An anti-CTLA-4 antibody and an anti-PD-1 antibody have been approved for treatment of metastatic melanoma and are currently in clinical trials for other cancers. Anti-PD-L1 antibody, directed to the ligand for PD-1, is also currently in clinical development.

[0010] Inhibition of FGFR signaling has been reported to improve anti-tumor immunity and impair metastasis in breast cancer. (*See, e.g.,* T. Ye et al., *Breast Cancer Res. Treat.* 143: 435-446 (2014).) Anti-FGFR2 antibodies have also been tested in models of gastric cancer, for example. However, whether co-administering an FGFR2 inhibitor with an immune checkpoint inhibitor such as a PD-1 or PD-L1 inhibitor would further improve treatment in a tumor model was unknown. The inventors herein have demonstrated that a combination of an FGFR2 inhibitory antibody and a PD-1 inhibitory antibody demonstrates at least additive effects in a mouse breast tumor model. The inventors further show that treatment with an FGFR2 inhibitory antibody alone leads to an increase in PD-L1-expressing cells, NK cells, and CD3+, CD8+, and CD4+ T cells in tumor tissue in a mouse breast tumor model, and results in an increased lymphoid to myeloid cell ratio in the tumor tissue. In addition, an FGFR2 inhibitor given alone has also benefitted a human bladder cancer subject. The results herein, taken together, indicate that FGFR2 inhibitors may alter the tumor microenvironment and may therefore enhance tumor-killing immune responses, either alone or in combination with a PD-1/PD-L1 inhibitor.

SUMMARY

[0011] In one aspect, there is provided use of an anti-fibroblast growth factor receptor 2 (FGFR2) antibody and a programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) inhibitor for preparation of a medicament for treatment of cancer in a subject. In another aspect, there is provided use of an anti-FGFR2 antibody for preparation of a medicament for increasing the number of NK cells and/or PD-L1 positive cells in a tumor tissue of a subject with cancer, wherein the anti-FGFR2 antibody is an antibody as defined in present invention. In another aspect, there is provided use of an anti-FGFR2 antibody for preparation of a medicament for increasing the number of one or more of PD-L1 positive cells, NK cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, and macrophages in tumor tissue of a cancer subject, wherein the anti-FGFR2 antibody is an antibody as defined in the present invention, and wherein the

antibody has enhanced ADCC activity. In a further aspect, there is provided use of an anti-FGFR2 antibody for preparation of a medicament for treatment of cancer in a subject, wherein, if the subject is determined to have an increased number of NK cells and/or PD-L1 positive cells and/or CD8+ T cells relative to a sample prior to administration of the antibody, the treatment comprises further administering at least one PD-1/PD-L1 inhibitor to the subject, wherein the anti-FGFR2 antibody is an antibody as defined in the present invention. In a further aspect, there is provided a composition comprising an anti-FGFR2-IIIb antibody and at least one PD-1/PD-L1 inhibitor. In yet further aspect, there is provided a method of determining whether a gastric or bladder cancer patient will be responsive to treatment with an FGFR2 inhibitor comprising determining whether the gastric or bladder cancer overexpresses FGFR2-IIIb by immunohistochemistry (IHC), wherein the overexpression is determined by an IHC signal of 1+, 2+, or 3+ in a sample of the cancer, and wherein the method optionally further comprises determining whether the FGFR2 gene is amplified. In some examples, methods of treating cancer in a subject are provided, comprising administering to the subject an FGFR2 inhibitor such as an anti-FGFR2 antibody or FGFR2 ECD or FGFR2 ECD fusion molecule in combination with at least one immune stimulating agent. In some examples, the immune stimulating agent is a PD-1/PD-L1 inhibitor such as an anti-PD-1 antibody, anti-PD-L1 antibody, PD-1 fusion molecule, or PD-1 polypeptide. In some examples, the immune stimulating agent comprises one or more of the agents described in the section below entitled "combinations with other immune stimulating agents." In some examples, the FGFR2 inhibitor is an antibody. In some examples, the FGFR2 inhibitor is an antibody that recognizes FGFR2-IIIb. In some examples, the FGFR2-IIIb antibody

binds with lower affinity to FGFR2-IIIc than to –IIIb or does not detectably bind to FGFR2-IIIc. In some examples, the FGFR2 inhibitor is an FGFR2 ECD. In some examples, the FGFR2 inhibitor is an FGFR2 ECD fusion molecule comprising an FGFR2 ECD and a fusion partner, such as an Fc domain, albumin, or polyethylene glycol (PEG). In some examples, where the at least one immune stimulating agent comprises a PD-1/PD-L1 inhibitor, the PD-1/PD-L1 inhibitor is an antibody. In some examples, the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody or an anti-PD-L1 antibody. In some examples, the PD-1/PD-L1 inhibitor is a PD-1 polypeptide, while in some examples, the PD-1/PD-L1 inhibitor is a PD-1 fusion molecule.

[0012] In any of the examples of the methods and compositions herein, the PD-1/PD-L1 inhibitor may have the following characteristics. In some examples, the inhibitor is an anti-PD-1 antibody comprising the heavy chain and light chain CDRs of an antibody selected from nivolumab, pidilizumab, and pembrolizumab. In some examples, the anti-PD-1 antibody comprises the heavy chain and light chain variable regions of an antibody selected from nivolumab, pidilizumab, and pembrolizumab. In some examples, the anti-PD-1 antibody is selected from nivolumab, pidilizumab, and pembrolizumab. In some examples, the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody. In some examples, the anti-PD-L1 antibody comprises the heavy chain and light chain CDRs of an antibody selected from, BMS-936559, MPDL3280A (atezolizumab), MEDI4736, and MSB0010718C (avelumab). In some examples, the anti-PD-L1 antibody comprises the heavy chain and light chain variable regions of an antibody selected from BMS-936559, MPDL3280A, MEDI4736, and MSB0010718C. In some examples, the anti-PD-L1 antibody is selected from BMS-936559, MPDL3280A, MEDI4736, and MSB0010718C. In some examples, the PD-1/PD-L1 inhibitor is a fusion molecule. In some examples, the fusion molecule is AMP-224. In some examples, the PD-1/PD-L1 inhibitor is a PD-1 polypeptide, such as AUR-012.

[0013] In any of the compositions or methods described herein involving an anti-PD-1 antibody, the anti-PD-1 antibody may be a humanized antibody. In any of the compositions or methods described herein, the anti-PD-1 antibody may be selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$. In any of the compositions or methods described herein, the anti-PD-1 antibody may be a chimeric antibody. In any of the compositions or methods

described herein, the anti-PD-1 antibody may be selected from an IgA, an IgG, and an IgD. In any of the compositions or methods described herein, the anti-PD-1 antibody may be an IgG. In any of the methods described herein, the antibody may be an IgG1 or IgG2.

[0014] In any of the compositions or methods described herein, the FGFR2 inhibitor may have the following characteristics. In some examples, the inhibitor is an FGFR2 antibody. In some examples, the FGFR2 antibody is an FGFR2-IIIb antibody (also denoted αFGFR2b herein). In some examples, the FGFR2-IIIb antibody binds to FGFR2-IIIb with higher affinity than to FGFR2-IIIc, or alternatively, does not bind detectably to FGFR2-IIIc. In some examples, the antibody inhibits binding of FGF2 and/or FGF7 to FGFR2.

[0015] In some examples, the FGFR2 antibody has the heavy and light chain hypervariable region (HVR) H1, H2, H3, L1, L2, and L3 amino acid sequences of monoclonal antibodies GAL-FR21, GAL-FR22, or GAL-FR23, described in U.S. Patent No. 8,101,723 B2. In some example the FGFR2-IIIb antibody heavy chain variable region comprises: (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 6; (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 7; and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 8; and the light chain variable region comprises: (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 11.

[0016] In some examples, the FGFR2 antibody comprises an FGFR2-IIIb antibody in which the heavy chain variable domain that is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:4, or that comprises the amino acid sequence of SEQ ID NO: 4. In some examples, the FGFR2 antibody comprises an FGFR2-IIIb antibody in which the light chain variable domain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:5, or that comprises the amino acid sequence of SEQ ID NO: 5. In some examples, the heavy chain variable domain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid

sequence of SEQ ID NO:4, or that comprises the amino acid sequence of SEQ ID NO: 4 and the light chain variable domain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:5, or that comprises the amino acid sequence of SEQ ID NO: 5. In some examples, the FGFR2 antibody comprises an FGFR2-IIIb antibody in which the heavy chain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 2, or that comprises the amino acid sequence of SEQ ID NO: 2. In some examples, the FGFR2 antibody comprises an FGFR2-IIIb antibody in which the light chain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:3, or that comprises the amino acid sequence of SEQ ID NO: 3. In some examples, the heavy chain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:2, or that comprises the amino acid sequence of SEQ ID NO: 2 and the light chain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:3, or that comprises the amino acid sequence of SEQ ID NO: 3.

[0017] In some examples the FGFR2-IIIb antibody heavy chain variable region comprises: (i) CDR1 comprising the amino acid sequence of SEQ ID NO: 40; (ii) CDR2 comprising the amino acid sequence of SEQ ID NO: 41; and (iii) CDR3 comprising the amino acid sequence of SEQ ID NO: 42; and the light chain variable region comprises: (iv) CDR1 comprising the amino acid sequence of SEQ ID NO: 44; (v) CDR2 comprising the amino acid sequence of SEQ ID NO: 45; and (vi) CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

[0018] In some examples, the FGFR2 antibody comprises an FGFR2-IIIb antibody in which the heavy chain variable domain that is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:39, or that comprises the amino acid sequence of SEQ ID NO: 39. In some examples, the FGFR2 antibody comprises an FGFR2-IIIb antibody in which the light chain variable domain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:43, or that comprises the amino acid sequence of SEQ ID NO: 43. In some examples, the heavy chain

variable domain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:39, or that comprises the amino acid sequence of SEQ ID NO: 39 and the light chain variable domain is at least 95%, such as at least 97%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:43, or that comprises the amino acid sequence of SEQ ID NO: 43.

[0019] In some examples the FGFR2 antibody is afucosylated. In some examples, the antibody lacks fucose at Asn297. In some examples, the antibody comprises a kappa light chain constant region. In some examples, the antibody comprises an IgG1 heavy chain constant region. In some examples, an afucosylated antibody has enhanced ADCC (antibody-dependent cell cytotoxic) activity *in vitro* and/or *in vivo* compared to an antibody having the same amino acid sequence that is fucosylated at Asn297. In some examples, an afucosylated antibody has enhanced affinity for Fc gamma RIIIA compared to an antibody having the same amino acid sequence that is fucosylated at position Asn297. In some examples, the afucosylated antibody is capable of increasing the number of one or more of PD-L1 positive cells, NK cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, and macrophages in tumor tissue in a mouse xenograft and/or syngeneic tumor model compared to a control (e.g. as compared to a control antibody that does not target FGFR2).

[0020] In some examples, the FGFR2 inhibitor is an FGFR2 ECD such as an FGFR2 ECD fusion molecule. FGFR2 ECD fusion molecules may comprise fusion partners such as an Fc domain, albumin, or PEG.

[0021] In some examples, and FGFR2 inhibitor is capable of binding to FGFR2 as well as to an FGFR2 mutant with an activating mutation, such as the FGFR2-S252W mutation, which is found in some cancer cells.

[0022] In any of the compositions or methods described herein involving an FGFR2 antibody, the FGFR2 antibody may be a humanized antibody. In any of the compositions or methods described herein, the FGFR2 antibody may be selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$. In any of the compositions or methods described herein, the FGFR2 antibody may be a chimeric antibody. In any of the compositions or methods described herein, the FGFR2 antibody may be selected from an IgA, an IgG, and an IgD. In any of the compositions or methods described herein, the

FGFR2 antibody may be an IgG. In any of the methods described herein, the antibody may be an IgG1, IgG2, IgG3, or IgG4.

[0023] In some examples, the FGFR2 inhibitor is administered at a dose of at least 0.1, 0.3, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 20, or 30 mg/kg, or within a range bounded by any two of those doses. In some examples, an PD-1/PD-L1 inhibitor is administered at a dose of at least 0.1, 0.3, 0.5, 1, 2, 3, 4, 5, or 10 mg/kg or at a range bounded by any two of these doses such as at a range of 0.5-10 mg/kg. In some examples, the FGFR2 inhibitor and the at least one immune stimulating agent, such as a PD-1/PD-L1 inhibitor, are administered at least once per 1, 2, 3, 4, or 5 weeks.

[0024] In some examples, the cancer overexpresses FGFR2IIIb either in the presence or in the absence of FGFR2 gene amplification. In some examples, the FGFR2IIIb overexpression is determined by immunohistochemistry (IHC). For example, the overexpression may be determined by an IHC signal of 1+, 2+, or 3+ in at least 10% of tumor cells, such as in at least 20%, 30%, 40%, or 50% of tumor cells.

[0025] In some examples, the cancer is selected from gastric cancer, breast cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, cholangiocarcinoma, esophageal cancer (including gastroesophageal junction adenocarcinoma), and endometrial cancer. In some examples, the cancer is recurrent or progressive after a therapy selected from surgery, chemotherapy, radiation therapy, or a combination thereof. In some examples, the subject is a PD-1/PD-L1 inhibitor inadequate responder. In some examples, the subject has previously received PD-1/PD-L1 inhibitor therapy.

[0026] In some examples, a method of treating cancer further comprises administering at least one additional therapeutic agent selected from a platinum agent, paclitaxel, ABRAXANE®, docetaxel, gemcitabine, capecitabine, irinotecan, epirubicin, FOLFOX, FOLFIRI, leucovorin, fluorouracil, mitomycin C, and doxorubicin hydrochloride. In some examples, the platinum agent is selected from cisplatin, oxaliplatin, and carboplatin. In some examples, a method of treating cancer further comprises administering paclitaxel. In some examples, a method of treating cancer further comprises administering cisplatin and/or 5-FU.

[0027] In some examples, the FGFR2 inhibitor and the PD-1/PD-L1 inhibitor are administered concurrently or sequentially. In some xamples, the FGFR2 inhibitor and the immune stimulating agent are administered concurrently. In some examples, one or more doses of immune stimulating agent are administered prior to administering an FGFR2 inhibitor. In some examples, the subject received a complete course of immune stimulating agent therapy prior to administration of the FGFR2 inhibitor. In some examples, the FGFR2 inhibitor is administered during a second course of immune stimulating agent therapy. In some examples, the subject received at least one, at least two, at least three, or at least four doses of immune stimulating agent prior to administration of the FGFR2 inhibitor. In some examples, at least one dose of immune stimulating agent is administered concurrently with the FGFR2 inhibitor. In some examples, one or more doses of the FGFR2 inhibitor are administered prior to administering an immune stimulating agent. In some examples, the subject may receive at least two, at least three, at least three, or at least four doses of the FGFR2 inhibitor prior to administration of immune stimulating agent. In some examples, at least one dose of the FGFR2 inhibitor is administered concurrently with immune stimulating agent.

[0028] In some examples, administration of the FGFR2 inhibitor and a PD-1/PD-L1 inhibitor in a mouse xenograft and/or syngeneic tumor model results in either additive or synergistic inhibition of tumor growth. In some examples, the model is a breast cancer model. In some examples, the model comprises 4T1 cells.

[0029] In any of the above method examples, the combination of the FGFR2 inhibitor and PD-1/PD-L1 inhibitor may inhibit tumor growth in a mouse xenograft and/or syngeneic tumor model over a period of at least 1 week, 10 days, or 2 weeks, for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%. In any of the above method examples, administration of the combination of the FGFR2 inhibitor and immune stimulating agent, such as PD-1/PD-L1 inhibitor, to the subject may reduce the volume of at least one tumor in the subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least

95%, for example, over a period of at least one month, two months, three months, six months, or one year.

[0030] In any of the above method examples, administration of the FGFR2 inhibitor in a xenograft and/or syngeneic tumor model may show an increase in NK cells, such as NKp46+ cells, an increase in PD-L1 expressing cells, an increase in macrophages such as F480+ macrophages, an increase in one or more of CD3+, CD8+, and CD4+ T cells, and/or an increase in the ratio of lymphoid to myeloid cells in tumor tissue compared to a control over a period of at least 1 day, at least 4 days, at least 1 week, at least 10 days, or at least 2 weeks, and for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%. In some examples, the mouse syngeneic tumor model is a 4T1 mammary tumor model. In some examples, the control is a vehicle or is an Ig-Fc molecule or another compound that does not inhibit tumor growth in the model.

[0031] Also provided herein are methods of increasing the number of NK cells, PD-L1 positive cells, and/or CD3+, CD8+, and/or CD8+ T cells and/or macrophages in a tumor tissue of a subject with cancer, and/or methods of increasing the lymphoid cell to myeloid cell ratio in a tumor tissue of a subject with cancer, comprising administering to said subject an effective amount of an FGFR2 inhibitory antibody, such as any of the FGFR2 antibodies described in the preceding paragraphs. An increase in one or more of CD3+, CD8+, and CD4+ T cells, and/or an increase in the ratio of lymphoid to myeloid cells in tumor tissue compared to a control over a period of at least 1 day, at least 4 days, at least 1 week, at least 10 days, or at least 2 weeks, and for example, by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%. In some examples, the antibody may have one or more of the following properties: (a) lacks a fucose at position Asn297; (b) comprises a κ light chain constant region; (c) comprises an IgG1 heavy chain constant region; (d) has enhanced ADCC activity *in vitro* compared to an antibody having the same amino acid sequence that is fucosylated at position Asn297; and (e) has enhanced affinity for Fc gamma RIIIA compared to an antibody having the same amino acid sequence that is fucosylated at position Asn297. In some examples, the afucosylated antibody is capable of increasing the number of one or more of PD-L1 positive cells, NK cells,

CD3+ T cells, CD4+ T cells, CD8+ T cells, and macrophages in tumor tissue in a mouse xenograft and/or syngeneic tumor model compared to a control (e.g. as compared to a control antibody that does not target FGFR2). In some examples, the method inhibits tumor growth or reduces volume of at least one tumor in the subject. In some examples, the subject suffers from breast cancer, gastric cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, cholangiocarcinoma, esophageal cancer (including gatroesophageal junction adenocarcinoma), and endometrial cancer. In some examples, the method further comprises, following administration of the FGFR2 antibody, obtaining at least one tumor sample from the subject and determining the number of NK cells, PD-L1 positive cells, and/or CD3+, CD8+, and/or CD4+ T cells in the sample, and, if the number of one or more of those types of cells is increased relative to a sample prior to FGFR2 antibody administration or relative to a non-tumor sample from the subject, administering a PD-1/PD-L1 inhibitor to the subject. In some examples, the method further comprises, following administration of the FGFR2 antibody, obtaining at least one tumor sample from the subject and determining the ratio of lymphoid cells to myeloid cells in the sample, and, if the ratio is increased relative to a sample prior to FGFR2 antibody administration or relative to a non-tumor sample from the subject, administering at least one to the subject. The at least one immune stimulating agent, such as at least one PD-1/PD-L1 inhibitor, of these methods may be any of those described in the preceding paragraphs or described in the section below entitled "combinations with other immune stimulating agents." In some examples, the patient may be administered a combination of FGFR2 inhibitor, PD-1/PD-L1 inhibitor, and at least one other immune stimulating agent.

[0032] Also provided herein are methods of treating cancer in a subject comprising administering to the subject an FGFR2 inhibitor and, if the subject is determined to have an increased number of NK cells, PD-L1 positive cells, macrophages, CD3+ T cells, CD8+ T cells, and/or CD4+ T cells relative to a control, such as a sample prior to FGFR2 antibody administration or relative to a non-tumor sample from the subject, administering at least one immune stimulating agent to the subject, such as a PD-

1/PD-L1 inhibitor. Also provided herein are methods of treating cancer in a subject comprising administering to the subject an FGFR2 inhibitor and, if the subject is determined to have an increased ratio of lymphoid cells to myeloid cells relative to a control, such as a sample prior to FGFR2 antibody administration or relative to a non-tumor sample from the subject, administering at least one immune stimulating agent to the subject, such as a PD-1/PD-L1 inhibitor. In such methods, the FGFR2 inhibitor and immune stimulating agent may be any of those described in the preceding paragraphs or in the section below entitled "combinations with other immune stimulating agents." Furthermore, the FGFR2 and immune stimulating agent administration may be according to the previously described methods in which administration of immune stimulating agent does not begin until at least one dose of FGFR2 inhibitor is administered. In such cases, a test to determine the number of NK cells, PD-L1 positive cells, macrophages, CD3+, CD8+, and/or CD4+ T cells, lymphoid, and/or myeloid cells may, for example, be conducted after FGFR2 inhibitor administration has begun but prior to the start of FGFR2 and immune stimulating agent combination administration.

[0033] Compositions comprising any of the FGFR2 inhibitors described herein and any of the immune stimulating agents described herein are also provided. In such compositions, the FGFR2 inhibitor and the at least one immune stimulating agent may be located in separate containers or separate compartments of the same container, or alternatively, they may be mixed together into the same container or compartment. Such compositions may be used, for example, for treatment of cancer, such as any of the cancers described above. In some examples, instructions for use may also be included, such as instructions for use in treating cancer.

[0034] Provided herein are also methods of increasing the number of one or more of PD-L1 positive cells, NK cells, macrophages, CD3+ T cells, CD4+ T cells, and CD8+ T cells in tumor tissue of a cancer subject, comprising administering an FGFR2 inhibitor, wherein the inhibitor is an FGFR2 antibody with enhanced ADCC activity. In some such examples, no immune stimulating agent is administered with the FGFR2 antibody. In some such examples, administration of the FGFR2 antibody in a mouse xenograft and/or syngeneic tumor model increases the number of one or more of PD-L1 positive cells, NK cells, macrophages, CD3+ T cells, CD8+ T

cells, and CD4+ T cells in tumor tissue compared to a control, and/or increases the ratio of lymphoid to myeloid cells in the tumor tissue. In some such examples, the subject suffers from breast cancer, gastric cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, cholangiocarcinoma, esophageal cancer (including gatroesophageal junction adenocarcinoma), or endometrial cancer, such as from bladder cancer.

[0035] In the above methods, the FGFR2 antibody may be an FGFR2-IIIb antibody, which may have one or more of the following properties: (a) binds to FGFR2-IIIb with higher affinity than to FGFR2-IIIc or does not detectably bind to FGFR2-IIIc; (b) inhibits binding of FGF2 and/or FGF7 to human FGFR2; (c) inhibits growth of a human tumor in a mouse xenograft and/or syngeneic tumor model; (d) induces an ADCC activity; (e) possesses enhanced ADCC activity; and (f) is afucosylated.

[0036] In some examples of the above method, the FGFR2 antibody comprises heavy chain and light chain variable regions, wherein the heavy chain variable region comprises: (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 6; (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 7; and(iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 8;
and the light chain variable region comprises: (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 11.

[0037] In some cases, the heavy chain variable domain of the FGFR2 antibody comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 4 and/or the light chain variable domain of the FGFR2 antibody comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 5. In some cases, the heavy chain variable domain of the FGFR2 antibody comprises the amino acid sequence of SEQ ID NO: 4 and/or the light chain variable domain of the FGFR2 antibody comprises the amino acid sequence of SEQ ID NO: 5. In some cases, the heavy chain of the FGFR2 antibody comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 2 and/or the light chain of the FGFR2 antibody comprises an amino acid sequence at least 95%

identical to the amino acid sequence of SEQ ID NO: 3. In some cases, the heavy chain of the FGFR2 antibody comprises the amino acid sequence of SEQ ID NO: 2 and/or the light chain of the FGFR2 antibody comprises the amino acid sequence of SEQ ID NO: 3. In some cases, the FGFR2 antibody is chimeric, humanized, or human. In some examples, the FGFR2 antibody is selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$. In some examples, the FGFR2 antibody has one or more of the following properties: (a) lacks a fucose at position Asn297; (b) comprises a κ light chain constant region; (c) comprises an IgG1 heavy chain constant region; (d) has enhanced ADCC activity *in vitro* compared to an antibody having the same amino acid sequence that is fucosylated at position Asn297; and (e) has enhanced affinity for Fc gamma RIIIA compared to an antibody having the same amino acid sequence that is fucosylated at position Asn297. In some examples, the afucosylated antibody is capable of increasing the number of one or more of PD-L1 positive cells, NK cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, and macrophages in tumor tissue in a mouse xenograft and/or syngeneic tumor model compared to a control (e.g. as compared to a control antibody that does not target FGFR2).

[0038] In any of the methods or uses described in this summary section, the cancer may have been previously determined to overexpress FGFR2IIIb, either in the presence or absence of amplification of the FGFR2 gene. Alternatively, any of the methods or uses described in this section, the method may further comprise testing the subject's cancer, for example prior to administration of an FGFR2 inhibitor, to determine if the cancer overexpresses FGFR2IIIb and/or to determine if the FGFR2 gene is amplified in tumor cells. In either case, FGFR2IIIb may optionally be determined by immunohistochemistry (IHC) and FGFR2 gene amplification may optionally be determined by fluorescence in situ hybridization (FISH), for example using probes for the FGFR2 gene locus and the centromere of chromosome 10 on which the FGFR2 gene is located. In some examples, an IHC signal of 1+, 2+, or 3+ in at least 10% of tumor cells, such as at least 20%, 30%, 40%, or 50% of tumor cells indicates overexpression of FGFR2IIIb. In some examples, an FGFR2 to centromere of chromosome 10 (CEN10) ratio of greater than or equal to 2 indicates FGFR2 gene amplification.

[0039] In some examples in which the patient suffers from gastric or bladder cancer, the subject may have been previously determined to have one of the following profiles or alternatively the method of treatment includes determining whether the patient fits one of the following profiles with respect to FGFR2 expression/gene amplification, and which may indicate the level of expected responsiveness to the treatment: a) in the case of a gastric cancer subject, an IHC signal of 3+ in at least 10% of tumor cells; b) in the case of a gastric cancer subject, an IHC signal of 3+ in at least 10% of tumor cells as well as amplification of the FGFR2 gene; c) in the case of a gastric cancer subject, an IHC signal of 3+ in at least 10% of tumor cells without amplification of the FGFR2 gene; d) in the case of a gastric cancer subject, an IHC signal of 1+ or 2+ in at least 10% of tumor cells; e) in the case of a bladder cancer subject, an IHC signal of 1+ in at least 10% of tumor cells; f) in the case of a bladder cancer subject, an IHC signal of 2+ in at least 10% of tumor cells; g) in the case of a bladder cancer subject, an H score of greater than 20; h) in the case of a bladder cancer subject, an H score of 10-19; i) in the case of a bladder cancer subject, an H score of less than 10.

[0040] This disclosure also provides methods of determining responsiveness to any of the FGFR2 inhibitors, treatments, and uses described above. Such methods may comprise testing the subject's cancer to determine if the cancer overexpresses FGFR2IIIb and/or to determine if the FGFR2 gene is amplified in tumor cells. Overexpression of FGFR2IIIb may optionally be determined by immunohistochemistry (IHC) and FGFR2 gene amplification may optionally be determined by fluorescence in situ hybridization (FISH), for example using probes for the FGFR2 gene locus and the centromere of chromosome 10 on which the FGFR2 gene is located. In some examples, an IHC signal of 1+, 2+, or 3+ in at least 10% of tumor cells, such as at least 20%, 30%, 40%, or 50% of tumor cells indicates overexpression of FGFR2IIIb. In some examples, an FGFR2 to centromere of chromosome 10 (CEN10) ratio of greater than or equal to 2 indicates FGFR2 gene amplification.

[0041] In some examples in which the patient suffers from gastric or bladder cancer, the method may comprise determining if the patient's cancer falls into one of the following categories, which may indicate responsiveness to the treatment or FGFR2 inhibitor composition: a) in the case of a gastric cancer subject, an IHC signal of 3+ in at least 10% of tumor cells; b) in the case of a gastric cancer subject, an IHC signal of 3+ in at

What is Claimed is:

1. Use of an anti-fibroblast growth factor receptor 2 (FGFR2) antibody and a programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) inhibitor for preparation of a medicament for treatment of cancer in a subject.

2. The use of claim 1, wherein the anti-FGFR2 antibody is an anti-FGFR2-IIIb antibody, and wherein the anti-FGFR2-IIIb antibody optionally has one or more of the following properties:

a. binds to FGFR2-IIIb with higher affinity than to FGFR2-IIIc or does not detectably bind to FGFR2-IIIc;

b. inhibits binding of FGF2 to human FGFR2;

c. inhibits binding of FGF7 to human FGFR2;

d. inhibits growth of a human tumor in a mouse tumor model;

e. induces an ADCC activity;

f. possesses enhanced ADCC activity;

g. is afucosylated; and

h. is capable of increasing the number of one or more of PD-L1 positive cells, NK cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, and macrophages in tumor tissue in a mouse tumor model compared to a control.

3. The use of claim 2, wherein the anti-FGFR2-IIIb antibody comprises heavy chain and light chain variable regions, wherein the heavy chain variable region comprises:

(i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 6;

(ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 7; and

(iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 8;

and the light chain variable region comprises:

(iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9;

(v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and

(vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 11.

4. The use of claim 3, wherein the heavy chain variable domain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 4 and the light chain variable

domain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 5.

5. The use of claim 4, wherein the heavy chain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 2 and the light chain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 3.

6. The use of any one of claims 1-5, wherein the anti-FGFR2 antibody is chimeric, humanized, or human.

7. The use of any one of claims 1-6, wherein the anti-FGFR2 antibody is afucosylated.

8. The use of any one of claims 1-4 and 6-7, wherein the anti-FGFR2 antibody is selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$.

9. The use any one of claims 2-8, wherein the anti-FGFR2-IIIb antibody is to be administered at a dose of 15 mg/kg every 2 weeks.

10. The use of any one of claims 1-9, wherein the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody.

11. The use of claim 10, wherein the anti-PD-1 antibody is selected from nivolumab, pidilizumab, and pembrolizumab.

12. The use of any one of claims 1-9, wherein the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody.

13. The use of any one of claims 1-12, wherein the anti-FGFR2 antibody and the PD-1/PD-L1 inhibitor are to be administered concurrently or sequentially.

14. The use of any one of claims 1-13, wherein the cancer is selected from breast cancer, gastric cancer, non-small cell lung cancer, melanoma, squamous cell carcinoma of the head and neck, ovarian cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, bladder cancer, cholangiocarcinoma, esophageal cancer, and endometrial cancer.

15. The use of claim 14, wherein the cancer is gastric or bladder cancer.

16. The use of any one of claims 1-15, wherein the cancer overexpresses FGFR2-IIIb.

17. The use of any one of claims 1-15, wherein the cancer has previously been determined to overexpress FGFR2-IIIb, either in the presence or in the absence of FGFR2 gene amplification.

18. The use of claim 17, wherein FGFR2-IIIb overexpression is determined by immunohistochemistry (IHC).

19. The use of claim 18, wherein the overexpression is determined by an IHC signal of 1+, 2+, or 3+ in a sample of the cancer.

20. The use of claim 17, 18, or 18, wherein FGFR2 gene amplification is determined by obtaining the ratio of FGFR2 to chromosome 10 centromere (CEN10) using fluorescence in situ hybridization (FISH), wherein the FGFR2 gene is considered amplified if the FGFR2/CEN10 ratio determined by FISH is greater than or equal to 2.

21. The use of any one of claims 1-20, wherein:

a) the cancer is gastric cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer;

b) the cancer is gastric cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer, and wherein the FGFR2 gene is amplified;

c) the cancer is gastric cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer, and wherein the FGFR2 gene is not amplified; or

d) the cancer is bladder cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer.

22. The use of any one of claims 1-21, wherein the subject is a PD-1/PD-L1 inhibitor inadequate responder.

23. The use of any one of claims 1-22, wherein administration of the anti-FGFR2 antibody and the PD-1/PD-L1 inhibitor in a mouse tumor model of the cancer results in either additive or synergistic inhibition of tumor growth.

24. The use of any one of claims 1-21, wherein administration of the anti-FGFR2 antibody in a mouse tumor model:

a. increases the number of NK cells in tumor tissue compared to a control,

b. increases the number of PD-L1 positive cells in tumor tissue compared to a control,

c. increases the number of CD3+, CD8+, and/or CD4+ T cells in tumor tissue compared to a control, and/or

d. increases the ratio of lymphoid cells to myeloid cells in tumor tissue compared to a control.

25. Use of an anti-FGFR2 antibody for preparation of a medicament for increasing the number of NK cells and/or PD-L1 positive cells in a tumor tissue of a subject with cancer, wherein the anti-FGFR2 antibody is an antibody as defined in any one of claims 1-8.

26. Use of an anti-FGFR2 antibody for preparation of a medicament for increasing the number of one or more of PD-L1 positive cells, NK cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, and macrophages in tumor tissue of a cancer subject, wherein the anti-FGFR2 antibody is an antibody as defined in any one of claims 1-8, and wherein the antibody has enhanced ADCC activity.

27. Use of an anti-FGFR2 antibody for preparation of a medicament for treatment of cancer in a subject, wherein, if the subject is determined to have an increased number of NK cells and/or PD-L1 positive cells and/or CD8+ T cells relative to a sample prior to administration of the antibody, the treatment comprises further administering at least one PD-1/PD-L1 inhibitor to the subject, wherein the anti-FGFR2 antibody is an antibody as defined in any one of claims 1-8.

28. The use of claim 27, wherein the PD-1/PD-L1 inhibitor is an inhibitor as defined in any one of claims 10-12.

29. A composition comprising an anti-FGFR2-IIIb antibody and at least one PD-1/PD-L1 inhibitor.

30. The composition of claim 29, wherein the FGFR2 inhibitor and the at least one PD-1/PD-L1 inhibitor are comprised within separate containers or compartments.

31. The composition of claim 29 or 30, further comprising instructions for use in cancer treatment.

32. The composition of claim 29, 30, or 31, wherein the anti-FGFR2-IIIb antibody comprises heavy chain and light chain variable regions, wherein the heavy chain variable region comprises:

(i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 6;

(ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 7; and

(iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 8;

and the light chain variable region comprises:

(iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9;

(v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and

(vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 11.

33. The composition of any one of claims 29-32, wherein the heavy chain variable domain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 4 and the light chain variable domain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 5.

34. The composition of claim 33, wherein the heavy chain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 2 and the light chain of the anti-FGFR2-IIIb antibody comprises the amino acid sequence of SEQ ID NO: 3.

35. The composition of any one of claims 29-34, wherein the PD-1/PD-L1 inhibitor is an anti-PD-1 antibody.

36. The composition of claim 35, wherein the anti-PD-1 antibody is selected from nivolumab, pidilizumab, and pembrolizumab.

37. The composition of any one of claims 29-34, wherein the PD-1/PD-L1 inhibitor is an anti-PD-L1 antibody.

38. The composition of any one of claims 29-37, wherein the anti-FGFR2-IIIb antibody is chimeric, humanized, or human.

39. The composition of any one of claims 29-38, wherein the anti-FGFR2-IIIb antibody is afucosylated.

40. The composition of any one of claims 29-33 or 35-39, wherein the anti-FGFR2-IIIb antibody is selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$.

41. A method of determining whether a gastric or bladder cancer patient will be responsive to treatment with an FGFR2 inhibitor comprising determining whether the gastric or bladder cancer overexpresses FGFR2-IIIb by immunohistochemistry (IHC), wherein the overexpression is determined by an IHC signal of 1+, 2+, or 3+ in a sample of

the cancer, and wherein the method optionally further comprises determining whether the FGFR2 gene is amplified.

42. The method of claim 41, wherein the patient is determined to be responsive to treatment with an FGFR2 inhibitor if:

a) the cancer is gastric cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer;

b) the cancer is gastric cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer, and wherein the FGFR2 gene is amplified;

c) the cancer is gastric cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer, and wherein the FGFR2 gene is not amplified; or

d) the cancer is bladder cancer, and the cancer has an FGFR2-IIIb IHC signal of 2+ or 3+ in a sample of the cancer.

43. The method of claim 41 or 42, wherein the FGFR2 inhibitor is an antibody according to any one of claims 1-8.

44. The method of claim 41, 42, or 43, wherein the treatment comprises an FGFR2 inhibitor and a PD-1/PD-L1 inhibitor.