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(54) **Title:** DOSAGE REGIMEN FOR COMBINATION THERAPY USING PD-1 AXIS BINDING ANTAGONISTS AND GPC3 TARGETING AGENT

(57) **Abstract:** Provided are dosage regimens for combination therapy using PD-1 axis binding antagonists and GPC3 targeting agent. For example, the dosage regimens comprise (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

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DESCRIPTION

Title of Invention

DOSAGE REGIMEN FOR COMBINATION THERAPY USING PD-1 AXIS BINDING ANTAGONISTS AND GPC3 TARGETING AGENT

Field of Invention

[0001]

This invention relates to effective dosage regimen for combination therapy using PD-1 axis binding antagonists and GPC3 targeting agent

Background of the Invention

[0002]

Hepatocellular cancer is reportedly the fifth leading cause of cancer deaths worldwide, accounting for approximately 600,000 deaths each year (Llovet JM, Burroughs A, Bruix J; Lancet (2003), 362, 1907-17). Most patients with hepatocellular cancer die within 1 year after being diagnosed with the disease. Unfortunately, hepatocellular cancer cases are frequently diagnosed at a late stage which rarely responds to curative therapies. Still, medical treatments including chemotherapy, chemoembolization, ablation, and proton beam therapy are insufficiently effective for such patients. Many patients exhibit recurrence of the disease with vascular invasion and multiple intrahepatic metastases, which rapidly progresses to the advanced stage. Their 5-year survival rates are only 7% (Bosch FX, Ribes J, Cleries R; Gastroenterology (2004), 127, S5-16). Patients with hepatocellular cancer amenable to the resection of local foci have relatively good prognosis, though their 5-year survival rates still remain at a level of 15% and 39% (Takenaka K, Kawahara N, Yamamoto K, Kajiyama K, Maeda T, Itasaka H, Shirabe K, Nishizaki T, Yanaga K, Sugimachi K; Arch Surg (1996), 131, 71-6). Thus, there has been a demand in the art for novel therapy for such a malignant disease hepatocellular cancer.

[0003]

Hepatocellular cancer is reportedly responsible for more than 90% of primary liver cancer cases in Japan. Medical methods for treating such hepatocellular cancer include, for example,

chemotherapy-based transcatheter arterial embolization (TAE) therapy, which involves inducing the selective necrosis of the hepatocellular cancer by the injection of a mixture of an oil-based contrast medium (Lipiodol), an anticancer agent, and an obstructing substance (Gelfoam) into the hepatic artery (which serves as a nutrient supply pathway to the tumor) resulting in the obstruction of the nutrient artery. In addition, invasive approaches are used, such as percutaneous ethanol injection, percutaneous microwave coagulation therapy, and radiofrequency ablation. Also, clinical trials have been conducted on systemic chemotherapy using chemotherapeutic agents such as fluorouracil (5-FU), uracil-tegafur (UFT), mitomycin C (MMC), mitoxantrone (DHAD), adriamycin (ADR), epirubicin (EPI), and cisplatin (CDDP) either alone or in combination with interferon (IFN) (Yeo W, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ; J Natl Cancer Inst (2005), 97, 1532-8).

[0004]

Meanwhile, an orally active form of sorafenib (Nexavar, BAY43-9006) has been approved, which is more advantageously effective than the chemotherapeutic agents described above in such a way that this agent blocks the growth of cancer cells by inhibiting Raf kinase in the Raf/MEK/ERK signal transduction while the agent exerts antiangiogenic effects by targeting VEGFR-2, VEGFR-3, and PDGFR-beta. tyrosine kinases. The efficacy of sorafenib has been studied in two phase-III multicenter placebo-controlled trials (Sorafenib HCC Assessment Randomized Protocol (SHARP) trial and Asia-Pacific trial) targeting advanced hepatocellular cancer. Sorafenib was confirmed to prolong survival durations, with HR of 0.68, in both of these trials. In the SHARP trial, sorafenib prolonged the survival duration to 10.7 months versus 7.9 months with the placebo. In the Asian trial, this agent prolonged the survival duration to 6.5 months versus 4.2 months with the placebo. The agent, however, had a low objective response rate and showed no prolongation of a time to symptomatic progression, though the agent prolonged a time to tumor progression (5.5 months versus 2.8 months in the European and American trial and 2.8 months versus 1.4 months in the Asian trial) on the images. The Asian cohorts exhibited a short duration of life prolongation, which is probably because their treatments were started at a slightly later stage during the disease process in the Asian region compared with Europe and the United States (Llovet J, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. Sorafenib in advanced hepatocellular carcinoma. New Eng. J. Med. (2008) 359, 378-90 and Cheng AL, Chen Z,

Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorefanib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomized, double-blind, placebo- controlled trial. Lancet Oncol. (2009) 10, 25-34).

[0005]

As liver cancer progresses, its specific symptoms associated with liver dysfunction are generally observed, such as anorexia, weight loss, general malaise, palpable right hypochondrial mass, right hypochondrial pain, sense of abdominal fullness, fever, and jaundice. The chemotherapeutic agents (e.g., sorafenib), however, have complications to be overcome, including their inherent adverse reactions such as diarrhea or constipation, anemia, suppression of the immune system to cause infection or sepsis (with lethal severity), hemorrhage, cardiac toxicity, hepatic toxicity, renal toxicity, anorexia, and weight loss.

[0006]

Although particular early-stage symptoms are not initially observed in liver cancer, its specific symptoms associated with liver dysfunction, such as anorexia, weight loss, general malaise, palpable right hypochondrial mass, right hypochondrial pain, sense of abdominal fullness, fever, and jaundice, are generally observed with progression of the liver cancer. According to clinical observation, such symptoms are enhanced by use of the chemotherapeutic agents. For example, anorexia in a patient with detectable liver cancer cells and symptoms such as weight loss associated with or independent of the anorexia may be more enhanced by the administration of the chemotherapeutic agents to the patient than without the use of the chemotherapeutic agents. In some cases, the use of the chemotherapeutic agents must be discontinued for the patient having such symptoms. These enhanced symptoms are impediments to treatments with the chemotherapeutic agents. Thus, there has been a demand for the establishment of excellent therapy from the viewpoint of, for example, improving therapeutic effects or improving QOL of patients to be treated.

[0007]

Glypican 3 (GPC3) is frequently expressed at a high level in liver cancer and as such, seems to be useful in the identification of its functions in liver cancer or as a therapeutic or diagnostic target of liver cancer.

[0008]

Under the circumstances described above, drugs are under development with GPC3 as a therapeutic target of liver cancer. A liver cancer drug comprising an anti-GPC3 antibody as an active ingredient has been developed, the antibody having antibody-dependent cellular cytotoxicity (hereinafter, referred to as "ADCC") activity and/or complement-dependent cytotoxicity (hereinafter, referred to as "CDC") activity against cells expressing GPC3 (WO2003/000883). Also, a GPC3-targeting drug comprising a humanized anti-GPC3 antibody having ADCC activity and CDC activity as an active ingredient has been developed (WO2006/006693). Further GPC3-targeting drugs have been developed, which comprise a humanized anti-GPC3 antibody with enhanced ADCC activity (WO2006/046751 and WO2007/047291) or an anti-GPC3 antibody having ADCC activity and CDC activity as well as improved plasma dynamics (WO2009/041062). These anti-GPC3 antibodies in combination therapy with the chemotherapeutic agents such as sorafenib have been found to attenuate the adverse reactions, for example, brought about by the sole therapy of the chemotherapeutic agents (e.g., sorafenib) and also found to exhibit synergistic effects based on these agents (WO2009/122667). Accordingly, excellent methods for treating liver cancer are in the process of being established using GPC3-targeting drugs as the nucleus from the viewpoint of, for example, improving therapeutic effects or improving QOL of patients to be treated.

[0009]

On the other hand, the provision of two distinct signals to T-cells is a widely accepted model for lymphocyte activation of resting T lymphocytes by antigen-presenting cells (APCs). Lafferty et al, Aust. J. Exp. Biol. Med. Sci 53: 27-42 (1975). This model further provides for the discrimination of self from non-self and immune tolerance. Bretscher et al, Science 169: 1042-1049 (1970); Bretscher, P.A., Proc. Nat. Acad. Sci. USA 96: 185-190 (1999); Jenkins et al, J. Exp. Med. 165: 302-319 (1987). The primary signal, or antigen specific signal, is transduced through the T-cell receptor (TCR) following recognition of foreign antigen peptide presented in the context of the major histocompatibility-complex (MHC). The second or co-stimulatory signal is delivered to T-cells by co-stimulatory molecules expressed on antigen-presenting cells (APCs), inducing T-cells to promote clonal expansion, cytokine secretion and effector function. Lenschow et al., Ann. Rev. Immunol. 14:233 (1996). In the absence of co-stimulation, T-cells can become refractory to antigen stimulation, do not

mount an effective immune response, and further may result in exhaustion or tolerance to foreign antigens.

[0010]

In the two-signal model T-cells receive both positive and negative secondary co-stimulatory signals. The regulation of such positive and negative signals is critical to maximize the host's protective immune responses, while maintaining immune tolerance and preventing autoimmunity. Negative secondary signals seem necessary for induction of T-cell tolerance, while positive signals promote T-cell activation. While the simple two-signal model still provides a valid explanation for naive lymphocytes, a host's immune response is a dynamic process, and co-stimulatory signals can also be provided to antigen-exposed T-cells. The mechanism of co-stimulation is of therapeutic interest because the manipulation of co-stimulatory signals has shown to provide a means to either enhance or terminate cell-based immune response. Recently, it has been discovered that T cell dysfunction or anergy occurs concurrently with an induced and sustained expression of the inhibitory receptor, programmed death 1 polypeptide (PD-1). As a result, therapeutic targeting of PD-1 and other molecules which signal through interactions with PD-1, such as programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest.

[0011]

PD-L1 is overexpressed in many cancers and is often associated with poor prognosis (Okazaki T et al., Intern. Immun. 2007 19(7):813) (Thompson RH et al., Cancer Res 2006, 66(7):3381). Interestingly, the majority of tumor infiltrating T lymphocytes predominantly express PD-1, in contrast to T lymphocytes in normal tissues and peripheral blood T lymphocytes indicating that up-regulation of PD-1 on tumor-reactive T cells can contribute to impaired antitumor immune responses (Blood 2009 114(8): 1537). This may be due to exploitation of PD-L1 signaling mediated by PD-L1 expressing tumor cells interacting with PD-1 expressing T cells to result in attenuation of T cell activation and evasion of immune surveillance (Sharpe et al., Nat Rev 2002) (Keir ME et al., 2008 Annu. Rev. Immunol. 26:677). Therefore, inhibition of the PD-L1/PD-1 interaction may enhance CD8+ T cell-mediated killing of tumors.

[0012]

Therapeutic targeting PD-1 and other molecules which signal through interactions with PD-1, such as programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest. The inhibition of PD-L1 signaling has been proposed as a means to enhance T cell immunity for the treatment of cancer (e.g., tumor immunity) and infection, including both acute and chronic (e.g., persistent) infection. An optimal therapeutic treatment may combine blockade of PD-1 receptor/ligand interaction with an agent that directly inhibits tumor growth. There remains a need for an optimal therapy for treating, stabilizing, preventing, and/or delaying development of various cancers.

[0013]

All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

Summary of the Invention

[0014]

The inventors have discovered unexpectedly much more effective combination therapy regimens using a PD-1 axis binding antagonist and a GPC3 targeting agent in treating or delaying progression of cancer in an individual.

[0015]

In one aspect, provided herein is a suitable dosage regimen of a PD-1 axis binding antagonist in combination with a GPC3 targeting agent in treating or delaying progression of cancer in an individual. In another aspect, provided herein is a suitable dosage regimen of a GPC3 targeting agent in combination with a PD-1 axis binding antagonist in treating or delaying progression of cancer in an individual. In one aspect, provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an effective amount of GPC3 targeting agent.

[0016]

The present invention provides:

[1] A pharmaceutical composition for treating or delaying progression of cancer in an individual for use in a combination therapy with a PD-1 axis binding antagonist, wherein said composition comprises a GPC3 targeting agent as an active ingredient,
wherein the combination therapy comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[2] The pharmaceutical composition according to [1] above, wherein the GPC3 targeting agent is administered at a dose of approximately 800 mg/body to approximately 3,200 mg/body once or more within the loading period.

[3] The pharmaceutical composition according to [1] or [2] above, wherein the loading period is 2 weeks or less, or 1 week or less.

[4] The pharmaceutical composition according to any one of [1] to [3] above, wherein the total amount of the GPC3 targeting agent administered within the loading period is approximately 1,600 mg/body to approximately 4,800 mg/body.

[5] The pharmaceutical composition according to any one of [1] to [4] above, wherein the GPC3 targeting agent is administered at a dose of approximately 1,600 mg/body once or twice within the loading period.

[6] The pharmaceutical composition according to any one of [1] to [5] above, wherein the last administration of the GPC3 targeting agent within the loading period is separated in time from the first administration of the PD-1 axis binding antagonist or the GPC3 targeting agent within the maintenance period by 2 to 4 days.

[7] The pharmaceutical composition according to any one of [1] to [6] above, wherein the PD-1 axis binding antagonist is administered at a dose of approximately 1,000 mg/body to approximately 1,400 mg/body once or more within the maintenance period, and wherein the GPC3 targeting agent is administered at a dose of approximately 800 mg/body to approximately 2,000 mg/body once or more within the maintenance period.

[8] The pharmaceutical composition according to any one of [1] to [7] above, wherein the PD-1 axis binding antagonist is administered at a dose of approximately 1,200 mg/body once per 3 weeks, and wherein the GPC3 targeting agent is administered at a dose of approximately 1,600 mg/body once per week or once per 3 weeks.

[9] The pharmaceutical composition according to any one of [1] to [7] above, wherein the maintenance period comprises treatment cycles, wherein one treatment cycle comprises
(1) administration of approximately 1,200 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and
(2) administration of approximately 1,600 mg/body of the GPC3 targeting agent once per week for 3 weeks, and the treatment cycle is repeated.

[10] The pharmaceutical composition according to any one of [1] to [9], wherein the administration of the PD-1 axis binding antagonist and/or the GPC3 targeting antibody within the loading period and/or the maintenance period are intravenous injection or infusion.

[11] The pharmaceutical composition according to any one of [1] to [9] above, wherein the administration of the PD-1 axis binding antagonist and/or the GPC3 targeting antibody within the loading period and/or the maintenance period are subcutaneous injection.

[12] The pharmaceutical composition according to any one of [1] to [11] above, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[13] The pharmaceutical composition according to any one of [1] to [12] above, wherein the PD-1 axis binding antagonist is
(1) an anti-PD-L1 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21 ; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24,
(2) an anti-PD-L1 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4, or

(3) Atezolizumab.

[14] The pharmaceutical composition according to any one of [1] to [13] above, wherein the GPC3 targeting agent is
(1) an anti-GPC3 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:34, HVR-H2 sequence of SEQ ID NO:35, and HVR-H3 sequence of SEQ ID NO:36; and a light chain comprising HVR-L1 sequence of SEQ ID NO:37, HVR-L2 sequence of SEQ ID NO:38, and HVR-L3 sequence of SEQ ID NO:39,
(2) an anti-GPC3 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:50 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:52, or
(3) Codrituzumab.

[15] The pharmaceutical composition according to any one of [1] to [14] above, wherein the cancer is selected from the group consisting of liver cancer, breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer and prostate cancer.

[0017]
[16] The pharmaceutical composition according to any one of [1] to [15], wherein the cancer is a GPC3-positive cancer.

[17] The pharmaceutical composition according to any one of [1] to [16] above, wherein the combination therapy further comprises administering an effective amount of a chemotherapeutic agent.

[18] The pharmaceutical composition according to [1] or [2] above, wherein the GPC3 targeting agent is administered to achieve a blood trough level of the GPC3 targeting agent of 200 μg/ml or higher in the individual within the loading period.

[19] The pharmaceutical composition according to any one of [1] to [6] above, wherein the GPC3 targeting agent is administered to achieve a blood trough level of the GPC3 targeting agent of 200 μg/ml or higher in the individual within the maintenance period.

[20] A pharmaceutical composition for treating or delaying progression of cancer in an individual for use in a combination therapy with a GPC3 targeting agent, wherein said composition comprises a PD-1 axis binding antagonist as an active ingredient,
wherein the combination therapy comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[21] A method for treating or delaying progression of cancer in an individual, comprising administering an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent, the method comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[22] The method according to [21] above, wherein the GPC3 targeting agent is administered at a dose of approximately 800 mg/body to approximately 3,200 mg/body once or more within the loading period.

[23] The method according to [21] or [22] above, wherein the loading period is 2 weeks or less, or 1 week or less.

[24] The method according to any one of [21] to [23] above, wherein the total amount of the GPC3 targeting agent administered within the loading period is approximately 1,600 mg/body to approximately 4,800 mg/body.

[25] The method according to any one of [21] to [24] above, wherein the GPC3 targeting agent is administered at a dose of approximately 1,600 mg/body once or twice within the loading period.

[26] The method according to any one of [21] to [25] above, wherein the last administration of the GPC3 targeting agent within the loading period is separated in time from the first administration of the PD-1 axis binding antagonist or the GPC3 targeting agent within the maintenance period by 2 to 4 days.

[27] The method according to any one of [21] to [26] above, wherein the PD-1 axis binding antagonist is administered at a dose of approximately 1,000 mg/body to approximately 1,400 mg/body once or more within the maintenance period, and wherein the GPC3 targeting agent is administered at a dose of approximately 800 mg/body to approximately 2,000 mg/body once or more within the maintenance period.

[28] The method according to any one of [21] to [27] above, wherein the PD-1 axis binding antagonist is administered at a dose of approximately 1,200 mg/body once per 3 weeks, and wherein the GPC3 targeting agent is administered at a dose of approximately 1,600 mg/body once per week or once per 3 weeks.

[29] The method according to any one of [21] to [27] above, wherein the maintenance period comprises treatment cycles, wherein one treatment cycle comprises
(1) administration of approximately 1,200 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and
(2) administration of approximately 1,600 mg/body of the GPC3 targeting agent once per week for 3 weeks, and the treatment cycle is repeated.

[30] The method according to any one of [21] to [29] above, wherein the administration of the PD-1 axis binding antagonist and/or the GPC3 targeting antibody within the loading period and/or the maintenance period are intravenous injection or infusion.

[31] The method according to any one of [21] to [29] above, wherein the administration of the PD-1 axis binding antagonist and/or the GPC3 targeting antibody within the loading period and/or the maintenance period are subcutaneous injection.

[32] The method according to any one of [21] to [31] above, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[33] The method according to any one of [21] to [32] above, wherein the PD-1 axis binding antagonist is Atezolizumab.

[34] The method according to any one of [21] to [33] above, wherein the GPC3 targeting agent is Codrituzumab.

[35] The method according to any one of [21] to [34] above, wherein the cancer is selected from the group consisting of liver cancer, breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer and prostate cancer.

[36] The method according to any one of [21] to [35] above, wherein the cancer is a GPC3-positive cancer.

[37] The method according to any one of [21] to [36] above, wherein the method further comprises administering an effective amount of a chemotherapeutic agent.

[38] The method according to [21] or [22] above, wherein the GPC3 targeting agent is administered to achieve a blood trough level of the GPC3 targeting agent of 200 μg/ml or higher in the individual within the loading period.

[39] The method according to any one of [21] to [26] above, wherein the GPC3 targeting agent is administered to achieve a blood trough level of the GPC3 targeting agent of 200 μg/ml or higher in the individual within the maintenance period.

[40] A kit comprising
(1) a pharmaceutical composition comprising a PD-1 axis binding antagonist as an active ingredient,
(2) a pharmaceutical composition comprising a GPC3 targeting agent as an active ingredient,
(3) a container, and
(4) a package insert or label comprising instructions for a combination therapy using the GPC3 targeting agent and the PD-1 axis binding antagonist, wherein the combination therapy comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.,

[41] A combination for treating or delaying progression of cancer in an individual comprising a PD-1 axis binding antagonist and a GPC3 targeting agent, wherein the combination comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[42] Use of a PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[43] Use of a GPC3 targeting agent in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the GPC3 targeting agent and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[44] A pharmaceutical composition for treating or delaying progression of cancer in an individual for use in a combination therapy with a PD-1 axis binding antagonist, wherein said composition comprises a GPC3 targeting agent as an active ingredient, wherein the combination therapy comprises
(1) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and
(2) administration of approximately 800 mg/body to approximately 2,000 mg/body of the GPC3 targeting agent once per week or once per 3 weeks.

[45] A pharmaceutical composition for treating or delaying progression of cancer in an individual for use in a combination therapy with a GPC3 targeting agent, wherein said composition comprises a PD-1 axis binding antagonist as an active ingredient, wherein the

combination therapy comprises

(1) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and

(2) administration of approximately 800 mg/body to approximately 2,000 mg/body of the GPC3 targeting agent once per week or once per 3 weeks.

[46] A method for treating or delaying progression of cancer in an individual, comprising administering an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent, the method comprises

(1) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and

(2) administration of approximately 800 mg/body to approximately 2,000 mg/body of the GPC3 targeting agent once per week or once per 3 weeks.

[47] A kit comprising

(1) a pharmaceutical composition comprising a PD-1 axis binding antagonist as an active ingredient,

(2) a pharmaceutical composition comprising a GPC3 targeting agent as an active ingredient,

(3) a container, and

(4) a package insert or label comprising instructions for a combination therapy using the GPC3 targeting agent and the PD-1 axis binding antagonist, wherein the combination therapy comprises

(a) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and

(b) administration of approximately 800 mg/body to approximately 2,000 mg/body of the GPC3 targeting agent once per week or once per 3 weeks.

[48] A combination for treating or delaying progression of cancer in an individual comprising a PD-1 axis binding antagonist and a GPC3 targeting agent, wherein the combination comprises

(1) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and

(2) administration of approximately 800 mg/body to approximately 2,000 mg/body of an

GPC3 targeting agent once per week or once per 3 weeks.

[49] Use of a PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises
(1) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and
(2) administration of approximately 800 mg/body to approximately 2,000 mg/body of an GPC3 targeting agent once per week or once per 3 weeks.

[50] Use of a GPC3 targeting agent in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the GPC3 targeting agent and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises
(1) administration of approximately 1,000 mg/body to approximately 1,400 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and
(2) administration of approximately 800 mg/body to approximately 2,000 mg/body of the GPC3 targeting agent once per week or once per 3 weeks.

[0018]
In another aspect, provided herein is a method of enhancing immune responses against tumor cells in an individual having cancer comprising administering an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent. For example, enhanced immune responses against tumor cells includes infiltration of immune cells including macrophages and multinucleated giant cells in to tumor tissues. For another example, enhanced immune responses against tumor cells includes increase of CD45-positive lymphocytes, CD3ε-positive lymphocytes and CD8-positive T lymphocytes in tumor infiltrated lymphocytes (TILs).

[0019]
In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist is selected from the group consisting of a PD-1

binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is an antibody. In some embodiments, the antibody is a humanized antibody, a chimeric antibody or a human antibody. In some embodiments, the antibody is an antigen binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of Fab, Fab', F(ab')2, scFv and Fv.

[0020]

In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is an antibody. In some embodiments, the PD-1 binding antagonist is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (pembrolizumab, lambrolizumab), CT-011 (pidilizumab), PDR001, REGN2810, BGB A317, SHR-1210, AMP-514 (MEDI0680), and AMP-224.

[0021]

In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an antibody. In some embodiments, the PD-L1 binding antagonist is selected from the group consisting of: YW243.55.S70, Atezolizumab, MPDL3280A, MDX-1105, avelumab, and MEDI4736 (durvalumab). In some embodiments, the anti-PD-L1 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and/or a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiment, the anti-PD-L1 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and/or a light

chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-PD-L1 antibody comprises the three heavy chain HVR sequences of antibody YW243.55.S70 and/or the three light chain HVR sequences of antibody YW243.55.S70 described in WO2010/077634 and U.S. Patent No. 8,217,149, which are incorporated herein by reference. In some embodiments, the anti-PD-L1 antibody comprises the heavy chain variable region sequence of antibody YW243.55.S70 and/or the light chain variable region sequence of antibody YW243.55.S70. In some embodiments, the anti-PD-L1 antibody is Atezolizumab.

[0022]

In some embodiments, the PD-1 axis binding antagonist is a PD-L2 binding antagonist. In some embodiments, the PD-L2 binding antagonist is an antibody. In some embodiments, the PD-L2 binding antagonist is an immunoadhesion.

[0023]

In some embodiments of the methods, uses, compositions, and kits described above and herein, the anti-GPC3 antibody is a humanized antibody, a chimeric antibody or a human antibody. In some embodiments, the antibody is an antigen binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of Fab, Fab', F(ab')2, scFv and Fv.

[0024]

In some embodiments, the anti-GPC3 antibody is GC33 or codrituzumab. In some embodiments, the anti-GPC3 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:42, HVR-H2 sequence of SEQ ID NO:43, and HVR-H3 sequence of SEQ ID NO:44; and/or a light chain comprising HVR-L1 sequence of SEQ ID NO:45, HVR-L2 sequence of SEQ ID NO:46, and HVR-L3 sequence of SEQ ID NO:47. In some embodiments, the anti-GPC3 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:50 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:51. In some embodiments, the anti-GPC3 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:50 and/or a light chain comprising the amino acid sequence of SEQ ID NO:52. In some embodiments that can be combined with any other embodiments, the anti-GPC3 antibody is not GC33 or

codrituzumab.

[0025]

In some embodiments, the antibody described herein (e.g., a PD-1 axis binding antagonist antibody or an anti-GPC3 antibody) comprises an aglycosylation site mutation. In some embodiments, the aglycosylation site mutation is a substitution mutation. In some embodiments, the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering). In some embodiments, the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A. In some embodiments, the substitution mutation is a D265A mutation and an N297G mutation. In some embodiments, the aglycosylation site mutation reduces effector function of the antibody. In some embodiments, the PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody) is a human IgG1 having Asn to Ala substitution at position 297 according to EU numbering.

[0026]

In some embodiments of the methods, uses, compositions and kits described above and herein, the cancer is a GPC3-positive cancer. In some embodiments, the cancer is liver cancer, breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, prostate cancer, or other cancers described herein. In some embodiments, the individual has cancer or has been diagnosed with cancer. In some embodiments, the cancer cells in the individual express PD-L1.

[0027]

In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment or administration of the human PD-1 axis binding antagonist and the anti-GPC3 antibody results in a sustained response in the individual after cessation of the treatment. In some embodiments, the anti-GPC3 antibody is administered before the PD-1 axis binding antagonist, simultaneous with the PD-1 axis binding antagonist, or after the PD-1 axis binding antagonist. In some embodiments, the PD-1 axis binding antagonist and the anti-GPC3 antibody are in the same composition. In some embodiments, the PD-1 axis binding antagonist and the anti-GPC3 antibody are in separate compositions.

[0028]

In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist and/or the anti-GPC3 antibody is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment further comprises administering a chemotherapeutic agent for treating or delaying progression of cancer in an individual. In some embodiments, the individual has been treated with a chemotherapeutic agent before the combination treatment with the PD-1 axis binding antagonist and the anti-GPC3 antibody. In some embodiments, the individual treated with the combination of the PD-1 axis binding antagonist and/or the anti-GPC3 antibody is refractory to a chemotherapeutic agent treatment. Some embodiments of the methods, uses, compositions, and kits described throughout the application, further comprise administering a chemotherapeutic agent for treating or delaying progression of cancer.

[0029]

In some embodiments of the methods, uses, compositions and kits described above and herein, CD8 T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity relative to prior to the administration of the combination. In some embodiments, the number of CD8 T cells is elevated relative to prior to administration of the combination. In some embodiments, the CD8 T cell is an antigen- specific CD8 T cell.

[0030]

It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

Brief Description of Drawings

[0031]

[FIG. 1]

FIG. 1 is a diagram showing the Hepa1-6 expressing human GPC3 tumor volume changes in each mouse treated three times weekly either by vehicle control, 1 mg/kg or 5 mg/kg of mGC33. Arrow indicates date of the injection. Five mice per each group were treated.

[FIG. 2A]

FIG. 2A is a diagram showing the images of hematoxylin and eosin staining (HE) or F4/80 immune-histochemical staining (IHC) of tissues isolated from the mice treated either by vehicle control or 5 mg/kg of mGC33.

[FIG. 2B]

FIG. 2B is a diagram showing the images of hematoxylin and eosin staining (HE) or PD-L1 immune-histochemical staining (IHC) of tissues isolated from the mice treated either by vehicle control or 5 mg/kg of mGC33. Arrow indicates PD-L1 immunoreactivity observed in cell membranes of infiltrated immune cells.

[FIG. 3A]

FIG. 3A is a diagram showing the CT26 expressing human GPC3 tumor volume changes in each mouse treated either by monotherapy (mGC33 or 10F.9G2 (anti-PD-L1)) or combination (mGC33 + 10F.9G2). Arrow indicates date of the injection. Five mice per group were treated. Average of tumor volume in each group and SD bar were plotted.

[FIG. 3B]

FIG. 3B is a diagram showing the progression free survival rate in CT26/hGPC3 bearing mice treated either by monotherapy (mGC33 or 10F.9G2 (anti-PD-L1)) or combination (mGC33 + 10F.9G2). Progression was defined when tumor size was reached more than 100 mm^3.

[FIG. 4A]

FIG. 4A is a diagram showing the CT26 expressing human GPC3 tumor volume changes in each mouse treated either by monotherapy or combination. Arrow indicates date of the injection. Five mice per group were treated. Average of tumor volume in each group and SD bar were plotted. Tumor growth inhibition values of 5 mg/kg or 25 mg/kg of mGC33, 10F.9G2, 5 mg/kg or 25 mg/kg of mGC33 + 10F.9G2 were 52%, 58%, 68%, 75% and 85%, respectively.

[FIG. 4B]

FIG. 4B is a diagram showing the individual tumor volume at day 29. Average (*) of tumor volume in each group was also plotted.

[FIG. 5A]

FIG. 5A is a diagram showing the Hepa1-6 expressing human GPC3 tumor volume changes in each mouse treated either by vehicle control, 1 mg/kg, 5 mg/kg or 25 mg/kg of mGC33, 10F.9G2 or combination of 5 mg/kg or 25 mg/kg of GC33 and 10F.9G2. Arrow indicates date of the injection. Five mice per each group were treated.

[FIG. 5B]

FIG. 5B is a diagram showing the individual tumor area or mean + SD of tumor area in each treated group at day 34. Tumor area (mm^2) was calculated by (length (mm) of long axis of tumor tissue) x (length (mm) of short axis of tumor tissue) after HE staining of tumor tissues isolated from each mice. And the results of pathological evaluation of each tumor tissues were added in the bottom of graphs. Pathological progression of disease (pPD) was defined as "no tumor regression was noted". Pathological partial regression (pPR) was defined as "degeneration and/or necrosis of tumor cells with immune cell infiltration was noted". Pathological complete regression (pCR) was defined as "no tumor cells were noted in the tumor implantation site".

[FIG. 6]

FIG. 6 is a schematic diagram showing the design of the clinical trial. In Cohort 1, three or six patients are enrolled. In the dose-escalation part of the clinical trial, if no dose limiting toxicity is observed in the patient, or if dose limiting toxicity is observed in one or less patient in the six patients, other six patients will be enrolled into Cohort 2. If dose limiting toxicity is observed in 2 or more in the three patients, or if dose limiting toxicity is observed in the six patients, other six patients will be enrolled into Cohort 3. After the dose-escalation part, at least nine other patients will be enrolled into Expansion Cohort, in which the patients will be treated at a higher dose which has been confirmed to be tolerable in the dose escalation part.

[FIG. 7A]

FIG. 7A is a diagram showing the change in trough concentration of GC33 in all of the patients enrolled in Cohort 1. The horizontal line in FIG. 7A shows 230 μg/mL as a desirable trough concentration in GC33 concentration.

[FIG. 7B]

FIG. 7B is a diagram showing the change in trough concentration of GC33 in all of the patients having the loading dose at day 4 in Cohort 1. The horizontal line in FIG. 7B shows 230 μg/mL as a desirable trough concentration in GC33 concentration.

[FIG. 8A]

FIG. 8A is a diagram showing the change in trough concentration of GC33 in all of the patients enrolled in Cohort 2. The horizontal line in FIG. 7A shows 230 μg/mL as a desirable trough concentration in GC33 concentration.

[FIG. 8B]

FIG. 8B is a diagram showing the change in trough concentration of GC33 in all of the patients having the loading dose at day 4 in Cohort 1. The horizontal line in FIG. 8B shows 230 μg/mL as a desirable trough concentration in GC33 concentration.

[FIG. 9]

FIG. 9 is a diagram showing the mean trough concentration of GC33. The solid line indicates the mean trough concentration of GC33 in 3 patients enrolled into the cohort 1. The dotted line indicates the mean trough concentration in 17 patients enrolled into the cohort 2 and the expansion cohort.

[FIG. 10A]

FIG. 10A is a diagram showing the percent change of AFP from baseline AFP value at day 8 from the initial dosing of GC33. Asterisk mark (*) indicates patient whose baseline AFP value is over 100 ng/mL.

[FIG. 10B]

FIG. 10B is a diagram showing the best AFP response during the treatment. The best AFP response is calculated either as the maximum percent change of AFP if the AFP value is decreased from the baseline by treatment or the minimum percent change of AFP if the AFP value is not decreased by treatment. Asterisk mark (*) indicates patient whose baseline AFP value is over 100 ng/mL..

[FIG.11A]

FIG. 11A is a diagram showing Kaplan-Meier plot for progression-free-survival (PFS) in the population for the efficacy evaluation. The dotted line indicates PFS for the population treated by 800 mg of GC33 and the solid line indicates PFS for the population treated by 1600 mg of GC33.

[FIG.11B]

FIG. 11B is a diagram showing Kaplan-Meier plot for overall survival (OS) in the population for the efficacy evaluation. The dotted line indicates OS for the population treated by 800 mg of GC33 and the solid line indicates OS for the population treated by 1600 mg of GC33.

[FIG.11C]

FIG. 11C is a diagram showing Kaplan-Meier plot for PFS in the population for the efficacy evaluation only having 1600 mg of GC33 dosing. The solid line indicates PFS for the population with a loading dose of GC33 at day 4 and the dotted line indicates PFS for the population without a loading dose of GC33 at day 4.

[FIG.11D]

FIG. 11D is a diagram showing Kaplan-Meier plot for OS in the population for the efficacy evaluation only having 1600 mg of GC33 dosing. The solid line indicates OS for the population with a loading dose of GC33 at day 4 and the dotted line indicates OS for the population without a loading dose of GC33 at day 4.

[FIG.11E]

FIG. 11E is a diagram showing Kaplan-Meier plot for PFS in the population for the efficacy evaluation only whose baseline AFP value is over 100 ng/mL. The dotted line indicates PFS for the population treated by 800 mg of GC33 and the solid line indicates PFS for the population treated by 1600 mg of GC33.

[FIG.11F]

FIG. 11B is a diagram showing Kaplan-Meier plot for OS in the population for the efficacy evaluation only whose baseline AFP value is over 100 ng/mL. The dotted line indicates OS for the population treated by 800 mg of GC33 and the solid line indicates OS for the population treated by 1600 mg of GC33.

[FIG.11G]

FIG. 11G is a diagram showing Kaplan-Meier plot for PFS in the population for the efficacy evaluation only having 1600 mg of GC33 dosing and whose baseline AFP value is over 100 ng/mL. The solid line indicates PFS for the population with a loading dose of GC33 at day 4 and the dotted line indicates PFS for the population without a loading dose of GC33 at day 4.

[FIG.11H]

FIG. 11H is a diagram showing Kaplan-Meier plot for OS in the population for the efficacy evaluation only having 1600 mg of GC33 dosing and whose baseline AFP value is over 100

ng/mL. The solid line indicates OS for the population with a loading dose of GC33 at day 4 and the dotted line indicates OS for the population without a loading dose of GC33 at day 4.

Detailed Description

[0032]

The data in the application show that dosage regimens for the combination of a GPC3 targeting agent with anti-PD-L1 immune therapy resulted in enhanced inhibition of tumor growth, increased response rates and durable responses. The inventors demonstrated the benefit of dosage regiments the combination therapy provided herein: administering a GPC3 targeting agent and a PD-1 axis binding antagonist according to dosage regimens provided herein results in enhanced therapeutic effects (e.g., enhanced tumor response, enhanced anti-tumor activity, larger decrease in alfa-feto protein (AFP), etc.) and/or durable long term responses.

[I. Definitions]

[0033]

Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0034]

As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0035]

The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0036]

It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0037]

The term "PD-1 axis binding antagonist" refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis - with a result being to restore or enhance T-cell function (e.g., proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[0038]

The term "PD-1 binding antagonist" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (lambrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is AMP-224 or AMP-514 (MEDI0680) described herein. In another specific aspect, a PD-1 antagonist is selected from the group consisting of PDR001,

REGN2810, BGB A317 and SHR-1210 described herein.

[0039]

The term "PD-L1 binding antagonist" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 or Atezolizumab described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In another specific aspect, an anti-PD-L1 antibody is avelumab described herein. In still another specific aspect, an anti-PD-L1 antibody is MPDL3280A described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 (durvalumab) described herein.

[0040]

The term "PD-L2 binding antagonist" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such

as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

[0041]

The term "dysfunction" in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both exhaustion and/or anergy in which antigen recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth.

[0042]

The term "dysfunctional", as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into down-stream T-cell effector functions, such as proliferation, cytokine production (e.g., IL-2) and/or target cell killing.

[0043]

The term "anergy" refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (e.g. increase in intracellular Ca^{+2} in the absence of ras-activation). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of costimulation. The unresponsive state can often be overridden by the presence of Interleukin-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

[0044]

The term "exhaustion" refers to T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or

memory T cells. Exhaustion prevents optimal control of infection and tumors. Exhaustion can result from both extrinsic negative regulatory pathways (e.g., immunoregulatory cytokines) as well as cell intrinsic negative regulatory (costimulatory) pathways (PD-1, B7-H3, B7-H4, etc.).

[0045]

"Enhancing T-cell function" means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include: increased secretion of γ-interferon from $CD8^+$ T-cells, increased proliferation, increased antigen responsiveness (e.g., viral, pathogen, or tumor clearance) relative to such levels before the intervention. In one embodiment, the level of enhancement is as least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

[0046]

A "T cell dysfunctional disorder" is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation. In a particular embodiment, a T-cell dysfunctional disorder is a disorder that is specifically associated with inappropriate increased signaling through PD-1. In another embodiment, a T-cell dysfunctional disorder is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In a specific aspect, the decreased responsiveness results in ineffective control of a pathogen or tumor expressing an immunogen. Examples of T cell dysfunctional disorders characterized by T-cell dysfunction include unresolved acute infection, chronic infection and tumor immunity.

[0047]

"Tumor immunity" refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

[0048]

"Immunogenicity" refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response. Examples of enhancing tumor immunogenicity include treatment with a PD-1 axis binding antagonist and a GPC3 targeting agent.

[0049]

"Sustained response" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the beginning of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5X, 2.0X, 2.5X, or 3.0X length of the treatment duration.

[0050]

The term "pharmaceutical composition" or “pharmaceutical formulation” refers to a composition or preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition or formulation would be administered. Such compositions or formulations are sterile. "Pharmaceutically acceptable" excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0051]

As used herein, the term "treatment" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, reducing tumor growth, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0052]

As used herein, "delaying progression of a disease" means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0053]

An "effective amount" is at least the minimum amount required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (i.e., slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or

pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0054]

As used herein, "in conjunction with" and "in combination with" refer to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" and "in combination with" refer to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0055]

A "disorder" is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

[0056]

The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer. In one embodiment, the cell proliferative disorder is a tumor.

[0057]

"Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", "cell proliferative disorder", "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

[0058]

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited

to, squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, gliblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma, breast carcinoma and hepatocellular carcinoma, including metastatic forms of those cancers.

[0059]

The term "cytotoxic agent" as used herein refers to any agent that is detrimental to cells (e.g., causes cell death, inhibits proliferation, or otherwise hinders a cellular function).

Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Exemplary cytotoxic agents can be selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signaling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. In one embodiment the cytotoxic agent is a taxane. In one embodiment the taxane is paclitaxel or docetaxel. In one embodiment the cytotoxic agent is a platinum agent. In one embodiment the cytotoxic agent is an antagonist of EGFR. In one embodiment the antagonist of EGFR is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (e.g., erlotinib). In one embodiment the cytotoxic agent is a RAF inhibitor. In one embodiment, the RAF inhibitor is a BRAF and/or CRAF inhibitor. In one embodiment the RAF inhibitor is vemurafenib. In one embodiment the cytotoxic agent is a PI3K inhibitor.

[0060]

"Chemotherapeutic agent" includes, but not limited to, Nitrogen mustard analogues, Alkyl sulfonates, Ethylene imines, Nitrosoureas, Epoxides, other alkylating agents, Folic acid analogues, Purine analogues, Pyrimidine analogues, other antimetabolic agents, Vinca alkaloids or analogues, Podophyllotoxin derivatives, Camptothecan analogs, Colchicine derivatives, Taxanes, other plant alkaloids or natural products, Actinomycines, Anthracyclines or related substances, other cytotoxic antibiotics, Platinum compounds, Methylhydrazines, Kinase inhibitors, Enzymes, Histone Deacetylase Inhibitors, Retinoids, Immune checkpoint inhibitors, antibodies and other molecular target drug.

[0061]

"Chemotherapeutic agent" also includes compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA(registered), Genentech/OSI Pharm.), bortezomib (VELCADE(registered), Millennium Pharm.),

disulfiram, epigallocatechin gallate, salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX(registered), AstraZeneca), sunitib (SUTENT(registered), Pfizer/Sugen), letrozole (FEMARA(registered), Novartis), imatinib mesylate (GLEEVEC(registered), Novartis), finasunate (VATALANIB(registered), Novartis), oxaliplatin (ELOXATIN(registered), Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE(registered), Wyeth), Lapatinib (TYKERB(registered), GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR(registered), Bayer Labs), gefitinib (IRESSA(registered), AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN(registered) cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5α-reductases including finasteride and dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (Angew Chem. Intl. Ed. Engl. 1994 33: 183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzino statin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN(registered) (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin,

mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK(registered) polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE(registered) (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, I11.), and TAXOTERE(registered) (docetaxel, doxetaxel; Sanofi-Aventis); chloranmbucil; GEMZAR(registered) (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE(registered) (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA(registered)); ibandronate; CPT-11 ; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0062]

Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath),

bevacizumab (AVASTIN(registered), Genentech); cetuximab (ERBITUX(registered), Imclone); panitumumab (VECTIBIX(registered), Amgen), rituximab (RITUXAN(registered), Genentech/Biogen Idec), pertuzumab (OMNITARG(registered), 2C4, Genentech), trastuzumab (HERCEPTIN(registered), Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG(registered), Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti-interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG1 λ antibody genetically modified to recognize interleukin-12 p40 protein.

[0063]

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. In one embodiment, growth inhibitory agent is growth inhibitory antibody that prevents or reduces proliferation of a cell expressing an antigen to which the antibody binds. In another embodiment, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-

fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE(registered), Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL(registered), Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0064]

By "radiation therapy" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0065]

A "subject" or an "individual" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[0066]

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0067]

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or

nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0068]

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0069]

The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[0070]

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain

may be referred to as "V_H." The variable domain of the light chain may be referred to as "V_L." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[0071]

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hyper variable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0072]

The "light chains" of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains.

[0073]

The term IgG "isotype" or "subclass" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[0074]

Depending on the amino acid sequences of the constant domains of their heavy chains,

antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, γ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[0075]

The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[0076]

A "naked antibody" for the purposes herein is an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0077]

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, scFv and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0078]

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0079]

"Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0080]

The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0081]

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, e.g., Pluckthuen, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0082]

The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9: 129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9: 129-134 (2003).

[0083]

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0084]

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler and Milstein, Nature, 256:495-97 (1975); Hongo et al, Hybridoma, 14 (3): 253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage-display technologies (see, e.g., Clackson et al, Nature, 352: 624-628 (1991); Marks et al, J. Mol. Biol. 222: 581-597 (1992); Sidhu et al, J. Mol. Biol. 338(2): 299-310 (2004); Lee et al, J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993) ; Bruggemann et al, Year in Immunol. 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., Bio/Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

[0085]

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, e.g., U.S. Patent No. 4,816,567; and Morrison et al, Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). Chimeric antibodies

include PRIMATTZED(registered) antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest.

[0086]

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hyper variable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al, Nature 321:522-525 (1986); Riechmann et al, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, e.g., Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1: 105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

[0087]

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal

antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol., 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice (see, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE(trade mark) technology). See also, for example, Li et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0088]

A "species-dependent antibody" is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody "binds specifically" to a human antigen (e.g., has a binding affinity (Kd) value of no more than about $1x10^{-7}$M, preferably no more than about $1x10^{-8}$M and preferably no more than about $1x10^{-9}$M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at least about 50 fold, or at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

[0089]

The term "hyper variable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hyper variable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (HI, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al., Immunity 13:37-45 (2000); Johnson and Wu, in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, e.g., Hamers-Casterman et al., Nature 363:446-448 (1993); Sheriff et al., Nature Struct. Biol. 3:733-736 (1996).

[0090]

A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

[0091]

[TABLE 1]

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0092]

HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions.

[0093]

"Framework" or "FR" residues are those variable domain residues other than the HVR residues as herein defined.

[0094]

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0095]

The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., *supra*). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0096]

The expression "linear antibodies" refers to the antibodies described in Zapata et al. (1995 Protein Eng, 8(10): 1057-1062). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0097]

As use herein, the term "binds", "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous

population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of ≤ 1 μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0098]

The term "loading period" herein refers to a time period within which one or more doses of a therapeutic agent or therapeutic agents are administered to an individual, and is followed by maintenance period or maintenance dosing period. Within the loading period of the combination therapy, only the GPC3 targeting agent or both the GPC3 targeting agent and the PD-1 axis binding antagonist may be administered. The amount of each dose of therapeutic agent(s) administered within the loading period exceeds or the same as the amount of each dose of therapeutic agent(s) administered within the maintenance period, and/or the dose(s) within the loading period are administered more frequently than the dose(s) within the maintenance period, so as to achieve the desired steady-state concentration of the therapeutic agent earlier than can be achieved within the maintenance period.

[0099]

The term "maintenance period" or "maintenance dosing period" herein refers to a time period within which one or more doses of a therapeutic agent or therapeutic agents administered to an individual. Usually, within the maintenance period, one or more doses of a therapeutic agent or therapeutic agents administered at spaced administration intervals as described herein. Maintenance period may be extended as long as appropriate, for example, the maintenance period is so long that dose(s) of a therapeutic agent or therapeutic agents may be administered 1 time, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times or more, 15 times or more, 20 times or more, 30 times or more, 40 times or more, 50 times or more, or 100 times or more.

[0100]

The term "administration interval" (an interval between individual administrations) herein refers an interval between administration of the nth dose (n is an integer of 1 or greater) and administration of the (n+1)th dose. The "nth dose" herein may be any dose administered within the loading period or any dose administered within maintenance period. In one embodiment, the administration interval of a PD-1 axis binding antagonist and/or a GPC3-targeting agent of the present disclosure is a minimum period of 1 day or longer and a maximum period of 3 months or shorter, and may specifically be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 10 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 1 month, 2 months, or 3 months, for example. The administration interval may also be expressed differently, and may be specified as, e.g., once daily, once weekly, or once in 3 weeks, or may be specified as, e.g., every day, every week, or every 3 weeks, for example. The administration interval may also be expressed differently, and may be specified as, e.g., on day 1 of 7-day cycle or on day 1 of 21-day cycle, for example.

[II. PD-1 Axis Binding Antagonists]

[0101]

Provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent. Also provided herein is a method of enhancing immune responses against tumor cells in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent. For example, enhanced immune responses against tumor cells includes infiltration of immune cells including macrophages and multinucleated giant cells in to tumor tissues. For another example, enhanced immune responses against tumor cells includes increase of CD45-positive lymphocytes, CD3ε-positive lymphocytes and CD8-positive T lymphocytes in tumor infiltrated lymphocytes (TILs). For example, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. PD-1 (programmed death 1) is also referred to in the art as "programmed cell death 1", PDCD1, CD279 and SLEB2. PD-L1 (programmed death ligand 1) is also referred to in the art as "programmed cell death 1 ligand 1", PDCD1LG1, CD274,

B7-H, and PD-L1. PD-L2 (programmed death ligand 2) is also referred to in the art as "programmed cell death 1 ligand 2", PDCD1LG2, CD273, B7-DC, Btdc, and PDL2. In some embodiments, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2.

[0102]

In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, the PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0103]

In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (lambrolizumab), and CT-011 (pidilizumab). In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence)). In some embodiments, the PD-1 binding antagonist is AMP-224 or AMP-514 (MEDI0680). In some embodiments, the PD-1 binding antagonist is selected from the group consisting of PDR001, REGN2810, BGB A317 SHR-1210, BI 754091, JNJ-63723283, MGA012, PF-06801591, JS-001, and TSR-042. In some embodiments, the PD-L1 binding antagonist is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 binding antagonist is selected from the group consisting of YW243.55.S70, Atezolizumab, MPDL3280A, MDX-1105, avelumab, MEDI4736 (durvalumab), KN035, CX-072, LY33000054, and FAZ053. Antibody YW243.55.S70 is an anti-PD-L1 described in WO2010/077634. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874. Avelumab is an anti-PDL1 antibody described in WO2013079174. MEDI4736 (durvalumab), is an anti-PD-L1 monoclonal antibody described in WO2011/066389 and US2013/034559. Nivolumab, also known as MDX-1106-

04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO(registered), is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA(registered), and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0104]

In some embodiments, the PD-1 axis binding antagonist is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PD-L1 antibody is a monoclonal antibody. In some embodiments, the anti-PD-L1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the anti-PD-L1 antibody is a humanized antibody. In some embodiments, the anti-PD-L1 antibody is a human antibody.

[0105]

Examples of anti-PD-L1 antibodies useful for the methods of this invention, and methods for making thereof are described in PCT patent application WO2010/077634, WO2007/005874, WO2011/066389, and US2013/034559, which are incorporated herein by reference. The anti-PD-L1 antibodies useful in this invention, including compositions containing such antibodies, may be used in combination with a GPC3 targeting agent to treat cancer.

Anti-PD-1 antibodies

[0106]

In some embodiments, the anti-PD-1 antibody is MDX-1106. Alternative names for "MDX-1106" include MDX-1106-04, ONO-4538, BMS-936558 or Nivolumab. In some embodiments, the anti-PD-1 antibody is Nivolumab (CAS Registry Number: 946414-94-4). In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:1 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:2. In a still further embodiment, provided is an

isolated anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence set forth in SEQ ID NO:1, and

(b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence set forth in SEQ ID NO:2.

Anti-PD-L1 antibodies

[0107]

In some embodiments, the antibody in the formulation comprises at least one tryptophan (e.g., at least two, at least three, or at least four) in the heavy and/or light chain sequence. In some embodiments, amino acid tryptophan is in the CDR regions, framework regions and/or constant regions of the antibody. In some embodiments, the antibody comprises two or three tryptophan residues in the CDR regions. In some embodiments, the antibody in the formulation is an anti-PD-L1 antibody. PD-L1 (programmed death ligand 1), also known as PD-L1, B7-H1, B7-4, CD274, and B7-H, is a transmembrane protein, and its interaction with PD-1 inhibits T-cell activation and cytokine production. In some embodiments, the anti-PD-L1 antibody described herein binds to human PD-L1. Examples of anti-PD-L1 antibodies that can be used in the methods described herein are Atezolizumab, or anti-PD-L1 antibodies described in PCT patent application WO 2010/077634 A1 and US 8,217,149, which are incorporated herein by reference.

[0108]

In some embodiments, the anti-PD-L1 antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PD-L1 antibody is a monoclonal antibody. In some embodiments, the anti-PD-L1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In some embodiments, the anti-PD-L1 antibody is a humanized antibody. In some embodiments, the anti-PD-L1 antibody is a human antibody.

[0109]

Anti-PD-L1 antibodies described in WO 2010/077634 A1 and US 8,217,149 may be used in the methods described herein. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain variable region sequence of SEQ ID NO:3 and/or a light chain variable region sequence of SEQ ID NO:4. In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence set forth in SEQ ID NO:3, and

(b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence set forth in SEQ ID NO:4.

[0110]

In one embodiment, the anti-PD-L1 antibody comprises a heavy chain variable region polypeptide comprising an HVR-H1, HVR-H2 and HVR-H3 sequence, wherein:

(a) the HVR-H1 sequence is $GFTFSX_1SWIH$ (SEQ ID NO:5);

(b) the HVR-H2 sequence is $AWIX_2PYGGSX_3YYADSVKG$ (SEQ ID NO:6);

(c) the HVR-H3 sequence is RHWPGGFDY (SEQ ID NO:7);

further wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S. In one specific aspect, X_1 is D; X_2 is S and X_3 is T.

[0111]

In another aspect, the polypeptide further comprises variable region heavy chain framework sequences juxtaposed between the HVRs according to the formula: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the framework sequences are VH subgroup III consensus framework. In a still further aspect, at least one of the framework sequences is the following:

HC-FR1 is EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:8)

HC-FR2 is WVRQAPGKGLEWV (SEQ ID NO:9)
HC-FR3 is RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)
HC-FR4 is WGQGTLVTVSA (SEQ ID NO:11).

[0112]

In a still further aspect, the heavy chain polypeptide is further combined with a variable region light chain comprising an HVR-L1, HVR-L2 and HVR-L3, wherein:

(a) the HVR-L1 sequence is $RASQX_4X_5X_6TX_7X_8A$ (SEQ ID NO:12);
(b) the HVR-L2 sequence is $SASX_9LX_{10}S$ (SEQ ID NO:13);
(c) the HVR-L3 sequence is $QQX_{11}X_{12}X_{13}X_{14}PX_{15}T$ (SEQ ID NO:14);

wherein: X_4 is D or V; X_5 is V or I; X_6 is S or N; X_7 is A or F; X_8 is V or L; X_9 is F or T; X_{10} is Y or A; X_{11} is Y, G, F, or S; X_{12} is L, Y, F or W; X_{13} is Y, N, A, T, G, F or I; X_{14} is H, V, P, T or I; X_{15} is A, W, R, P or T. In a still further aspect, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H; X_{15} is A.

[0113]

In a still further aspect, the light chain further comprises variable region light chain framework sequences juxtaposed between the HVRs according to the formula: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the framework sequences are VL kappa I consensus framework. In a still further aspect, at least one of the framework sequence is the following:

LC-FR1 is DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:15)
LC-FR2 is WYQQKPGKAPKLLIY (SEQ ID NO:16)
LC-FR3 is GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:17)
LC-FR4 is FGQGTKVEIKR (SEQ ID NO:18).

[0114]

In another embodiment, provided is an isolated anti-PD-L1 antibody or antigen binding fragment comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises and HVR-H1, HVR-H2 and HVR-H3, wherein further:
(i) the HVR-H1 sequence is $GFTFSX_1SWIH$; (SEQ ID NO:5)
(ii) the HVR-H2 sequence is $AWIX_2PYGGSX_3YYADSVKG$ (SEQ ID NO:6)

(iii) the HVR-H3 sequence is RHWPGGFDY, and (SEQ ID NO:7)

(b) the light chain comprises and HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is $RASQX_4X_5X_6TX_7X_8A$ (SEQ ID NO: 12)

(ii) the HVR-L2 sequence is $SASX_9LX_{10}S$; and (SEQ ID NO: 13)

(iii) the HVR-L3 sequence is $QQX_{11}X_{12}X_{13}X_{14}PX_{15}T$; (SEQ ID NO: 14)

wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S; X_4 is D or V; X_5 is V or I; X_6 is S or N; X_7 is A or F; X_8 is V or L; X_9 is F or T; X_{10} is Y or A; X_{11} is Y, G, F, or S; X_{12} is L, Y, F or W; X_{13} is Y, N, A, T, G, F or I; X_{14} is H, V, P, T or I; X_{15} is A, W, R, P or T. In a specific aspect, X_1 is D; X_2 is S and X_3 is T. In another aspect, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H; X_{15} is A. In yet another aspect, X_1 is D; X_2 is S and X_3 is T, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H and X_{15} is A.

[0115]

In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, III or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0116]

In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3, IgG4. In a still further specific aspect, the human

constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0117]

In yet another embodiment, provided is an anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, or

(b) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

[0118]

In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, III or IV subgroup sequence. In

a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0119]

In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0120]

In another further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence set forth in SEQ ID NO:25, and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence set forth in SEQ ID NO:4.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more

of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSS (SEQ ID NO:27).

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, III or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0121]

In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect, the minimal effector function results from production in prokaryotic cells. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0122]

In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences is the following:

HC-FR1 is	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	(SEQ ID NO:29)
HC-FR2 is	WVRQAPGKGLEWVA	(SEQ ID NO:30)

HC-FR3 is RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)
HC-FR4 is WGQGTLVTVSS (SEQ ID NO:27).

[0123]

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, III or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences is the following:

LC-FR1 is DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 15)
LC-FR2 is WYQQKPGKAPKLLIY (SEQ ID NO: 16)
LC-FR3 is GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 17)
LC-FR4 is FGQGTKVEIK (SEQ ID NO: 28).

[0124]

In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0125]

In yet another embodiment, provided is an anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(c) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, and/or

(d) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence

having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

[0126]

In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSSASTK (SEQ ID NO:31).

[0127]

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, III or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18. In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0128]

In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence set forth in SEQ ID NO:26, or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence set forth in SEQ ID NO:4.

[0129]

In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:4. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 26. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

[0130]

In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence set forth in SEQ ID NO:32, and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence set forth in SEQ ID NO:33.

In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence set forth in SEQ ID NO: 55, and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence set forth in SEQ ID NO:33.

[0131]

In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32 or 55. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32 or 55.

[0132]

In some embodiments, the isolated anti-PD-L1 antibody is aglycosylated. Glycosylation of

antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites form an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or threonine residue within the glycosylation site another amino acid residue (e.g., glycine, alanine or a conservative substitution).

[0133]

In any of the embodiments herein, the isolated anti-PD-L1 antibody can bind to a human PD-L1, for example a human PD-L1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof.

[0134]

In a still further embodiment, provided is an isolated nucleic acid encoding any of the antibodies described herein. In some embodiments, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-PD-L1 antibodies. In a still further specific aspect, the vector is in a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a still further specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese hamster ovary (CHO) cell.

[0135]

The antibody or antigen binding fragment thereof, may be made using methods known in the art, for example, by a process comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-PD-L1 antibodies or antigen-binding fragment

in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

[III. GPC3 targeting agents]

[0136]

Provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent. Also provided herein is a method of enhancing immune responses against tumor cells in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent. For example, enhanced immune responses against tumor cells includes infiltration of immune cells including macrophages and multinucleated giant cells into tumor tissues. For another example, enhanced immune responses against tumor cells includes increase of CD45-positive lymphocytes, CD3ε-positive lymphocytes and CD8-positive T lymphocytes in tumor infiltrated lymphocytes (TILs).

[0137]

In some embodiments, the GPC3 targeting agent is an anti-GPC3 antibody. Provided herein are antibodies that bind to a human glypican 3 (GPC3). Alternative names for "GPC3" include SGB, DGSX, MXR7, SDYS, SGBS, OCI-5, SGBS1 and GTR2-2. The term "GPC3" as used herein, refers to any native GPC3 from any human source. The term encompasses "full-length" and unprocessed GPC3 as well as any form of GPC3 that results from processing in the cell (e.g., mature protein), including, but not limited to a C-terminal peptide of GPC3. The term also encompasses naturally occurring variants and isoforms of GPC3, e.g., splice variants or allelic variants. For example, descriptions of GPC3 and sequences are provided at UniProtKB/Swiss-Prot Accession No. P51654.1.

[0138]

In some embodiments, the anti-GPC3 antibody binds to GPC3 and inhibits cell proliferation or growth of cancer cells. In some embodiments, the anti-GPC3 antibody is codrituzumab.

[0139]

In some embodiments, an anti-GPC3 antibody can include an antibody-drug conjugate

(ADC) (WO2007/137170) comprising a 1G12 antibody (WO2003/100429) (sold under catalog No. B0134R by BioMosaics Inc.) conjugated with a cytotoxic substance.

[0140]

In some embodiments, an anti-GPC3 antibody is a humanized anti-GPC3 antibody described in WO2006/006693 or WO2007/047291.

[0141]

In some embodiments, an anti-GPC3 antibody include a humanized anti-GPC3 antibody described in WO2006/006693 or WO2009/041062. In some embodiments, provided is a humanized anti-GPC3 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises and HVR-H1, HVR-H2 and HVR-H3, wherein further:

(i) the HVR-H1 sequence is DYSMH (SEQ ID NO:34)

(ii) the HVR-H2 sequence is WINTETGEPTYADDFKG (SEQ ID NO:35)

(iii) the HVR-H3 sequence is LY (SEQ ID NO:36)

(b) the light chain comprises and HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is KSSQSLLHSDGKTFLN (SEQ ID NO:37)

(ii) the HVR-L2 sequence is LVSRLDS (SEQ ID NO:38)

(iii) the HVR-L3 sequence is CQGTHFPRT (SEQ ID NO:39).

In a specific aspect, the anti-GPC3 antibody is humanized. The humanized anti-GPC3 antibody can be prepared using, as templates for humanization, appropriately selected human framework sequences having high sequence identity to a heavy chain framework sequence represented by SEQ ID NO:40 or a light chain framework sequence represented by SEQ ID NO:41.

[0142]

In some embodiments, provided here is an anti-GPC3 chimeric antibody or a humanized anti-GPC3 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises and HVR-H1, HVR-H2 and HVR-H3, wherein further:

(i) the HVR-H1 sequence is DYEMH (SEQ ID NO:42)

(ii) the HVR-H2 sequence is ALDPKTGDTAYSQKFKG (SEQ ID NO:43)

(iii) the HVR-H3 sequence is FYSYTY (SEQ ID NO:44)

(b) the light chain comprises and HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is RSSQSLVHSNRNTYLH (SEQ ID NO:45)

(ii) the HVR-L2 sequence is KVSNRFS (SEQ ID NO:46)

(iii) the HVR-L3 sequence is SQNTHVPPT (SEQ ID NO:47).

The humanized anti-GPC3 antibody can be prepared using, as templates for humanization, appropriately selected human framework sequences having high sequence identity to a heavy chain framework sequence represented by SEQ ID NO:48 or a light chain framework sequence represented by SEQ ID NO:49.

[0143]

In a further embodiment, provided here is a humanized anti-GPC3 antibody capable of binding to an epitope to which a second antibody can bind, wherein said second antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises and HVR-H1, HVR-H2 and HVR-H3, wherein further:

(i) the HVR-H1 sequence is DYEMH (SEQ ID NO:42)

(ii) the HVR-H2 sequence is ALDPKTGDTAYSQKFKG (SEQ ID NO:43)

(iii) the HVR-H3 sequence is FYSYTY (SEQ ID NO:44)

(b) the light chain comprises and HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is RSSQSLVHSNRNTYLH (SEQ ID NO:45)

(ii) the HVR-L2 sequence is KVSNRFS (SEQ ID NO:46)

(iii) the HVR-L3 sequence is SQNTHVPPT (SEQ ID NO:47).

[0144]

In a further embodiment, provided is a humanized anti-GPC3 antibody comprising a heavy chain variable region selected from the group of heavy chain variable regions represented by SEQ ID NOs:50 and a light chain variable region represented by SEQ ID NO:51. In a further alternative non-limiting aspect, provided is a humanized anti-GPC3 antibody comprising a heavy chain variable region selected from the group of heavy chain variable regions represented by SEQ ID NO:50 and a light chain variable region selected from the group of light chain variable regions represented by SEQ ID NO:52.

[0145]

In a still further embodiment, provided is a humanized anti-GPC3 antibody comprising a heavy chain variable region represented by SEQ ID NO:53 and a light chain variable region represented by SEQ ID NO:54.

[0146]

Alternative examples of the anti-GPC3 antibody of the present invention include an anti-GPC3 antibody having cytotoxic activity. In the present invention, non-limiting examples of the cytotoxic activity include antibody-dependent cell-mediated cytotoxicity or antibody-dependent cellular cytotoxicity (ADCC) activity, complement-dependent cytotoxicity (CDC) activity, and cytotoxic activity based on T cells. In the present invention, the CDC activity means cytotoxic activity brought about by the complement system. On the other hand, the ADCC activity means the activity of damaging target cells by, for example, immunocytes, through the binding of the immunocytes via Fcγ receptors expressed on the immunocytes to the Fc regions of antigen-binding molecules comprising antigen-binding domains capable of binding to membrane molecules expressed on the cell membranes of the target cells. Whether or not the antigen-binding molecule of interest has ADCC activity or has CDC activity can be determined by a method known in the art (e.g., Current protocols in Immunology, Chapter 7. Immunologic studies in humans, Coligan et al., ed. (1993)).

[0147]

In some embodiments, alternative examples of the anti-GPC3 antibody of the present invention include an anti-GPC3 antibody conjugated with a cytotoxic substance. Such an anti-GPC3 antibody-drug conjugate (ADC) is specifically disclosed in, for example, WO2007/137170, though the conjugate of the present invention is not limited to those described therein. Specifically, the cytotoxic substance may be any of chemotherapeutic agents listed below or may be a compound disclosed in Alley et al. (Curr. Opin. Chem. Biol. (2010) 14, 529-537) or WO2009/140242. Antigen-binding molecules are conjugated with these compounds via appropriate linkers or the like.

[0148]

In some embodiments, alternative examples of the anti-GPC3 antibody of the present invention include an anti-GPC3 antibody comprises an FcγR-binding modified Fc region having higher binding activity against Fcγ receptors than that of the Fc region of native

human IgG against Fcγ receptors. The modification can include amino acid modification at any position as long as the resulting Fc region has higher binding activity against Fcγ receptors than that of the native human IgG Fc region against Fcγ receptors. When the antigen-binding molecule contains a human IgG1 Fc region as a human Fc region, the modification preferably allows the Fc region to contain a fucose-containing sugar chain as a sugar chain bound to position 297 (EU numbering) and is effective for producing higher binding activity against Fcγ receptors than that of the native human IgG Fc region against Fcγ receptors. Such amino acid modification has been reported in, for example, International Publication Nos. WO2007/024249, WO2007/021841, WO2006/031370, WO2000/042072, WO2004/029207, WO2004/099249, WO2006/105338, WO2007/041635, WO2008/092117, WO2005/070963, WO2006/020114, WO2006/116260, WO2006/023403, and WO2014/097648.

[0149]

In some embodiments, the Fc region contained in the anti-GPC3 antibody provided by the present invention can also include an Fc region modified such that a higher proportion of fucose-deficient sugar chains is bound to the Fc region or a higher proportion of bisecting N-acetylglucosamine is added to the Fc region in the composition of sugar chains bound to the Fc region. WO2006/046751 and WO2009/041062 disclose specific examples of the anti-GPC3 antibody comprising the Fc region modified such that a higher proportion of fucose-deficient sugar chains is bound to the Fc region or a higher proportion of bisecting N-acetylglucosamine is added to the Fc region in the composition of sugar chains bound to the Fc region.

[0150]

In some embodiments, the anti-GPC3 antibody that may be used in the present invention include an anti-GPC3 antibody having an amino acid residue modified to alter its isoelectric point (pI). Preferred examples of the "alteration of the electric charge of an amino acid residue" in the anti-GPC3 antibody are described in WO2009/041062.

[0151]

In some embodiments, the anti-GPC3 antibody includes a modified form of the antibody that has received a posttranslational modification of the polypeptide constituting the primary

structure of the anti-GPC3 antibody. The posttranslational modification of a polypeptide refers to chemical modification given to the polypeptide translated during polypeptide biosynthesis. For example, an anti-GPC3 antibody that has received the modification of N-terminal glutamine to pyroglutamic acid by pyroglutamylation is also included in the anti-GPC3 antibody of the present invention, as a matter of course. Also, for example, a posttranslationally modified anti-GPC3 antibody comprising heavy and light chains or heavy chains linked via a "disulfide bond", which means a covalent bond formed between two sulfur atoms is included in the anti-GPC3 antibody of the present invention. A thiol group contained in an amino acid cysteine can form a disulfide bond or crosslink with a second thiol group. In general IgG molecules, CH1 and CL regions are linked via a disulfide bond, and two polypeptides constituting heavy chains are linked via a disulfide bond between cysteine residues at positions 226 and 229 based on the EU numbering. A posttranslationally modified anti-GPC3 antibody having such a linkage via a disulfide bond is also included in the anti-GPC3 antibody of the present invention.

[0152]

In a still further embodiment, provided is an isolated nucleic acid encoding any of the antibodies described herein. In some embodiments, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-GPC3 antibodies. In a still further specific aspect, the vector is in a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a still further specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese hamster ovary (CHO) cell.

[0153]

The antibody or antigen binding fragment thereof, may be made using methods known in the art, for example, by a process comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-GPC3 antibodies or antigen-binding fragment in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

[IV. Antibody Preparation]

[0154]

The antibody described herein is prepared using techniques available in the art for generating antibodies, exemplary methods of which are described in more detail in the following sections.

[0155]

The antibody is directed against an antigen of interest (i.e., PD-L1 (such as a human PD-L1) or GPC3 (such as a human glypican 3)). Preferably, the antigen is a biologically important polypeptide and administration of the antibody to a mammal suffering from a disorder can result in a therapeutic benefit in that mammal.

[0156]

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 150nM, ≤ 100nM, ≤ 50nM, ≤ 10nM, ≤ 1nM, ≤ 0.1nM, ≤ 0.01nM, or ≤ 0.001nM (e.g., 10^{-8}M or less, e.g., from 10^{-8}M to 10^{-13}M, e.g., from 10^{-9}M to 10^{-13}M).

[0157]

In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER(registered) multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest. The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20(registered)) in PBS. When the plates have dried, 150μl/well of scintillant (MICROSCINT-20(trade mark); Packard) is added, and the plates are counted on a

TOPCOUNT(trade mark) gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0158]

According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE(registered)-2000 or a BIACORE(registered)-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20(trade mark)) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE (registered) Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds $10^6 M^{-1} s^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO(trade mark) spectrophotometer (ThermoSpectronic) with a stirred cuvette.

(i) Antibody Fragments

[0159]

Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances, there are advantages of using

antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

[0160]

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form F(ab')2 fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, F(ab') 2 fragments can be isolated directly from recombinant host cell culture. Fab and F(ab') 2 fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO93/16185; U.S. Patent Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Patent No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

(ii) Single-Domain Antibodies

[0161]

In some embodiments, an antibody of the invention is a single-domain antibody. A single-domain antibody is a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody

(Domantis, Inc., Waltham, Mass.; see, e.g., U.S. Patent No. 6,248,516). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

(iii) Antibody Variants

[0162]

In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

(iv) Substitution, Insertion, and Deletion Variants

[0163]

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "Preferred Substitutions." More substantial changes are provided in Table 1 under the heading of "Exemplary Substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0164]

[TABLE 2] Exemplary Substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0165]

Amino acids may be grouped according to common side-chain properties:

a. hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

b. neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

c. acidic: Asp, Glu;

d. basic: His, Lys, Arg;

e. residues that influence chain orientation: Gly, Pro;

f. aromatic: Trp, Tyr, Phe.

[0166]

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0167]

One type of substitutional variant involves substituting one or more hyper variable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0168]

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0169]

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody

to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0170]

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0171]

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

(v) Glycosylation variants

[0172]

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such

that one or more glycosylation sites is created or removed.

[0173]

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0174]

In one embodiment, antibody variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, antibodies are contemplated herein that have reduced fusose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (e.g., a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, the antibody is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In certain embodiments, the antibody is one wherein none of the N-linked glycans thereon comprise fucose, i.e., wherein the antibody is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence

variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US2003/0157108 (Presta, L.); US2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US2003/0157108; WO2000/61739; WO2001/29246; US2003/0115614; US2002/0164328; US2004/0093621; US2004/0132140; US2004/0110704; US2004/0110282; US2004/0109865; WO2003/085119; WO2003/084570; WO2005/035586; WO2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87:614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pattent Publication No. US2003/0157108, Presta, L; and WO2004/056312, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. Biotech. Bioeng. 87:614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107).

[0175]

Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); US2005/0123546 (Umana et al.), and Ferrara et al., Biotechnology and Bioengineering, 93(5):851-861 (2006). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO1997/30087 (Patel et al.); WO1998/58964 (Raju, S.); and WO1999/22764 (Raju, S.).

[0176]

In certain embodiments, the antibody variants comprising an Fc region described herein are capable of binding to an FcγRIII. In certain embodiments, the antibody variants comprising an Fc region described herein have ADCC activity in the presence of human effector cells or have increased ADCC activity in the presence of human effector cells compared to the

otherwise same antibody comprising a human wild-type IgG1 Fc region.

(vi) Fc region variants

[0177]

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0178]

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. Proc. Nat'lAcad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I. et al., Proc. Nat'lAcad. Sci. USA 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI(trade mark) non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96(registered) non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. Proc. Nat'lAcad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q

and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO2006/029879 and WO2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M.S. et al., Blood 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., Int'l. Immunol. 18(12):1759-1769 (2006)).

[0179]

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0180]

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO2004/056312, and Shields et al., J. Biol. Chem. 9(2):6591-6604 (2001).)

[0181]

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In an exemplary embodiment, the antibody comprising the following amino acid substitutions in its Fc region: S298A, E333A, and K334A.

[0182]

In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO99/51642, and Idusogie et al. J. Immunol. 164:4178-4184 (2000).

[0183]

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934 (Hinton et al.)). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants.

(vii) Antibody Derivatives

[0184]

The antibodies of the invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. In certain embodiments, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

(viii) Vectors, Host Cells, and Recombinant Methods

[0185]

Antibodies may also be produced using recombinant methods. For recombinant production of an anti-antigen antibody, nucleic acid encoding the antibody is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(ix) Selecting Biologically Active Antibodies

[0186]

Antibodies produced as described above may be subjected to one or more "biological activity" assays to select an antibody with beneficial properties from a therapeutic perspective or selecting formulations and conditions that retain biological activity of the antibody. The antibody may be tested for its ability to bind the antigen against which it was raised. For example, methods known in the art (such as ELISA, Western Blot, etc.) may be used.

[0187]

For example, for an anti-PD-L1 antibody, the antigen binding properties of the antibody can be evaluated in an assay that detects the ability to bind to PD-L1. In some embodiments, the binding of the antibody may be determined by saturation binding; ELISA; and/or competition assays (e.g. RIA's), for example. Also, the antibody may be subjected to other biological activity assays, e.g., in order to evaluate its effectiveness as a therapeutic. Such assays are known in the art and depend on the target antigen and intended use for the antibody. For example, the biological effects of PD-L1 blockade by the antibody can be assessed in CD8+T cells, a lymphocytic choriomeningitis virus (LCMV) mouse model and/or a syngeneic tumor model e.g., as described in US Patent 8,217,149.

[0188]

To screen for antibodies which bind to a particular epitope on the antigen of interest (e.g., those which block binding of the anti-PD-L1 antibody of the example to PD-L1), a routine cross-blocking assay such as that described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping, e.g. as described in Champe et al., J. Biol. Chem. 270:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

[V. Pharmaceutical Compositions and Formulations]

Pharmaceutical Compositions and Formulations for the combination therapy

[0189]

Also provided herein are pharmaceutical compositions and formulations comprising a PD-1 axis binding antagonist (such as an anti-PD-L1 antibody) and/or a GPC3 targeting therapy (such as an anti-GPC3 antibody) as an active ingredient and a pharmaceutically acceptable carrier.

[0190]

"Combination" as described herein refers to a combination of active ingredients for combination use, and includes both modes where separate substances are used in combination upon administration or where they are provided as a mixture (combination preparation).

[0191]

"Combination therapy" as described herein refers to co-administrating more than one active ingredients to an individual, and includes both modes where separate substances are used in combination upon administration or where they are provided as a mixture (combination preparation). In certain embodiments, the combination therapy is a combination therapy using a PD-1 axis binding antagonist and a GPC3-targeting agent. In other embodiments, the combination therapy optionally includes one or more secondary active ingredients, e.g., chemotherapeutic agents, as described herein. The combination therapy can be performed according to a specific dosage regimen, for example, one as described below.

[0192]

Pharmaceutical compositions and formulations as described herein can be prepared by mixing active ingredients (such as an anti-PD-L1 antibody and/or an anti-GPC3 antibody) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX(registered), Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0193]

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer. In some embodiments, the anti-PD-L1 antibody described herein is in a formulation comprising the antibody in a concentration of about 60 mg/mL, histidine acetate in a concentration of about 20 mM, sucrose in a concentration of about 120 mM, and polysorbate (e.g., polysorbate 20)

in a concentration of 0.04% (w/v), and the formulation has a pH of about 5.8. In some embodiments, the anti-PD-L1 antibody described herein is in a formulation comprising the antibody in a concentration of about 125 mg/mL, histidine acetate in a concentration of about 20 mM, sucrose is in a concentration of about 240 mM, and polysorbate (e.g., polysorbate 20) in a concentration of 0.02% (w/v), and the formulation has a pH of about 5.5.

[0194]

The composition and formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0195]

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0196]

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

Dosage regimen for the combination therapy

[0197]

In the present disclosure, the dosage (dose) may be expressed not only in terms of a fixed dose (mg/body), but also a dose calculated in terms of body weight (mg/kg), corresponding to a dose calculated per patient body.

[0198]

In certain embodiments, the dosage of PD-1 axis binding antagonist will be in the range of about 0.01 to about 50 mg/kg of patient body weight whether by one or more administrations. In some embodiments, the antibody used is about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In some embodiments, the antibody is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, an anti-PD-L1 antibody described herein is administered to a human at a dose of about 100 mg to about 1400 mg, or about 1000 mg to about 1400mg, for example, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles. The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. The progress of this therapy is easily monitored by conventional techniques.

[0199]

In certain embodiments, the dosage of GPC3-targeting agent of the present invention can be determined to achieve a blood trough level equal to or higher than a predetermined level in the patient. Preferred examples of the blood trough level can include 150 mg/mL or higher, 160 mg/mL or higher, 170 mg/mL or higher, 180 mg/mL or higher, 190 mg/mL or higher, 200 mg/mL or higher, 210 mg/mL or higher, 220 mg/mL or higher, 230 mg/mL or higher, 240 mg/mL or higher, 250 mg/mL or higher, 260 mg/mL or higher, 270 mg/mL or higher, 280 mg/mL or higher, 290 mg/mL or higher, 300 mg/mL or higher, and 400 mg/mL or higher. More preferred examples thereof can include 200 mg/mL or higher.

[0200]

In some embodiments, dosage regimens of the combination therapy using a PD-1 axis binding antagonist and a GPC3-targeting agent may include a loading period and a maintenance period. In some embodiments, the GPC3-targeting agent only, or, both the PD-

1 axis binding agent and the GPC3-targeting agent are administered within the loading period. In some embodiments, both the PD-1 axis binding agent and the GPC3-targeting agent are administered within the loading period.

[0201]

In some embodiments, dosage regimens of the combination therapy using a PD-1 axis binding antagonist and a GPC3-targeting agent may include (i) a loading period within which the GPC3-targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered. In other embodiments, dosage regimens of the combination therapy using the PD-1 axis binding antagonist and the GPC3 targeting agent may include the maintenance period only. The appropriate dosage regimen may be determined by considering multiple factors, such as efficacy and/or safety. Also, the dosage regimen may be determined by considering convenience of patients, as long as it does not impair efficacy and safety of the combination therapy.

[0202]

In some embodiments, a pharmaceutical composition or formulation comprising a GPC3 targeting agent is administered intravenously or subcutaneously to an individual having cancer at a dose of 100 mg to 2,500 mg/body, preferably 500 mg to 2,000 mg/body, 1,000 mg to 2,000 mg/body, 1,300 mg to 1,900 mg/body, 1,400 mg to 1,800 mg/body, 1,500 mg to 1,700 mg/body within a loading period. In the most preferred embodiment, the pharmaceutical composition or formulation comprising the GPC3 targeting agent is administered intravenously or subcutaneously at a dose of 1,600 mg/body once or more times within a loading period. For the sake of avoiding any doubt, it is expressly stated that, for example, the recitation "100 mg to 2,500 mg/body" is intended to mean that all the dosages included within 100 mg and 2,500 mg/body are specifically and individually recited herein, with a variation of 0.1 mg/body, for example, 100 mg/body, 100.1 mg/body, 100.2 mg/body, 100.3 mg/body, ... 2499.8 mg/body, 2499.9 mg/body, and 2,500 mg/body.

[0203]

In some embodiments, the administration of the last dose within a loading period is separated in time from the administration of the first dose within a maintenance period by 2

days or more, 3 days or more, 4 days or more, 5 days or more, 6 days or more, 1 week or more, or 2 weeks or more. Preferably, the administration of the last dose within the loading period is separated in time from the administration of the first dose within the maintenance period by 2 days, 3 days or 4 days, preferably 3 days. In some embodiments, a GPC3 targeting agent antibody is administered once, twice, three times or more within the loading period, followed by the maintenance period. In other embodiments, the loading period is 3 weeks or less, preferably 2 weeks or less, more preferably 1 week or less. In the most preferred embodiment, the loading period is 1 week and the GPC3 targeting agent is administered twice within the loading period, followed by the maintenance period. Administration intervals within the loading period may be determined as appropriate, for example, 1 day, 2 days, 3 days, 4 days, 5 days or 6 days. Preferably, administration intervals within the loading period is 3 days or 4 days, more preferably, 3 days (i.e., a GPC3 targeting agent is administered on day 1, day 4, day 8, ... within the loading period).

[0204]

In some embodiments, a pharmaceutical composition or formulation comprising a PD-1 axis binding antagonist is administered to an individual having cancer in combination with a GPC3 targeting agent, and is administered intravenously or subcutaneously at a dose of 100 mg to 2,000 mg/body, preferably 900 mg to 1,500 mg/body, 1,000 mg to 1,400 mg/body, 1,100 mg to 1,300 mg/body within a maintenance period. In the most preferred embodiment, the pharmaceutical composition or formulation comprising the PD-1 axis binding antagonist is administered in combination with the GPC3 targeting agent, and is administered intravenously or subcutaneously at a dose of 1,200 mg/body within the maintenance period. For the sake of avoiding any doubt, it is expressly stated that, for example, the recitation "100 mg to 2,000 mg/body" is intended to mean that all the dosages included within 100 mg and 2,000 mg/body are specifically and individually recited herein, with a variation of 0.1 mg/body, for example, 100 mg/body, 100.1 mg/body, 100.2 mg/body, 100.3 mg/body, ... 1999.8 mg/body, 1999.9 mg/body, and 2,000 mg/body.

[0205]

In some embodiments, a pharmaceutical composition or formulation comprising a PD-1 axis binding antagonist is administered to an individual having cancer in combination with a GPC3 targeting agent, and is administered intravenously or subcutaneously at an

administration interval (interval between administrations) of 3 to 42 days within a maintenance period. An adequate administration interval of intravenous or subcutaneous injection may be determined within the range according to a condition of an individual. Specific administration interval of intravenous or subcutaneous injection is 3 days, 4 days, 5 days, 6 days, 7 days (1 week), 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days (2 weeks), 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days (3 weeks), 22 days, 23days, 24 days, 25 days, 26 days, 27 days, 28 days (4 weeks), 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days (5 weeks), 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, or 42 days (6 weeks). In preferred embodiment, the administration interval is 4 days to 35 days (5 weeks). In further preferred embodiment, the administration interval is 5 days to 31 days or 1 month. In the most preferred embodiment, the administration interval is 21 days (3 weeks).

[0206]

In some embodiments, a pharmaceutical composition or formulation comprising a GPC3 targeting agent is administered to an individual having cancer in combination with a PD-1 axis binding antagonist, and is administered intravenously or subcutaneously at a dose of 100 mg to 2,500 mg/body, preferably 500 mg to 2,000 mg/body, 1,000 mg to 2,000 mg/body, 1,300 mg to 1,900 mg/body, 1,400 mg to 1,800 mg/body, 1,500 mg to 1,700 mg/body within a maintenance period. In the most preferred embodiment, the pharmaceutical composition or formulation comprising the GPC3 targeting agent is administered in combination with the PD-1 axis binding antagonist, and is administered intravenously or subcutaneously at a dose of 1,600 mg/body in the maintenance period. For the sake of avoiding any doubt, it is expressly stated that, for example, the recitation "100 mg to 2,500 mg/body" is intended to mean that all the dosages included within 100 mg and 2,500 mg/body are specifically and individually recited herein, with a variation of 0.1 mg/body, for example, 100 mg/body, 100.1 mg/body, 100.2 mg/body, 100.3 mg/body, ... 2499.8 mg/body, 2499.9 mg/body, and 2,500 mg/body.

[0207]

In some embodiments, a pharmaceutical composition or formulation comprising a GPC3 targeting agent is administered to an individual having cancer in combination with a PD-1 axis binding antagonist, and is administered intravenously or subcutaneously at

administration interval (interval between administrations) of 3 to 42 days within a maintenance period. An adequate administration interval of intravenous or subcutaneous injection can be determined within the range according to a condition of an individual. Specific administration interval of intravenous or subcutaneous injection is 3 days, 4 days, 5 days, 6 days, 7 days (1 week), 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days (2 weeks), 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days (3 weeks), 22 days, 23days, 24 days, 25 days, 26 days, 27 days, 28 days (4 weeks), 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days (5 weeks), 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, or 42 days (6 weeks). In preferred embodiment, the administration interval is 4 days to 35 days (5 weeks). In further preferred embodiment, the administration interval is 5 days to 31 days or 1 month. In the most preferred embodiment, the administration interval is 7 days (1 week) or 21 days (3 weeks).

[0208]

In one embodiment,

(i) a loading period is 1 week or 2 weeks and a total amount of a GPC3 targeting agent administered within the loading period is approximately 1,600 mg/body to approximately 4,800 mg/body,

(ii) followed by a maintenance period within which a PD-1 axis binding antagonist is administered at a dose of 1,200 mg/body once per 3 weeks and the GPC3 targeting agent is administered at a dose of 1,600 mg/body once per 1 week or once per 3 weeks.

[0209]

In one embodiment,

(i) a loading period is 2 weeks and a GPC3 targeting agent is administered at a dose of 1,600 mg/body twice within the loading period,

(ii) followed by a maintenance period within which a PD-1 axis binding antagonist is administered at a dose of 1,200 mg/body once per 3 weeks and the GPC3 targeting agent is administered at a dose of 1,600 mg/body once per 1 week or once per 3 weeks.

[0210]

In one embodiment,

(i) a loading period is 1 week and a GPC3 targeting agent is administered at a dose of

1,600 mg/body twice within the loading period,

(ii) followed by a maintenance period within which a PD-1 axis binding antagonist is administered at a dose of 1,200 mg/body once per 3 weeks and the GPC3 targeting agent is administered at a dose of 1,600 mg/body once per 1 week or once per 3 weeks.

[0211]

In one embodiment,

(i) a loading period is 1 week and a GPC3 targeting agent is administered at a dose of 1,600 mg/body three times within the loading period,

(ii) followed by a maintenance period within which a PD-1 axis binding antagonist is administered at a dose of 1,200 mg/body once per 3 weeks and the GPC3 targeting agent is administered at a dose of 1,600 mg/body once per 1 week or once per 3 weeks.

[0212]

In one embodiment,

(i) a loading period is 2 weeks and a GPC3 targeting agent is administered at a dose of 1,600 mg/body three times within the loading period,

(ii) followed by a maintenance period within which a PD-1 axis binding antagonist is administered at a dose of 1,200 mg/body once per 3 weeks and the GPC3 targeting agent is administered at a dose of 1,600 mg/body once per 1 week or once per 3 weeks.

[0213]

In other embodiments, a maintenance period may include a treatment cycle. In some embodiments, the treatment cycle is a 3-week cycle in which a PD-1 axis binding antagonist is administered once per 3 weeks (i.e., once in one treatment cycle) and a GPC3 targeting agent is administered once per 1 week (i.e., 3 times in one treatment cycle). The 3-week treatment cycle may be repeated as many times as appropriate, for example, to achieve partial or full tumor response, tumor shrinkage, or disappearance of the tumor.

[0214]

In the most preferred embodiment, a PD-1 axis binding antagonist and a GPC3 targeting agent are administered as described in Table 3 or Table 4.

[0215]

[TABLE 3]

Loading Period (1 week)	GPC3 targeting agent: 1,600 mg/body twice (preferably, on Day 1 and Day 4)
Maintenance Period	GPC3 targeting agent: 1,600 mg/body once per 1 week PD-1 axis binding antagonist: 1,200 mg/body once per 3 weeks

[0216]

[TABLE 4]

Loading Period (1 week)	GPC3 targeting agent: 1,600 mg/body once
Maintenance Period	GPC3 targeting agent: 1,600 mg/body once per 1 week PD-1 axis binding antagonist: 1,200 mg/body once per 3 weeks

[0217]

In one embodiment, there is a maintenance period only. During the maintenance period, a PD-1 axis binding antagonist is administered at a dose of 1,200 mg/body once per 3 weeks and the GPC3 targeting agent is administered at a dose of 1,600 mg/body once per 1 week or once per 3 weeks.

[0218]

In other embodiments, there is a maintenance period only. During the maintenance period, a PD-1 axis binding antagonist and a GPC3 targeting agent are administered according to a 3-week treatment cycle within which the PD-1 axis binding antagonist is administered once per 3 weeks (i.e., once in one treatment cycle) and the GPC3 targeting agent is administered once per 1 week (i.e., 3 times in one treatment cycle). The 3-week treatment cycle may be repeated as many times as appropriate, for example, to achieve partial or full tumor response, tumor shrinkage, or disappearance of the tumor.

[0219]

In some embodiments, a PD-1 axis binding antagonist and a GPC3 targeting agent may be administered on the same day, different days, sequentially (at different times) or concurrently

(at the same time). When the PD-1 axis binding antagonist and the GPC3 targeting agent are administered on the same day but at different times, the PD-1 axis binding antagonist may be administered prior to the GPC3 targeting agent, or the GPC3 targeting agent may be administered prior to the PD-1 axis binding antagonist. In some embodiments, a PD-1 axis binding antagonist is in a separate composition as a GPC3 targeting agent. In some embodiments, a PD-1 axis binding antagonist is in the same composition as a GPC3 targeting agent.

[0220]

In some embodiments, where two or more different PD-1 axis binding antagonists are available, one PD-1 axis binding antagonist administered to an individual may be changed to another PD-1 axis binding antagonist anytime during the combination therapy using a PD-1 axis binding antagonist and a GPC3-targeting agent.

[0221]

In some embodiments, where two or more different GPC-3 targeting agents are available, one GPC3-targeting agent administered to an individual may be changed to another GPC3-targeting agent anytime during the combination therapy using a PD-1 axis binding antagonist and a GPC3-targeting agent, wither during a loading period or a maintenance period. Also, different GPC3-targeting agents may be used in a loading period and a maintenance period, respectively.

[0222]

In some embodiments, an individual may receive any adequate premedication before the administration of a GPC3-targeting agent and/or a PD-1 axis binding antagonist.

[VI. Methods of Treatment]

[0223]

Provided herein are methods for treating, preventing or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and a GPC3 targeting agent. In some embodiments, the methods comprising administering to the individual a PD-1 axis binding antagonist and a GPC3 targeting agent in accordance with dosage regimens provided herein.

[0224]

In some embodiments, the individual is a human. In some embodiments, the individual has GPC3 positive cancer. In some embodiments, GPC3 positive cancer is liver cancer, breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, or prostate cancer. In some embodiments the liver cancer is a hepatocellular carcinoma. In some embodiments, the breast cancer is a breast carcinoma or a breast adenocarcinoma. In some embodiments, the breast carcinoma is an invasive ductal carcinoma. In some embodiments, the lung cancer is a lung adenocarcinoma. In some embodiments, the colon cancer is a colorectal adenocarcinoma. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the cancer cells in the individual express GPC3 protein at a level that is detectable (e.g., detectable using methods known in the art). In some embodiments, an individual to be treated in the invention can be one who has been determined to express GPC3 protein at a level that is detectable.

[0225]

The PD-1 axis binding antagonist and the GPC3 targeting agent may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the GPC3 targeting agent is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. An effective amount of the PD-1 axis binding antagonist and the GPC3 targeting agent may be administered for prevention or treatment of disease. The appropriate dosage of the PD-1 axis binding antagonist and/or the GPC3 targeting agent may be determined based on the type of disease to be treated, the type of the PD-1 axis binding antagonist and GPC3 targeting agent, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[0226]

In some embodiments, the methods may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery (e.g., lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (e.g., agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PI3K/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents described herein.

[VII. Articles of Manufacture or Kits]

[0227]

In another embodiment of the invention, an article of manufacture or a kit is provided comprising a PD-1 axis binding antagonist and/or a GPC3 targeting agent. In some embodiments, the article of manufacture or kit further comprises package insert comprising instructions for administrating the PD-1 axis binding antagonist in conjunction with a GPC3 targeting agent to treat or delay progression of cancer in an individual or to enhance immune responses against tumor cells of an individual having cancer. In some embodiments, the package insert comprises instruction for administering the PD-1 axis binding antagonist and the GPC3 targeting agent in accordance with dosage regimens provided herein.

[0228]

In some embodiments, the PD-1 axis binding antagonist and the GPC3 targeting agent are in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as

glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, the article of manufacture further includes one or more of another agent (e.g., a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

[0229]

The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

Example 1

[0230]

Mouse cell lines expressing human Glypican-3 (GPC3)

Mouse cancer cell lines, Hepa1-6 (ATCC No. CRL-1830) and CT26 (ATCC No. CRL-2638) were transfected with human GPC3 expression vector, pCXND2/hGPC3(FL)[Ishiguro T. et al., Cancer Res. 2008; 68: 9832-9838] using FuGENE6 (Roche Diagnostics Corp) and selected with 1 mg/mL G418 (Invitrogen). Cells that grew even in the presence of G418 were collected, and the colonies were isolated by limiting dilution. Expression of human GPC3 were confirmed by FACS using anti-human GPC3 antibody, GC33 [Ishiguro T. et al., Cancer Res. 2008; 68: 9832-9838]. Representative clones were selected and used for the experiments.

Example 2

[0231]

Anti-tumor activity of anti-GPC3 antibody in syngenic mouse model using Hepa1-6 cell line expressing human GPC3

Hepa1-6/hGPC3 cells were cultured using cell culture flasks in an incubator (set at 37°C and 5% CO2). The cells were detached from the flasks with trypsin and washed with D MEM containing 10% (v/v) FBS, 0.6 mg/mL G418. Then the cells were re-suspended in D-MEM (2×10^8 cells/mL), and an equal volume of Matrigel was added. The cell concentrations for implantation were 1×10^8 cells/mL. The cells were inoculated subcutaneously into the right flank of each C57BL/6J mouse (Charles River Laboratories Japan) (1×10^7 cells/mouse). Once palpable tumors were established, animals were randomized into testing groups so that each group had similar mean tumor volumes when the study started. Either 1 or 5 mg/kg of mouse GC33 anti-human GPC3 monoclonal antibody [WO2006/006693] diluted in PBS, or PBS as a vehicle control was injected at day 14, 21 and 28 intravenously after tumor inoculation. Mouse GC33 showed inhibition of tumor growth with dose dependency compared to vehicle control (Figure 1).

Example 3

[0232]

Pathological changes induced by anti-GPC3 antibody in syngenic mouse model using Hepa1-6 cell line expressing human GPC3

To assess the changes in the Hepa1-6 tumor tissue by mouse GC33 treatment, tumor tissue isolated either after 3 or 7 days from the single injection either of mouse GC33 antibody or vehicle control was used for the pathological examination. Tumor tissues were fixed by 4% parafolmaldehyde (PFA) and embedded in paraffin by the AMeX method [Suzuki et al, J Toxicol Sci. 2002; 27:165-172, Watanabe et al, J Toxicol Pathol. 2015; 28: 43-49]. Three micro-meter paraffin sections were stained with hematoxylin and eosin (HE) or immunohistochemically (IHC). IHC staining was performed according to the labeled streptavidin-biotin (LSAB) method (RTU horseradish peroxidase streptavidin). Antibodies against F4/80 (marker antigen of murine macrophages; A3-1, BioLegend), PD-L1 (marker antigen of mouse B7-H1/PD-L1; AF1019, R&D systems) were used as the primary antibodies. The positive signals were visualized by the peroxidase-diaminobenzidine reaction, and the sections were counterstained with hematoxylin.

By mouse GC33 injection, immune cell infiltrations and tumor cell death were observed in the peripheral regions of the tumor tissues. Also increased infiltration of F4/80 positive macrophage cells were observed in the area in which tumor cell death was observed in the tumor tissues treated by mouse GC33, while F4/80 positive cells mainly observed in

the stromal regions in the tumor tissue with vehicle control (Figure 2A). Subsequently, PD-L1 expression was increased by mouse GC33 especially on the infiltrated immune cells, compared to vehicle control, which suggested that PD-L1 might be induced to suppress the anti-tumor activity by mouse GC33 (Figure 2B).

Example 4

[0233]

Anti-tumor activity in combination with the anti-GPC3 (GC33) and anti-PD-L1 antibodies (10F.9G2) in syngenic mouse model using CT26 cell line expressing human GPC3

To evaluate anti-tumor activities of anti-GPC3 or anti-PD-L1 monotherapies or combination in mouse CT26/hGPC3 model, either mouse GC33 antibody and/or 500 μg of anti-mouse PD-L1 rat antibody, 10F.9G2 (purchased from BioXCell) were injected either from day 3 (early treatment model) or day 15 (established model) after subcutaneously inoculation of $1x10^6$ of CT26/hGPC3 cells. In the early treatment model, either 5 or 25 mg/kg of mouse GC33 was injected at day 3, 6, 10 and 13 intravenously, and 500 μg of anti-PD-L1 antibody, 10F.9G2, was injected at same schedule as single agent or combination. In establishment model, either 5 or 25 mg/kg of mouse GC33 was injected at day 15 and 18 intravenously, and 500 μg of anti-PD-L1 antibody, 10F.9G2, was injected at same schedule as single agent or combination.

In both models, combination of 25 mg/kg of GC33 and 10F.9G2 showed most potent anti-tumor activity compared to those by each monotherapy (Figure 3 and 4).

Example 5

[0234]

Anti-tumor activity in combination with the anti-GPC3 (GC33) and anti-PD-L1 antibodies (10F.9G2) in syngenic mouse model using Hepa1-6 cell line expressing human GPC3

Either 1, 5, or 25 mg/kg of mouse GC33 antibody once a week for 3 weeks or 200 μg of anti-mouse PD-L1 rat antibody, 10F.9G2 followed by 100 μg weekly for 2 weeks as a single agent or combination were injected intravenously to Hepa1-6 bearing mice as same as above. Pathological examination was conducted with HE staining sections which prepared with conventional methods described above. As to the IHC, 1st antibodies listed in the Table 2 were used for each marker and visualized either by LSAB methods described above or ENV+ method. IHC was conducted for 3 representative animals from each group.

[0235]

[TABLE 5] List of antibodies used in the IHC staining

Marker	1st Antibody		Visualization system
	Clone / isotype	Source	
GPC3	GC33 / mouse IgG2a	in house	LSAB
PD1	- / goat poly IgG	R&D Systems, Inc.	LSAB*
PD-L1	- / goat poly IgG	R&D Systems, Inc.	LSAB*
F4/80	Cl:A3-1 / rat IgG2b	BioLegend, Inc.	LSAB
CD204	SRA-E5 / mouse IgG1	TransGenic, Inc.	LSAB
CD206	MR5D3 / rat IgG2a	GeneTex, Inc.	LSAB
CD163	- / rabbit poly IgG	Santa Cruz Biotechnology, Inc.	ENV+
CD11b	EPR1344 / rabbit IgG	Novus Biologicals	ENV+
CD11c	- / rabbit poly IgG	Proteintech	ENV+
CD3	SP7 / rabbit IgG	GeneTex, Inc.	ENV+
FoxP3	FJK-16s / rat IgG2a	eBioscience, Inc.	LSAB
CD45R	RA3-6B2 / rat IgG2a	Santa Cruz Biotechnology, Inc.	LSAB
ICOS	C398.4A / hamster IgG	BioLegend, Inc.	LSAB
CD34	MEC14.7 / rat IgG2a	BioLegend, Inc.	LSAB

* Biotinylated Rabbit Anti-Goat IgG Antibody (Vector Laboratoties, Inc.)
LSAB, Streptavidin, Horseradish Peroxidase, R.T.U. (Vector Laboratoties, Inc. or Dako)
ENV+, EnVision+ System- HRP. Labelled Polymer. Anti-Rabbit (Dako)

While mouse GC33 or 10F.9G2 showed inhibition of tumor growth compared to vehicle control, mouse GC33 and 10F.9G2 combination showed the strongest anti-tumor activity (Figure 5A). Five days after 3rd injection, all mice were necropsied and tumor tissues were evaluated pathologically. In tumor tissues examined, no viable tumor cells were observed in all mice treated with 25 mg/kg of mouse GC33 in combination with PD-L1 antibody and in 4 out of 5 mice treated with 5 mg/kg of mouse GC33 in combination with PD-L1 antibody (Figure 5B).

Pathological examination of each treated tumor tissues revealed that increase in number of F4/80-positive cells, PD-L1 expression on the multinucleated giant cells (MNGC) and CD3-positive cells infiltrated into tumor tissues and decrease in number of CD206, CD163, and CD11b-positive cells and PD-L1 expression on the mononuclear cells (MNC) by each treatment compared to vehicle control. By combination, infiltration of CD3-positive T cells was increased than each monotherapy which tend to be related to anti-tumor activities (Table 6).

[0236]

[TABLE 6] Pathological evaluations of treated tumors

	Vehicle (n=3)	mGC33 5 mg/kg (n=3)	mGC33 1 mg/kg (n=3)	10F.9G2 (n=3)	mGC33 5 mg/kg + 10F.9G2 (n=3)
Tumor cell					
GPC3	3*	2	1~3	2	-~1
PD1	1	-~1	1~2	-~1	-
PDL1	2	1~2	1~2	1~2	1
Immune cell infiltration					
F4/80	3	3~4	3~4	3~4	3~4
CD204	3	3~4	3~4	3~4	3~4
CD206	2	1	1	1	-~1
CD163	2	1~2	1~2	1	1
CD11b	3	2	3	2	2
CD11c	1~2	1~2	1	1~2	2
CD3	2	2~3	1~2	2~3	3
FoxP3	1	1	1	1	1
B220	1	1	1	1	1
ICOS	1	1~2	1	1~2	1~2
PD1	2	2	1~2	1	1~2
PDL1 (MNC)	2	1~2	1	1	1
PDL1 (MNGC)	1	1~3	-~3	2~3	1~3
Vasculature					
CD34	3	1	1	1	1

MNC, mononuclear cell; MNGC, multinucleated giant cell
*Severity of lesion: 1, very slight; 2, slight; 3, moderate; 4, marked.

Example 6

[0237]

GC33 (or codrituzumab) is a recombinant humanized IgG1 monoclonal antibody capable of binding to human GPC3 with high affinity (WO2006/006693). MPDL3280A (or atezolizumab) is a recombinant humanized IgG1 monoclonal antibody capable of binding to Programmed death-ligand 1 (PD-L1) and inhibiting PD-L1 binding to Programmed death-1 (PD-1) molecule. MPDL3280A incorporates an amino acid substitution (asparagine to alanine) at position 298 in CH2 domain of each heavy chain resulting in a non-glycosylated antibody that has minimal binding to Fcγ receptor (WO2010/077634). GC-207JG study was designed as a phase-Ib multicenter clinical trial (hereinafter, "the study" or "the clinical trial") and carried out in order to evaluate the safety, tolerability, anti-tumor activity and pharmacokinetics (PK) of GC33 in combination with MPDL3280A in patients with locally advanced or metastatic hepatocellular carcinoma (HCC) to determine dosage regimen for this combination. In this test aimed at evaluating safety and/or tolerability in the patients with locally advanced or metastatic HCC, the pharmacokinetic profiles of GC33 and MPDL3280A, and antitumor effects, and searching for biomarkers, dose-escalation part for GC33 consisting of up to 3 cohorts and expansion part were carried out (Figure 6). Dose-escalation part was tested by 3+3 design. Intravenous (IV) administration of GC33 started at indicated dose on Day 1 and 4 of week 1 followed by weekly (QW) from the second week

and afterwards (Table 7). The i.v. administration of MPDL3280A at 1,200 mg every 3 weeks started from Day 8. Expansion part was planned to be initiated at the higher dose confirmed to be tolerable in the dose-escalation part.

[0238]

[TABLE 7]

Cohort	Dosage
1	GC33:800 mg QW from Day 8 (+ 2 loading doses: 800 mg at Day 1 and 4) MPDL3280A: 1,200 mg Q3W from Day 8
2	GC33:1,600 mg QW from Day 8 (+ 2 loading doses: 1,600 mg at Day 1 and 4) MPDL3280A: 1,200 mg Q3W from Day 8
3	GC33:400 mg QW from Day 8 (+ 2 loading doses: 400 mg at Day 1 and 4) MPDL3280A: 1,200 mg Q3W from Day 8

[0239]

The HCC patients subjected to the administration had histologically confirmed advanced or metastatic HCC (except for fibrolamellar type) unsuitable for curative treatment (surgical resection, liver transplantation, etc.) or exacerbated after curative treatment and had a past history of treatment based on systemic therapy with at least one agent. Eligible patients were at least 18 years old with GPC3 high expression (2+ or 3+) in the tumor samples measured by GPC3 immunohistochemistry and exhibited Eastern Cooperative Oncology Group Performance Status of 0 (see Table 10) or 1 and Child-Pugh score 5-7 (see Tables 11 and 12). The patients also had at least one tumor lesion that was measurable at baseline. The measurability of tumor lesions at baseline were determined according to the response evaluation criteria in solid tumors (RECIST v1.1. See, e.g., Eisenhauer et al., Eur J Cancer. 45(2):228-47 (2009)). Appropriate hematopoietic functions (absolute neutrophil count $\geq$ 1,500/μL, platelet $\geq$ 75,000/μL, hemoglobin $\geq$ 9.0 g/dL), hepatic functions (total bilirubin $\leq$ 2.0 mg/dL, aspartate aminotransferase and alanine aminotransferase $\leq$ 5 times the upper limit

of the normal level), and renal functions (calculated creatinine clearance ≥ 50 mL/min) were evaluated as other criteria. For patients with HBV infection, HBV DNA is < 500 IU/mL within 3 months prior to enrollment regardless of treatment for HBV. Registrable female subjects were premenopausal female patients confirmed to be negative for a serum pregnancy test conducted within 14 days before the clinical trial registration, women without the possibility of pregnancy as a result of surgical contraception or after a lapse of 1 year or longer after menopause, and female patients other than the postmenopausal women (12-month or longer absence of menstruation) or the surgically contracepted women (resection of the ovary and/or the uterus), who consented to use two types of appropriate fertility control methods during clinical trial treatment and for at least 5 months after the completion of administration of the study drugs. Registrable male subjects were patients who consented to use fertility control based on the barrier method during the clinical trial treatment and for at least 90 days after the completion of administration of the study drugs.

[0240]

The expression of GPC3 proteins in HCC tumor tissues was evaluated by GPC3 immunohistochemical staining (GPC3-IHC). Scores were determined according to the "immunohistochemical staining score" (e.g., composite score 2 in WO2015/170480) as shown in Table 8, or according to the simplified GPC3 algorithm as shown in Table 9.

[0241]

[TABLE 8] Immunohistochemical Staining Score

Score	Description
0	1. Absent membrane staining. 2. Cytoplasmic staining of any intensity in <10% of tumor cells
1+	1. Membrane staining of any intensity in <10% of tumor cells AND/OR 2. Cytoplasmic staining of any intensity ≥10% of tumor cells (note that strong cytoplasmic staining, if present, must be in <50% of tumor cells)
2+	1. Presence of weak to moderate membrane staining in ≥10% of tumor cells (note that strong membrane staining, if present, must be in <10% of tumor cells) WITH OR WITHOUT: 2. Cytoplasmic staining of any intensity ≥10% of tumor cells (note that strong cytoplasmic staining, if present, must be in <50% of tumor cells)
3+	1. Strong membrane staining in ≥10% of tumor cells with or without cytoplasmic staining OR 2. Strong cytoplasmic staining in ≥50% of tumor cells

[0242]

[TABLE 9] Simplified GPC3 Algorithm

Status	Descriptions
Negative (0/1+)	1. Membrane staining of any intensity in < 10% of tumor cells AND/OR 2. Cytoplasmic staining of any intensity in < 50% of tumor cells 3. Weak or moderate cytoplasmic staining in >50% of tumor cells
Positive (2+/3+)	1. Membrane staining of any intensity in ≥ 10% of tumor cells AND/OR 2. Strong cytoplasmic staining in ≥ 50% of tumor cells

[0243]

[TABLE 10] Eastern Cooperative Oncology Group Performance Status Scale

Grade	Description
0	Fully active, able to carry on all predisease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature; e.g., light housework or office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about >50% of waking hours
3	Capable of only limited self-care, confined to a bed or chair >50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

[0244]

[TABLE11] Child-Pugh Classification of Severity of Liver Disease

Modified Child-Pugh classification of severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy.

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin, mg/dL	<2	2-3	>3
Albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time			
Seconds over control	1-3	4-6	>6
INR	<1.8	1.8-2.3	>2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

[0245]

[TABLE12] Child-Pugh Classification of Severity of Liver Disease

A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year patient survival.

Grade	Points	One-year patient survival (%)	Two-year patient survival (%)
A: well-compensated disease	5-6	100	85
B: significant functional compromise	7-9	80	60
C: decompensated disease	10-15	45	35

[0246]

On the other hand, the registered subjects excluded patients who received major surgical operation within 4 weeks before the first administration of the GC33, patients confirmed to have brain or leptomeningeal metastasis, patients having a past history of malignant tumor within the last 5 years, patients having active infection requiring treatment except for hepatitis B or hepatitis C, patients having any history of clinically meaningful variceal bleeding within the last three months before the clinical trial registration or evidence of varices at high risk for bleeding, patients having a past history of organ transplantation including liver transplantation, patients who were scheduled to receive or were receiving the administration of an anticancer agent other than the agents to be administered in this test, patients who received major surgery, local therapy for HCC, chemotherapy, radiotherapy, hormone therapy, immunotherapy, or another investigation drug within the last 4 weeks before the first administration of GC33, patients who did not completely get over adverse reactions associated with the preceding locoregional or systemic therapy of hepatocellular cancer, patients received interferon therapy within the last 4 weeks before the first administration of GC33, patients having prior treatment with anti-PD-1 or anti-PD-L1 therapeutic antibody or pathway-targeting agents, patients who have had anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody within the last 6 weeks before to the first administration of GC33, severe immune-related adverse effects from anti-CTLA-4 antibody were observed, patients who received the administration of an anticoagulant or a

thrombolytic agent for therapeutic purposes within the last 2 weeks before the first administration of the GC33 (except for the administration of the agent at a low dose for the purpose of removing clogs in a catheter or for preventive purposes), pregnant or lactating patients, HIV-positive patients or patients having an AIDS-related disease, patients having a past history of hypersensitivity for similar agents (monoclonal antibodies, protein-containing preparations, and Chinese hamster ovary-derived preparations), and patients having a serious comorbidity judged by a principal investigator or a sub-investigator as being possibly worsened due to the study drug.

[0247]

The protocol of GC-207JG study was carried out according to the guideline of the Good Clinical Practice (GCP) and approved by each participating ethical committee on clinical trials. All patients signed their names on written informed consent before registration. The patients received the continuous administration of GC33 and MPDL3280A unless the disease progressed or unacceptable toxicity appeared.

Example 7

[0248]

Tumor response (or size of tumor lesion) is evaluated on the basis of a baseline and evaluated after 7 weeks from the start of administration and then evaluated repetitively every 6 weeks until the disease progressed, according to RECIST v1.1. (See, e.g., Eisenhauer et al., Eur J Cancer. 45(2):228-47 (2009)). The state of the disease (e.g., complete response (CR), partial response (PR), progressive disease (PD), or stable disease (SD), as defined by RECIST v1.1. (see, e.g., Eisenhauer et al., supra.) is evaluated by principal investigators.

[0249]

The expression of GPC3 proteins in HCC tumor tissues is evaluated by GPC3 immunohistochemical staining (GPC3-IHC). The measurement of GPC3-IHC is carried out by Ventana Medical Systems, Inc. (USA). Unstained slides of HCC tumor tissues prepared from tumor blocks formalin-fixed and paraffin-embedded after excision by needle biopsy or archival resected specimens in each hospital are subjected to immunohistochemical staining. The antibody used is a mouse GC33 antibody (Ventana Medical Systems Inc. Catalog Number: 790-4564). In the dose-escalation part and expansion part, patients whose HCC

tissues are GPC3 positive measured by GPC3-IHC were enrolled.

Example 8

[0250]

In GC-207JG study, efficacy by these combination is measured as:

1) frequency of complete response (CR), partial response (PR), and stable response (SR) for over 3 months as assessed by RECIST v1.1(See, e.g., Eisenhauer et al., Eur J Cancer. 45(2):228-47 (2009)),

2) duration of response as assessed by RECIST v1.1 (See, e.g., Eisenhauer et al., supra.),

3) overall response rate (defined as CR or PR) and disease control rate (defined as CR, PR or SD over 3 months) assessed by RECIST v1.1 (See, e.g., Eisenhauer et al., supra.),

4) time-to-progression (TTP) and progression-free-survival (PFS) as assessed by RECIST v1.1 (See, e.g., Eisenhauer et al., supra.), and

5) overall survival.

[0251]

In addition, change of alfa-feto protein (AFP) or serum GPC3 after administration of the study drugs from baseline levels is also evaluated as anti-tumor activity.

Example 9

[0252]

In GC-207JG study, 3 subjects have been enrolled into the cohort 1, 7 subjects have been enrolled into the cohort 2 to evaluate dose limiting toxicity (DLT) by this combination. No DLT was observed neither in the cohort 1 nor the cohort 2, then the dose levels for the expansion cohort was determined to be the same as cohort 2, which is 1600 mg qw of GC33 with loading doses at day 1 and 4 and 1200 mg q3w of MPDL3280A. At least 9 subjects were enrolled into the expansion cohort.

[0253]

Serum concentrations of GC33 (and MPDL3280A) were measured available as of April 21st, 2017. In the cohort 1, trough concentration of GC33 was over 230 μg/mL from 2 weeks in 1 subject but not in other 2 subjects (Figure 7). On the other hand, in the cohort 2, all 7 subjects' GC33 trough concentrations were over 230 μg/mL from 2 weeks after the first

infusion as expected (Figure 8A). Furthermore, the loading dose of GC33 at day 4 enabled the trough concentration of GC33 to rapidly increase to exceed 230 μg/mL, compared to the trough concentrations in subjects without the loading dose of GC33 at day 4 (Figure 8B). Average body weight for these 10 subjects was 67.96 kg and the standard deviation was 17.03 kg (45.2 – 103.8 kg).

[0254]

Among subjects enrolled into the cohort 1 and 2, 2 subjects have been excluded for the evaluation of anti-tumor activity because 1 subject withdrew the consent and 1 another subject's baseline GPC3-IHC score was revealed as negative though its archival tumor tissue was GPC3 positive for the eligibility. On the other hand, 1 subject enrolled into the cohort 1 (-31.8% at Week 19 tumor assessment) and 1 subject enrolled into the cohort 2 (-28.6% at Week 13 tumor assessment) showed tumor shrinkage assessed as of July 12th, 2017, by RECIST v1.1 (See, e.g., Eisenhauer et al., Eur J Cancer. 45(2):228-47 (2009)). (Whereas there was only 1 subject showed partial response (PR) by RECIST v1.1 in the GC33 arm in the phase II of GC33 monotherapy (NP27884 study (N=125 in total and N=67 with GPC3-IHC 2+ or 3+)) (Abou-Alfa GA et al., J Hepatol. 2016;65(2):289-295),), which suggested that the combination of either 800 mg or 1600 mg qw regimen of GC33 and 1200 mg q3w regimen of MPDL3280A might show better efficacy than each monotherapy.

[0255]

To evaluate anti-tumor activity of GC33 monotherapy, AFP changes at day 8 from baseline values were compared. In addition to 8 subjects above (3 subjects in cohort 1 and 7 subjects in cohort 2, excluding 2 subjects who have been excluded for the evaluation.), 3 subjects enrolled into the expansion cohort were added to this evaluation because they have had AFP data both at baseline and day 8 (with 2-week allowance) as of July 12th, 2017. Subjects having the infusion of 1600 mg of GC33 at Day 1 (cohort 2 and expansion cohort) tended to show much larger AFP decrease than subjects having the infusion of 800 mg of GC33 at Day 1 (cohort 1) (p=0.035 by t-test, Table 13). A total of 3 subjects from cohort 2 and expansion cohort skipped the loading dose of GC33 at day 4 because of some adverse events. When compared the AFP changes between subjects with 2 loading doses of GC33 in the 1st week and others with only one loading dose of GC33, no statistically significant difference observed with or without the loading dose of GC33 at day 4 in the 1st week (p=0.587 by t-

test, Table 14).

[0256]

[TABLE 13] AFP change from baseline at day 8 in dose-escalation or expansion cohorts

GC33 dose level	**Number of subject**	**AFP changes (%) at day 8 Average ± SD**
800 mg qw	3	5.94 ± 14.08
1600 mg qw	8	-22.08 ± 17.34

[0257]

[TABLE 14] AFP change from baseline at day 8 in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**AFP changes (%) at day 8 Average ± SD**
With loading dose at day 4	5	-19.22 ± 20.37
Without loading dose at day 4	3	-26.86 ± 12.94

Example 10

[0258]

In the expansion cohort, 10 subjects were enrolled and dosed then the total number of subjects enrolled into GC-207JG study was 20 subjects. Of a total of 20 subjects who were enrolled in the GC-207JG study either dose escalation cohorts or expansion cohort, all subjects received treatment with GC33 and MPDL3280A, and were evaluated on safety profile of this treatment. All subjects in the study reported at least one adverse event: 30 adverse events reported in 3 subjects who received GC33 800 mg and MPDL3280A 1200 mg, 153 adverse events reported in 17 subjects who received GC33 1600 mg and MPDL3280A 1200 mg. Adverse events with an incidence of ≥10% in all patient were pyrexia (80.0%), fatigue (50.0%), decreased appetite (30.0%), aspartate aminotransferase

increased, lymphocyte count decreased, and cough (25.0% each), constipation and nasopharyngitis (15.0% each), chest pain, alanine aminotransferase increased, blood bilirubin increased, C-reactive protein increased, ascites, stomatitis, hemoptysis, oropharyngeal pain, hypokalaemia, myalgia, pain in extremity, upper respiratory tract infection, dizziness, eczema, pruritus, rash and hypertension (10.0% each). Adverse events reported at ≥Grade 3 were reported in 14 subjects overall; 2 subjects received GC33 800 mg and MPDL3280A 1200 mg and 12 subjects received GC33 1600 mg and MPDL3280A 1200 mg. Adverse events reported at ≥Grade 3 with an incidence of ≥10% in all subject were aspartate aminotransferase increased and lymphocyte count decreased (20.0% each) and ascites (10.0%). As shown in Table 15, safety profiles were comparable among cohorts.

[0259]

[TABLE 15] Safety summary

Adverse Events	**Cohort 1 (N=3)**	**Cohort 2 and Expansion cohort (N=17)**	**Total (N=20)**
Any grade, n (%)	3 (100.0%)	17 (100.0%)	20 (100.0%)
Treatment-related	2 (66.7%)	17 (100.0%)	19 (95.0%)
Grade 3-4, n (%)	2 (66.7%)	11 (64.7%)	13 (65.0%)
Treatment-related	2 (66.7%)	6 (35.3%)	7 (35.0%)
Grade 5, n (%)	0	1 (5.9%)	1 (5.0%)
Treatment-related	0	0	0
Serious AE, n (%)	1 (33.3%)	5 (29.4%)	6 (30.0%)
Treatment-related	0	1 (5.9%)	1 (5.0%)

Example 11

[0260]

The mean trough concentrations of GC33 of subjects either having 800 mg of GC33 or 1600 mg of GC33 were shown in Figure 9. The trough concentration of subjects with 1600

mg qw dose of GC33 was quickly reached above 230 μg/m and maintained the trough concentration over 230 μg/mL.

[0261]

Among 20 subjects enrolled and dosed, 2 subjects who didn't have any assessment of response by CT scan or MRI have been excluded from the evaluation of efficacy such as objective response rate (ORR), disease control rate (DCR), progression-free-survival (PFS) and overall survival (OS). In addition to these 2 subjects, 2 other subjects have been excluded from the evaluation of anti-tumor activity based on AFP changes because 1 subject's baseline GPC3-IHC score was revealed as negative and 2 other subject who didn't have AFP data at day 8 (with 2 weeks allowance) but these subjects were included in the evaluation for the best AFP response, largest AFP decrease from baseline during the treatment period.

[0262]

To evaluate anti-tumor activity of GC33 monotherapy and combination with MPDL3280A, AFP changes at day 8 from baseline value and best AFP response. As shown in Table 16, subjects having the infusion of 1600mg of GC33 showed a trend of larger AFP decrease at day 8 than subjects having the infusion of 800 mg of GC33 (p=0.071 by t-test). When compared the AFP changes between subjects with 2 loading doses of 1600 mg of GC33 in the 1st week and others with only one loading dose of 1600 mg of GC33, no statistically significant difference observed with or without the loading dose of GC33 at day 4 in the 1st week (p=0.555 by t-test, Table 17). When compared the best AFP response by GC33 dose levels, no statistically significant difference was observed regardless of GC33 dose levels and regardless of whether subjects having the infusion of 1600 mg of GC33 were received the loading dose of GC33 at day 4 (p=0.458 or 0.914, respectively by t-test, Table 18). If subjects whose baseline AFP value was over 100 ng/mL were applied to the evaluation by AFP best response, most of the subjects having 1600 mg of GC33 and 1200 mg of MPDL3280A showed AFP decrease, but one subject having 800 mg of GC33 and 1200 mg of MPDL3280A didn't show AFP decrease (Figure 10), which suggested that higher dose level of GC33 and MPDL3280A combination might show strong AFP response against the HCC patients with high value of AFP.

[0263]

[TABLE 16] AFP change from baseline at day 8 in dose-escalation or expansion cohorts

GC33 dose level	**Number of subject**	**AFP changes (%) at day 8 Average ± SD**
800 mg qw	3	5.94 ± 14.08
1600 mg qw	12	-20.12 ± 21.46

[0264]

[TABLE 17] AFP change from baseline at day 8 in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**AFP changes (%) at day 8 Average ± SD**
With loading dose at day 4	9	-17.87 ± 23.85
Without loading dose at day 4	3	-26.86 ± 12.94

[0265]

[TABLE 18] AFP best response in dose-escalation or expansion cohorts

GC33 dose level	**Number of subject**	**AFP changes (%) at day 8 Average ± SD**
800 mg qw	3	-19.61 ± 43.99
1600 mg qw	14	-36.40 ± 32.93

[0266]

[TABLE 19] AFP best response in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**AFP changes (%) at day 8 Average ± SD**
With loading dose at day 4	10	-37.04 ± 38.07
Without loading dose at day 4	4	-34.81 ± 18.57

[0267]

The ORR and DCR for the subjects who received 800 mg of GC33 and 1200 mg of MPDL3280A (cohort 1) were 0% and 66.7% (two SDs, respectively. Among the subjects who received 1600 mg of GC33 and 1200 mg of MPDL3280A (cohort 2 of the dose escalation part and the expansion part), 15 of 17 subjects who had tumor assessment after starting study treatment, were included in the population for the efficacy evaluation. The ORR and DCR for these 15 subjects was 6.7% (one PR) and 53.3% (1 PR and 7 SDs), respectively. Tumor response according to RECIST v1.1 was only seen in cohorts with 1600 mg of GC33.

[0268]

In addition to the tumor response, DCR either 6 or 9 months from the first infusion of GC33 were also evaluated. DCR was the percentage of subjects without progression of disease at 6 or 9 months. The DCR at 6 months were comparable between 800 mg of GC33 and 1600 mg of GC33, but higher DCR was observed in cohorts with 1600 mg of GC33 than that in cohort 1 (Table 20) and in subjects having 1600 mg of GC33 with loading dose than those without loading dose at day 4 (Table 21). Among the subjects whose baseline AFP level was over 100 ng/mL, only in the subject group having 1600 mg of GC33 with loading dose at day 4, subjects with disease control both at 6 and 9 months were observed (Table 22 and 23).

[0269]

[TABLE 20] Disease control rate (DCR) in dose-escalation or expansion cohorts

GC33 dose level	**Number of subject**	**DCR at 6 months**	**DCR at 9 months**
800 mg qw	3	33.3%	0.0%
1600 mg qw	15	40.0%	20.0%

[0270]

[TABLE 21] Disease control rate (DCR) in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**DCR at 6 months**	**DCR at 9 months**
With loading dose at day 4	11	36.4%	27.3%
Without loading dose at day 4	4	50.0%	0.0%

[0271]

[TABLE 22] Disease control rate (DCR) in subjects whose baseline AFP value was over 100 ng/mL

GC33 dose level	**Number of subject**	**DCR at 6 months**	**DCR at 9 months**
800 mg qw	1	0.0%	0.0%
1600 mg qw	9	33.3%	33.3%

[0272]

[TABLE 23] Disease control rate (DCR) in subjects whose baseline AFP value was over 100 ng/mL in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**DCR at 6 months**	**DCR at 9 months**
With loading dose at day 4	7	42.9%	42.9%
Without loading dose at day 4	2	0.0%	0.0%

[0273]

Median progression-free survival (PFS) and overall survival (OS) were estimated by Kaplan-Meier method. Median PFS was 4.4 months and median OS was 13.5 months in the population for the efficacy evaluation including both cohort 1, 2 and expansion cohort, where the median PFS and OS were 2.6 months and 8.7 months in the GC33 arm in the phase II of GC33 monotherapy, NP27884 study, which suggested that the combination of GC33 and MPDL3280A might show better clinical efficacy compared to GC33 monotherapy.

[0274]

Median PFS and OS either in cohort 1 or cohort 2 and expansion were shown in Table 24 and median PFS and OS either for the subjects having 1600 mg of GC33 with or without day 4 loading dose were shown in Table 25. Median PFS and OS for the subjects whose baseline AFP level was over 100 ng/mL were shown in Table 26 and 27. Median OS for subjects having 1600 mg of GC33 tend to be longer than that for subjects having 800 mg of GC33, but there was no difference between the subject groups with and without loading dose at day 4. In addition, in the population whose AFP was over 100 ng/mL as baseline value, median PFS and OS were much longer in the subjects having 1600 mg of GC33 compared to those with 800 mg of GC33 dosing. Kaplan-Meier curves were shown in Figure 11.

[0275]

[TABLE 24] Median PFS and OS in dose-escalation or expansion cohorts

GC33 dose level	**Number of subject**	**Median PFS**	**Median OS**
800 mg qw	3	5.6 months	8.6 months
1600 mg qw	15	4.2 months	13.5 months

[0276]

[TABLE 25] Median PFS and OS in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**Median PFS**	**Median OS**
With loading dose at day 4	11	4.0 months	13.5 months
Without loading dose at day 4	4	5.7 months	Not reached

[0277]

[TABLE 26] Median PFS and OS in subjects whose baseline AFP value was over 100 ng/mL

GC33 dose level	**Number of subject**	**Median PFS**	**Median OS**
800 mg qw	1	1.0 months	1.9 months
1600 mg qw	9	4.0 months	13.5 months

[0278]

[TABLE 27] Median PFS and OS in subjects whose baseline AFP value was over 100 ng/mL in cohort 2 or expansion cohort with or without loading dose at day 4

GC33 dose level	**Number of subject**	**Median PFS**	**Median OS**
With loading dose at day 4	7	4.0 months	13.5 months
Without loading dose at day 4	2	3.1 months	Not reached

[0279]

From the above, GC33 in combination with MPDL3280A was considered to be well tolerated in subjects with locally advanced or metastatic HCC. And the combination of GC33, especially 1600 mg weekly infusion of GC33, and MPDL3280A was expected to have certain efficacy for locally advanced or metastatic HCC.

[0280]

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety

Industrial Applicability

[0281]

The present invention contributes to improvement in the efficacy of the combination therapy using GPC3-targeting agent and/or PD-1 axis binding antagonist, improvement in QOL of a patient to be treated, and is useful in the treatment of cancer including liver cancer.

CLAIMS

[Claim 1]

A pharmaceutical composition for treating or delaying progression of cancer in an individual for use in a combination therapy with a PD-1 axis binding antagonist, wherein said composition comprises a GPC3 targeting agent as an active ingredient,
wherein the combination therapy comprises (i) a loading period within which the GPC3 targeting agent is administered, followed by (ii) a maintenance period within which the PD-1 axis binding antagonist and the GPC3 targeting agent are administered.

[Claim 2]

The pharmaceutical composition according to [Claim 1], wherein the GPC3 targeting agent is administered at a dose of approximately 800 mg/body to approximately 3,200 mg/body once or more within the loading period.

[Claim 3]

The pharmaceutical composition according to [Claim 1 or 2], wherein the loading period is 2 weeks or less, or 1 week or less.

[Claim 4]

The pharmaceutical composition according to [any one of Claims 1 to 3], wherein the total amount of the GPC3 targeting agent administered within the loading period is approximately 1,600 mg/body to approximately 4,800 mg/body.

[Claim 5]

The pharmaceutical composition according to [any one of Claims 1 to 4], wherein the GPC3 targeting agent is administered at a dose of approximately 1,600 mg/body once or twice within the loading period.

[Claim 6]

The pharmaceutical composition according to [any one of Claims 1 to 5], wherein the last administration of the GPC3 targeting agent within the loading period is separated in

time from the first administration of the PD-1 axis binding antagonist or the GPC3 targeting agent within the maintenance period by 2 to 4 days.

[Claim 7]

The pharmaceutical composition according to [any one of Claims 1 to 6], wherein the PD-1 axis binding antagonist is administered at a dose of approximately 1,000 mg/body to approximately 1,400 mg/body once or more within the maintenance period, and wherein the GPC3 targeting agent is administered at a dose of approximately 800 mg/body to approximately 2,000 mg/body once or more within the maintenance period.

[Claim 8]

The pharmaceutical composition according to [any one of Claims 1 to 7], wherein the PD-1 axis binding antagonist is administered at a dose of approximately 1,200 mg/body once per 3 weeks, and wherein the GPC3 targeting agent is administered at a dose of approximately 1,600 mg/body once per week or once per 3 weeks.

[Claim 9]

The pharmaceutical composition according to [any one of Claims 1 to 7], wherein the maintenance period comprises treatment cycles, wherein one treatment cycle comprises

(1) administration of approximately 1,200 mg/body of the PD-1 axis binding antagonist once per 3 weeks, and

(2) administration of approximately 1,600 mg/body of the GPC3 targeting agent once per week for 3 weeks, and the treatment cycle is repeated.

[Claim 10]

The pharmaceutical composition according to [any one of Claims 1 to 9], wherein the administration of the PD-1 axis binding antagonist and/or the GPC3 targeting antibody within the loading period and/or the maintenance period are intravenous injection or infusion.

[Claim 11]

The pharmaceutical composition according to [any one of Claims 1 to 9] wherein the administration of the PD-1 axis binding antagonist and/or the GPC3 targeting antibody

within the loading period and/or the maintenance period are subcutaneous injection.

[Claim 12]

The pharmaceutical composition according to [any one of Claims 1 to 11], wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[Claim 13]

The pharmaceutical composition according to [any one of Claims 1 to 12], wherein the PD-1 axis binding antagonist is

(1) an anti-PD-L1 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21 ; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24,

(2) an anti-PD-L1 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4, or

(3) Atezolizumab.

[Claim 14]

The pharmaceutical composition according to [any one of Claims 1 to 13], wherein the GPC3 targeting agent is

(1) an anti-GPC3 antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:42, HVR-H2 sequence of SEQ ID NO:43, and HVR-H3 sequence of SEQ ID NO:44; and a light chain comprising HVR-L1 sequence of SEQ ID NO:45, HVR-L2 sequence of SEQ ID NO:46, and HVR-L3 sequence of SEQ ID NO:47,

(2) an anti-GPC3 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:50 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:52, or

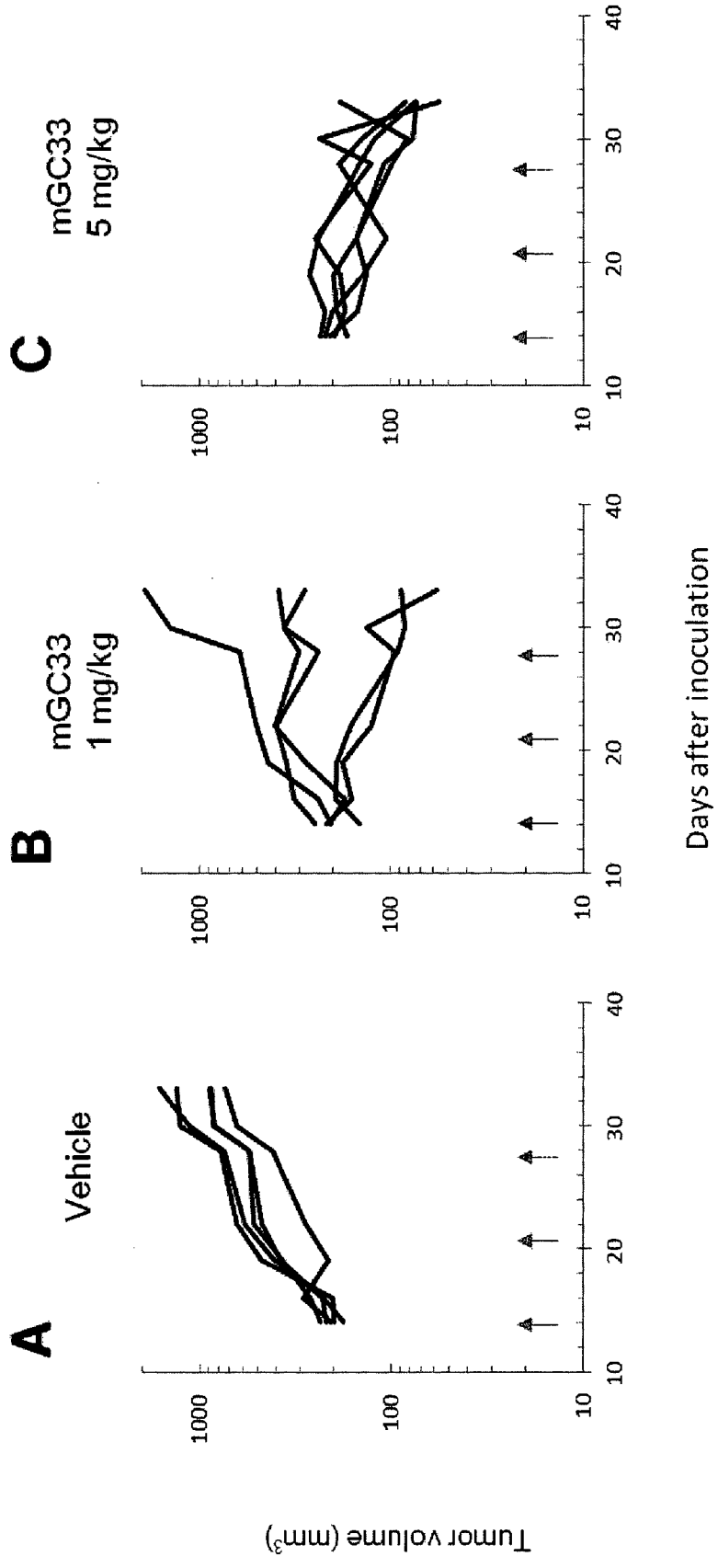
(3) Codrituzumab.

[Claim 15]

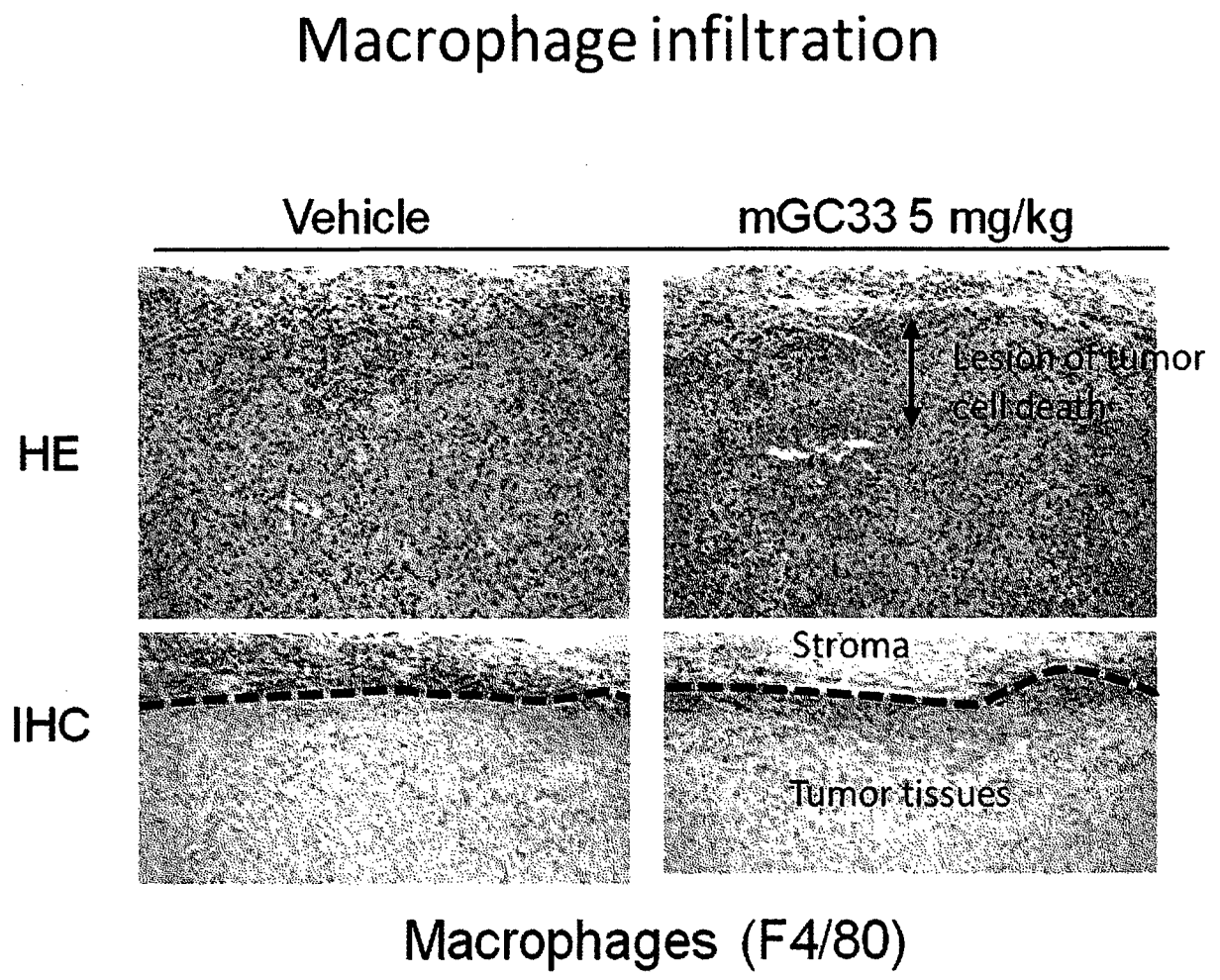
The pharmaceutical composition according to [any one of Claims 1 to 14], wherein the

cancer is selected from the group consisting of liver cancer, breast cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer and prostate cancer.

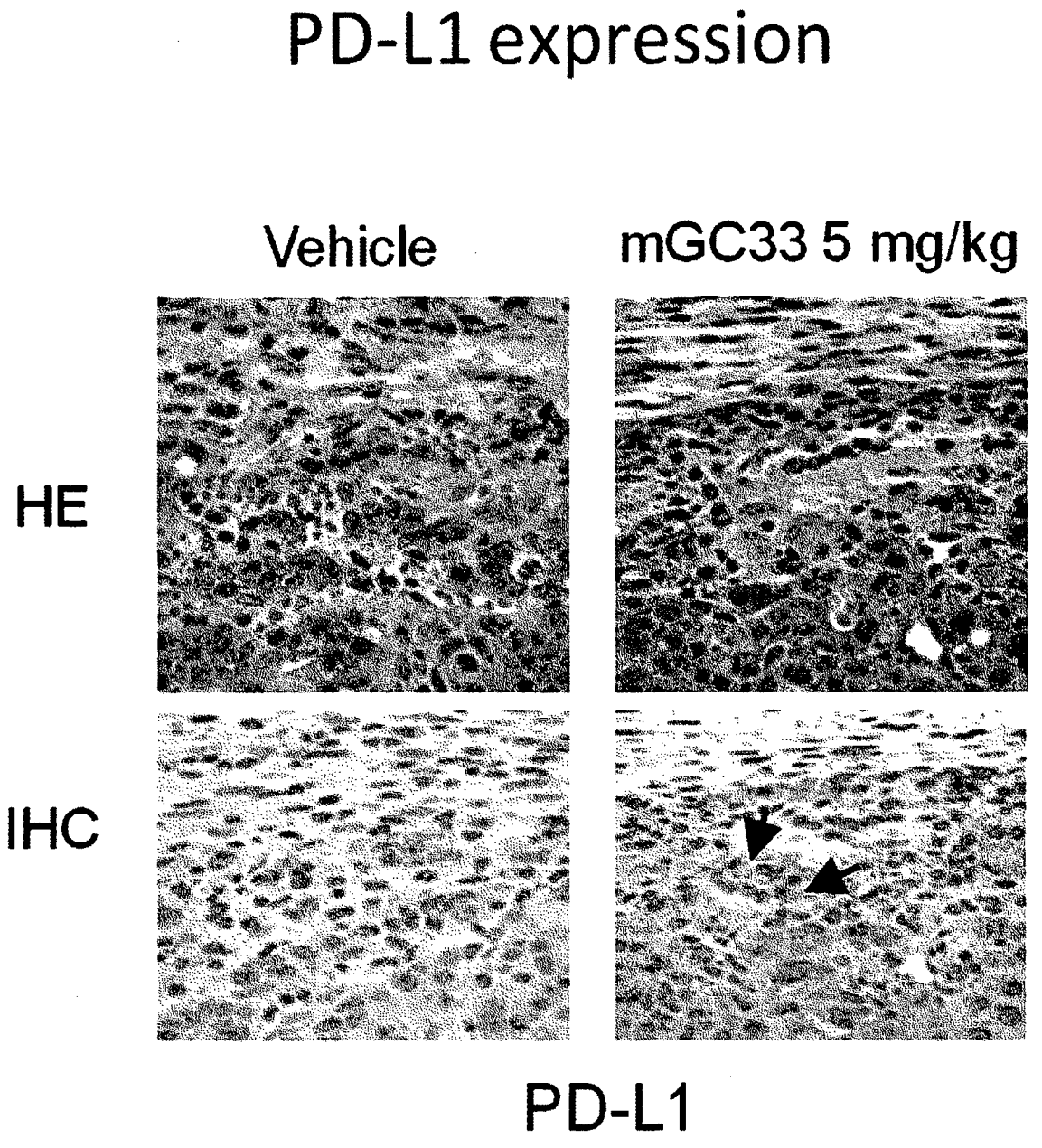
[Fig. 1]


A
Vehicle
B
mGC33
1 mg/kg
C
mGC33
5 mg/kg
Tumor volume (mm3)
1000
100
10
10
20
30
40
Days after inoculation

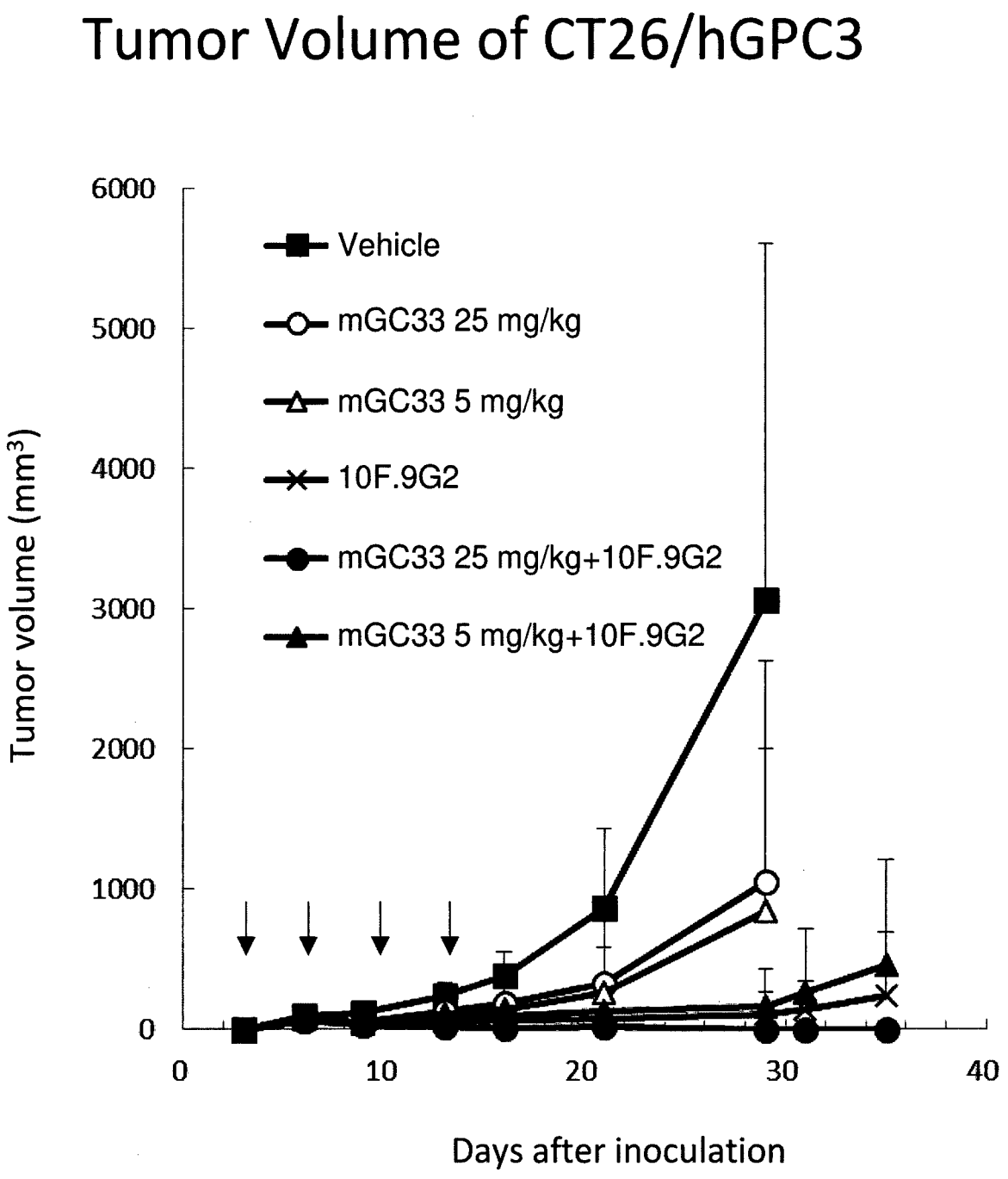
[Fig. 2A]


Macrophage infiltration
Vehicle
mGC33 5 mg/kg
HE
IHC
Lesion of tumor cell death
Stroma
Tumor tissues
Macrophages (F4/80)

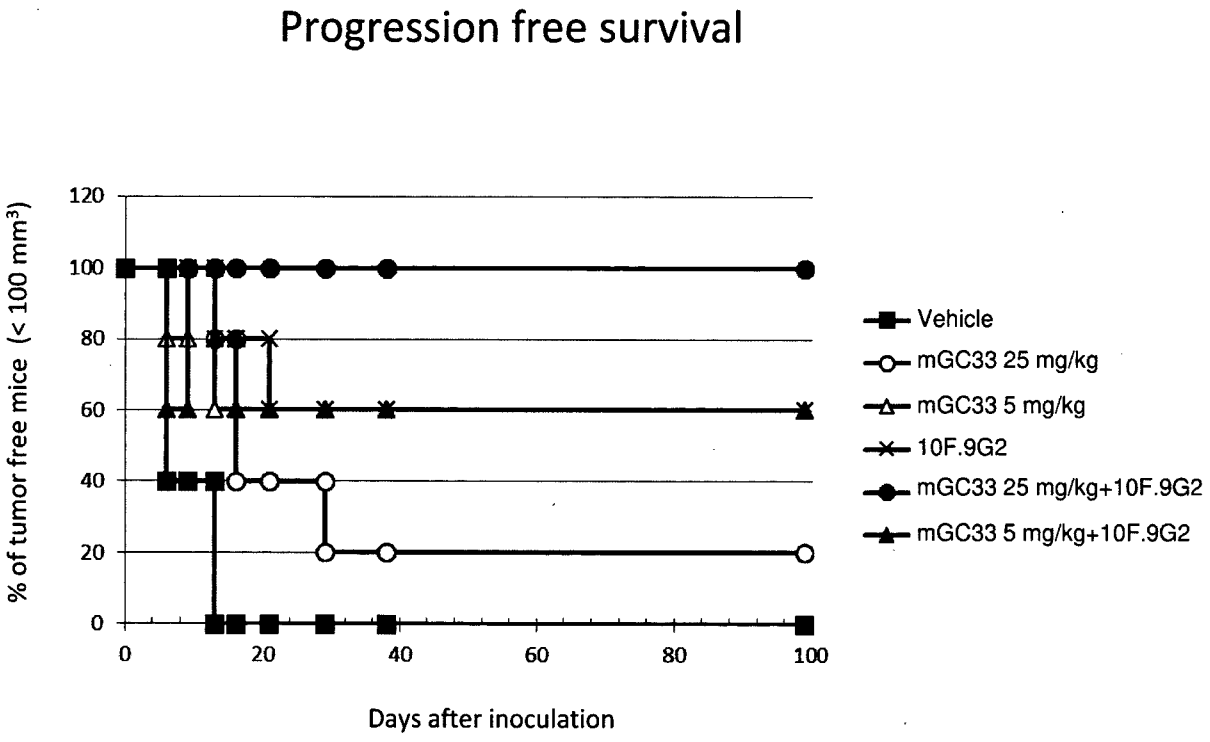
[Fig. 2B]



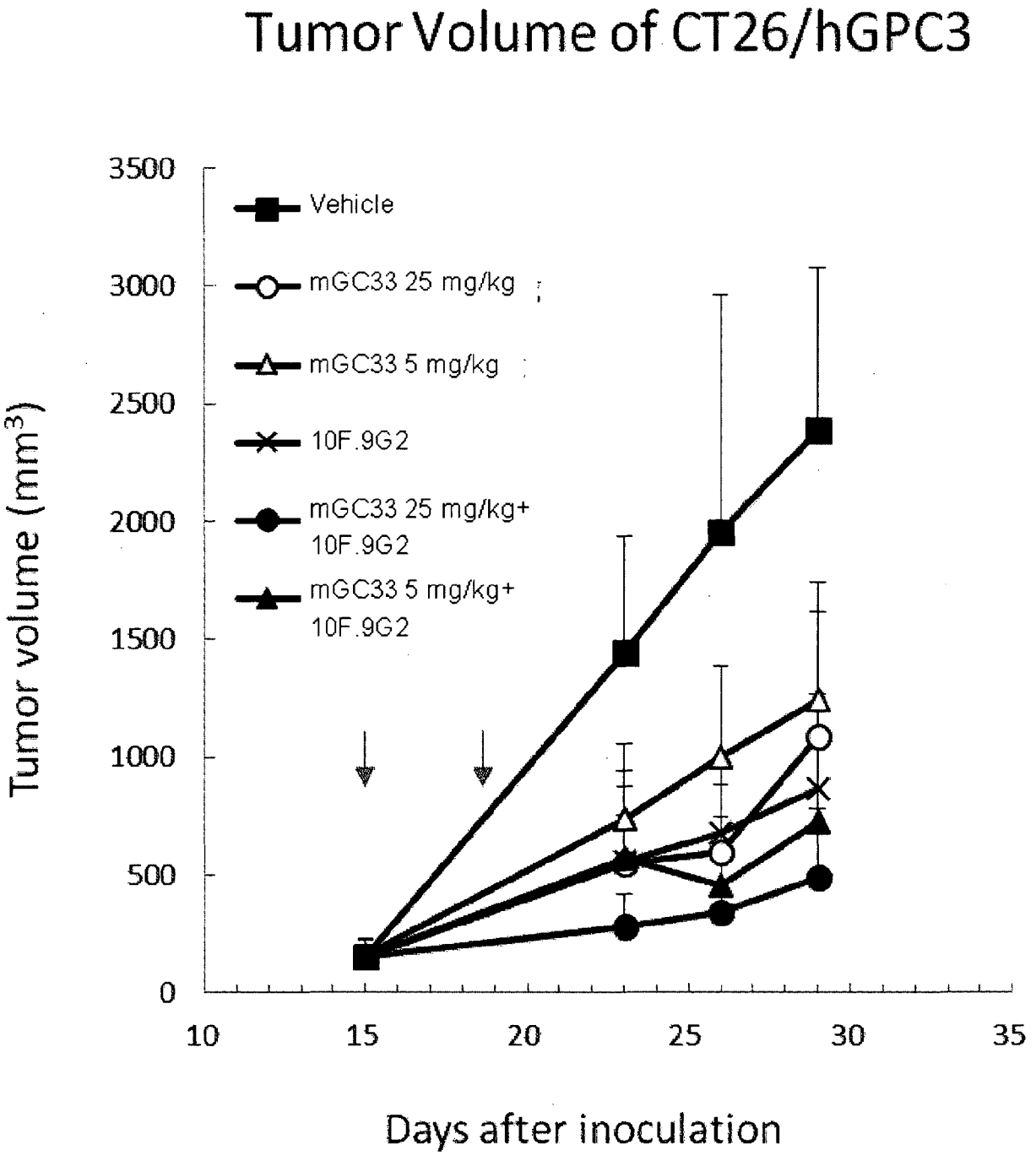
[Fig. 3A]


Tumor Volume of CT26/hGPC3
Tumor volume (mm³)
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
Days after inoculation
Vehicle
mGC33 25 mg/kg
mGC33 5 mg/kg
10F.9G2
mGC33 25 mg/kg+10F.9G2
mGC33 5 mg/kg+10F.9G2

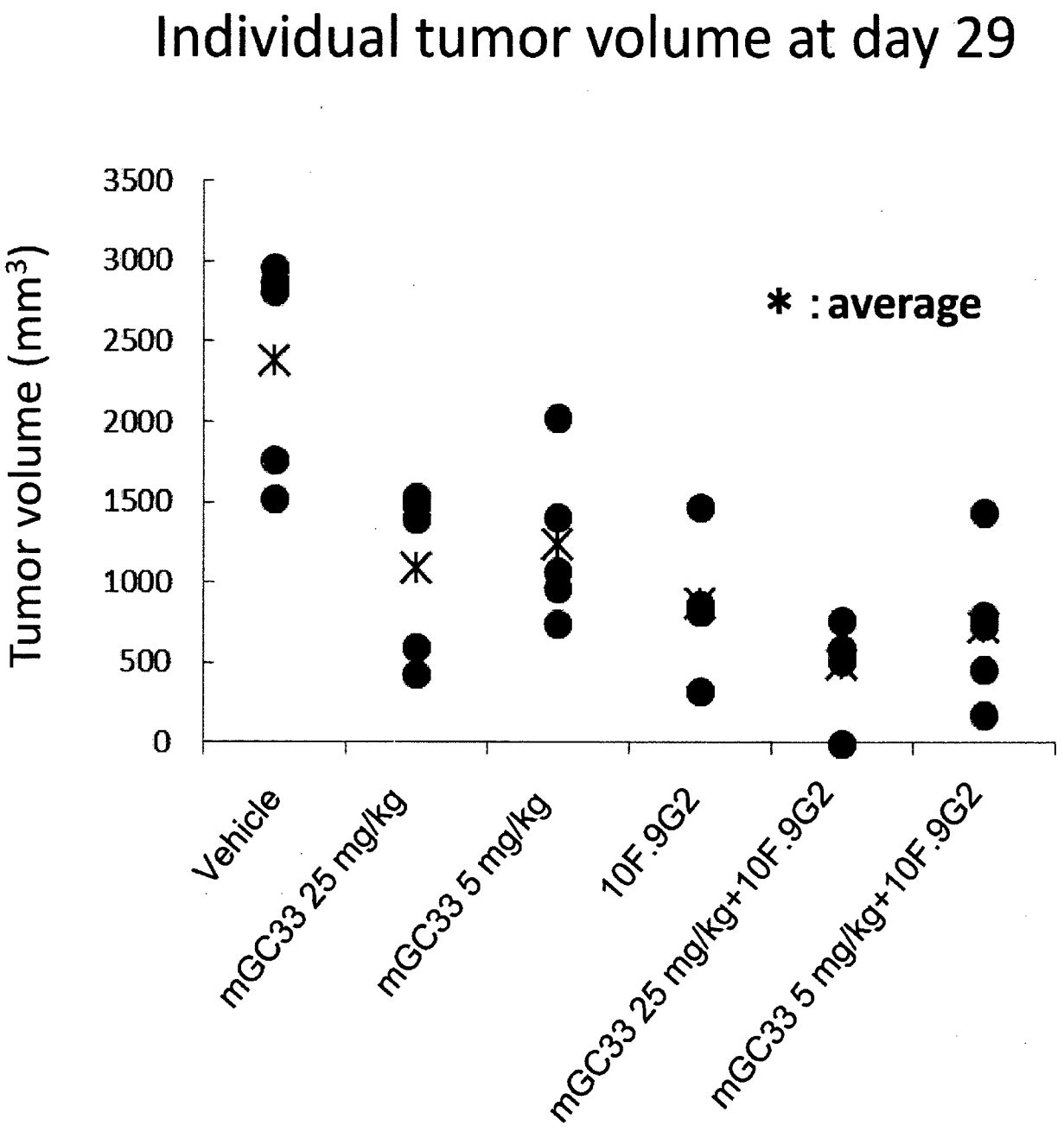
[Fig. 3B]


Progression free survival
% of tumor free mice (< 100 mm³)
120
100
80
60
40
20
0
0
20
40
60
80
100
Days after inoculation
Vehicle
mGC33 25 mg/kg
mGC33 5 mg/kg
10F.9G2
mGC33 25 mg/kg+10F.9G2
mGC33 5 mg/kg+10F.9G2

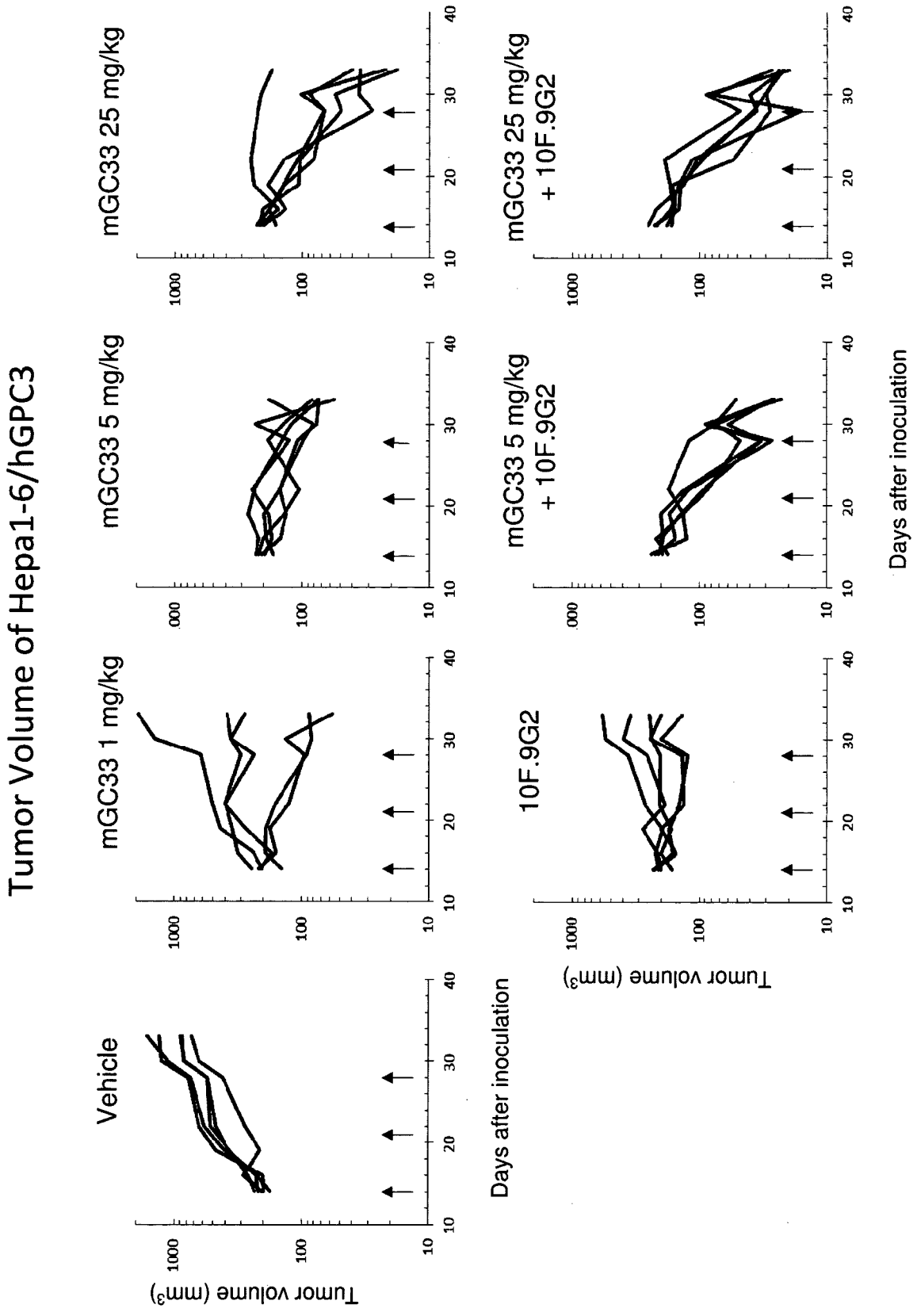
[Fig. 4A]


Tumor Volume of CT26/hGPC3
Tumor volume (mm3)
3500
3000
2500
2000
1500
1000
500
0
Vehicle
mGC33 25 mg/kg
mGC33 5 mg/kg
10F.9G2
mGC33 25 mg/kg+ 10F.9G2
mGC33 5 mg/kg+ 10F.9G2
10
15
20
25
30
35
Days after inoculation

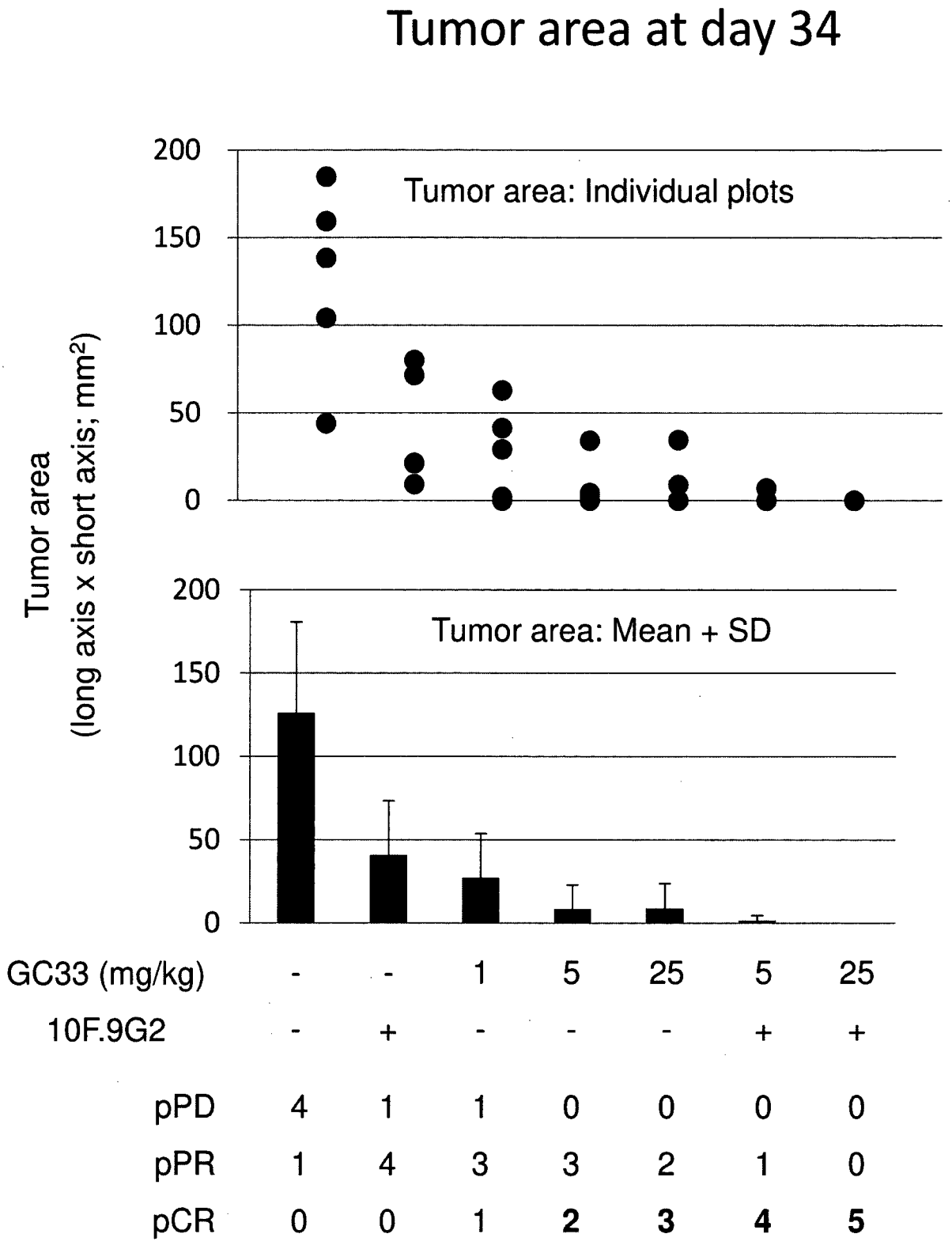
[Fig. 4B]


Individual tumor volume at day 29
Tumor volume (mm3)
3500
3000
2500
2000
1500
1000
500
0
* : average
Vehicle
mGC33 25 mg/kg
mGC33 5 mg/kg
10F.9G2
mGC33 25 mg/kg+10F.9G2
mGC33 5 mg/kg+10F.9G2

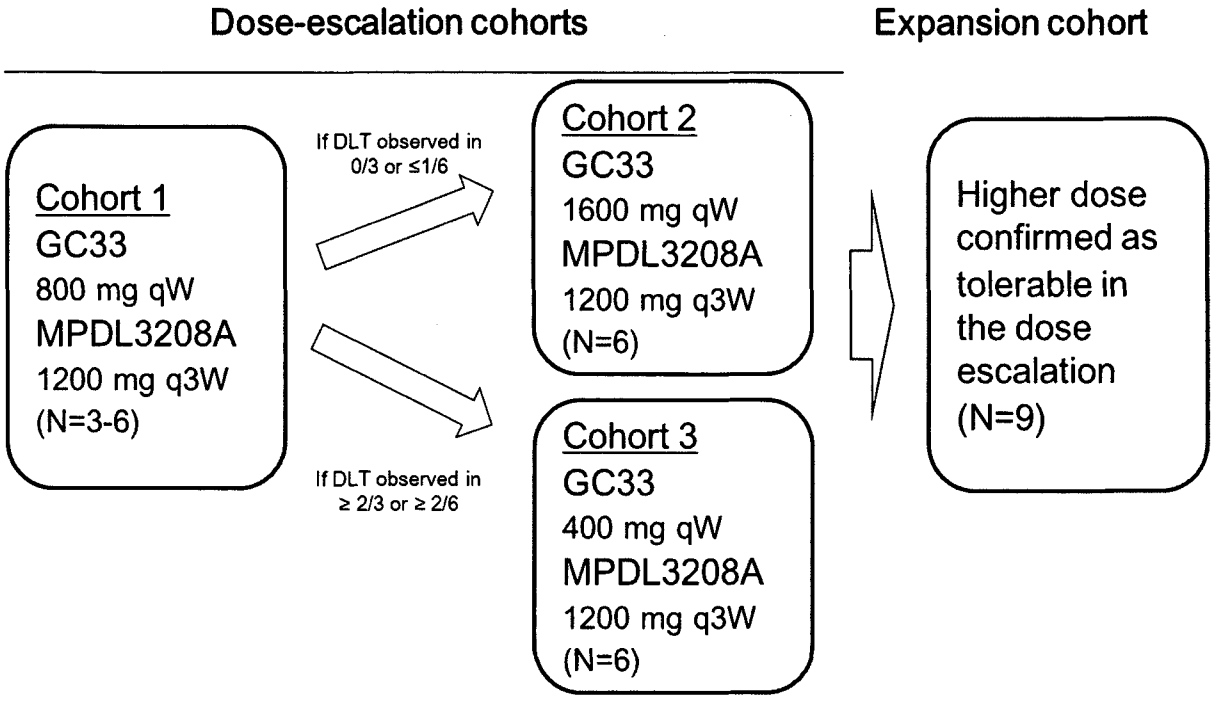
[Fig. 5A]


Tumor Volume of Hepa1-6/hGPC3
Vehicle
mGC33 1 mg/kg
mGC33 5 mg/kg
mGC33 25 mg/kg
10F.9G2
mGC33 5 mg/kg + 10F.9G2
mGC33 25 mg/kg + 10F.9G2
Tumor volume (mm3)
Days after inoculation
1000
100
10
10
20
30
40

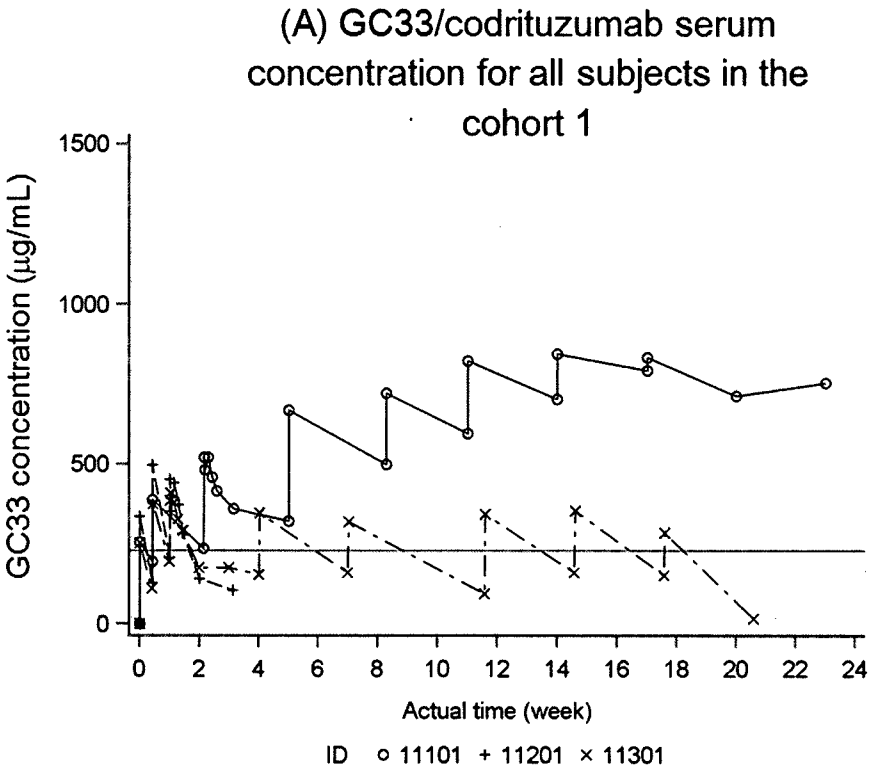
[Fig. 5B]


Tumor area at day 34
Tumor area: Individual plots
Tumor area: Mean + SD
Tumor area (long axis x short axis; mm²)
200
150
100
50
0
GC33 (mg/kg) - - 1 5 25 5 25
10F.9G2 - + - - - + +
pPD 4 1 1 0 0 0 0
pPR 1 4 3 3 2 1 0
pCR 0 0 1 2 3 4 5

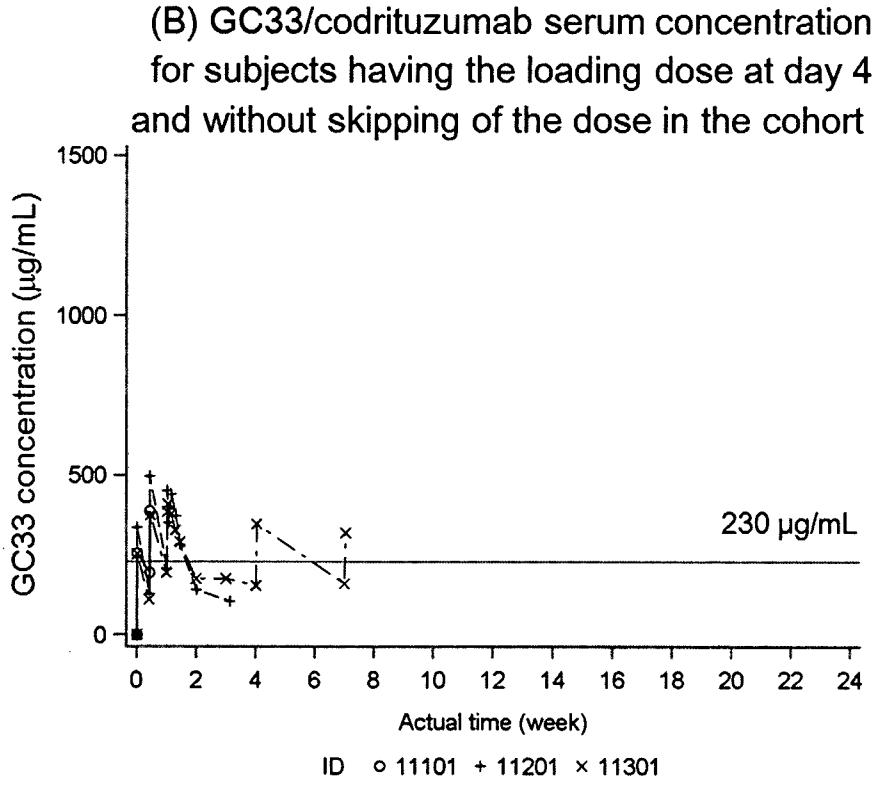
[Fig 6]


Dose-escalation cohorts
Expansion cohort
Cohort 1
GC33
800 mg qW
MPDL3208A
1200 mg q3W
(N=3-6)
If DLT observed in 0/3 or ≤1/6
If DLT observed in ≥ 2/3 or ≥ 2/6
Cohort 2
GC33
1600 mg qW
MPDL3208A
1200 mg q3W
(N=6)
Cohort 3
GC33
400 mg qW
MPDL3208A
1200 mg q3W
(N=6)
Higher dose confirmed as tolerable in the dose escalation
(N=9)

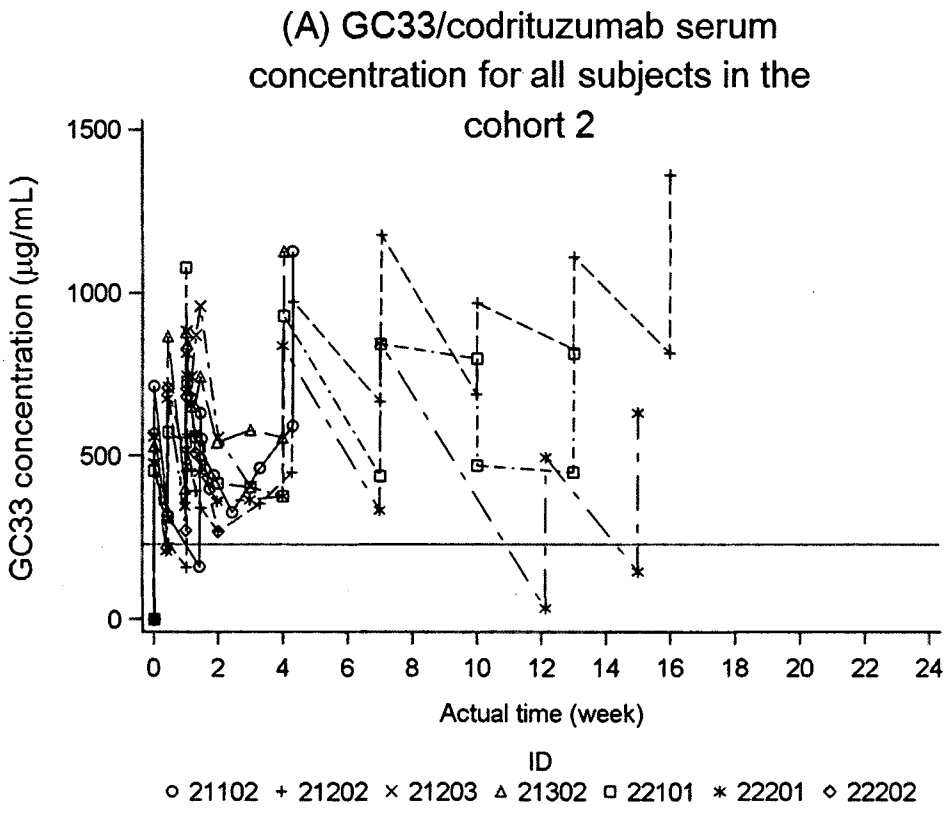
[Fig 7A]


(A) GC33/codrituzumab serum concentration for all subjects in the cohort 1
GC33 concentration (μg/mL)
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Actual time (week)
ID ○ 11101 + 11201 × 11301

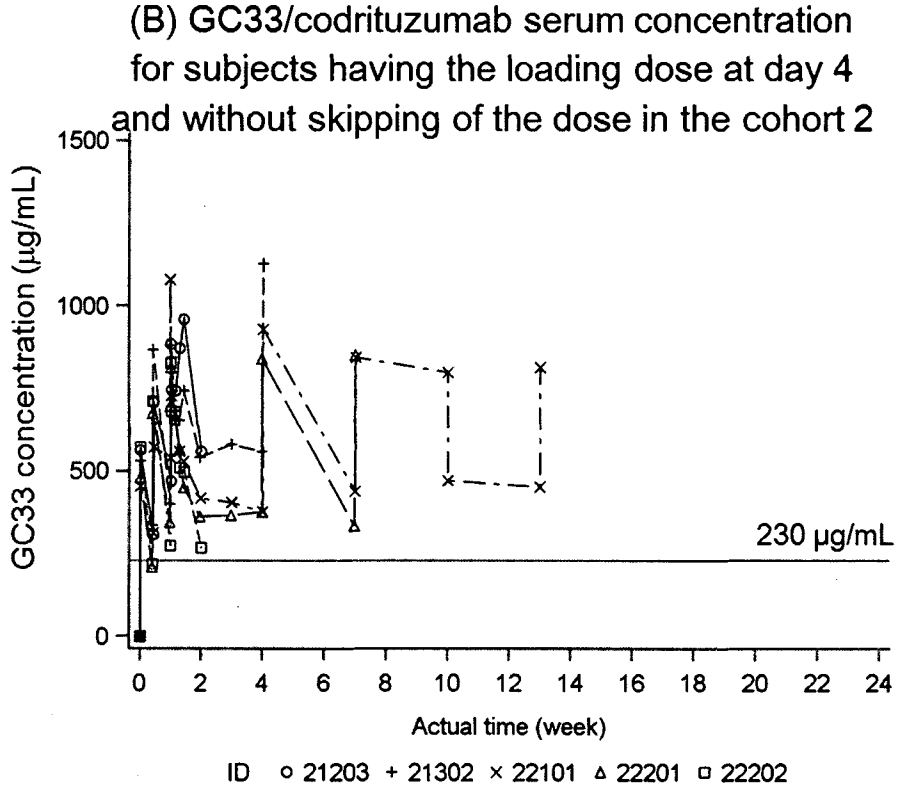
[Fig 7B]


(B) GC33/codrituzumab serum concentration for subjects having the loading dose at day 4 and without skipping of the dose in the cohort 1
GC33 concentration (μg/mL)
1500
1000
500
0
230 μg/mL
0 2 4 6 8 10 12 14 16 18 20 22 24
Actual time (week)
ID ○ 11101 + 11201 × 11301

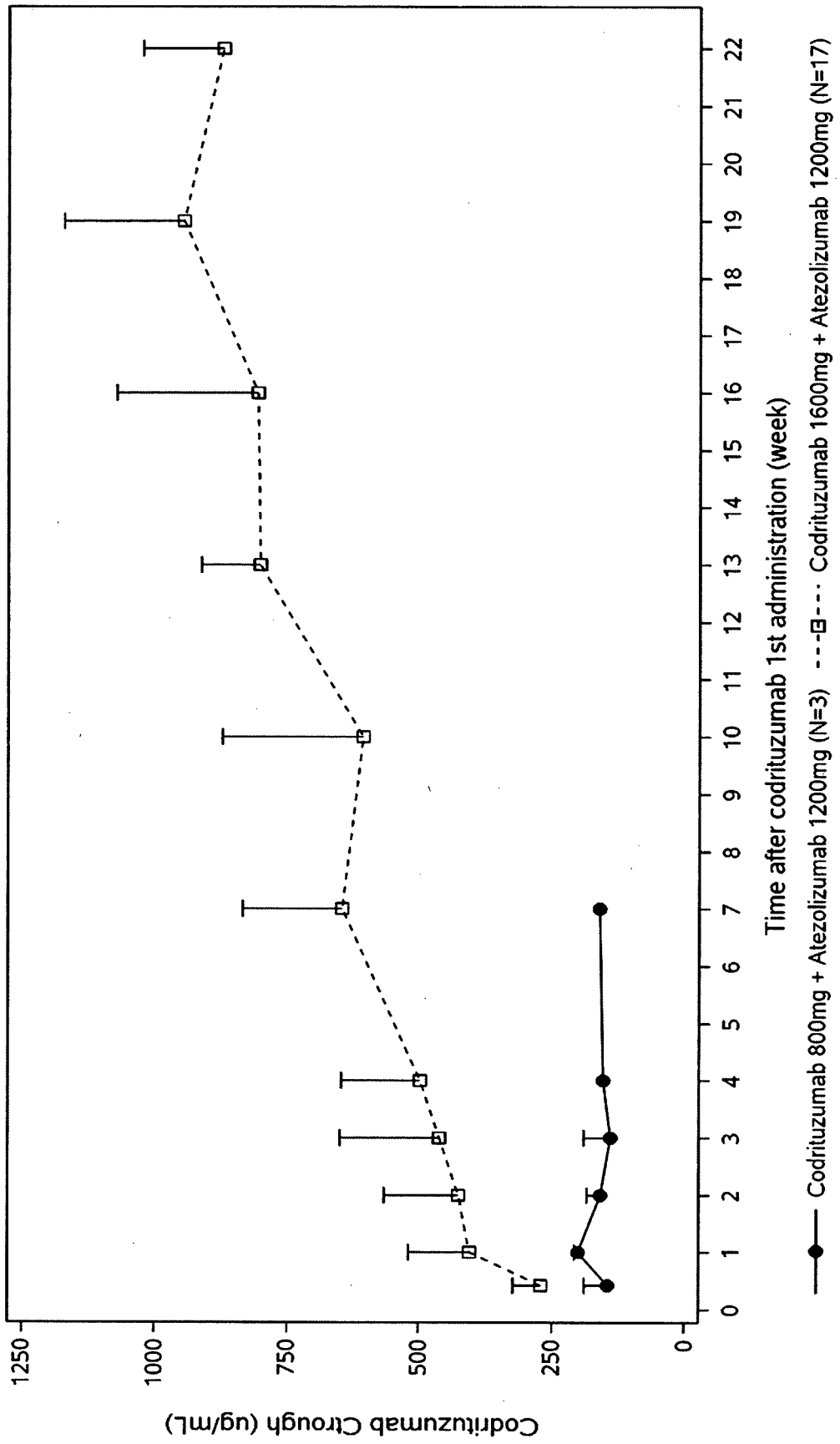
[Fig 8A]


(A) GC33/codrituzumab serum concentration for all subjects in the cohort 2
GC33 concentration (μg/mL)
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Actual time (week)
ID
○ 21102 + 21202 × 21203 △ 21302 □ 22101 ✳ 22201 ◇ 22202

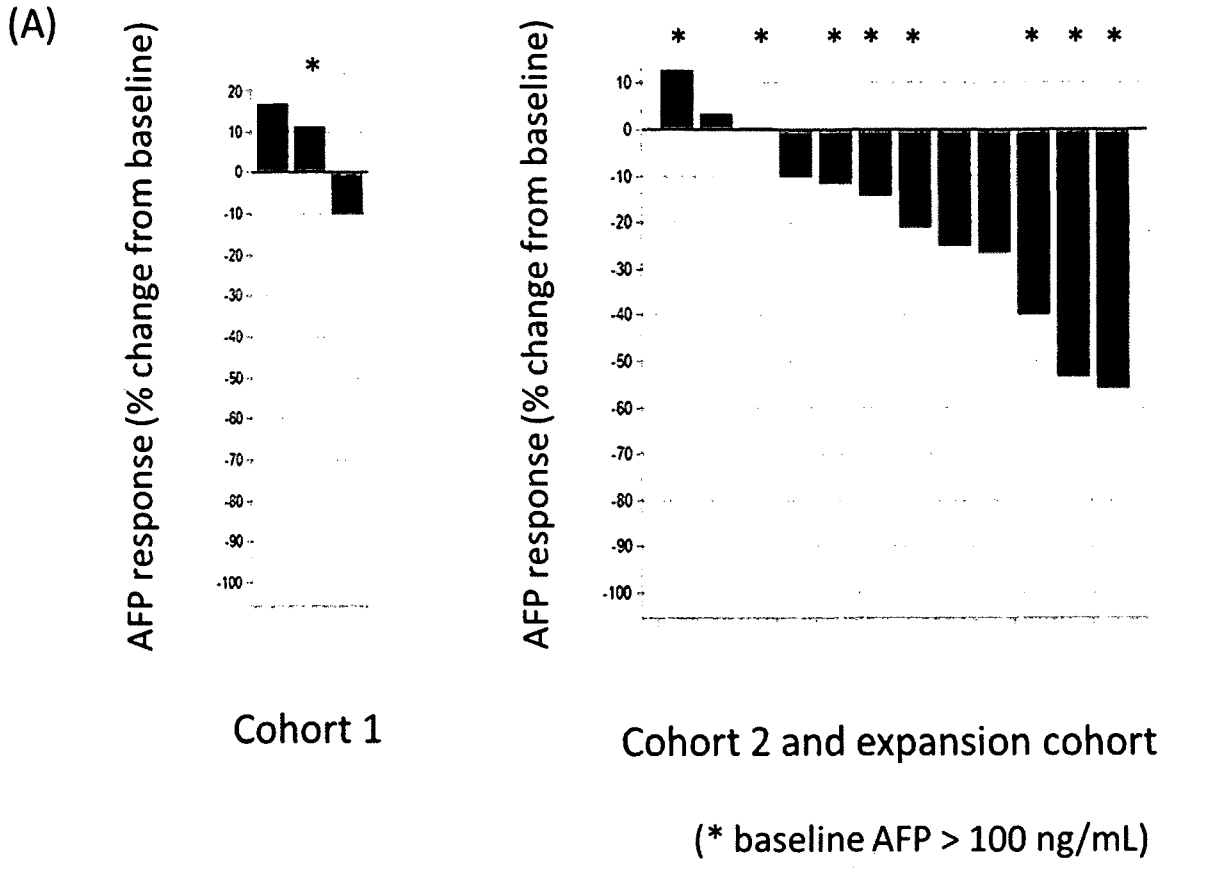
[Fig 8B]


(B) GC33/codrituzumab serum concentration for subjects having the loading dose at day 4 and without skipping of the dose in the cohort 2
GC33 concentration (μg/mL)
1500
1000
500
0
230 μg/mL
0 2 4 6 8 10 12 14 16 18 20 22 24
Actual time (week)
ID ○ 21203 + 21302 × 22101 △ 22201 □ 22202

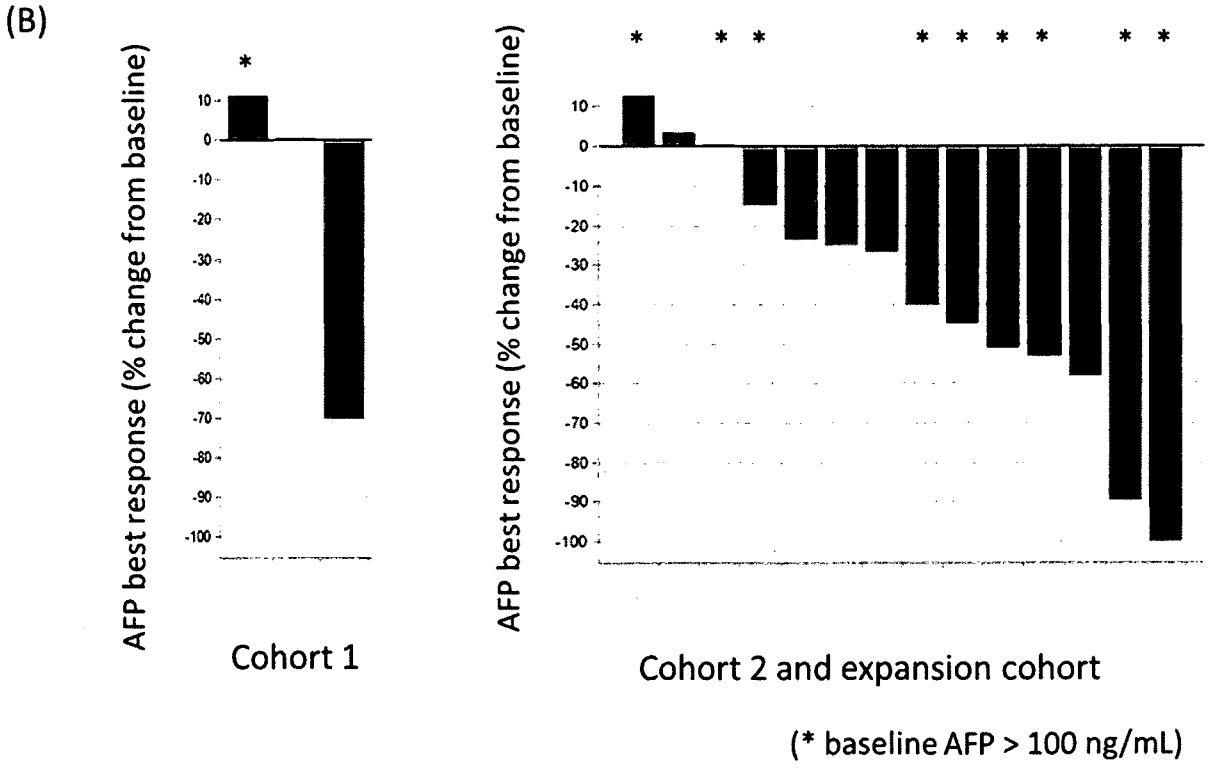
[Fig 9]


Codrituzumab Ctrough (ug/mL)
0
250
500
750
1000
1250
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Time after codrituzumab 1st administration (week)
Codrituzumab 800mg + Atezolizumab 1200mg (N=3)
Codrituzumab 1600mg + Atezolizumab 1200mg (N=17)

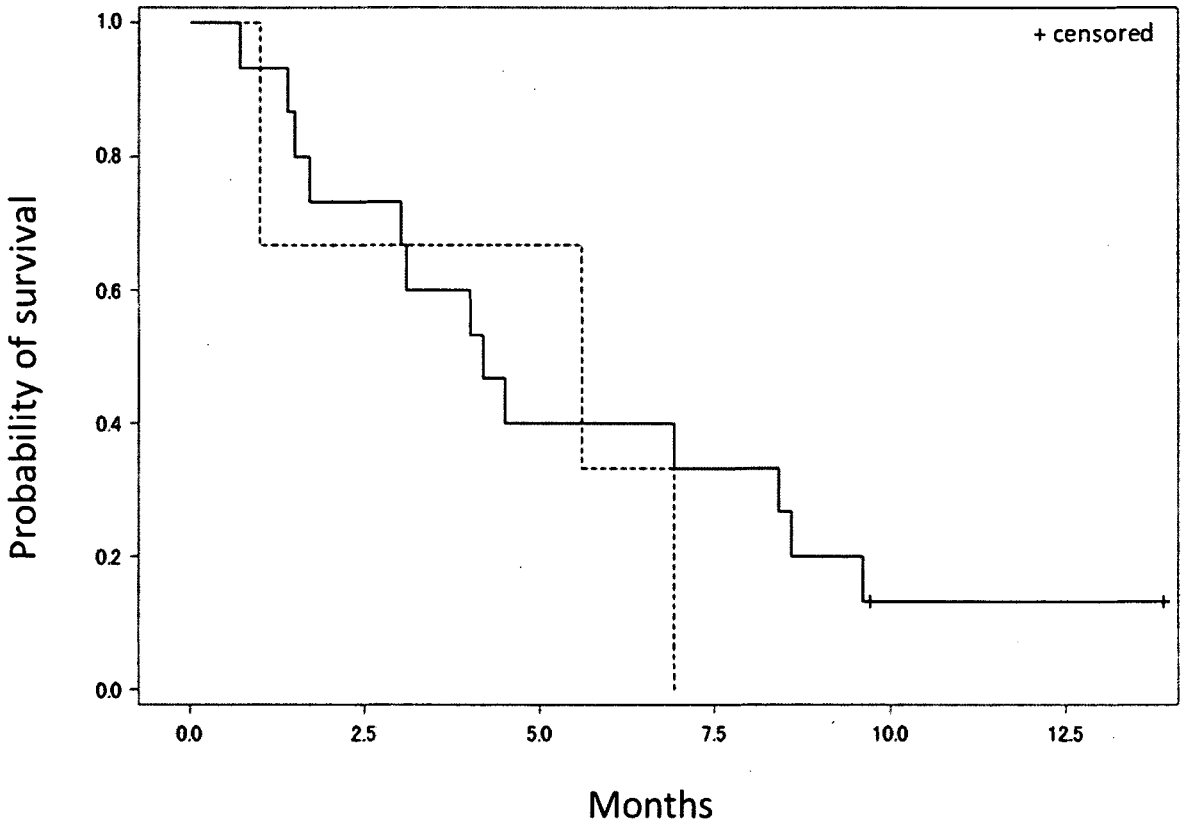
[Fig 10A]



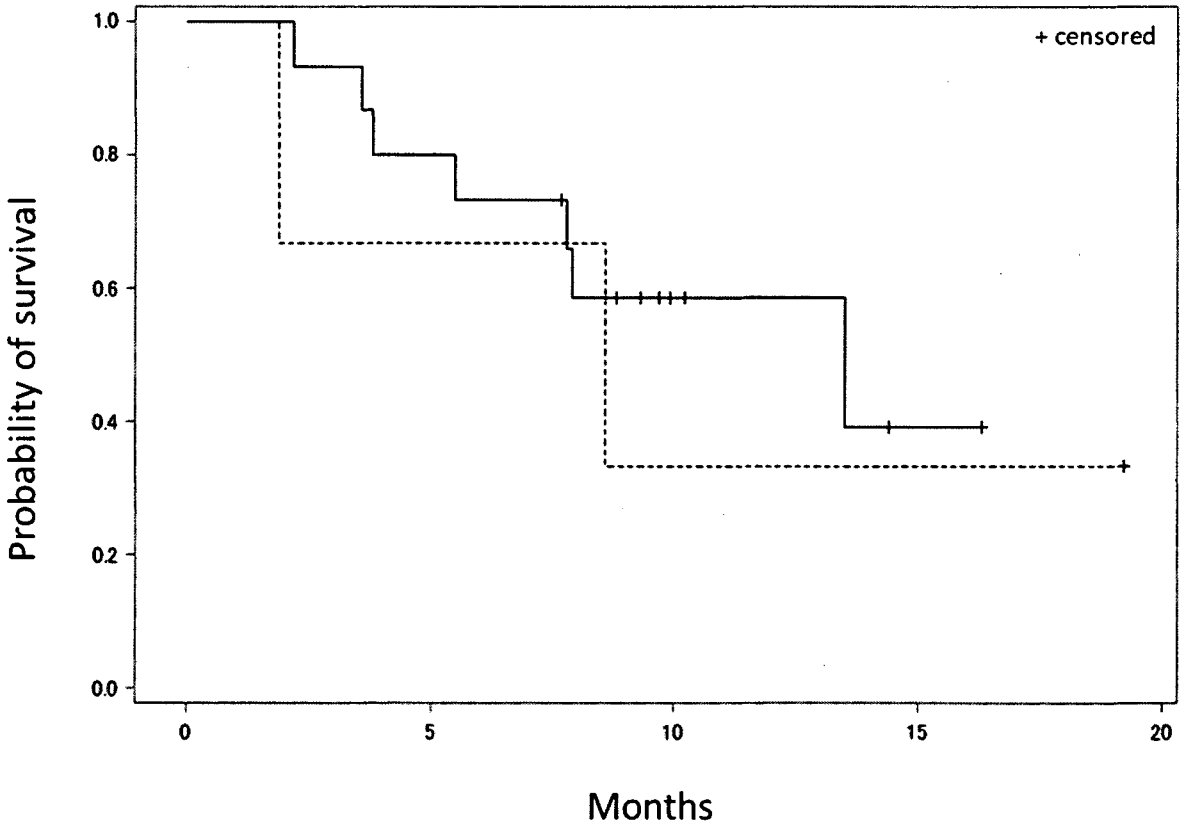
[Fig 10B]



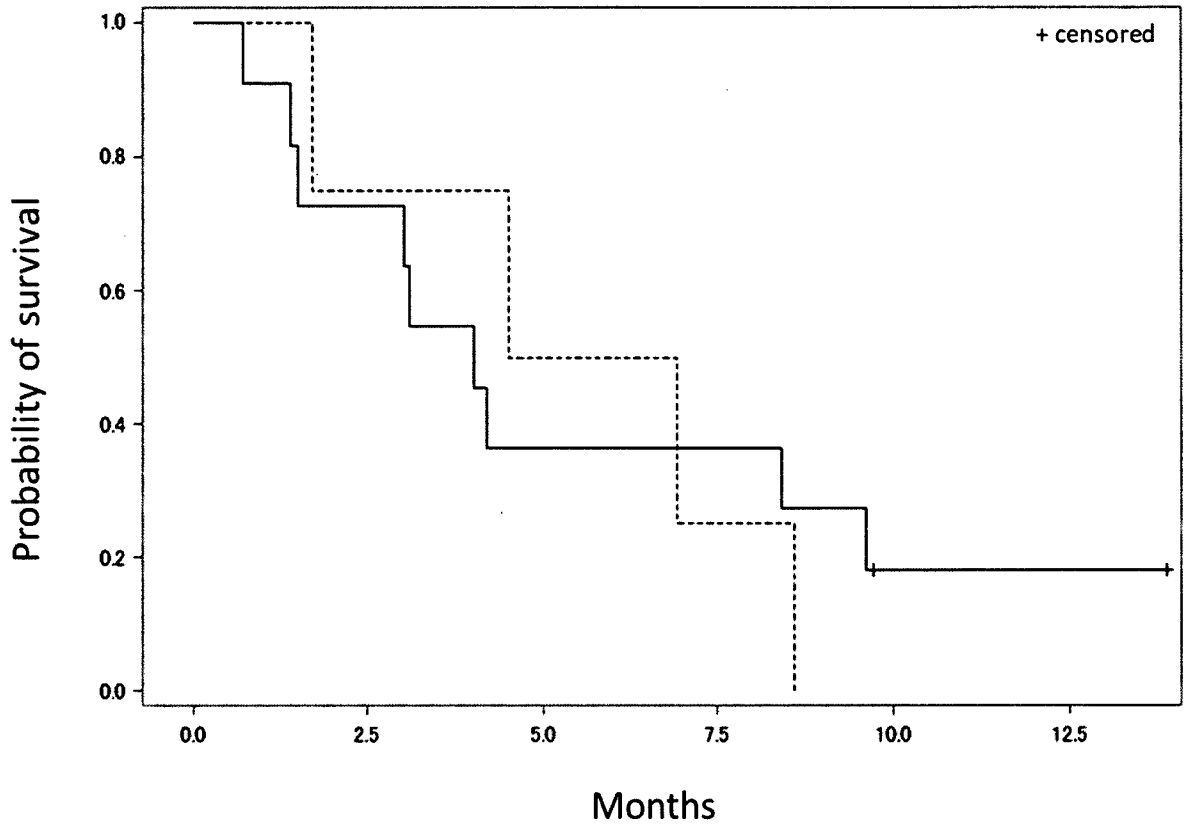
[Fig 11A]



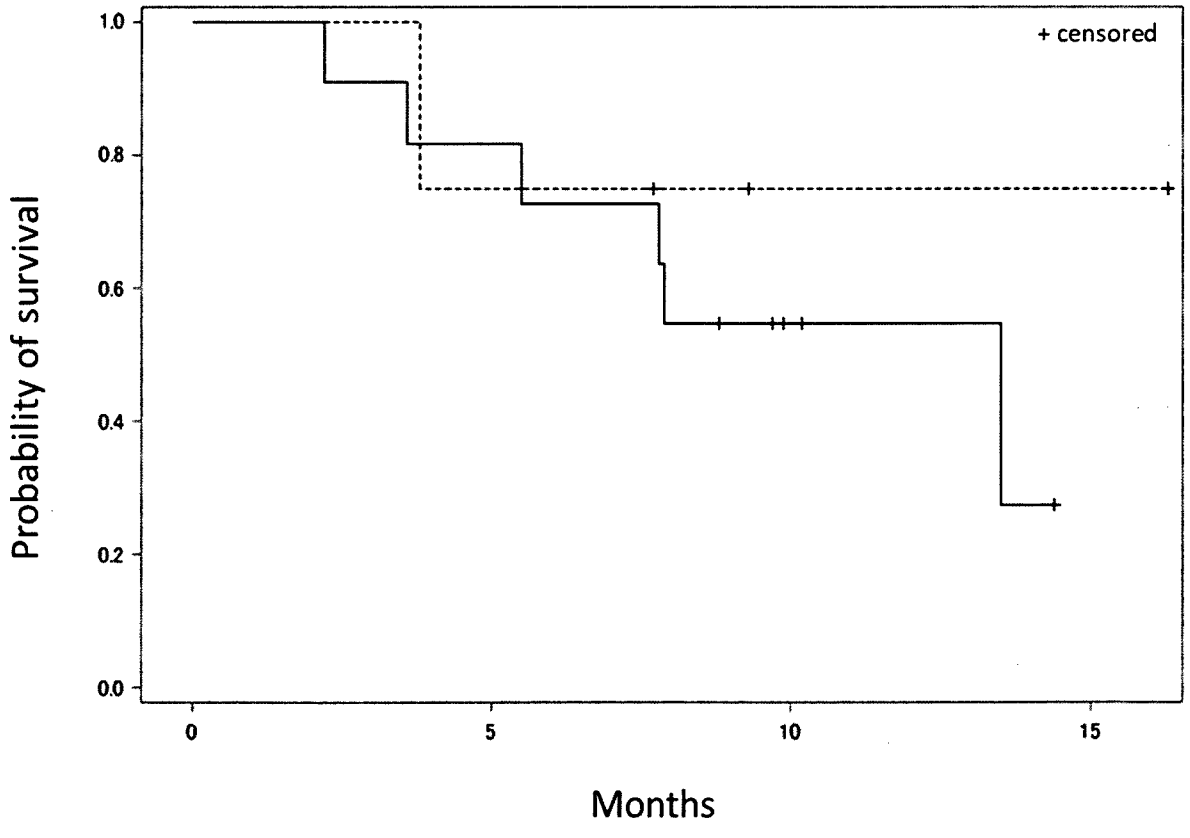
[Fig 11B]



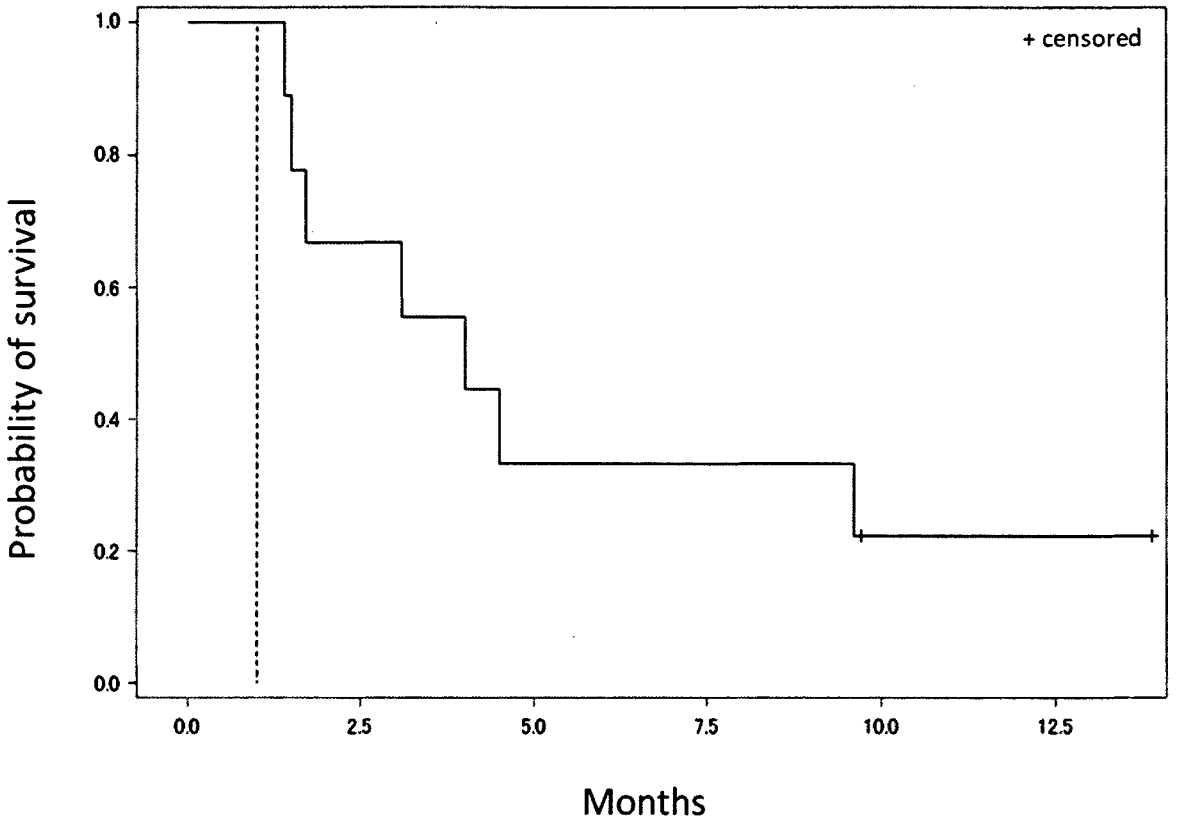
[Fig 11C]



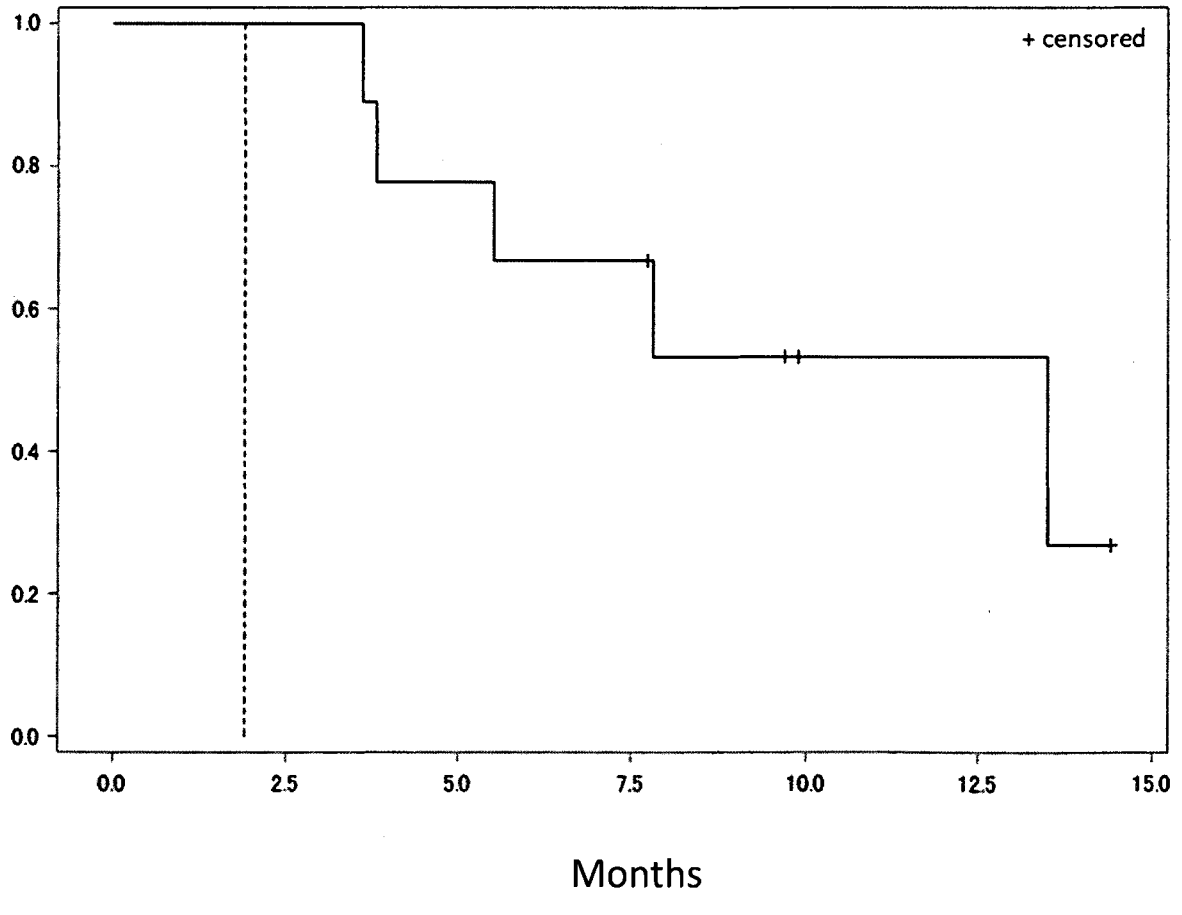
[Fig 11D]



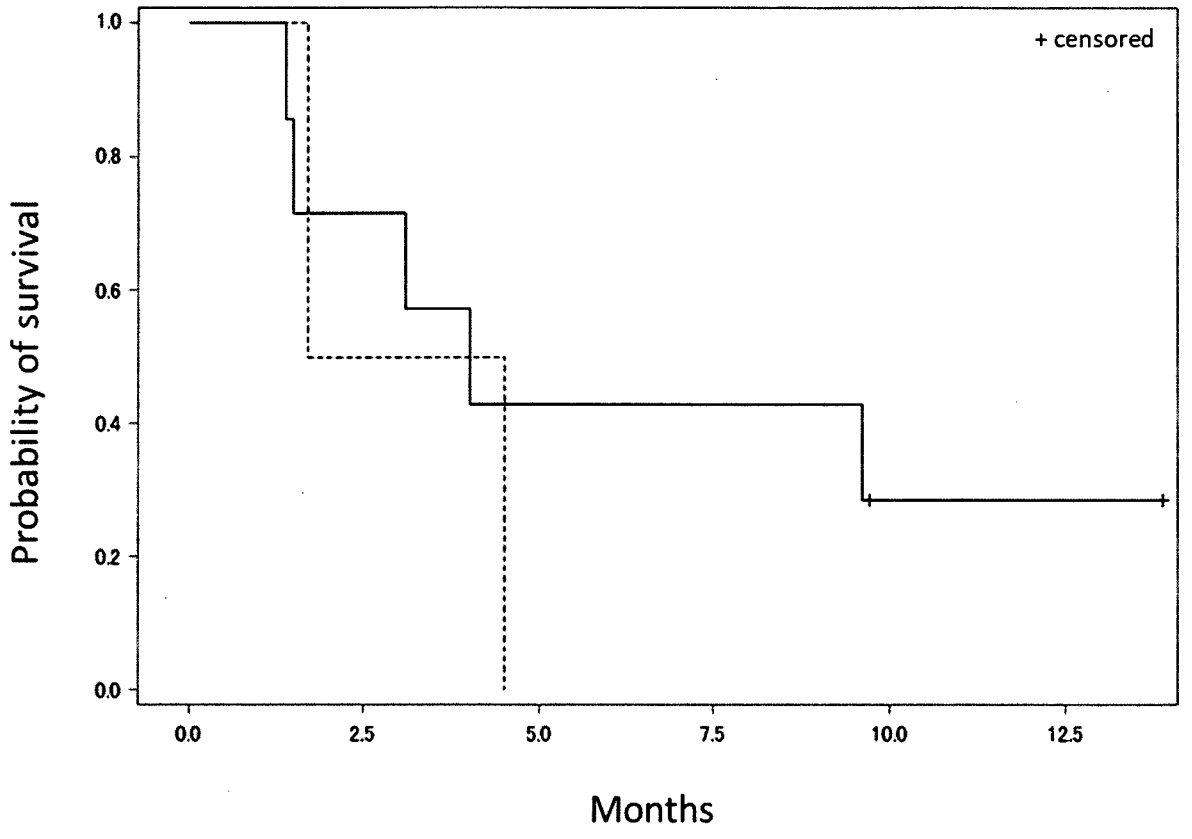
[Fig 11E]



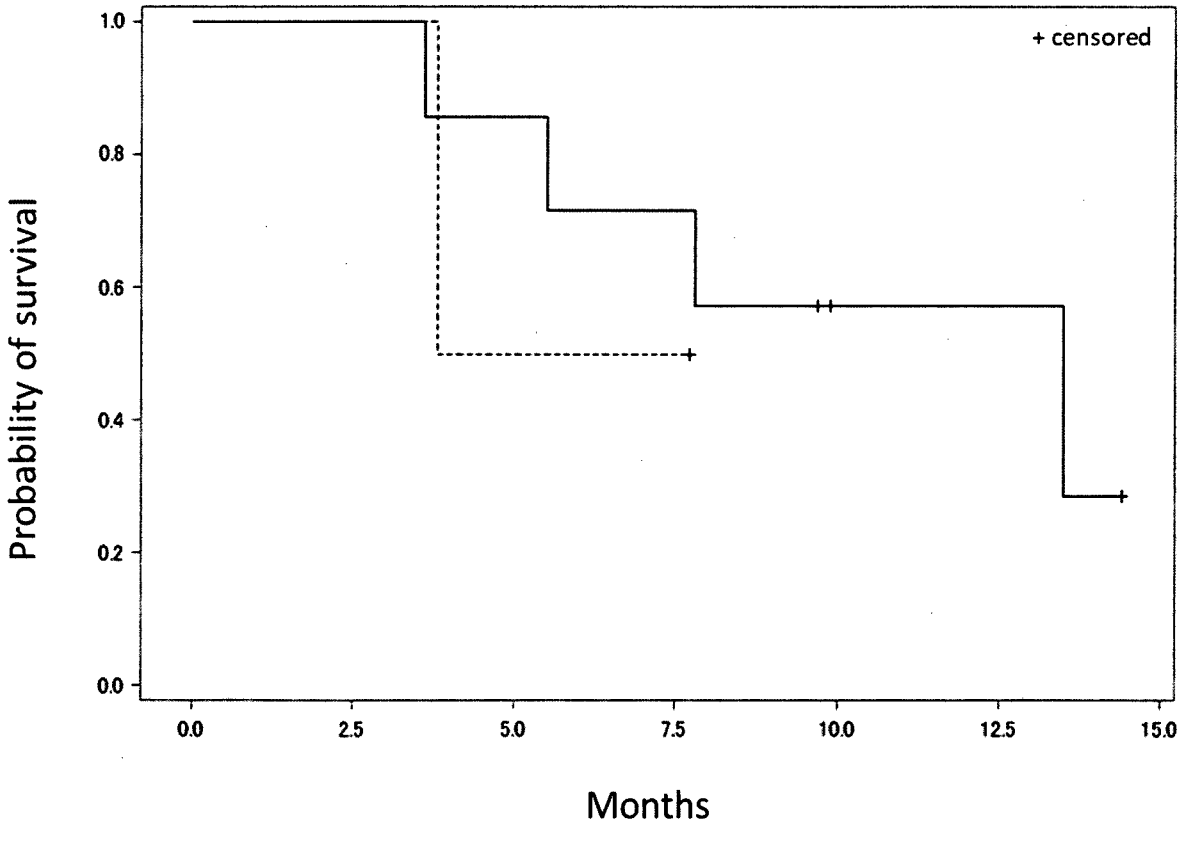
[Fig 11F]



[Fig 11G]



[Fig 11H]



INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2018/036161

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K45/06 A61K39/395 A61P35/00
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)
A61K A61P C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2017/159699 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]; GENENTECH INC [US]) 21 September 2017 (2017-09-21) paragraph [0260] - paragraph [0263]; claims 1-15; examples 4,5,6	1,2,4,5, 7,10-15
X	WO 2017/005771 A1 (UNIV DE BORDEAUX [FR]; INST NAT SANTE RECH MED [FR]) 12 January 2017 (2017-01-12)	1,2,4,5, 7,10-13, 15
Y	page 2, line 25; examples	1-15

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 January 2019	15/02/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Winger, Rudolf

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ABOU-ALFA GHASSAN K ET AL: "Phase Ib study of codrituzumab in combination with sorafenib in patients with non-curable advanced hepatocellular carcinoma (HCC)", CANCER CHEMOTHERAPY AND PHARMACOLOGY, SPRINGER VERLAG, BERLIN, vol. 79, no. 2, 24 January 2017 (2017-01-24), pages 421-429, XP036149357, ISSN: 0344-5704, DOI: 10.1007/S00280-017-3241-9 [retrieved on 2017-01-24]	1,2,4,5, 7,10-15
Y	page 426	1-15
Y	Anonymous ET AL: "A PHASE I STUDY OF CODRITUZUMAB, IN COMBINATION WITH ATEZOLIZUMAB IN PATIENTS WITH HEPATOCELLULAR CARCINOMA", , 22 July 2016 (2016-07-22), pages 1-2, XP055371632, Retrieved from the Internet: URL:http://rctportal.niph.go.jp/en/detail?trial_id=JapicCTI-163325 [retrieved on 2017-05-11] the whole document	1-15
X	WO 2015/112805 A1 (REGENERON PHARMA [US]) 30 July 2015 (2015-07-30)	1,2,4,5, 7,10-12, 14,15
Y	paragraph [0141]; claims	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/JP2018/036161

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017159699 A1	21-09-2017	AU 2017235097 A1	20-09-2018
		CA 3016552 A1	21-09-2017
		CN 109153719 A	04-01-2019
		EP 3430054 A1	23-01-2019
		JP 6430025 B2	28-11-2018
		JP 2018516842 A	28-06-2018
		KR 20180120063 A	05-11-2018
		TW 201740975 A	01-12-2017
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WO 2017005771 A1	12-01-2017	NONE	
WO 2015112805 A1	30-07-2015	AU 2015209238 A1	21-07-2016
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		SG 11201605908W A	30-08-2016
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		US 2018186883 A1	05-07-2018
		UY 35965 A	31-08-2015
		WO 2015112805 A1	30-07-2015