) following the manufacturer's protocol.

Immunohistochemical analysis

Deparaffinization and antigen retrieval was performed using an automated DAKO PT-link. Paraffin-embedded human and mouse PDA tumors were immunostained using the DAKO envision+ system-HRP. Tissue sections were incubated for 1 hour at room temperature with primary antibodies against phospho-Insulin (R&D, AF2507, used 1:50 after high pH antigen retrieval), CD68 (DAKO, clone KP1, M081401-2 used 1:2000 after high pH antigen retrieval) CD163 (abcam, ab74604 pre-diluted after low pH antigen retrieval), αSMA (abcam, Ab 5694 used 1:100 after low pH antigen retrieval), CD206 (abcam, ab8919 used 1:50 after low pH antigen retrieval) followed by secondary-HRP conjugated antibody (from DAKO envision kit) for 30 minutes at room temperature. All antibodies were prepared in antibody diluent from Dako envision kit. Staining was developed using diaminobenzidine and counterstained with hematoxylin.

Immunofluorescence

Human and mouse PDA frozen tissue sections were fixed with cold acetone, permeabilized in 0.1% Triton, blocked in 8% goat serum and incubated overnight at 4°C with anti-phospho Insulin/IGFR (R&D) (1:200), CD68 (DAKO, clone KP1) (1:200), CK19 (abcam) (1:200), αSMA (abcam) (1:100), EpCAM (BD Pharmingen) (1:100) and cleaved caspase 3 (Cell signaling) (1:300). The appropriate fluorescently labelled secondary antibodies were co-incubated with the DNA dye TOPRO-3 (Invitrogen).

Statistical Methods

All error bars indicate standard deviations of 3-5 replicates (in vitro studies) or 9-12 replicates (animal studies). Statistical significance was assessed with the Student t test or ANOVA coupled with posthoc tests using GraphPad Prism 5. A value of $P < 0.05$ was considered significant and signified by an asterisk. A value of $P < 0.01$ was considered highly significant and signified by two asterisks.

Institutional approvals

All studies involving human tissues were approved by the University of Liverpool and were considered exempt according to national guidelines. Human pancreas carcinoma samples were obtained from the Liverpool Tissue Bank and patients consented to use the surplus material for research purposes. All experiments on live animals were performed in accordance with institutional and national guidelines and regulations, under approval by the University of Liverpool BSU.

Results and Discussion

Macrophage derived IGFs activate Insulin receptor signaling on cancer cells and induce chemoresistance

TAMs promote chemoresistance of cancer cells (De Palma and Lewis, 2013; Mantovani and Allavena, 2015; Mielgo and Schmid, 2013; Noy and Pollard, 2014; Ruffell and Coussens, 2015), however the molecular mechanism(s) by which macrophages induce cancer chemoresistance remain elusive. To understand whether macrophages can directly impact on cancer cells' response to chemotherapy, we co-cultured pancreatic and breast cancer cells with primary macrophages or macrophage conditioned media (MCM) in the presence or absence of gemcitabine or paclitaxel, and assessed tumor cell death by flow cytometry. Co-culture of breast and pancreatic cancer cells with primary macrophages or MCM resulted in resistance of cancer cells to chemotherapy (Figures 20 and Figure 49). MCM was generated from primary human and mouse macrophages cultured under standard conditions in the presence of macrophage colony stimulating factor 1 (M-CSF1). We found that, cultured under these standard conditions, macrophages had rather an M2-like phenotype and expressed CD206 but not IL-12 (Figures 50 and 51), suggesting that M2-like macrophages are capable of inducing chemoresistance in pancreatic cancer cells (Figures 20 and Figure 49). To further confirm this, we polarized macrophages into M1 (with LPS and IFN γ) or M2 macrophages and cultured breast cancer cells with M1 or M2 MCM in the presence or absence of paclitaxel. Interestingly, only MCM from M2-like (but not M1-like) polarized macrophages was capable of inducing resistance of cancer cells to chemotherapy (Figure 20c). Mechanistically, a phospho-receptor tyrosine kinase array and immunoblotting analysis of pancreatic cancer cells cultured in the presence or absence of MCM revealed that MCM induces the phosphorylation and activation of three receptor tyrosine kinases (RTKs), Insulin/IGF receptor (number 1), AXL receptor (number 2) and Ephrin receptor (number 3) (Figures 21 and 22). We only focus on the Insulin receptor because only blockade of the Insulin receptor signaling pathway was found to

have an effect on macrophage-mediated chemoresistance. Activation of Insulin and IGF receptors by macrophages was further confirmed by detection of tyrosine phosphorylation on Insulin and IGF receptor immunoprecipitated from Suit-2 pancreatic cancer cells cultured in the presence or absence of MCM (Figure 23). To understand, how

5 macrophages activate Insulin/IGF receptor signaling in cancer cells, we assessed the expression levels of the three ligands Insulin, IGF-1 and IGF-2 in primary human and mouse macrophages. Interestingly, M2-like (but not M1-like) macrophages express IGF-1 and IGF-2 but not Insulin (Figures 24-26). In addition, expression of IGF-1 was significantly increased when macrophages were exposed to tumor conditioned media (TCM) from

10 pancreatic or breast cancer cells, while IGF-2 levels remained generally unchanged, except for the murine macrophages treated with the TCM from Py230 breast cancer cells (Figures 24 and 26). Immunoblotting analysis of Suit-2 human pancreatic cancer cells, KPC-derived primary murine pancreatic cancer cells and Py230 primary murine breast cancer cells cultured in the presence or absence of MCM or recombinant IGF, further

15 confirmed that MCM (similarly to recombinant IGF) activates Insulin/IGFR signaling in pancreatic and breast cancer cells (Figure 27). Importantly, blockade of IGF ligands with an IGF neutralizing antibody was able to prevent activation of Insulin/IGF receptor and its downstream effectors IRS1, IRS2 and AKT by MCM (Figure 28) and to inhibit macrophage-induced chemoresistance of cancer cells (Figures 29-31). In contrast, in the

20 absence of MCM, IGF blockade did not further increase the response of cancer cells to chemotherapy confirming that, at least in this co-cultured system, macrophages (and not tumor cells) are the source of IGF that mediates chemoresistance (Figure 30). In addition, recombinant IGF was sufficient to mediate resistance of pancreatic and breast cancer cells to gemcitabine and nab-paclitaxel (Figures 30-33). Treatment of tumor cells with MCM,

25 IGF blocking antibody or recombinant IGF alone did not alter the survival or proliferation of cancer cells, (Figures 52 and 53). Taken together, these results indicate that, while under non-stress conditions, tumor cells do not depend on IGF to survive and proliferate, when challenged with chemotherapy, IGF secreted by macrophages activates the Insulin/IGF receptor signaling pathway, which becomes essential for cancer cells' resistance to

30 chemotherapy.

Insulin/IGF receptors are activated on tumor cells in biopsies from pancreatic and breast cancer patients and this positively correlates with increased M2-like macrophage infiltration in tumors and tumor progression.

35 On the basis of these findings, which indicate an important role for M2-like macrophage-derived IGF in activating the insulin/IGF receptor survival signaling pathway in cancer cells, we evaluated whether the Insulin receptor is activated in biopsies from pancreatic

cancer patients and whether this correlates with increased macrophage infiltration.

Immunofluorescent and Immunohistochemical staining of phospho-Insulin/IGFR in frozen and paraffin embedded human PDA samples revealed that, indeed Insulin/IGFR was activated on the ductal epithelial pancreatic cancer cells in 30 out of 54 (~56%) consented

5 patients (Figures 34-36 and Figure 54). Similarly, immunofluorescent and immunohistochemical staining of CD68 and CD163 in the same frozen and paraffin embedded human PDA samples showed that pancreatic tumors are infiltrated by macrophages that surround the ductal epithelial pancreatic cancer cells (Figures 37, 38, 55 and 56). Since we previously found that M2-like (but not M1-like) macrophages
10 enhance chemoresistance by activating Insulin/IGFR on the tumor cells, we subsequently analyzed by immunohistochemistry serial sections from the consented 54 PDA patients for the co- presence of M2-like macrophages (CD163+) and phospho-Insulin/IGFR+ tumor cells. Importantly, we found that activation of Insulin/IGF receptor positively correlates with increased infiltration of M2-like macrophages (Chi-square = 9.272; $p = 0.002$) (Figures 39,
15 40 and Figure 57) and that co-expression of both phospho-Insulin/IGFR+ tumor cells and CD163+ M2-like macrophages positively correlates with tumor stage ($p = 0.018$) (Figure 41).

Since we previously found that M2-like macrophages promote chemoresistance of not only
20 pancreatic cancer cells, but also breast cancer cells, by activating the Insulin/IGFR signaling pathway on tumor cells, we analysed serial sections of 75 consented breast cancer patient samples for the co-expression of phospho-Insulin/IGFR on tumor cells and macrophages (CD68+ and CD163+). Similar to what we observed in PDA patients, we found that breast cancer cells positive for phospho-Insulin/IGFR were
25 surrounded by macrophages, especially M2-like CD163+ macrophages (Figure 42 and 43). 56 out of the 75 (~75%) analysed breast cancer samples were positive for phospho-Insulin/IGFR expression on tumor cells (Figure 44 and Figure 58) and activation of the Insulin/IGFR positively correlated with increased M2-like macrophage (CD163+) infiltration (Chi-square = 4.37; $p = 0.04$) (Figure 4f)

Intra-tumoral M2-like macrophages and α SMA+ stromal cells are the main sources of IGF in the pancreatic tumor microenvironment

Primary murine PDA cells were isolated from a genetically engineered mouse model of pancreatic cancer $Kras^{G12D}; Trp53^{R172H}; Pdx1-Cre$ (KPC) mice. KPC-derived cells were
35 maintained on collagen coated plates at a minimal passage number ($< p8$) to allow initial expansion. KPC cells were then orthotopically implanted into isogenic immune- competent recipient mice (Figure 46a). H&E and immunohistochemical staining of these tumors with

the proliferation marker Ki-67 confirmed that these mice developed PDA (Figure 46b). Similar to human PDA and to the genetically engineered KPC mouse model, these tumors are rich in α SMA+ myofibroblasts, are infiltrated by CD68+ macrophages and CD206+ M2-like macrophages and show activation of Insulin/IGFR (Figure 46b and Figures 59-62).

To determine whether in vivo TAMs express IGFs, we first isolated TAMs (F4/80+ cells) from established KPC tumors and assessed the expression levels of Insulin, IGF-1 and IGF-2. In agreement with our previous in vitro findings (Figure 20), we found that intra-tumoral TAMs isolated from murine pancreatic tumors express IGF-1 and IGF-2 and are a major source of these growth factors within the tumor microenvironment, expressing 3-10 times more IGF ligands compared to other (F4/80-) cells within the tumor microenvironment (Figure 62b, c).

To further investigate which cell population(s) within the tumor are the main producers of IGF, we used an alternative mouse model in which we orthotopically implanted into the pancreas of syngeneic recipient mice KPC-derived GFP+ cells (Figure 46c). Tumors were harvested at day 23 and CD45-/GFP+ tumor cells, CD45-/GFP- non-immune stromal cells, CD45+/F4/80+/CD206- M1-like macrophages and CD45+/F4/80+/CD206+ M2-like macrophages were sorted by flow cytometry (Figure 64) and analysed for the expression of IGF1 and IGF2 (Figure 46c,d). This model further confirmed that M2-like (but not M1-like) macrophages express IGF, but interestingly, also revealed non-immune α SMA+ stromal cells (also known as myofibroblasts) as an additional source of IGF production (Figure 46d, e and Figure 65). To further confirm myofibroblasts as an additional source of IGF production, we isolated primary pancreatic stellate cells from naïve mice pancreas. Pancreatic stellate cells when activated become myofibroblasts and express α SMA (Figure 66). We found that primary pancreatic myofibroblasts expressed IGF-1 and 2 and the expression of both IGF ligands further increased when myofibroblasts were exposed to KPC-derived TCM (Figures 67-68).

Blockade of IGFs improves response to chemotherapy in a pre-clinical tumor model of pancreatic cancer

α SMA+ stromal cells together with macrophages are the most abundant non-malignant stromal cells within the tumor microenvironment. The fact that these two stromal cell types are the main sources of IGF in the tumor microenvironment and that IGF signaling protects tumor cells from response to chemotherapy suggests that inhibiting IGF, in vivo, could increase tumor response to chemotherapy.

To test this hypothesis, we treated mice bearing established orthotopic pancreatic tumors with control IgG antibody, gemcitabine alone, IGF blocking antibody (BI 836845) alone or gemcitabine with IGF blocking antibody. Treatments were administered intraperitoneally, twice a week for two weeks (Figure 47a). As previously shown (Mitchem et al., 2013; Torres et al., 2013; Zhu et al., 2014) and similar to what is observed in PDA patients, gemcitabine alone had no effect on tumor growth (Figure 47b). Treatment with IGF blocking antibody alone only show a modest effect on tumor growth. In contrast, combination of gemcitabine with IGF blocking antibody significantly reduced tumor growth (Figure 47b). Analysis of immune cells populations within the different treatment groups showed an increase in F4/80+ macrophages in gemcitabine and gemcitabine+ IGF antibody treated tumors (Figure 47c), however the ratio of M1-like (CD206-) and M2-like (CD206+) macrophages within tumors remained the same in all treatment groups with approximately 1/3 of the intra-tumoral macrophages being M1-like macrophages and 2/3 being M2-like macrophages (Figure 47d). These findings suggest that while gemcitabine seems to trigger an increase in macrophage infiltration, none of the treatments seems to alter macrophage polarization. In addition, the percentage of inflammatory monocytes (Ly6C+/Ly6G-/CD11b+), Neutrophils/Myeloid derived suppressor cells (Gr1+, CD11b+) and cytotoxic CD8+ T cells (CTLs) remained the same in all treatment groups (Figure 47c). Immunohistochemical analysis of tumors, showed that phospho-Insulin/IGFR expression was decreased in tumors treated with IGF blocking antibody. Importantly, immunohistochemical staining of cleaved caspase-3 revealed higher levels of cell death in tumors treated with the combination of gemcitabine and the IGF blocking antibody, compared to those treated with an IgG irrelevant antibody, gemcitabine or IGF antibody alone. A similar in vivo experiment, using a different KPC-derived cell line and a different IGF blocking antibody (ab9572) yielded very similar results (Figures 69, 70).

Discussion

The data presented herein describe, for the first time, intra-tumoral stromal-derived IGF as a critical inducer of chemoresistance in pancreatic and breast cancer and provide pre-clinical data that support the rational of using IGF neutralizing antibodies with chemotherapy for the treatment of pancreatic cancer (Figure 48). We report a direct role for M2-like macrophages on chemoresistance of pancreatic and breast cancer cells and a paracrine signaling loop in which macrophage-derived IGF activates the Insulin/IGFR survival signalling pathway and blunts response of pancreatic and breast cancer cells to gemcitabine and paclitaxel. While IGFR inhibitors failed in the clinics (Guha, 2013; Pollak, 2012), two IGF blocking antibodies, MEDI-573 and BI836845, are currently being

evaluated in phase 2 and phase 1 clinical trials for metastatic breast cancer and various solid cancers, respectively. Our mouse tumor studies described here provide the proof-of-principle for the use of IGF-blocking antibodies in combination with chemotherapeutic agents such as gemcitabine for the treatment of patients with pancreatic cancer, and
5 suggest that inhibiting IGF ligands, and thereby preventing signaling through both Insulin and IGF receptors, may be a better therapeutic strategy. In addition, the fact that IGF is also able to increase resistance of breast and pancreatic cancer cells to paclitaxel and nab-paclitaxel, suggests that IGFs blockade may also increase efficacy of other cytotoxic agents in a variety of cancer types associated with a dominant presence of TAMs. In
10 agreement with this, autocrine IGF1-signaling was recently found to mediate chemoresistance in melanoma (Obenauf et al., 2015).

In addition, we found that in humans, the Insulin/IGFR signaling pathway is activated in 57% and 75% of pancreatic and breast cancer patient samples respectively, and this
15 correlates with a rich stromal compartment with increased M2-like macrophage infiltration and with advanced tumor stage. These observations suggest that combining chemotherapy with IGF blockade could be beneficial for patients in which the Insulin/IGF receptor is activated, and that Insulin/IGFR activation together with increased M2-like macrophage infiltration could be used in the future as a predictive biomarker for response
20 to therapy enabling patient stratification for treatment with anti-IGF therapy in combination with chemotherapy.

Despite extensive efforts invested in the clinical development of therapies against PDA, current standard treatments only exert a modest efficacy and targeting only the tumor cells
25 has not resulted in a significant improvement of PDA treatment. Similarly, triple negative breast cancer has a very poor survival rate and better strategic therapies are urgently needed. The rich stromal compartment present in both PDA and breast cancer is not just a bystander but is a source of a variety of non-malignant cells and extracellular matrix proteins, which support tumor progression, resistance to cytotoxic agents and metastasis.
30 Thus, therapies that target both the neoplastic cells and the tumor microenvironment, will achieve better therapeutic response (Bailey and Leach, 2012; Costello et al., 2012; Feig et al., 2012; Hidalgo et al., 2015; Paulson et al., 2013; Werner et al., 2013). Macrophages and myofibroblasts are the two main stromal cell types within the tumor microenvironment in solid cancers. Interestingly, we found that in tumors, both M2-like
35 macrophages and myofibroblasts are the main sources of IGF production and that inhibiting IGF increased the response of tumor cells to chemotherapy in a pre-clinical

model of PDA. Thus, these studies provide a new combinatorial therapeutic opportunity to treat these devastating diseases.

TAMs can enhance or limit the efficacy of chemotherapy depending on the tumor model and/or the chemotherapeutic agent used (De Palma and Lewis, 2013; Mantovani and Allavena, 2015; Mielgo and Schmid, 2013; Noy and Pollard, 2014; Ruffell and Coussens, 2015). In fact, chemoresistance is increased when cytotoxic agents increase M2-like macrophage infiltration via CCL2 (Nakasone et al., 2012) or CSF1 (DeNardo et al., 2011). Macrophages can also impair host responses to chemotherapy by expressing cathepsins that activate chemoprotective T cells (Bruchard et al., 2013; Shree et al., 2011) or by inducing the upregulation of cytidine deaminase, an enzyme that metabolizes nucleoside analogs (Weizman et al., 2014). TAMs demonstrate a high degree of plasticity, and can be polarized into M1-like anti-tumorigenic and M2-like pro-tumorigenic macrophages. Previous studies targeting signaling pathways necessary for the recruitment of macrophages or specific chemotactic factors (CCL2/PI3K γ /CSF1) have provided proof of concept that macrophages represent an attractive target to reduce tumor growth (DeNardo et al., 2011; Mitchem et al., 2013; Pyonteck et al., 2013; Ruffell and Coussens, 2015; Schmid et al., 2011a; Schmid et al., 2011b; Schmid et al., 2013; Zhu et al., 2014). However, dependent on the microenvironmental cytokine milieu, macrophages are not only promoting tumor growth, but they can also critically orchestrate an anti-tumor immune response (Mantovani and Allavena, 2015). Thus, therapies that aim to specifically inhibit the pro-tumorigenic functions of macrophages while sparing their tumoricidal activity could act as an alternative, and perhaps, more efficient approach than therapies that completely block macrophage recruitment to the tumor (Bronte and Murray, 2015; Quail and Joyce, 2013). In this regard, our in vivo studies indicate that IGF blockade induces tumors' response to gemcitabine without affecting immune cell infiltration, including macrophage infiltration or macrophage polarization. In conclusion, our studies suggest that M2-like macrophages and myofibroblasts can blunt host responses to chemotherapy via a IGFs-Insulin/IGFR paracrine signaling axis, and that IGF blockade improves gemcitabine efficacy in a pancreatic cancer model providing the rationale for moving this type of combinatorial treatment forward in the clinic.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to", and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is

used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

5 Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at
10 least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

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The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

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Claims

1. A pharmaceutical composition comprising a first composition comprising an IGF inhibitor and a pharmaceutically acceptable excipient, adjuvant, diluent or carrier, and a second composition comprising a chemotherapeutic agent and a pharmaceutically-acceptable excipient, adjuvant, diluent or carrier, wherein the IGF inhibitor directly inhibits the biological activity of IGF.
2. The pharmaceutical composition according to claim 1, wherein the first and second compositions are provided in a form which is suitable for sequential, separate and/or simultaneous administration.
3. The pharmaceutical composition according to claim 1 or 2, further comprising a PDL-1 and/or CD80 inhibitor.
4. A pharmaceutical composition according to any one of claims 1 to 3 for use in treating a proliferative disorder in a subject.
5. Use of the pharmaceutical composition according to any one of claims 1 to 3 in the treatment of a proliferative disorder in a subject.
6. A combination comprising an IGF inhibitor and a chemotherapeutic agent for use in treating a proliferative disorder in a subject, wherein the IGF inhibitor directly inhibits the biological activity of IGF.
7. The combination for use according to claim 6, wherein the combination further comprises a PDL-1 inhibitor and/or a CD80 inhibitor.
8. An IGF inhibitor for use in treating a proliferative disorder in a subject in combination with a chemotherapeutic agent, wherein the IGF inhibitor directly inhibits the biological activity of IGF.
9. The IGF inhibitor for use according to claim 8, wherein the IGF inhibitor is for use in combination with (i) the chemotherapeutic agent and (ii) a PDL-1 inhibitor and/or a CD80 inhibitor.

10. A chemotherapeutic agent for use in treating a proliferative disorder in a subject in combination with an IGF inhibitor, wherein the IGF inhibitor directly inhibits the biological activity of IGF.
11. The chemotherapeutic agent for use according to claim 10, wherein the chemotherapeutic agent is for use in combination with (i) the IGF inhibitor and (ii) a PDL-1 inhibitor and/or a CD80 inhibitor.
12. The pharmaceutical composition for use, the use of the pharmaceutical composition, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any of claims 4 to 11, wherein the proliferative disorder is cancer.
13. The pharmaceutical composition for use, the use of the pharmaceutical composition, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to claim 12, wherein the cancer is selected from pancreatic cancer, lung cancer, breast cancer, melanoma, colorectal cancer, ovarian cancer, gastric cancer, thyroid cancer, liver cancer, and prostate cancer.
14. The pharmaceutical composition for use, the use of the pharmaceutical composition, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to claim 11 or claim 12, wherein a tumor sample isolated from the patient has increased levels of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level
15. The pharmaceutical composition for use, the use of the pharmaceutical composition, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according claim 13 or claim 14, wherein the proliferative disorder is pancreatic cancer.
16. The pharmaceutical composition for use, the use of the pharmaceutical composition, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according any of claims 4 to 15, wherein said subject is susceptible to developing IGF-induced resistance to said chemotherapeutic agent.
17. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the IGF inhibitor inhibits at least one IGF selected from IGF-1 and IGF-2.

18. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the IGF inhibitor inhibits binding of at least one IGF to the insulin receptor, IGFR or hybrid receptors.

19. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according claim 17, wherein the IGF inhibitor inhibits binding of IGF-1 to the insulin receptor, IGFR or hybrid receptors and/or inhibits binding of IGF-2 to the insulin receptor, IGFR or hybrid receptors.

20. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the IGF inhibitor is an anti-IGF antibody or an antigen binding fragment thereof.

21. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the chemotherapeutic agent is selected from the group consisting of a nucleoside analogue, a topoisomerase inhibitor, a platinum complex, and combinations thereof.

22. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to claim 21, wherein the nucleoside analogue is gemcitabine or fluorouracil.

23. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the IGF inhibitor is an anti-IGF antibody or antigen binding fragment thereof, and wherein the chemotherapeutic agent is gemcitabine or fluorouracil.

24. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the

chemotherapeutic agent for use according to any preceding claim, wherein the PDL-1 inhibitor inhibits binding of PDL-1 to a PD-1 receptor.

25. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the PDL-1 inhibitor is an anti-PDL-1 antibody or an antigen binding fragment thereof.

26. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the CD80 inhibitor inhibits binding of CD80 to a CTLA-4 receptor.

27. The pharmaceutical composition, the use of the pharmaceutical composition, the pharmaceutical composition for use, the combination for use, the IGF inhibitor for use, or the chemotherapeutic agent for use according to any preceding claim, wherein the CD80 inhibitor is an anti-CD80 antibody or an antigen binding fragment thereof.

28. Use of a M2-like macrophage as a biomarker to select a patient population responsive to or sensitive cancer treatment with an IGF inhibitor and a chemotherapeutic agent.

29. Use of a M2-like macrophage as a biomarker to predict select a patient population at risk of chemoresistance to a chemotherapeutic agent.

30. A method of increasing the sensitivity rate (efficacy rate) of a combination of an IGF inhibitor and a chemotherapeutic agent to treat cancer in a patient population said method comprising selecting a sub population having a M2-like macrophage biomarker.

31. A method of identifying a subject having increased likelihood of responsiveness or sensitivity to a combination of an IGF inhibitor and a chemotherapeutic agent comprising:

- a. Determining the level of M2-like macrophages in a tumor sample isolated from the subject; and
- b. comparing the level of M2-like macrophages in the patient sample with the level of M2-like macrophages in a control sample or with a predetermined reference level for M2-like macrophages

wherein an increased level of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level is prognostic of an increased likelihood of responsiveness or sensitivity to an IGF inhibitor and a chemotherapeutic agent in the subject.

32. A method of identifying a subject having responsiveness or sensitivity to an IGF inhibitor comprising:

- a. Determining the level of M2-like macrophages in a tumor sample isolated from the subject; and
- b. comparing the level of M2-like macrophages in the patient sample with the level of M2-like macrophages in a control sample or with a predetermined reference level for M2-like macrophages

wherein an increased level of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level is prognostic of an increased likelihood of responsiveness or sensitivity to an IGF inhibitor.

33. A method of treating a subject having cancer comprising:

- a. Determining the level of M2-like macrophages in a tumor sample isolated from the subject; and
- b. comparing the level of M2-like macrophages in the patient sample with the level of M2-like macrophages in a control sample or with a predetermined reference level for M2-like macrophages
- b. administering a therapeutically effective amount of a chemotherapeutic agent and an IGF inhibitor when there is an increased level of M2-like macrophages in the tumor sample compared to the control sample or compared to the predetermined reference level.

34. Use of a M2-like macrophage in accordance with claim 28 or 29 or a method in accordance with any one of claims 30- 33, wherein the M2-like macrophage is CD163+.

35. Use of a M2-like macrophage in accordance with claim 28 or 29 or a method in accordance with any one of claims 30- 33, wherein the predetermined reference level of M2-like macrophages is at least 20 M2-like macrophages in the tumour core sample.

36. Use of a M2-like macrophage in accordance with claim 28 or 29 or a method in accordance with any one of claims 30- 33, wherein the cancer is pancreatic cancer or breast cancer.

37. A method or use in accordance with any one of claims 28 to 36 wherein the chemotherapeutic agent is gemcitabine or fluorouracil.
38. A kit for identifying a patient population which would benefit from a combined treatment with an IGF inhibitor and a chemotherapeutic agent comprising:
- a. a detectably labelled agent that specifically binds to a M2-like CD163+ macrophage; and
 - b. reagents for the assay.
39. An assay device comprising a compound or agent capable of detecting a M2-like macrophage.
40. A pharmaceutical composition as hereinbefore described with reference to the accompanying drawings.
41. A pharmaceutical composition for use in treating a proliferative disorder in a subject as herein before described with reference to the accompanying drawings.
42. Use of the pharmaceutical composition in the treatment of a proliferative disorder in a subject as herein before described with reference to the accompanying drawings.
43. A combination comprising an IGF inhibitor and a chemotherapeutic agent for use in treating a proliferative disorder in a subject as herein before described with reference to the accompanying drawings.
44. An IGF inhibitor for use in treating a proliferative disorder in a subject in combination with a chemotherapeutic agent, as herein before described with reference to the accompanying drawings.
45. A chemotherapeutic agent for use in treating a proliferative disorder in a subject in combination with an IGF inhibitor as herein before described with reference to the accompanying drawings.

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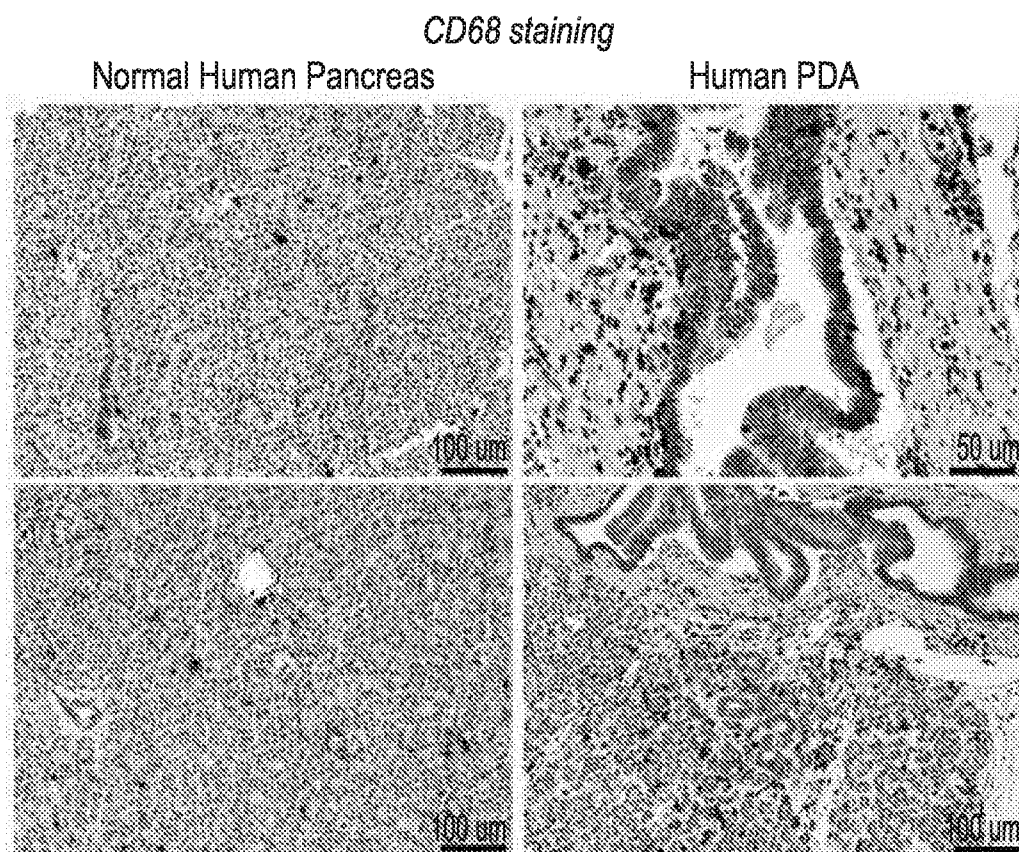


FIG. 1

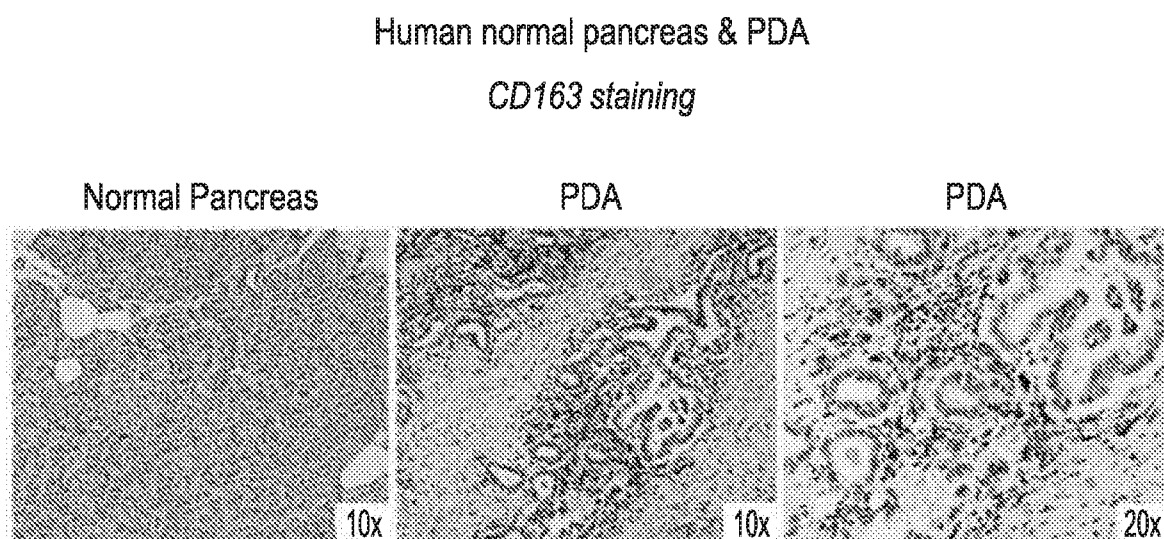
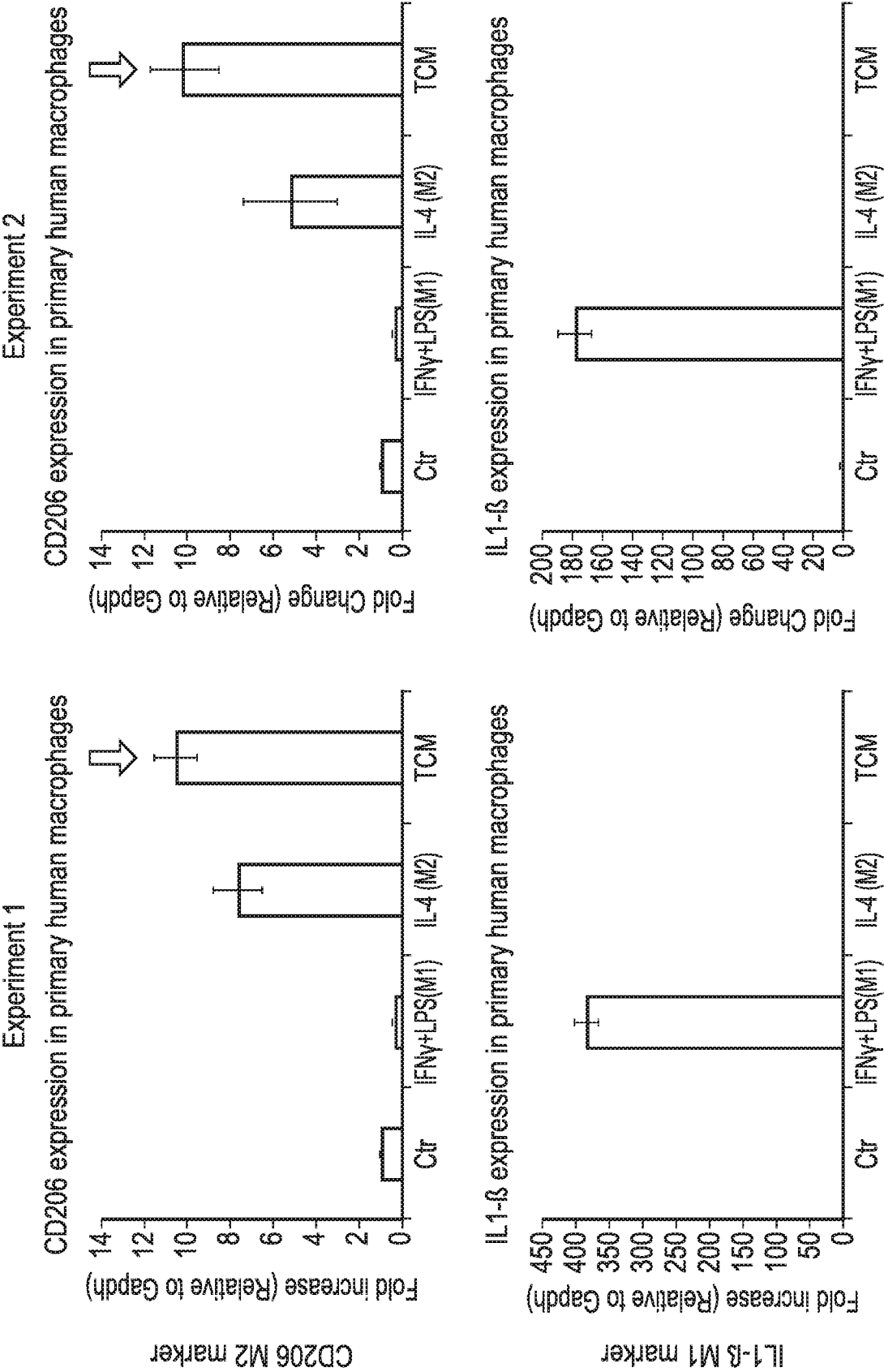


FIG. 2



TCM = Tumour conditioned media

FIG. 3

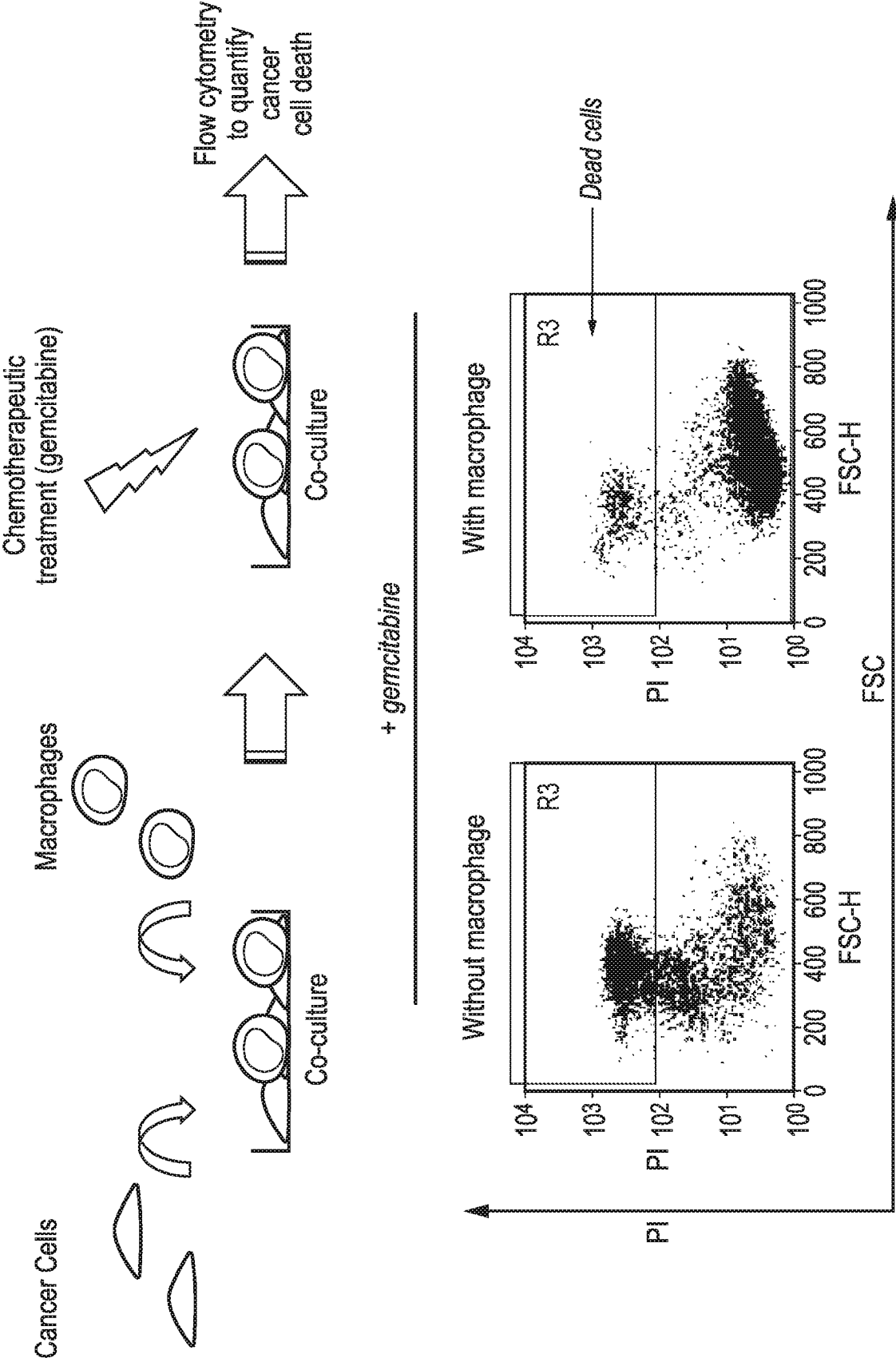


FIG. 4

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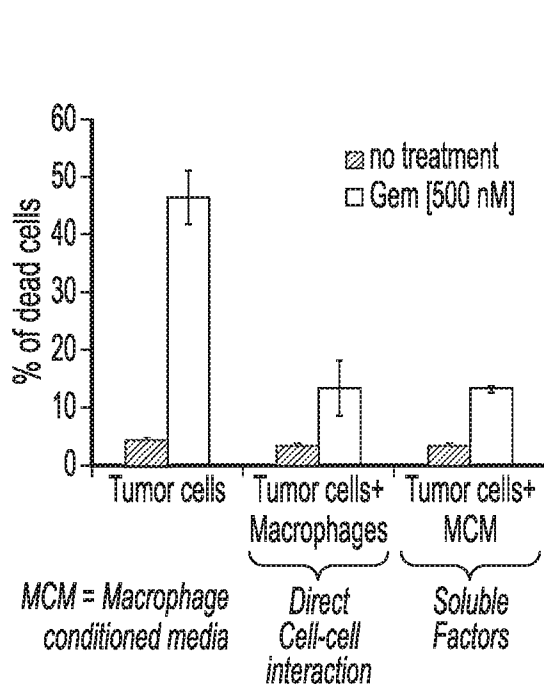


FIG. 5

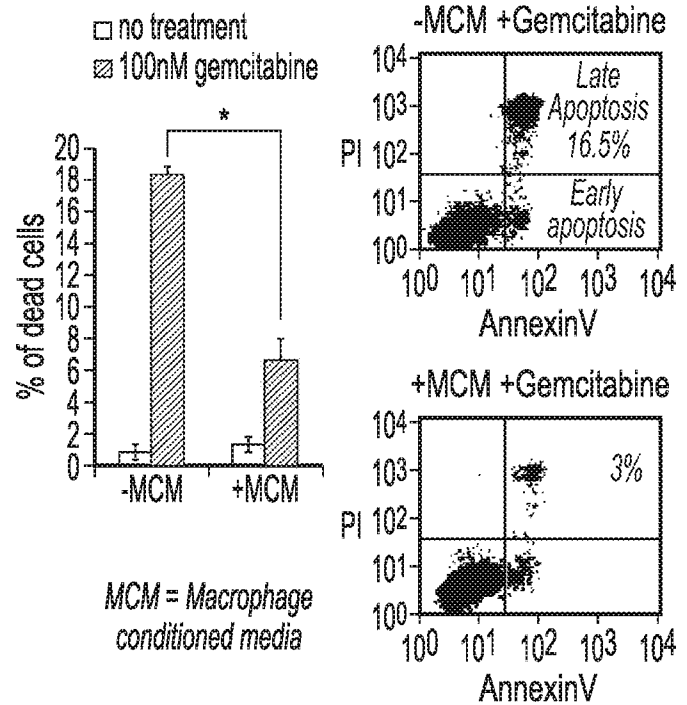
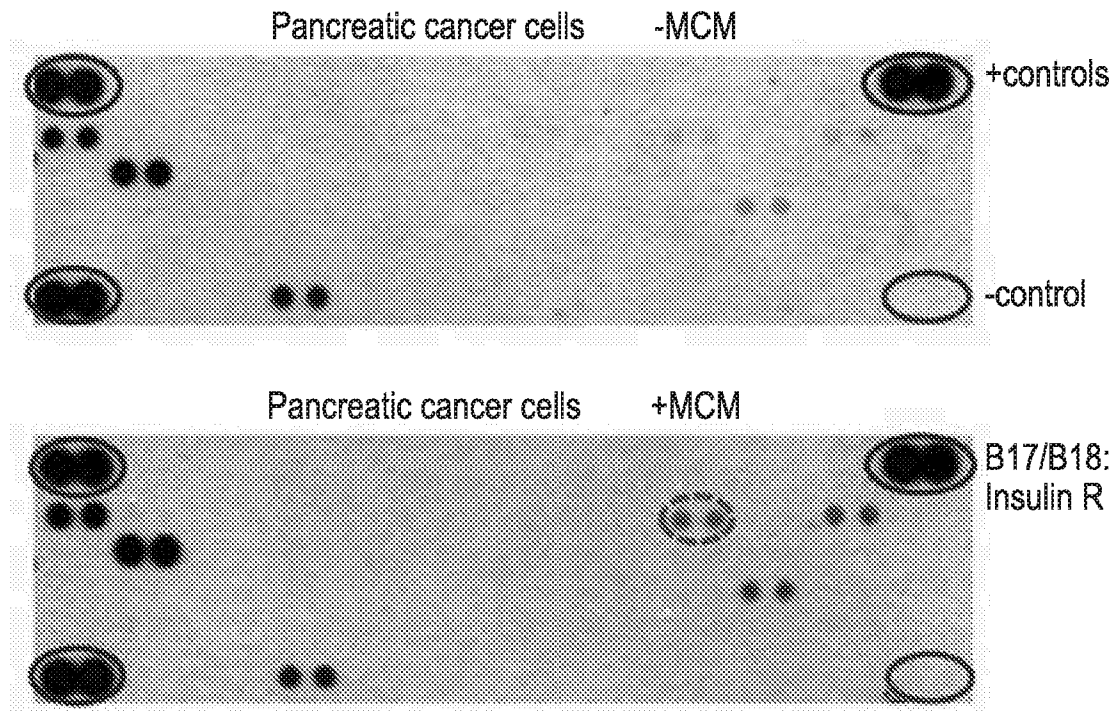


FIG. 6



Note: B19/B20 = IGF-1R (no differences observed)

FIG. 7

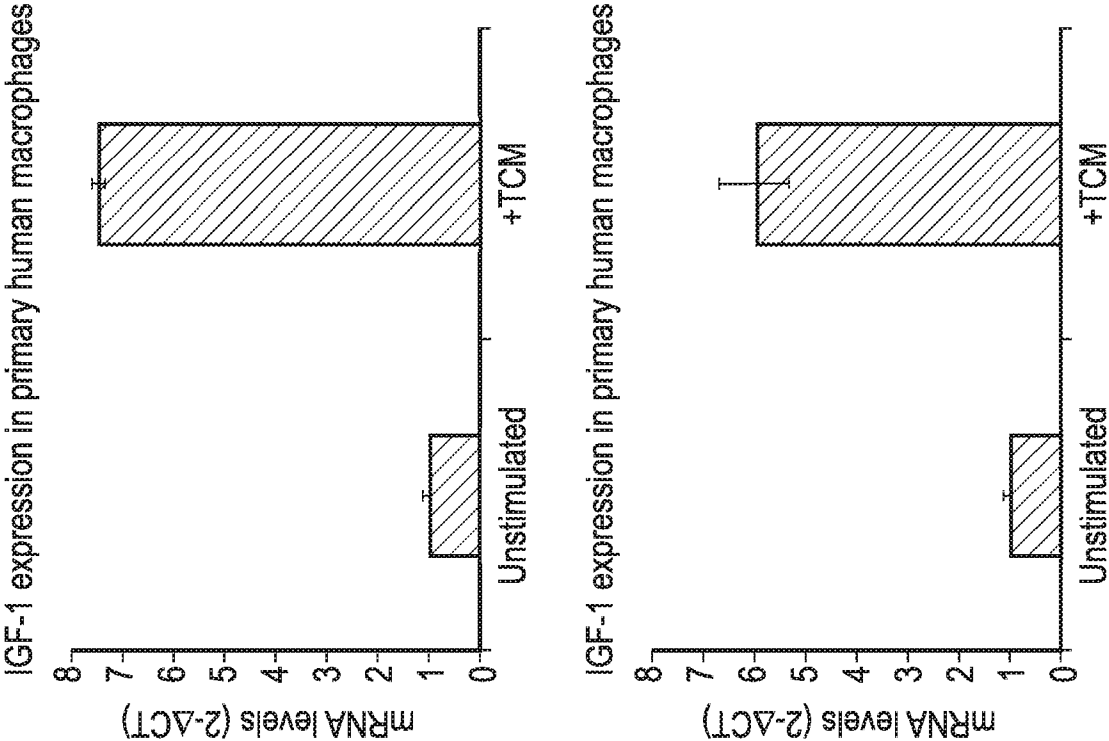


FIG. 9

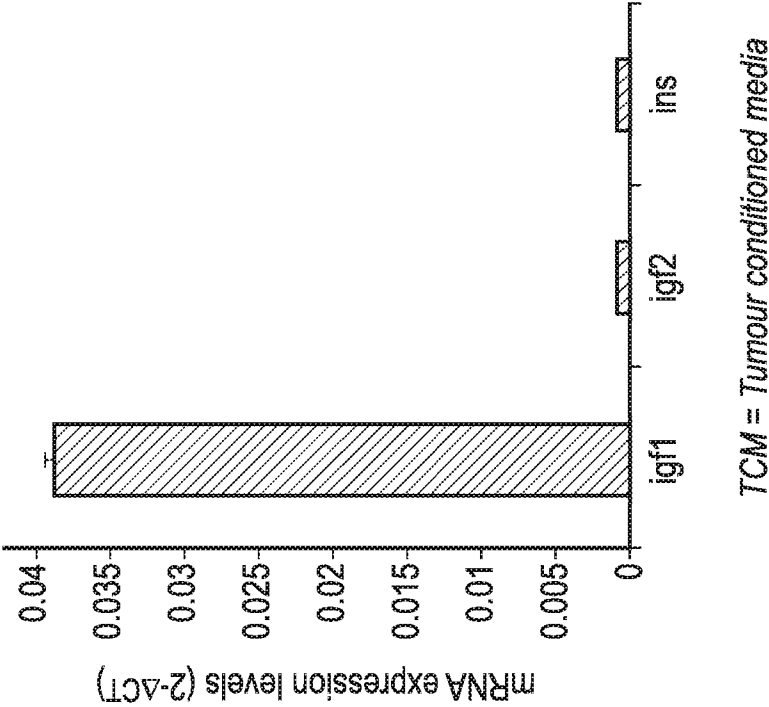


FIG. 8

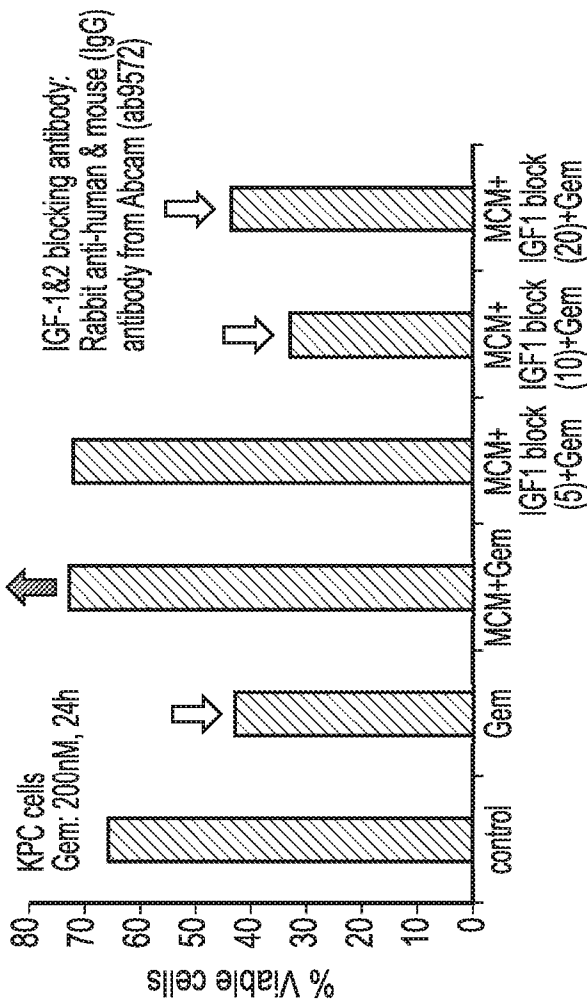


FIG. 11

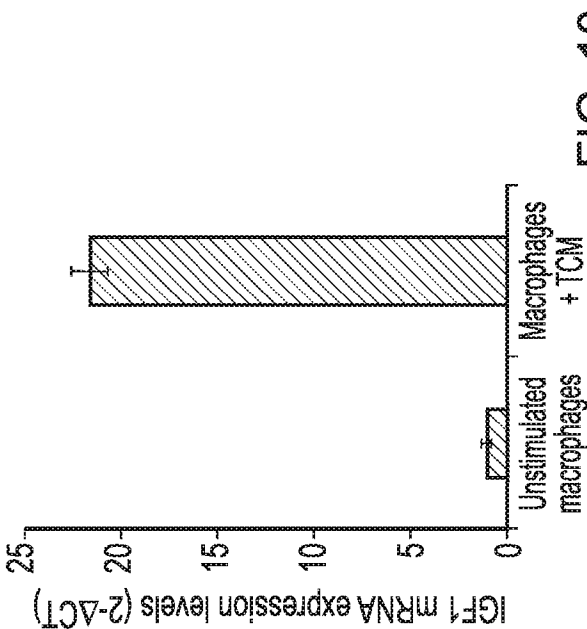


FIG. 10

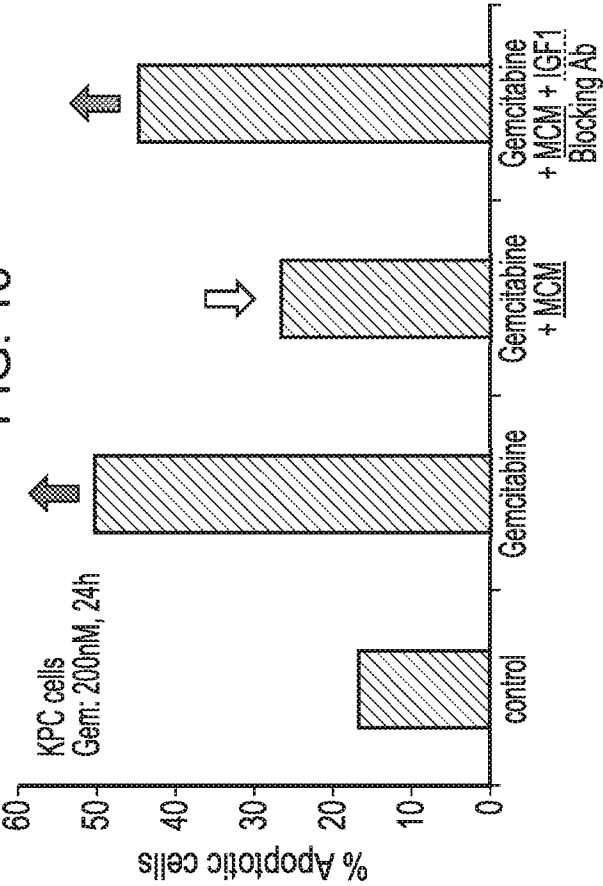


FIG. 12

In the absence of Macrophages, IGF blocking antibody has no effect on Tumour cell's response to Gemcitabine

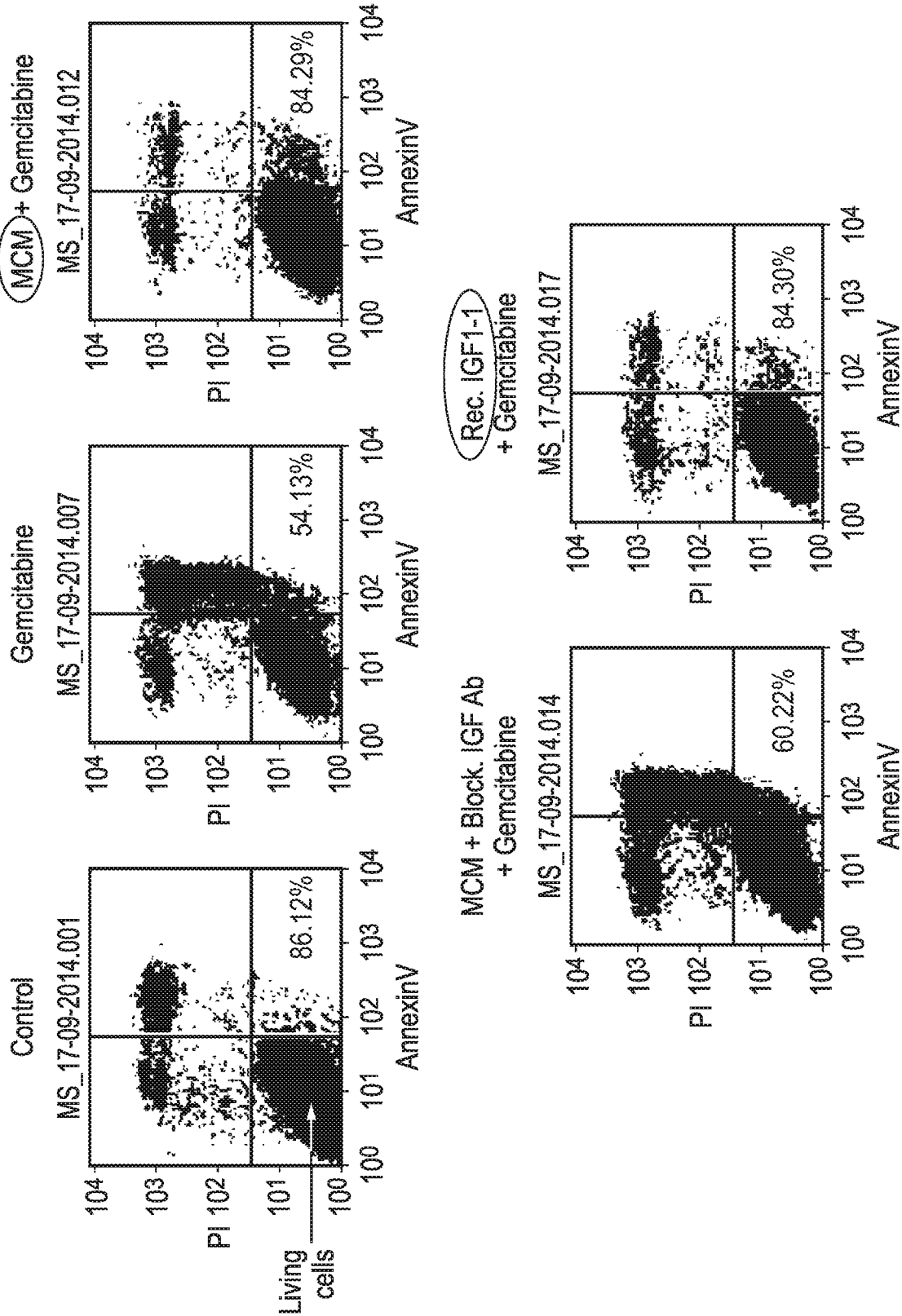


FIG. 13

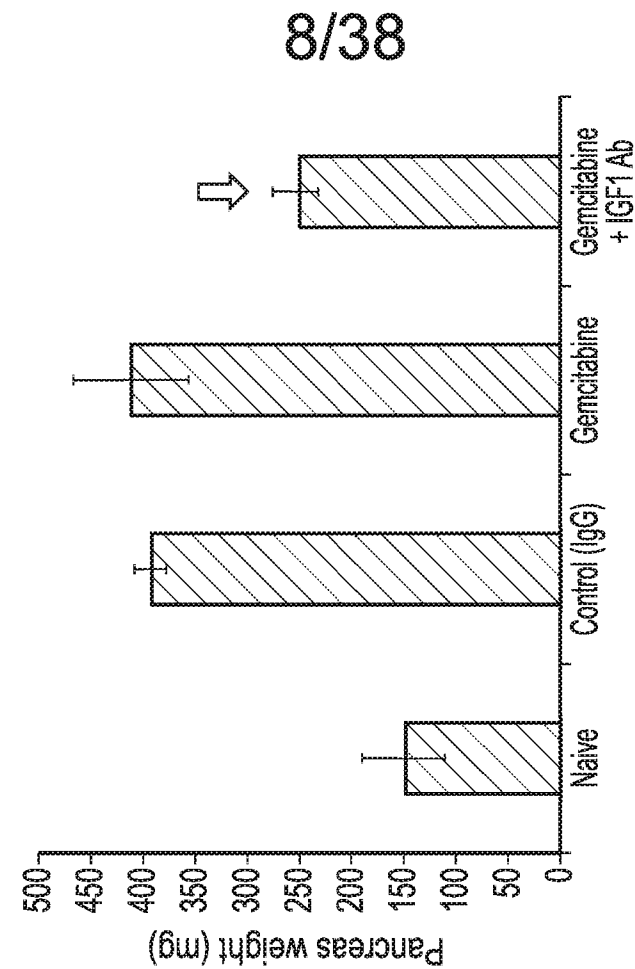


FIG. 15

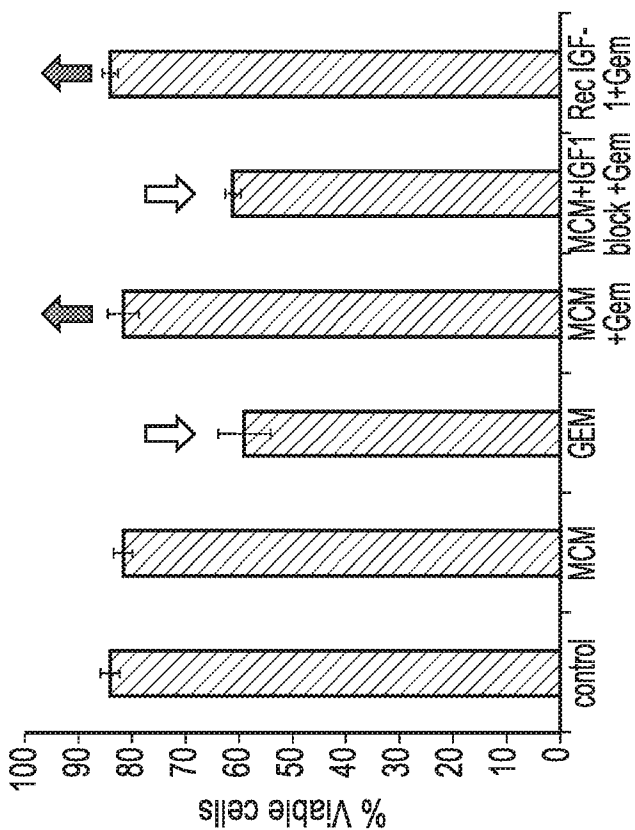


FIG. 14

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KPC tumour cells:

- MCM

+ MCM

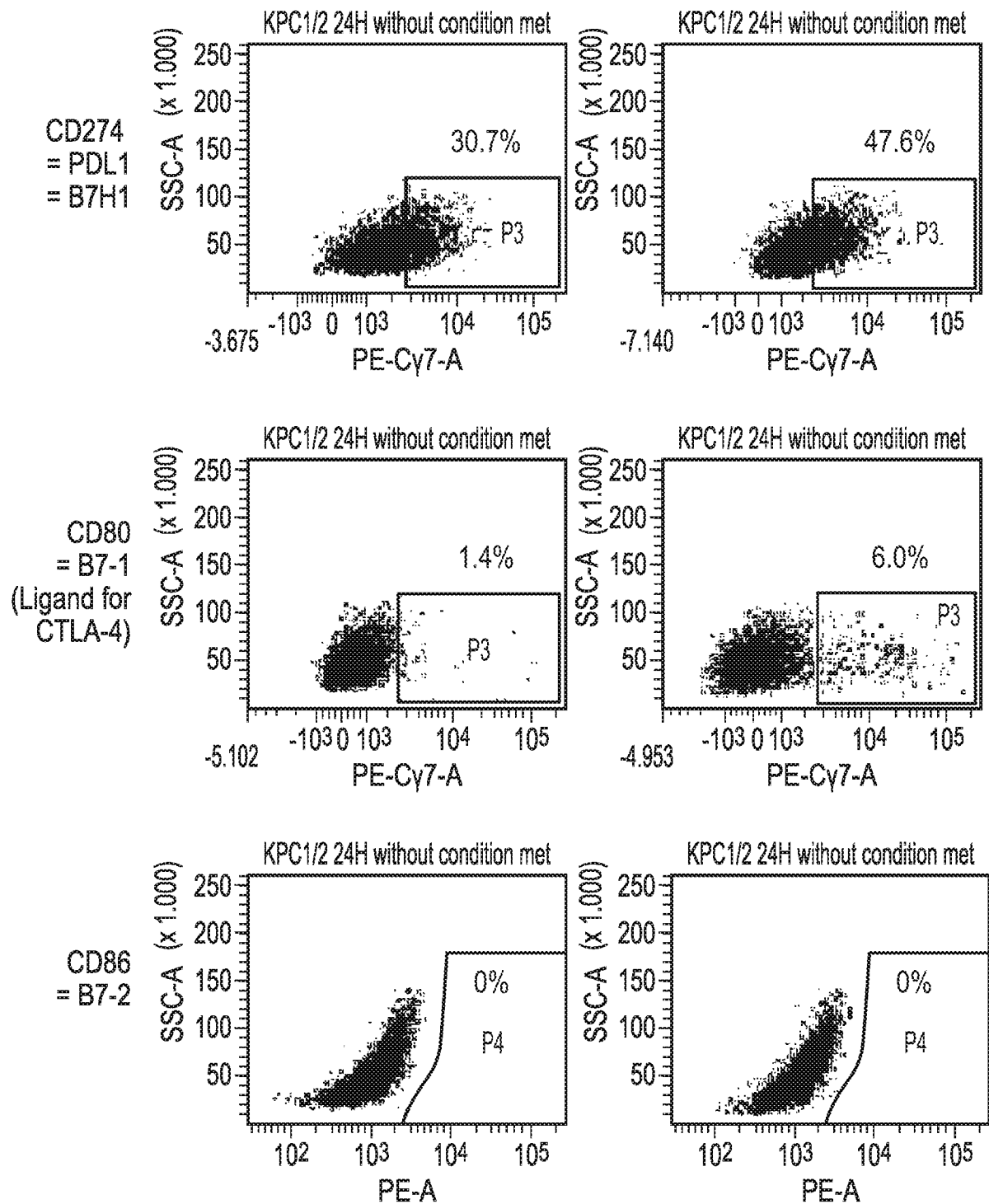


FIG. 16

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Human PDA

PDL1 staining

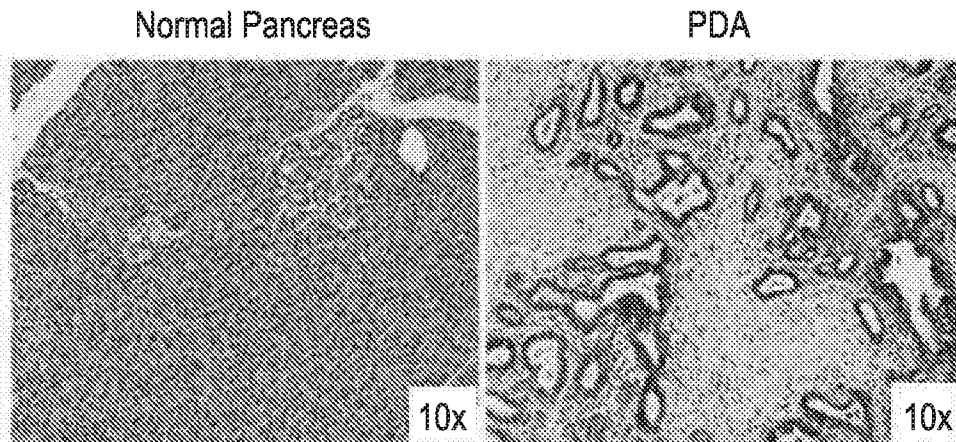


FIG. 17

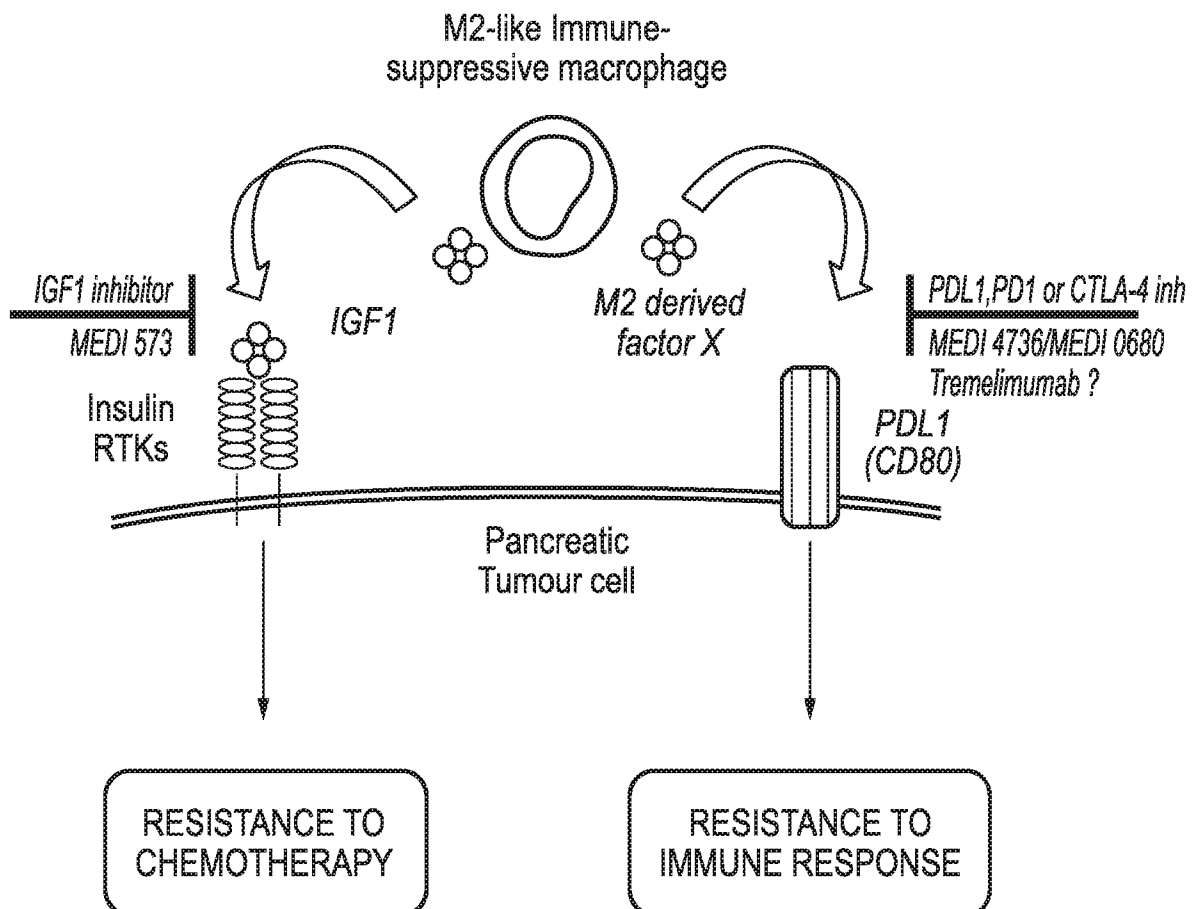
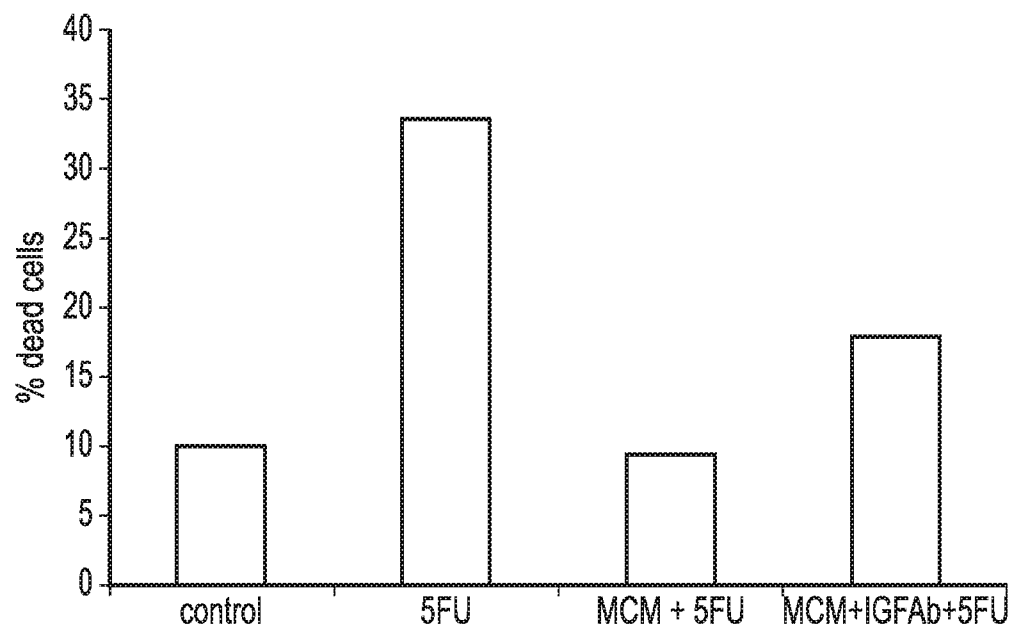


FIG. 18

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KPC pancreatic cancer cells treated with 5-FU (25 μ M for 48 hours)
IGF blocking antibody abcam 9572 (10 μ g/ml)

FIG. 19

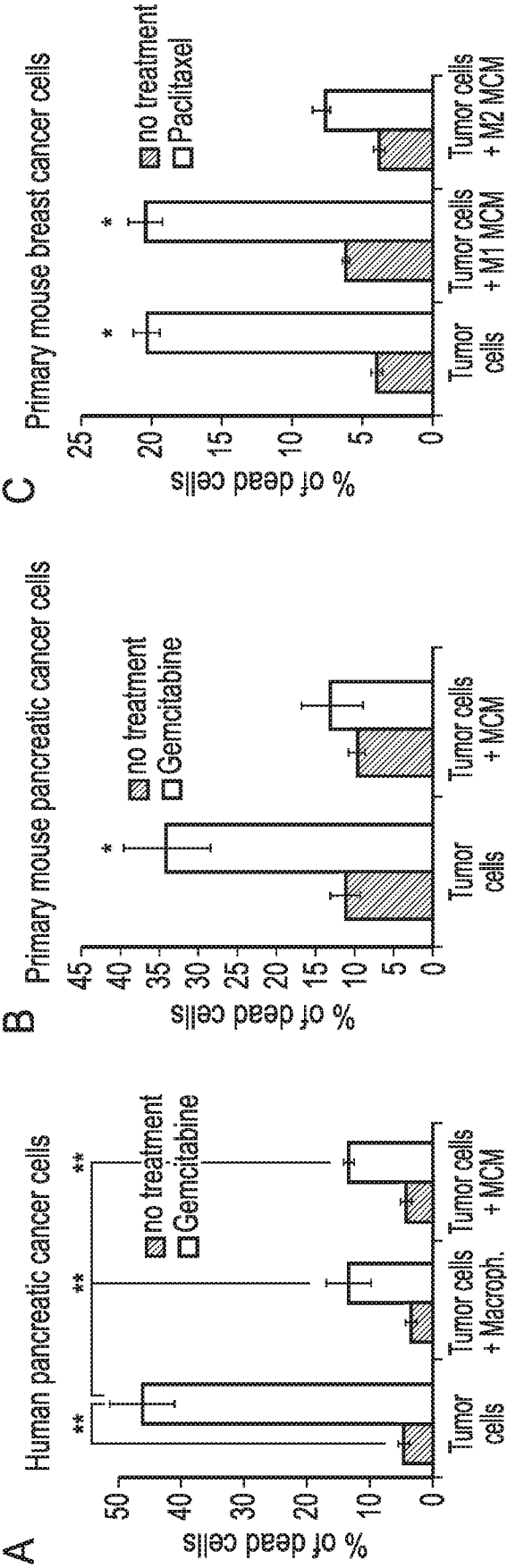
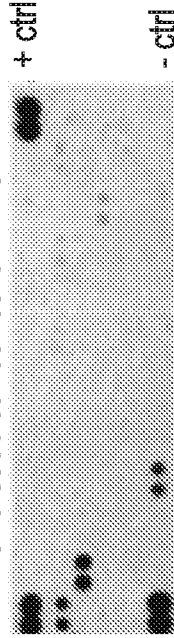


FIG. 20

Phospho-RTK antibody array

Pancreatic cancer cells - MCM



Pancreatic cancer cells + MCM

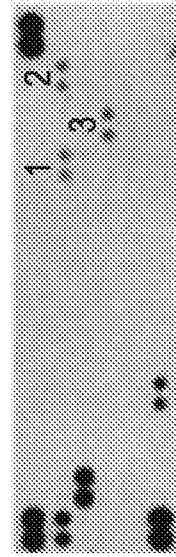


FIG. 21

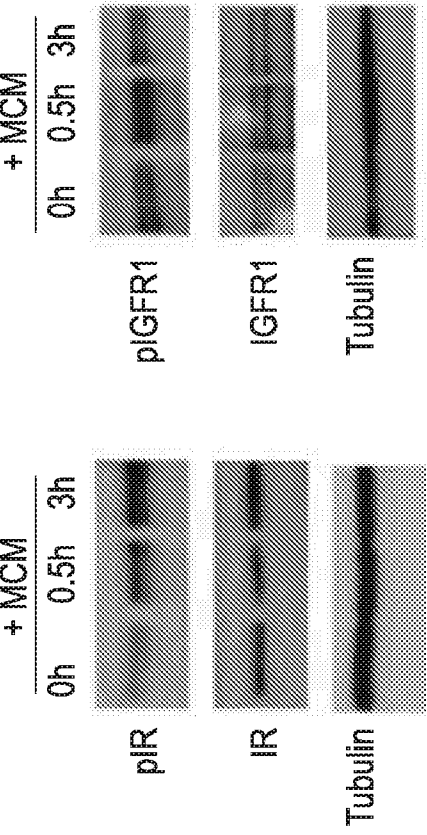


FIG. 22

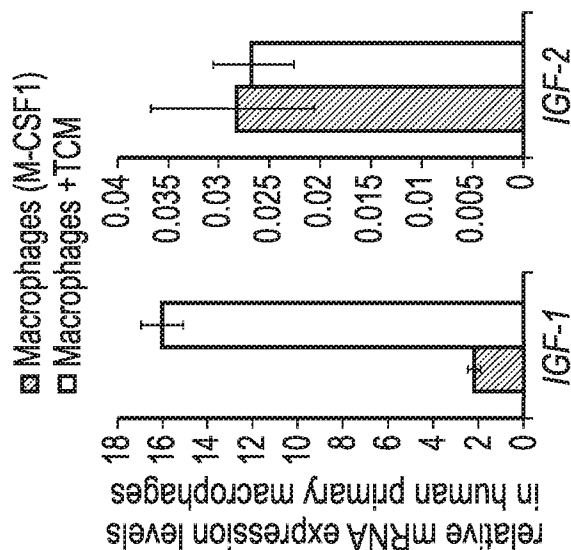


FIG. 24

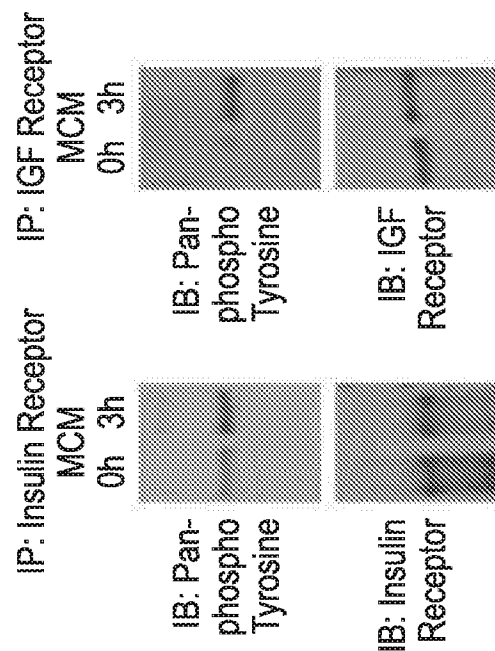


FIG. 23

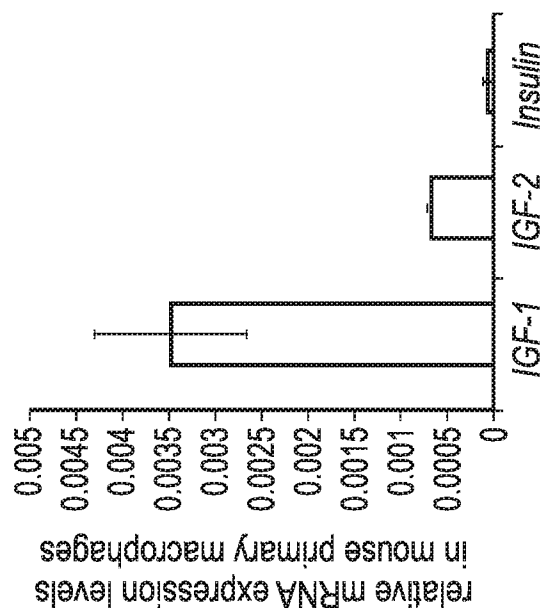


FIG. 25

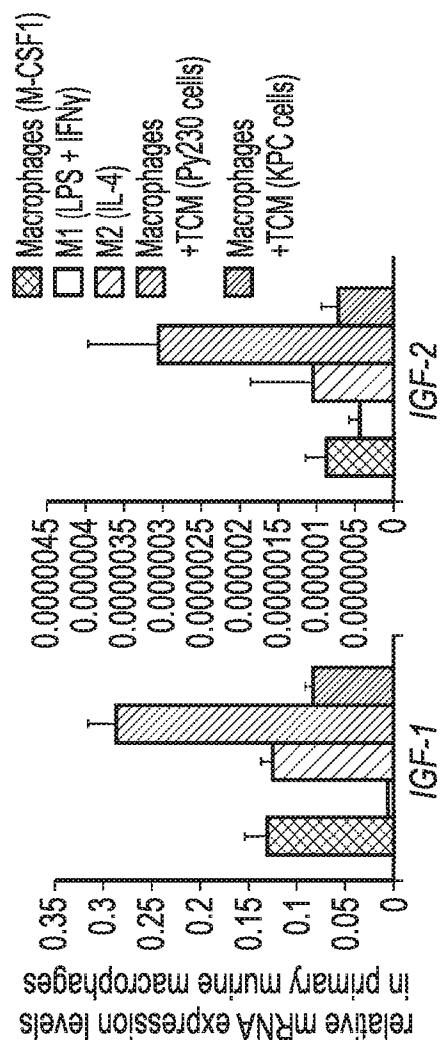
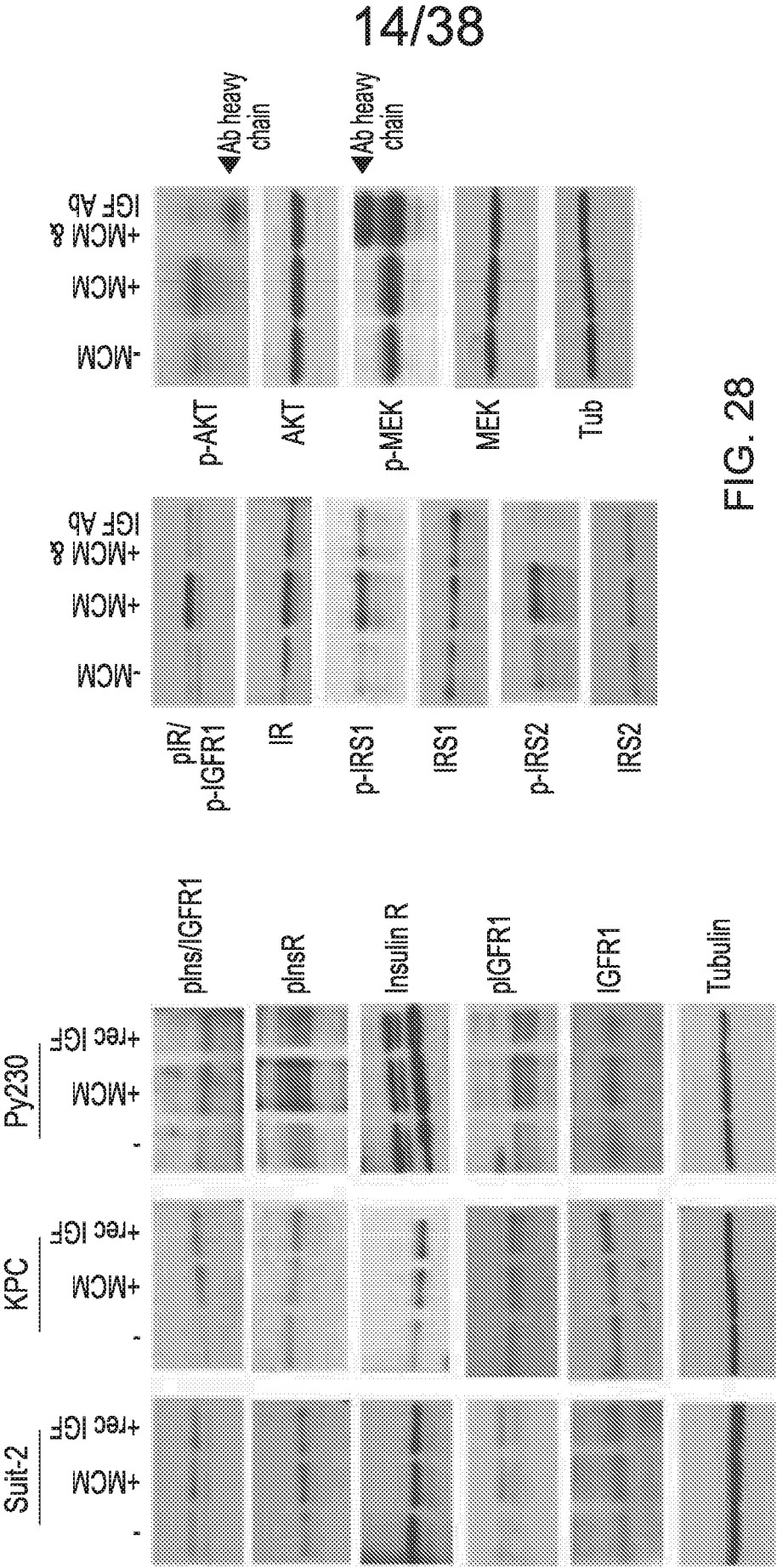
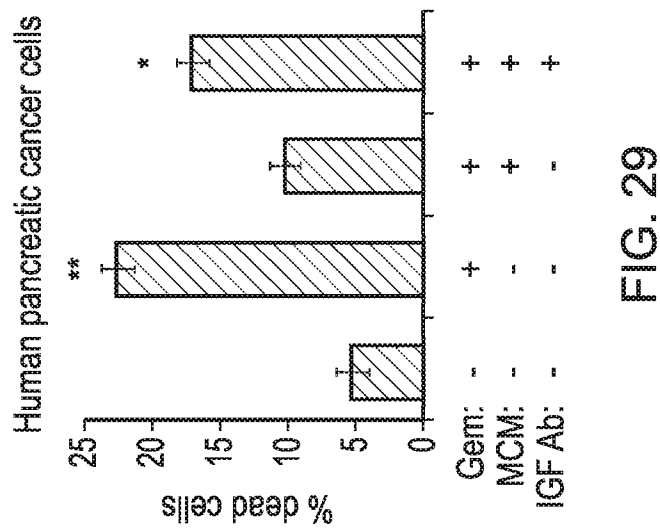
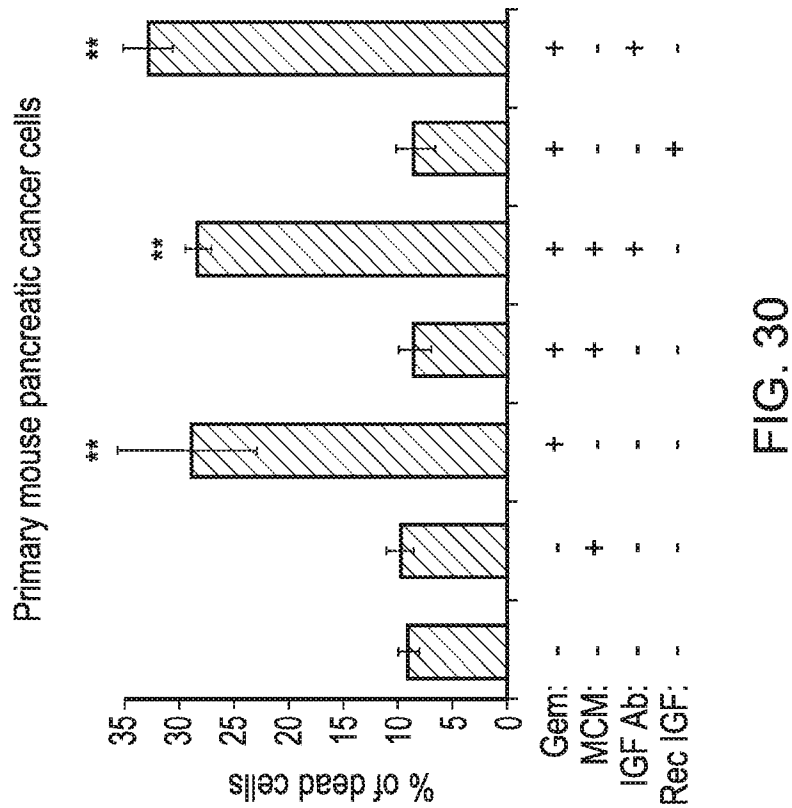


FIG. 26



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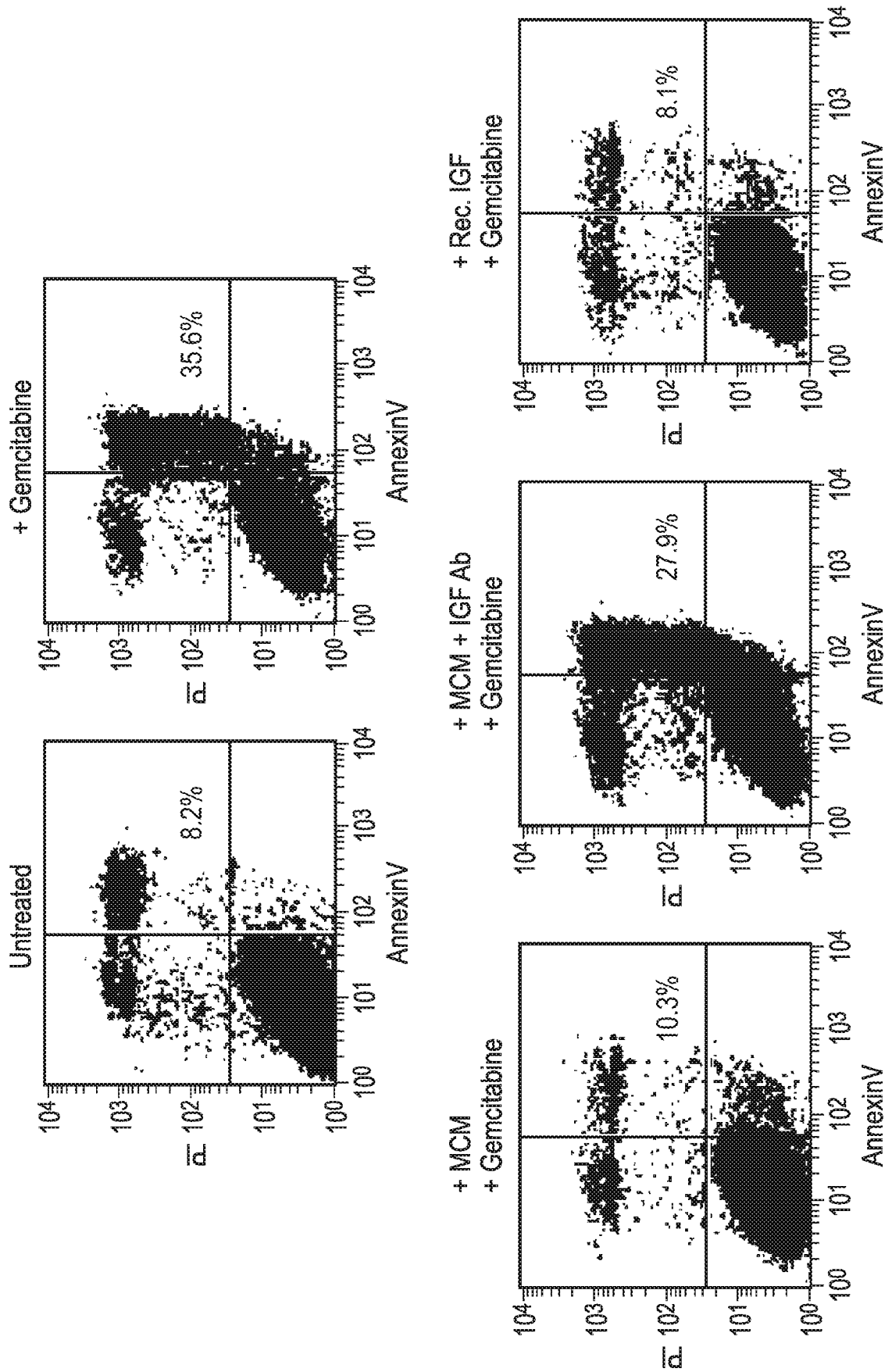


FIG. 31

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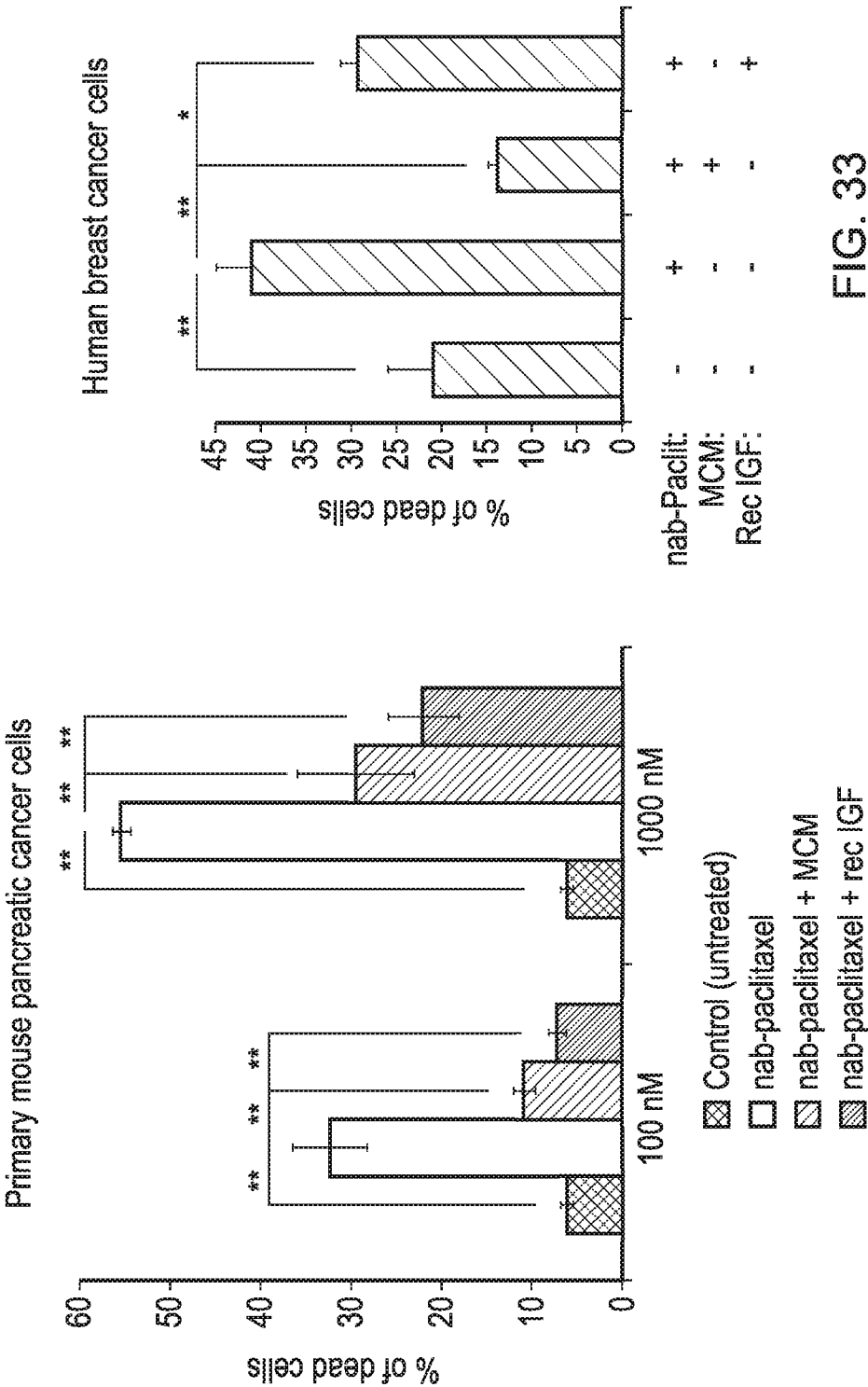


FIG. 32

FIG. 33

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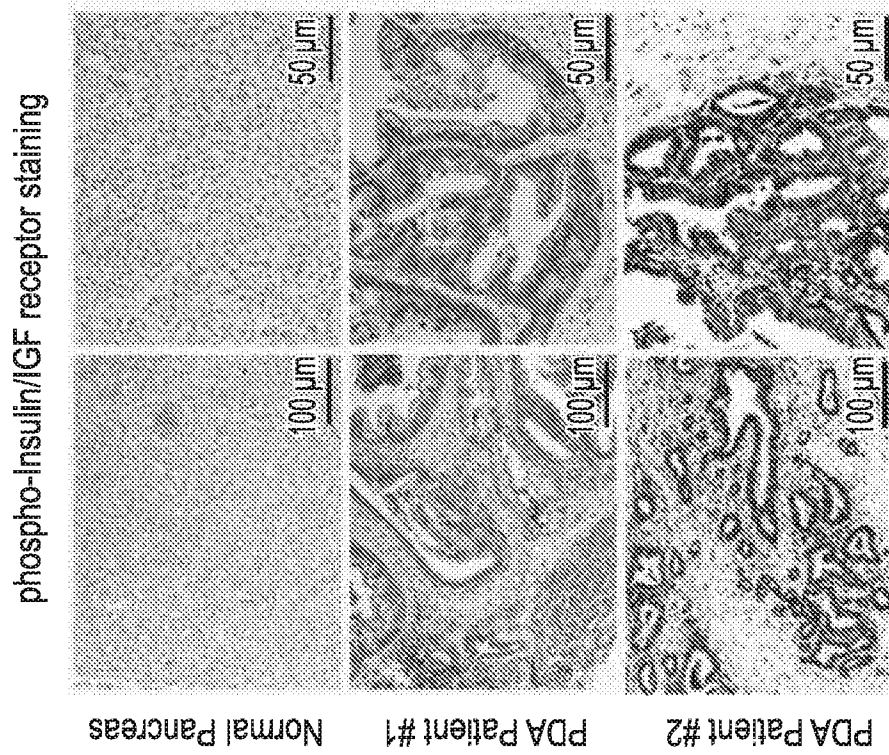


FIG. 35

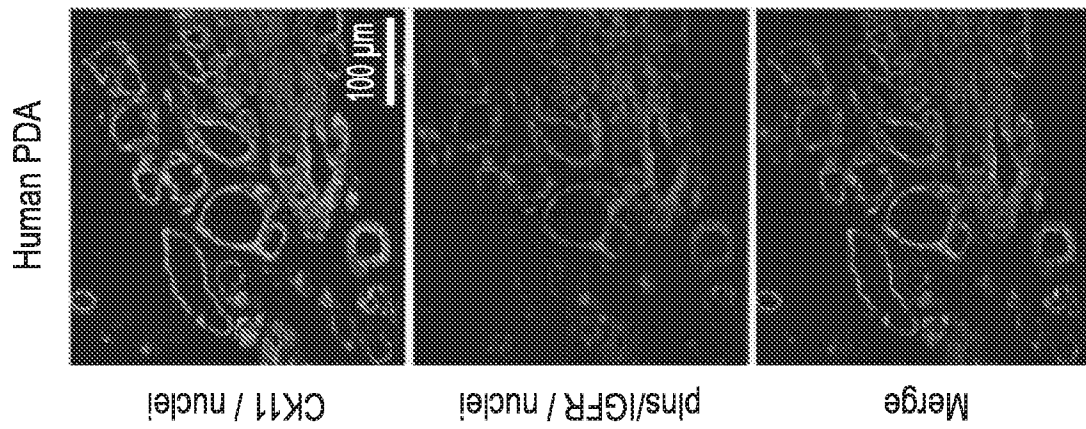
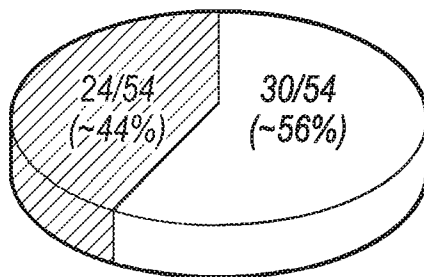


FIG. 34

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Phospho - Insulin/IGF receptor
expression in pancreatic
cancer biopsies



○ p-Insulin/IGFR + tumors
▨ p-Insulin/IGFR - tumors

FIG. 36

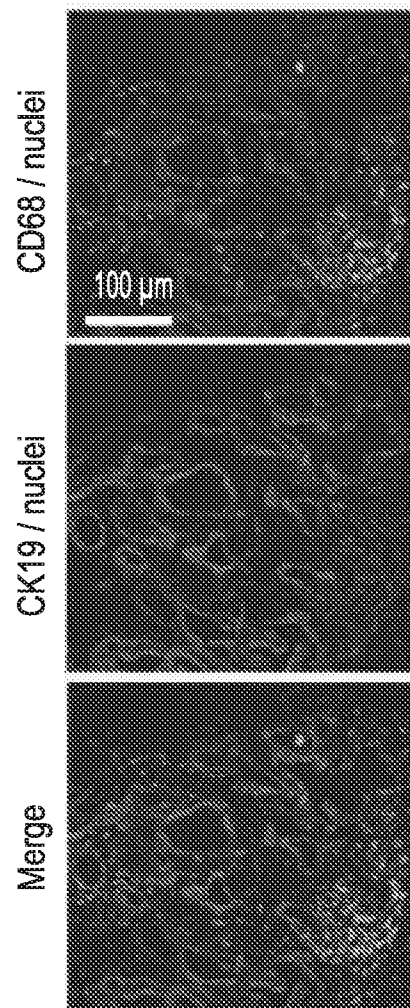


FIG. 37

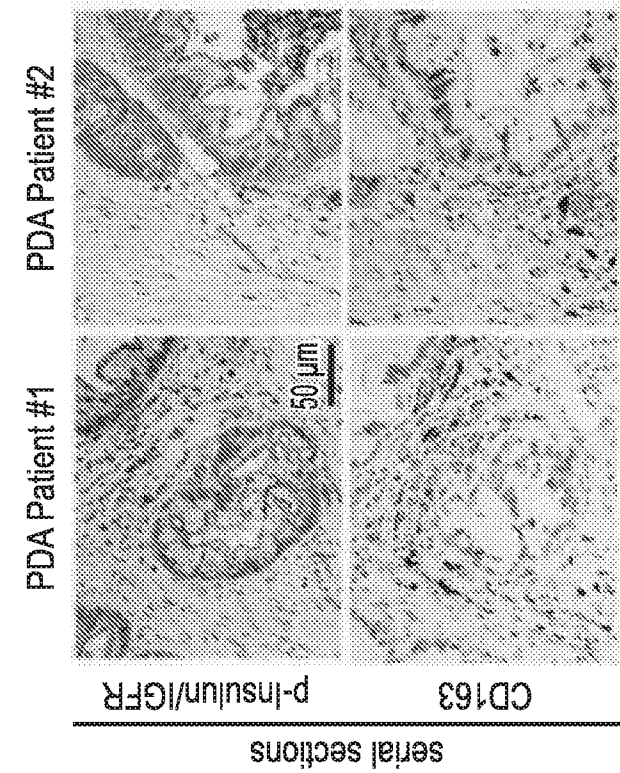


FIG. 39

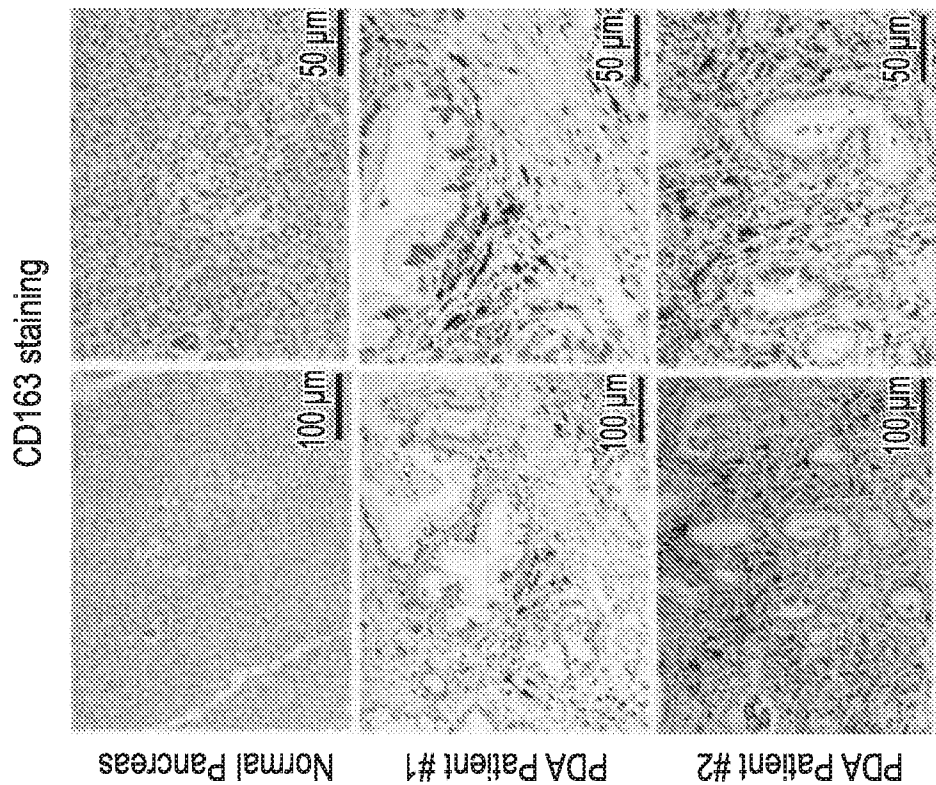


FIG. 38

Positive correlation between p-Insulin/IGFR & CD163 +/- tumors and tumor stage

	p-Ins/IGFR/CD163 +/- tumors	Other tumors	Total
Tumor stage I or II	9	0	9
Tumor stage III or IV	24	19	43
Total	33	19	52

Fisher Exact test; p = 0.018

FIG. 41

Positive correlation between p-Insulin/IGFR expression and CD163+ intra-tumoral macrophage numbers

	p-Insulin/IGFR - tumors	p-Insulin/IGFR + tumors	Total
CD163+ None/Low	18	10	28
CD163+ Moderate/High	6	20	26
Total	24	30	54

Chi-square = 9.272; p = 0.002

FIG. 40

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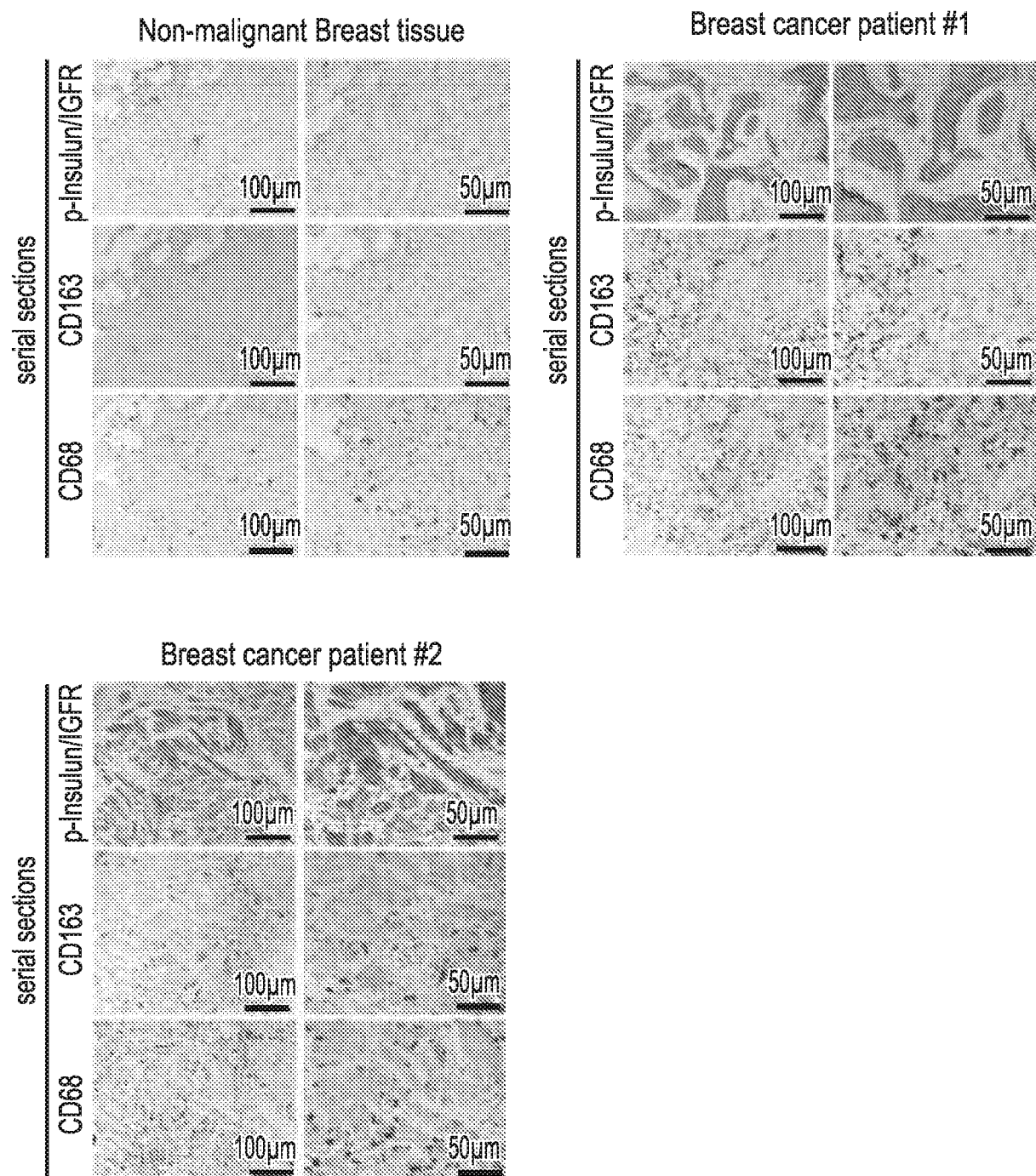


FIG. 42

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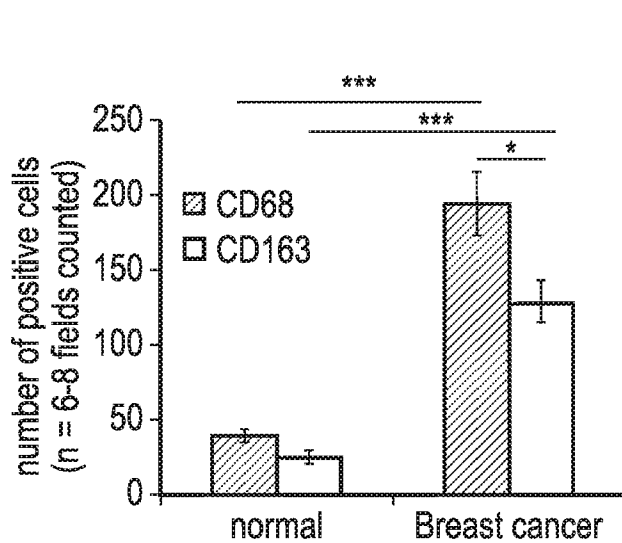


FIG. 43

Phospho - Insulin/IGF receptor
expression in breast
cancer biopsies

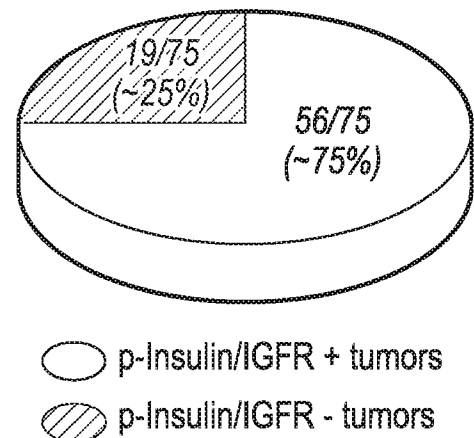


FIG. 44

Positive correlation between p-Insulin/IGFR expression
and CD163+ intra-tumoral macrophage numbers

	p-Insulin/IGFR - tumors	p-Insulin/IGFR + tumors	Total
CD163+ None/Low	12	20	32
CD163+ Moderate/High	7	36	43
Total	19	56	75

Chi-square = 4.37; p = 0.04

FIG. 45

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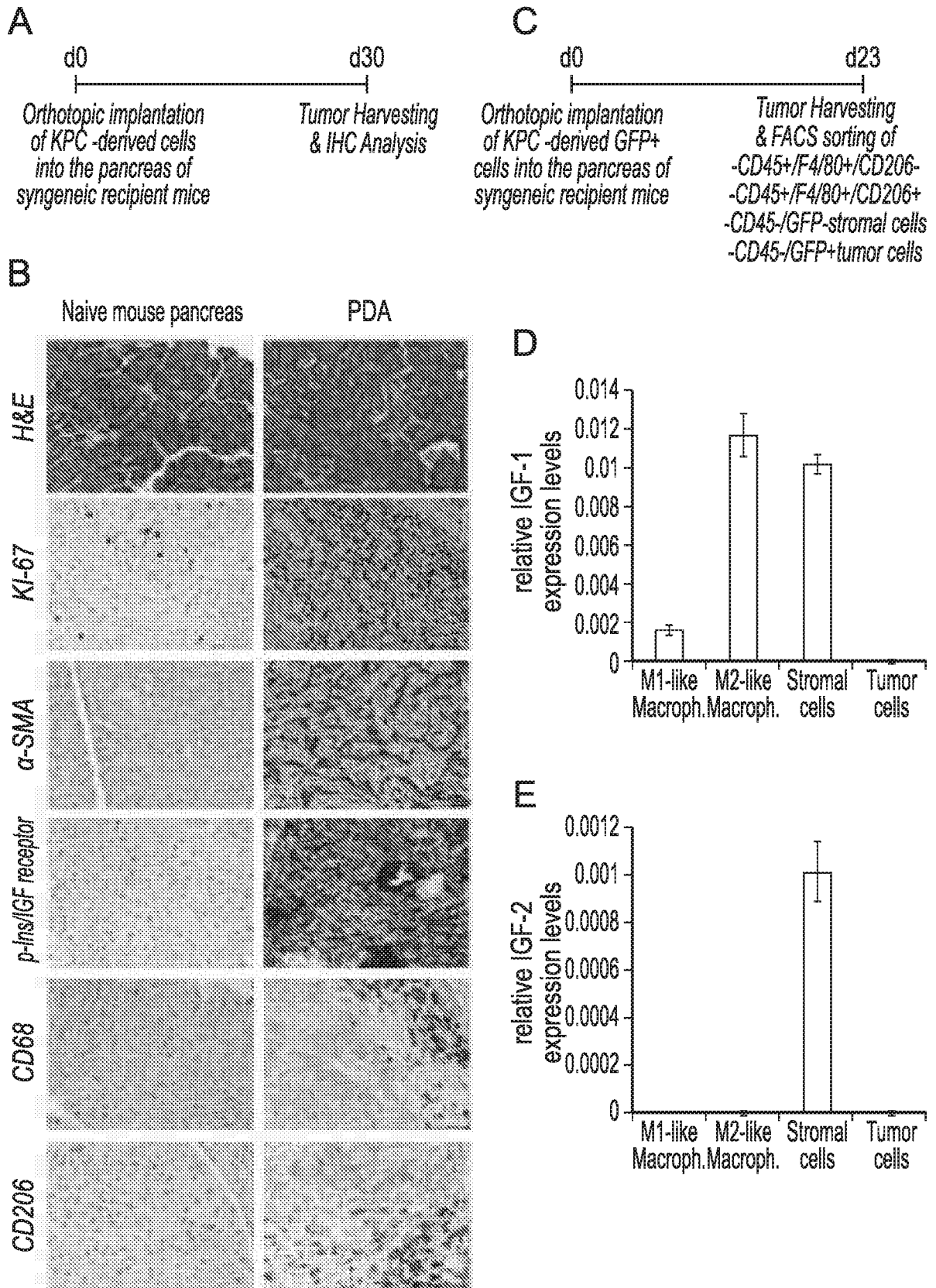


FIG. 46

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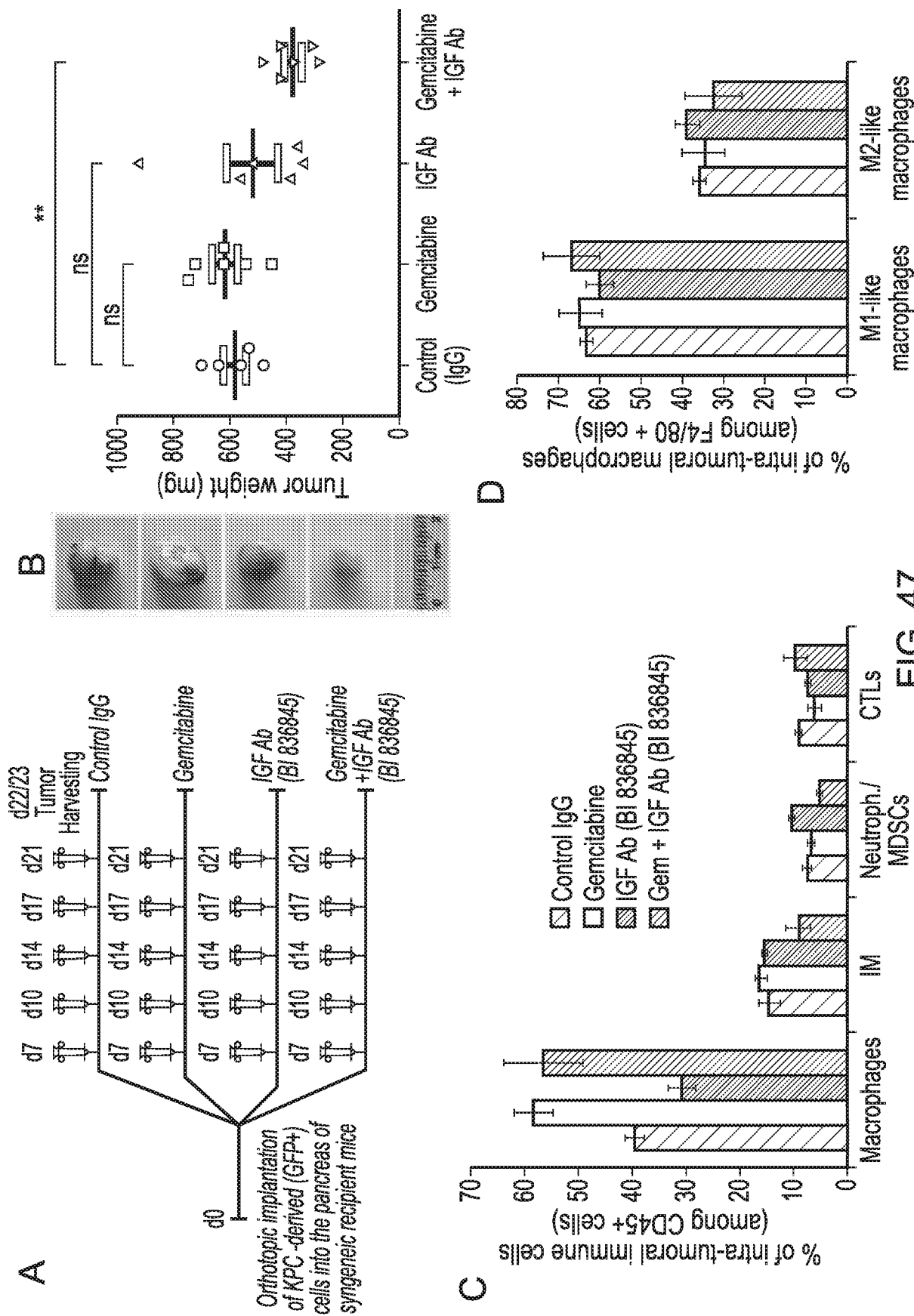


FIG. 47

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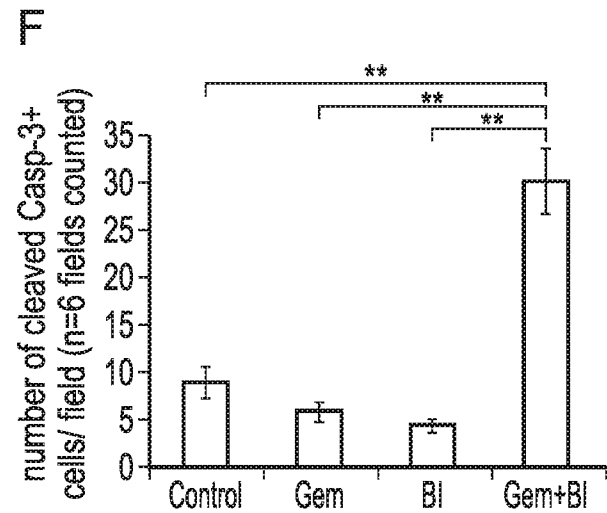
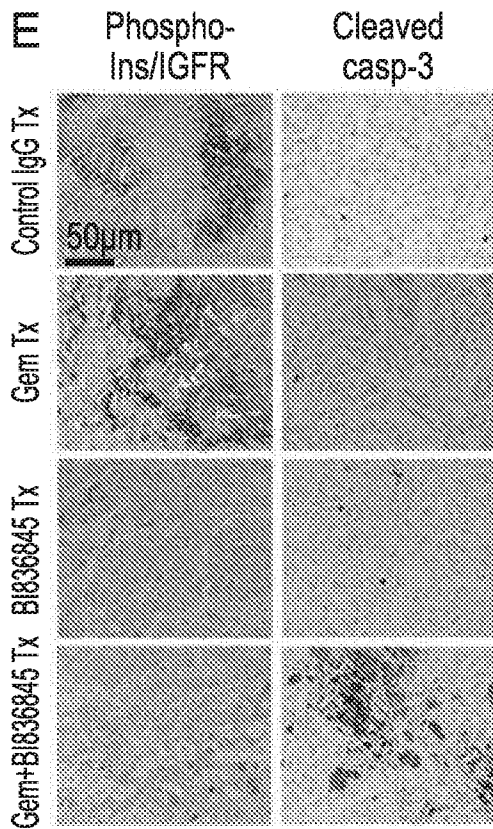


FIG. 47 (Continued)

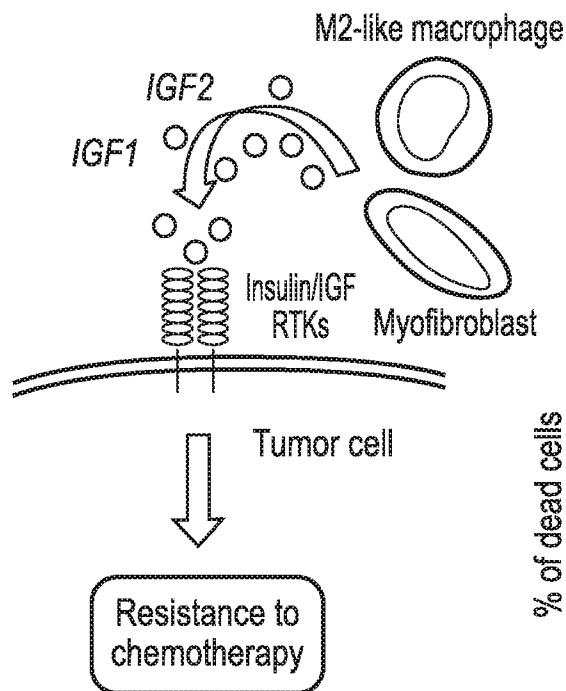


FIG. 48

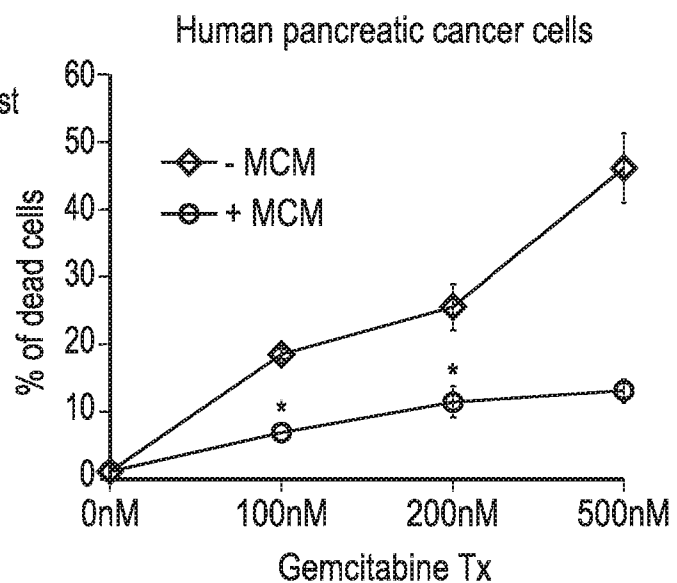


FIG. 49

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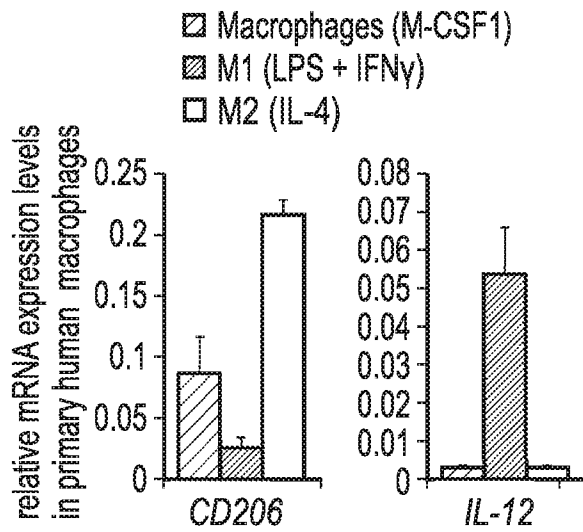


FIG. 50

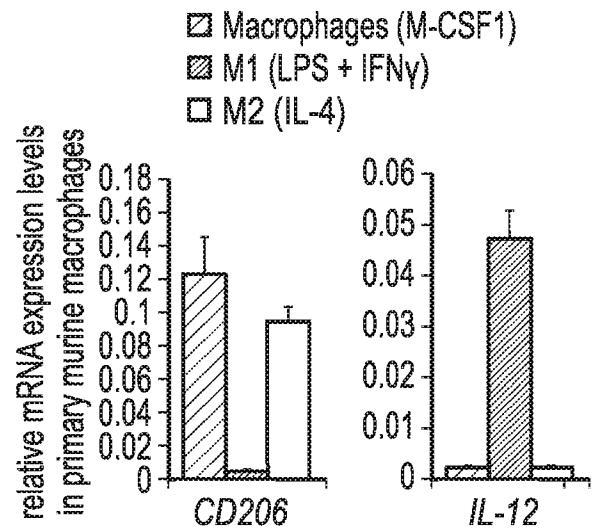


FIG. 51

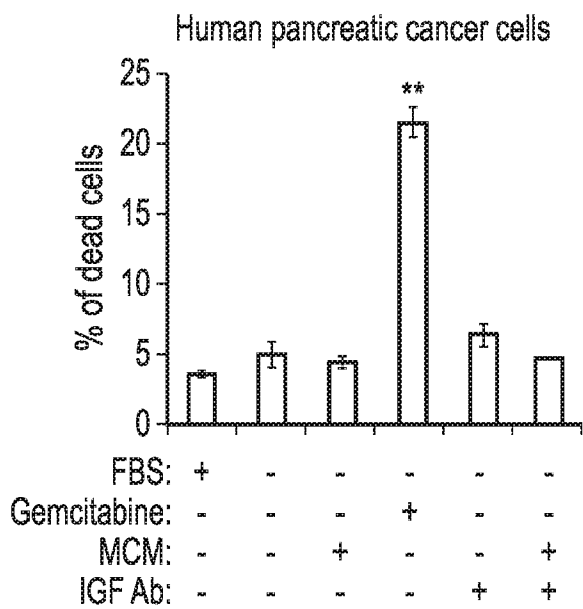


FIG. 52

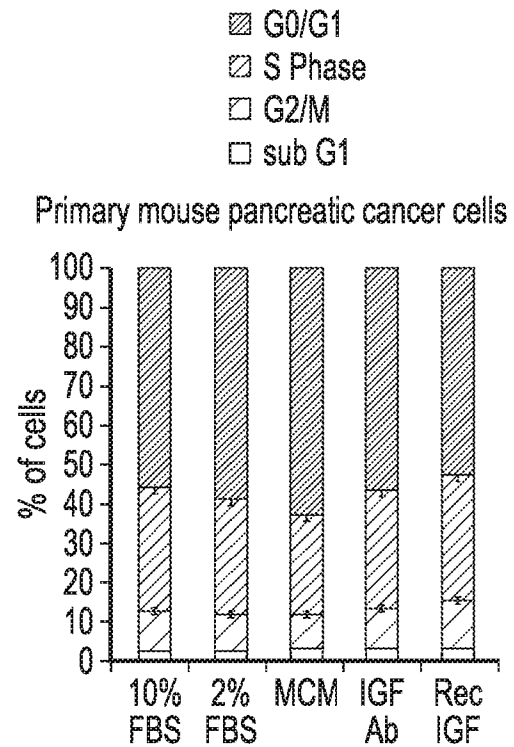


FIG. 53

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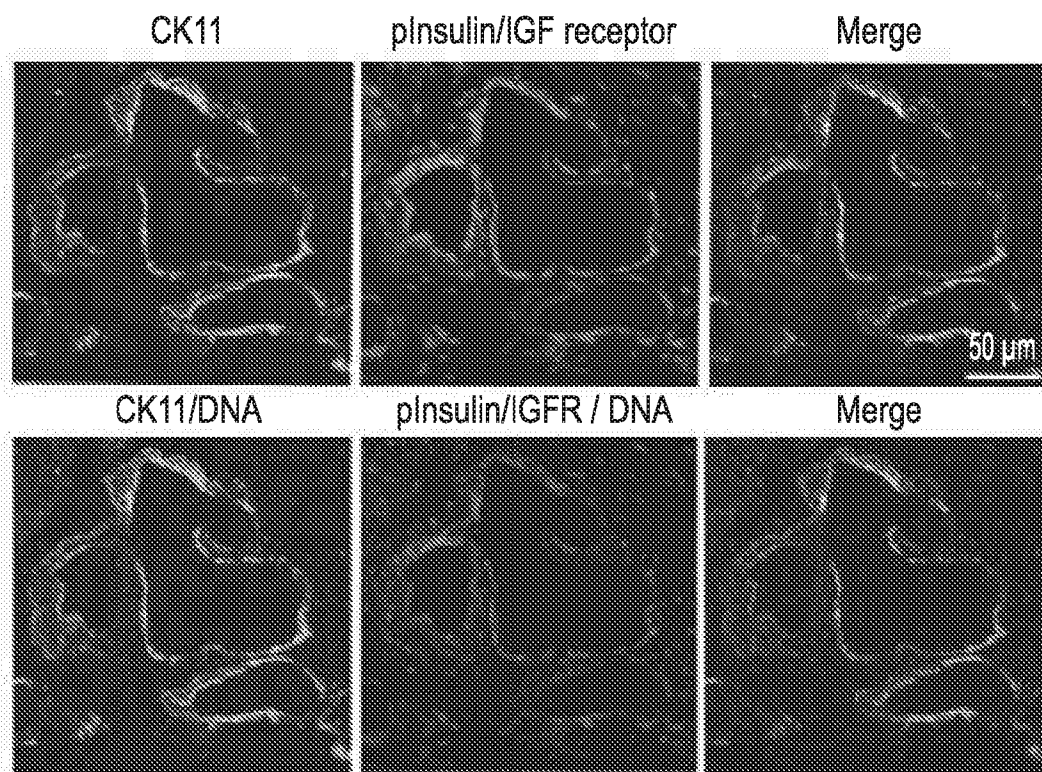
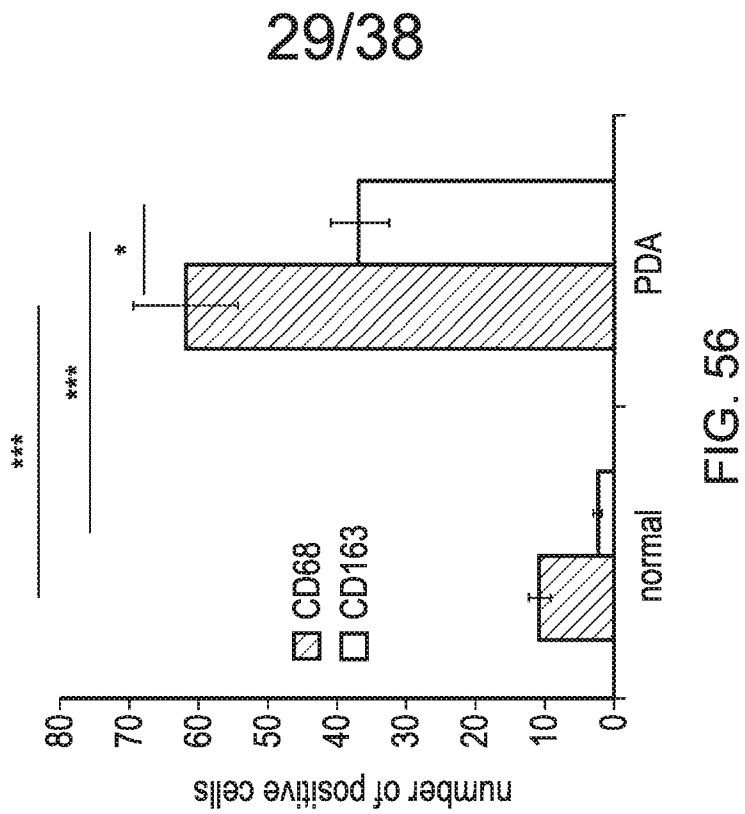
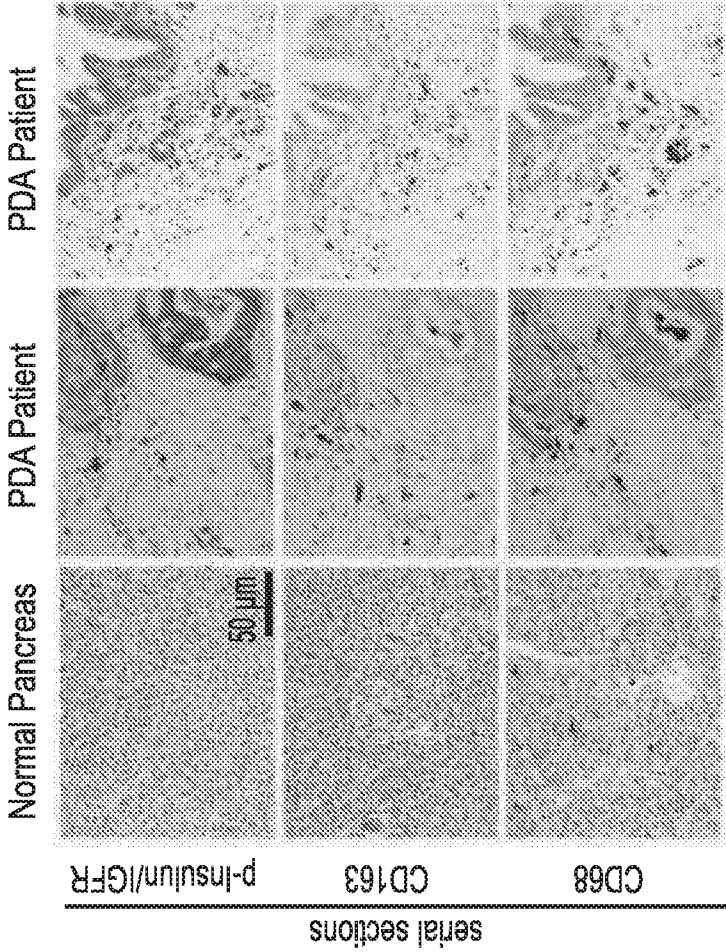


FIG. 54



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Clinical information from pancreatic cancer tissue samples

Number	Diagnosis	T stage	N stage	M stage	Gender	Age at surgery
1	PDA	2	1	x	Male	63
2	PDA	2	0	x	Female	54
3	PDA	3	1	x	Female	80
4	PDA	3	2	x	Male	56
5	PDA	1	1	x	Male	70
6	PDA	3	1	x	Male	68
7	PDA	1	0	x	Male	65
8	PDA	4	1	x	Female	60
9	PDA	3	1	x	Female	52
10	PDA	3	2	x	Male	59
11	PDA	1	0	x	Female	64
12	PDA	3	1	x	Male	58
13	PDA	3	1	x	Male	70
14	PDA	3	1	x	Female	57
15	PDA	Unrecorded	Unrecorded	Unrecorded	Female	69
16	PDA	3	1	x	Male	60
17	PDA	3	2	x	Male	55
18	PDA	3	1b	x	Male	52
19	PDA	3	1b	x	Male	71
20	PDA	3	1	x	Male	56
21	PDA	2	1b	x	Male	68
22	PDA	3	1b	x	Male	73
23	PDA	3	1b	x	Female	61
24	PDA	3	1	x	Female	76
25	PDA	4	1b	x	Male	68
26	PDA	3	1a	x	Female	74

 pInsulin/IGFR -/CD163-
 pInsulin/IGFR -/CD163+
 pInsulin/IGFR +/CD163-
 pInsulin/IGFR +/CD163+

FIG. 57

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FIG. 57
(Continued)

27	PDA	2	0	x	Male	73
28	PDA	3	1b	x	Female	75
29	PDA	1	1a	x	Male	57
30	PDA	3	0	x	Male	73
31	PDA	3	1b	x	Female	63
32	PDA	2	1b	x	Male	77
33	PDA	3	1b	x	Male	71
34	PDA	3	1	x	Female	78
35	PDA	3	1b	x	Female	67
36	PDA	3	1b	x	Female	59
37	PDA	3	1	x	Male	64
38	PDA	3	0	x	Male	71
39	PDA	3	0	x	Female	70
40	PDA	3	1	x	Female	62
41	PDA	3	1a	x	Male	77
42	PDA	3	1b	x	Female	65
43	PDA	3	1	x	Male	72
44	PDA	3	1b	x	Male	79
45	PDA	3	1b	x	Female	79
46	PDA	3	1b	x	Female	61
47	PDA	3	1	x	Female	73
48	PDA	3	0	x	Female	64
49	PDA	3	1b	x	Female	58
50	PDA	3	1b	x	Female	61
51	PDA	3	1	x	unrecorded	unrecorded
52	PDA	unrecorded	unrecorded	unrecorded	unrecorded	unrecorded
53	PDA	3	1	unrecorded	unrecorded	unrecorded
54	pda	3	0	unrecorded	unrecorded	unrecorded

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Clinical information from breast cancer tissue samples

Number	T stage	N stage	M stage	Tumor stage	αSMA+fibroblasts
1	1c	1bi	x	2A	Yes
2	2	0	x	2A	Yes
3	2	1bii	x	2B	Yes
4	1c	1bi	x	2A	Yes
5	2	0	x	2A	Yes
6	2	0	x	2A	Yes
7	2	1bi	x	2B	Yes
8	3	0	x	2B	Yes
9	1c	1bi	x	2A	Yes
10	3	1bi	x	3A	Yes
11	2	0	x	2A	Yes
12	1c	0	x	1	Yes
13	1c	1bi	x	2A	Yes
14	1c	0	x	1	Yes
15	1b	0	x	1	no
16	1c	1bi	x	2A	Yes
17	2	1bi	x	2B	Yes
18	1c	x	x	Cannot stage	Yes
19	2	1bii	x	2B	Yes
20	1c	0	x	1	Yes
21	2	1bi	x	2B	Yes
22	3	1bii	x	3A	Yes
23	1c	x	x	Cannot stage	Yes
24	1c	0	x	1	Yes
25	2	1bi	x	2B	Yes
26	2	0	x	2A	Yes
27	1c	0	x	1	Yes
28	1c	0	x	1	Yes
29	2	0	x	2A	no
30					Yes
31	4	1bii	x	3B	Yes
32	3	1bi	x	3A	Yes
33	1c	0	x	1	Yes
34	3	1bi	x	3A	Yes
35	2	0	x	2A	Yes
36	2	0	x	2A	Yes
37	2	1bii	x	2B	Yes
38	2	1bi	x	2B	Yes

FIG. 58

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39	1c	0	x	1	Yes
40	1c	0	x	1	Yes
41	3	1biii	x	3A	Yes
42	2	1bi	x	2B	Yes
43	2	1bii	x	2B	Yes
44	1c	0	x	1	Yes
45	1c	0	x	1	Yes
46	1c	0	x	1	Yes
47	2	0	x	2A	Yes
48	1b	0	x	1	Yes
49					Yes
50	Recurrent cancer therefore not staged			Not staged	Yes
51	1c	1bii	x	2A	Yes
52	2	1bi	x	2B	Yes
53	2	0	x	2A	Yes
54	1c	0	x	1	Yes
55	2	1a	x	2B	Yes
56	2	0	x	2A	Yes
57	1c	1biii	x	2A	Yes
58	2	x	x	Cannot stage	Yes
59	1c	0	x	1	Yes
60	2	1bii	x	2B	Yes
61	2	1biii	x	2B	Yes
62	2	1bi	x	2B	Yes
63	3	1biii	x	3A	Yes
64	2	0	x	2A	Yes
65					Yes
66	3	1bii	x	3A	Yes
67	2	1biii	x	2B	Yes
68	2	0	x	2A	Yes
69	1c	0	x	1	Yes
70	3	0	x	2B	Yes
71	2	0	x	2A	Yes
72	2	0	x	2A	Yes
73	1c	0	x	1	Yes
74	1c	0	x	1	Yes
75					Yes

 plnsulin/IGFR -/CD163-

 plnsulin/IGFR +/CD163-

 plnsulin/IGFR -/CD163+

 plnsulin/IGFR +/CD163+

FIG. 58 (Continued)

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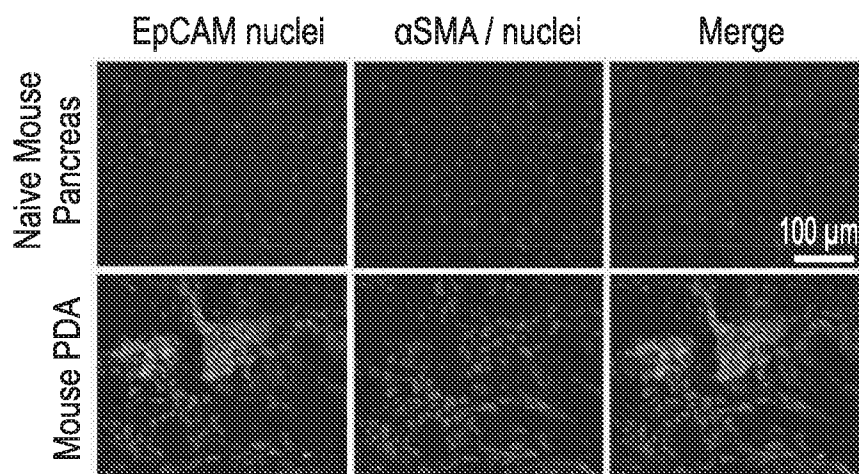


FIG. 59

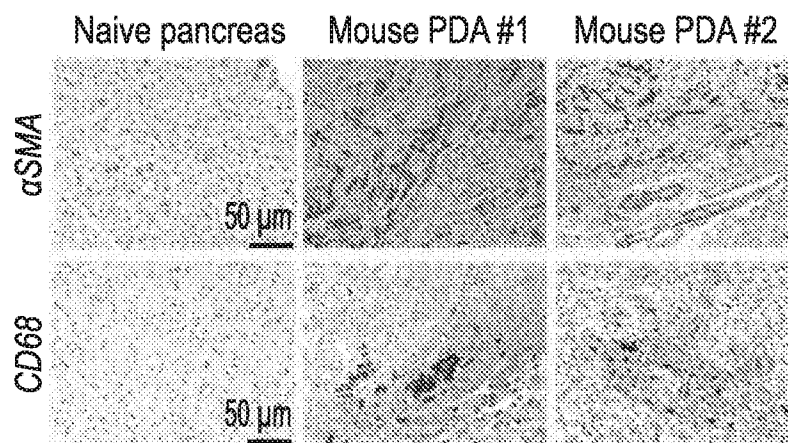


FIG. 60

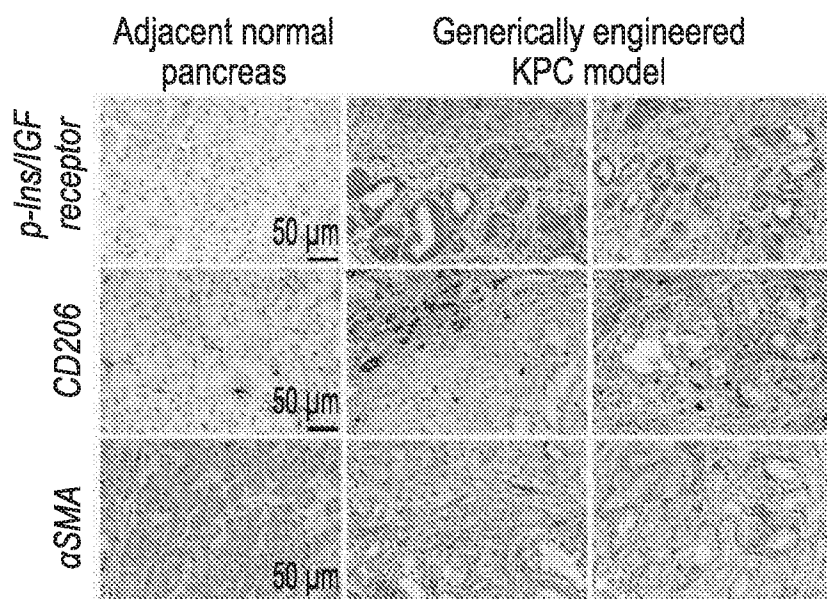


FIG. 61

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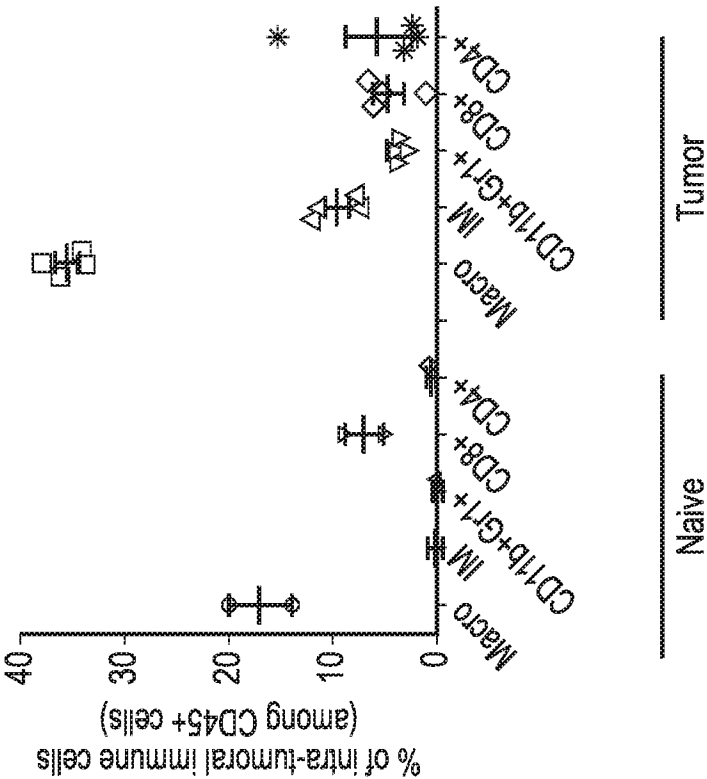
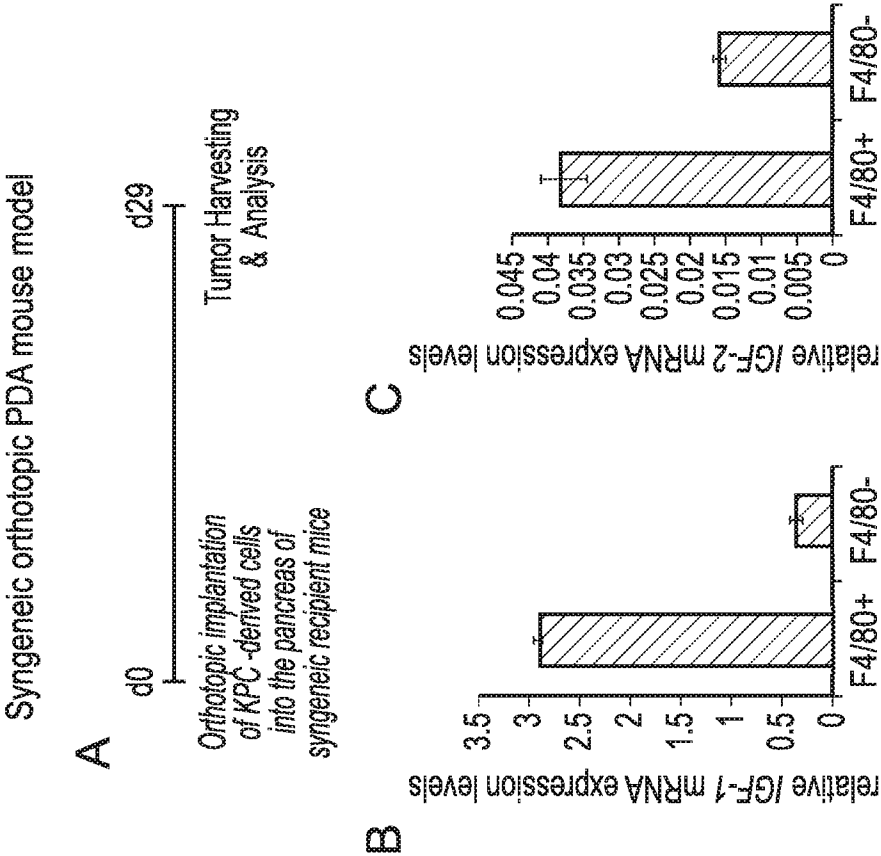


FIG. 63



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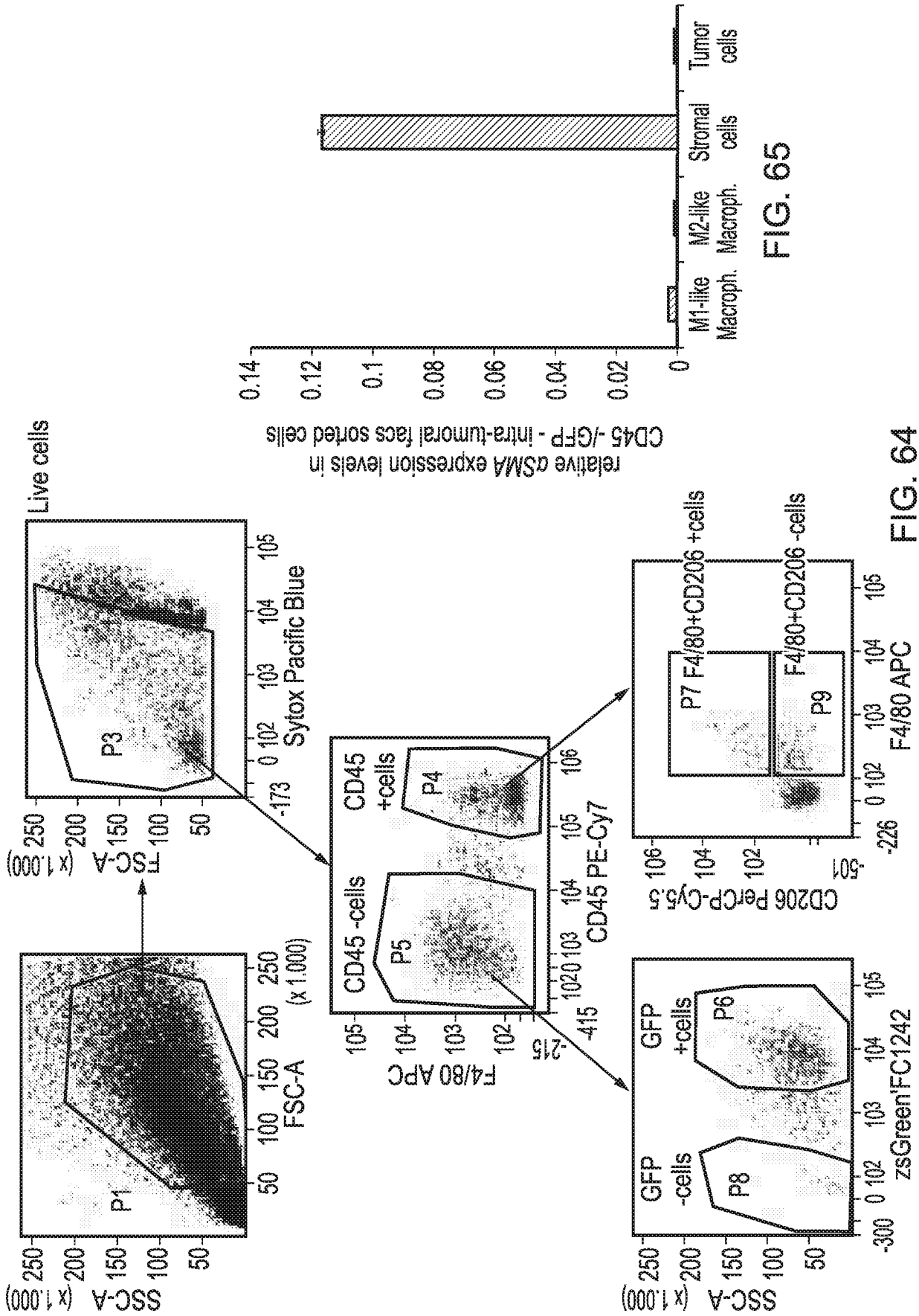


FIG. 65

FIG. 64

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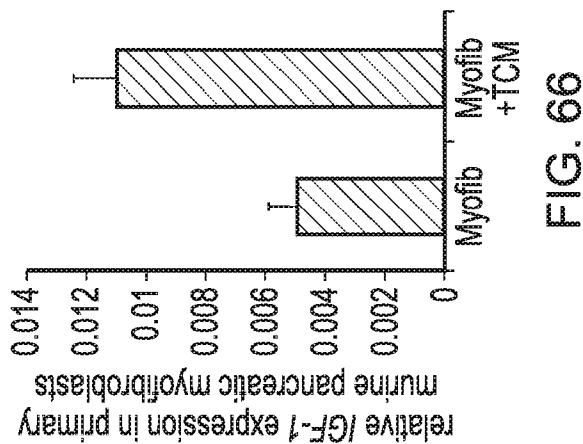
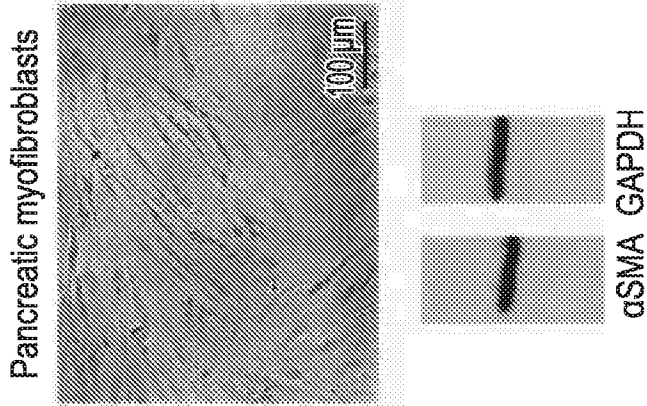


FIG. 66

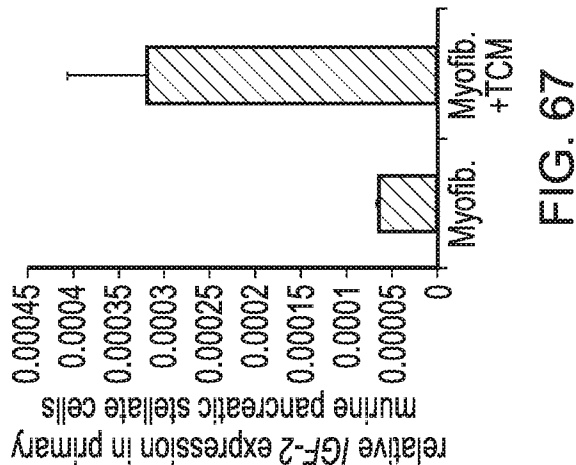


FIG. 67

FIG. 68

Syngeneic orthotopic KPC model

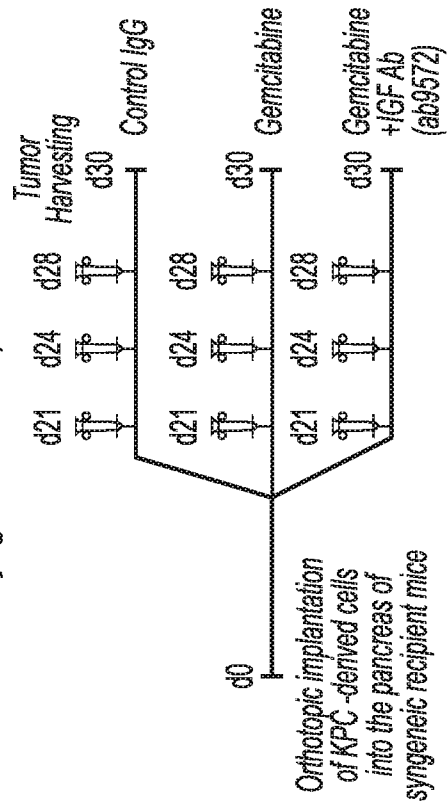
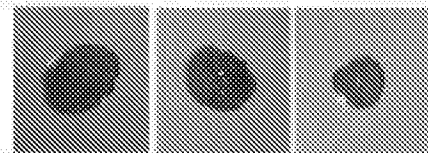


FIG. 69



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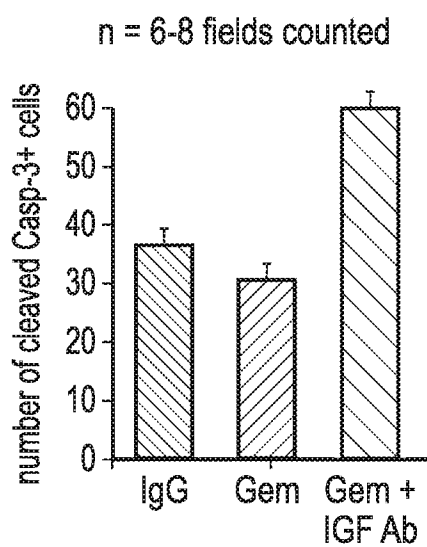
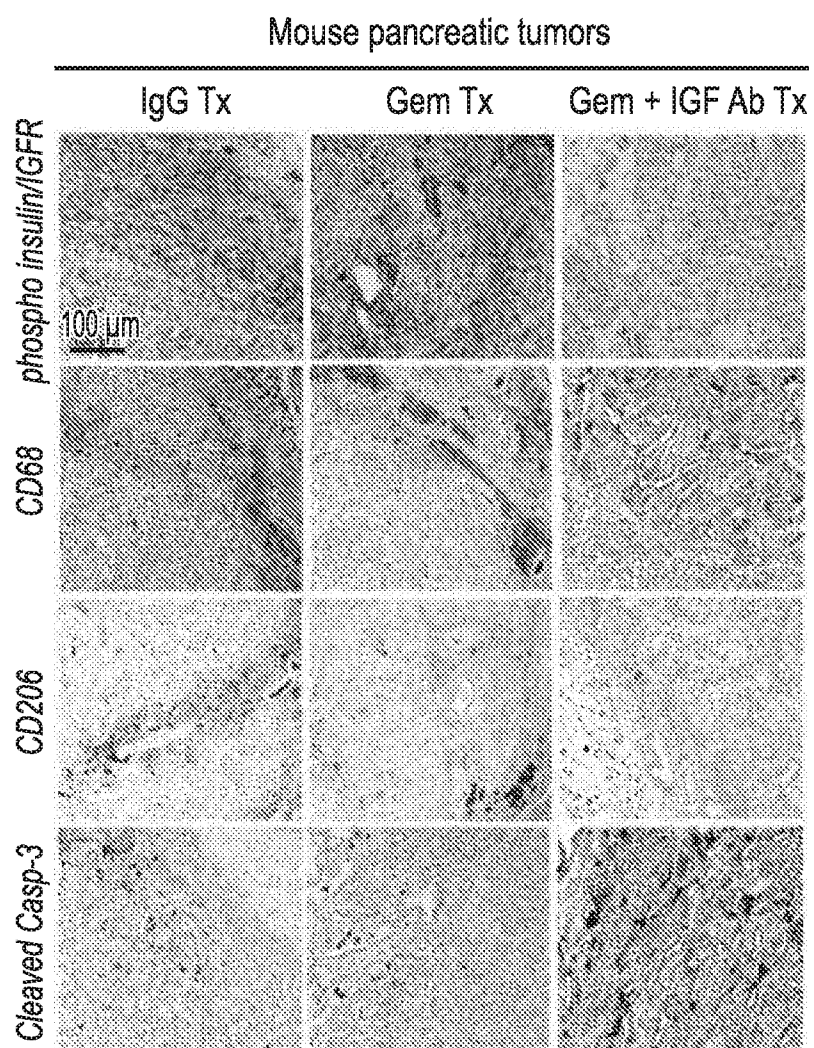


FIG. 70

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/050092

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 C07K16/22 A61P35/00 G01N33/569 A61K31/337
A61K31/7068

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K G01N

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	K. FRIEDBICHLER ET AL: "Pharmacodynamic and Antineoplastic Activity of BI 836845, a Fully Human IGF Ligand-Neutralizing Antibody, and Mechanistic Rationale for Combination with Rapamycin", MOLECULAR CANCER THERAPEUTICS, vol. 13, no. 2, 1 February 2014 (2014-02-01), pages 399-409, XP055261314, US	1-12, 17-20, 24-27, 40-45
Y	ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-13-0598 figure 5 page 400, left-hand column, paragraphs 1, 2 page 405, left-hand column, last paragraph - right-hand column, paragraph 1 ----- -/-	14,30, 33-37



Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

30 March 2016

Date of mailing of the international search report

19/04/2016

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Fax: (+31-70) 340-3016

Authorized officer

Brouns, Gaby

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2016/050092

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/135611 A1 (BOEHRINGER INGELHEIM INT [DE]) 12 September 2014 (2014-09-12) example 6 -----	1-13, 16-20, 24-27, 40-45
X	WO 2010/066868 A2 (BOEHRINGER INGELHEIM INT [DE]; ADAM PAUL [DE]; BORGES ERIC [DE]) 17 June 2010 (2010-06-17) examples 6, 7 figures 9, 10 claims 31, 32, 38, 39 -----	1-13, 15-21, 24-27, 40-45
X	WO 2004/024179 A1 (INSMED INC [US]; LEYLAND-JONES BRIAN [US]) 25 March 2004 (2004-03-25) examples 2, 4, 5 figures 3, 4 -----	1-13, 17-19, 22-27, 40-45
X	WO 02/24216 A2 (BIOEXPERTISE LLC [US]; MASCARENHAS DESMOND [US]) 28 March 2002 (2002-03-28) examples 2, 3 tables 1-3 figure 3 -----	1-13, 17-19, 22-27, 40-45
X	Jurriaan Brouwer-Visser ET AL: "Insulin-Like Growth Factor 2 Silencing Restores Taxol Sensitivity in Drug Resistant Ovarian Cancer", PLOS One, 1 June 2014 (2014-06-01), XP055261278, San Francisco DOI: 10.1371/journal.pone.0100165 Retrieved from the Internet: URL: http://journals.plos.org/plosone/article/asset?id=10.1371/journal.pone.0100165 .PDF figures 5C, 5D ----- -/--	1-13,16, 17, 24-27, 40-45

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2016/050092

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	N. AWASTHI ET AL: "BMS-754807, a Small-Molecule Inhibitor of Insulin-like Growth Factor-1 Receptor/Insulin Receptor, Enhances Gemcitabine Response in Pancreatic Cancer", MOLECULAR CANCER THERAPEUTICS, vol. 11, no. 12, 9 October 2012 (2012-10-09), pages 2644-2653, XP055260696, US ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-12-0447 figures 5, 6 -----	14,30, 33-37
A	MENG FANBIN ET AL: "Interaction between pancreatic cancer cells and tumor-associated macrophages promotes the invasion of pancreatic cancer cells and the differentiation and migration of macrophages.", IUBMB LIFE DEC 2014, vol. 66, no. 12, December 2014 (2014-12), pages 835-846, XP002755913, ISSN: 1521-6551 page 837, paragraph 4 -----	14,30, 33-37
A	A. MANTOVANI ET AL: "Tumor-Associated Macrophages as a Paradigm of Macrophage Plasticity, Diversity, and Polarization: Lessons and Open Questions", ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY., vol. 33, no. 7, 12 June 2013 (2013-06-12), pages 1478-1483, XP055261231, US ISSN: 1079-5642, DOI: 10.1161/ATVBAHA.113.300168 page 1481, left-hand column, paragraph 2 -----	14,30, 33-37
A	SAHITYA K. DENDULURI ET AL: "Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance", GENES & DISEASES, vol. 2, no. 1, 15 November 2014 (2014-11-15), pages 13-25, XP055261185, ISSN: 2352-3042, DOI: 10.1016/j.gendis.2014.10.004 table 1 ----- -/--	1-27,30, 33-37, 40-45

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/050092

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YUICHIRO MATSUOKA ET AL: "The tumour stromal features are associated with resistance to 5-FU-based chemoradiotherapy and a poor prognosis in patients with oral squamous cell carcinoma", APMIS, vol. 123, no. 3, 31 December 2014 (2014-12-31), pages 205-214, XP055261024, DK ISSN: 0903-4641, DOI: 10.1111/apm.12344 figures 1, 2 page 212, last paragraph -----	29,34, 35,37
X	KING HELEN ET AL: "Can we unlock the potential of IGF-1R inhibition in cancer therapy?", CANCER TREATMENT REVIEWS, SAUNDERS, US, vol. 40, no. 9, 4 August 2014 (2014-08-04) , pages 1096-1105, XP029064440, ISSN: 0305-7372, DOI: 10.1016/J.CTRV.2014.07.004 table 1 -----	32
X	SPRINZL MARTIN FRANZ ET AL: "Sorafenib inhibits macrophage-induced growth of hepatoma cells by interference with insulin-like growth factor-1 secretion", JOURNAL OF HEPATOLOGY, vol. 62, no. 4, 22 November 2014 (2014-11-22), pages 863-870, XP029205810, ISSN: 0168-8278, DOI: 10.1016/J.JHEP.2014.11.011 page 864, last paragraph -----	38,39
X	"Product Flyer SEPSIS AND INFLAMMATION Soluble CD163 ELISA assay for measuring Macrophage and Monocyte Activation Macro163 (TM) Product Flyer Background information", 1 January 2005 (2005-01-01), XP055261046, Retrieved from the Internet: URL: http://www.iqproducts.nl/downloads/sepsis/makro/Flyer_Macro163_V3.pdf [retrieved on 2016-03-24] the whole document ----- -/--	38,39

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2016/050092

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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