(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 June 2015 (11.06.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/084808 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01) *A61K 38/00* (2006.01)

(21) International Application Number:
PCT/US2014/068097

(22) International Filing Date:
2 December 2014 (02.12.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/910,663 2 December 2013 (02.12.2013) US
61/975,339 4 April 2014 (04.04.2014) US

(71) Applicant: **ONCOMED PHARMACEUTICALS, INC.** [US/US]; 800 Chesapeake Drive, Redwood, California 94063-4748 (US).

(72) Inventors: **KAPOUN, Ann M.**; 686 Ehrhorn Avenue, Mountain View, California 94041 (US). **ZHANG, Chun**; 570 Ashton Avenue, Palo Alto, California 94306 (US).

(74) Agents: **CALVO, Paul A.** et al.; Sterne, Kessler, Goldstein & Fox, PLLC, 1100 New York Ave., NW, Washington, District of Columbia 20005 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

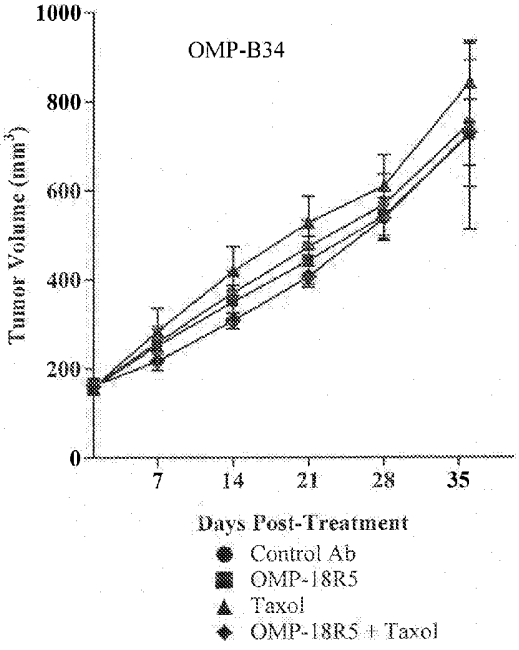
Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) Title: IDENTIFICATION OF PREDICTIVE BIOMARKERS ASSOCIATED WITH WNT PATHWAY INHIBITORS

Fig. 1A



(57) Abstract: The present invention provides biomarkers for identifying tumors likely to respond to treatment with Wnt pathway inhibitors. Also provided are methods for identifying tumors and/or patients that are likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor. Methods for treating a patient with cancer are provided, wherein the cancer is predicted to respond to a Wnt pathway inhibitor.

WO 2015/084808 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

IDENTIFICATION OF PREDICTIVE BIOMARKERS ASSOCIATED WITH WNT PATHWAY INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATONS

[0001] This application claims priority benefit of U.S. Provisional Application No. 61/910,663, filed December 2, 2013, and U.S. Provisional Application No. 61/975,339, filed April 4, 2014, each of which are hereby incorporated by reference herein in their entirety.

FIELD OF INVENTION

[0002] The present invention relates to the field of cancer treatment. More particularly, the invention provides methods for identifying tumors that are likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor. In addition, the invention provides methods for identifying, selecting, and/or treating patients with cancer who are likely to respond to treatment with a Wnt pathway inhibitor, either alone or in combination with other therapeutic agents.

BACKGROUND OF THE INVENTION

[0003] Cancer is one of the leading causes of death in the developed world, with approximately 1.6 million people diagnosed with cancer and over 550,000 deaths per year in the United States alone. Overall it is estimated that more than 1 in 3 people will develop some form of cancer during their lifetime. There are more than 200 different types of cancer, four of which - breast, lung, colorectal, and prostate—account for almost half of all new cases in the United States (Siegel et al., 2012, *CA: A Cancer J. for Clin.*, 62:10-29).

[0004] Signaling pathways normally connect extracellular signals to the nucleus leading to the expression of genes that directly or indirectly control cell growth, differentiation, survival, and death. However, in a wide variety of cancers signaling pathways are dysregulated and may be linked to tumor initiation and/or progression. Signaling pathways implicated in human oncogenesis include, but are not limited to, the Wnt pathway, the Ras-Raf-MEK-ERK or MAPK pathway, the PI3K-AKT pathway, the CDKN2A/CDK4 pathway, the Bcl-2/TP53 pathway, and the NOTCH pathway.

[0005] The Wnt signaling pathway is one of several critical regulators of embryonic pattern formation, post-embryonic tissue maintenance, and stem cell biology. More specifically, Wnt signaling plays an important role in the generation of cell polarity and cell fate specification including self-renewal by stem cell populations. Unregulated activation of the Wnt pathway is associated with numerous human cancers where it is believed the activation can alter the developmental fate of cells. It is believed that the activation of the Wnt pathway may maintain tumor cells in an undifferentiated state and/or lead to uncontrolled proliferation. This may allow carcinogenesis to proceed by

overtaking homeostatic mechanisms which control normal development and tissue repair (reviewed in Reya & Clevers, 2005, *Nature*, 434:843-50; Beachy et al., 2004, *Nature*, 432:324-31).

[0006] The Wnt signaling pathway was first elucidated in the Drosophila developmental mutant wingless (wg) and from the murine proto-oncogene int-1, now Wnt1 (Nusse & Varmus, 1982, *Cell*, 31:99-109; Van Ooyen & Nusse, 1984, *Cell*, 39:233-40; Cabrera et al., 1987, *Cell*, 50:659-63; Rijsewijk et al., 1987, *Cell*, 50:649-57). Wnt genes encode lipid-modified glycoproteins which are secreted and 19 different Wnt proteins have been identified in mammals. These secreted ligands activate a receptor complex consisting of a Frizzled (FZD) receptor family member and low-density lipoprotein (LDL) receptor-related protein 5 or 6 (LRP5/6). The FZD receptors are members of the G-protein coupled receptor (GPCR) superfamily and contain seven transmembrane domains and a large extracellular N-terminal ligand binding domain. The N-terminal ligand binding domain contains 10 conserved cysteines and is known as a cysteine-rich domain (CRD) or a "Fri domain". There are ten human FZD receptors, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. Different FZD CRDs have different binding affinities for specific Wnt proteins (Wu & Nusse, 2002, *J. Biol. Chem.*, 277:41762-9). In addition, FZD receptors may be grouped into those that activate the canonical β-catenin pathway and those that activate non-canonical pathways (Miller et al., 1999, *Oncogene*, 18:7860-72).

[0007] A role for Wnt signaling in cancer was first uncovered with the identification of Wnt1 (originally int1) as an oncogene in mammary tumors transformed by the nearby insertion of a murine virus (Nusse & Varmus, 1982, *Cell*, 31:99-109). Since these early observations additional evidence for the role of Wnt signaling in breast cancer has continued to accumulate. For example, over-expression of β-catenin in the mammary glands of transgenic mice results in hyperplasias and adenocarcinomas (Imbert et al., 2001, *J. Cell Biol.*, 153:555-68; Michaelson & Leder, 2001, *Oncogene*, 20:5093-9) whereas loss of Wnt signaling disrupts normal mammary gland development (Tepera et al., 2003, *J. Cell Sci.*, 116:1137-49; Hatsell et al., 2003, *J. Mammary Gland Biol. Neoplasia*, 8:145-58). In human breast cancer, β-catenin accumulation implicates activated Wnt signaling in over 50% of carcinomas, and though specific mutations have not been identified, up-regulation of Frizzled receptor expression has been observed (Brennan & Brown, 2004, *J. Mammary Gland Biol. Neoplasia*, 9:119-31; Malovanovic et al., 2004, *Int. J. Oncol.*, 25:1337-42).

[0008] Activation of the Wnt pathway is also associated with colorectal cancer, lung cancer, pancreatic cancer, and melanoma. Approximately 5-10% of all colorectal cancers are hereditary with one of the main cancer types being familial adenomatous polyposis (FAP). FAP is an autosomal dominant disease in which about 80% of affected individuals contain a germline mutation in the adenomatous polyposis coli (APC) gene. Mutations have also been identified in other Wnt pathway components including Axin and β-catenin. Individual adenomas are clonal outgrowths of epithelial cells containing a second inactivated allele, and the large number of FAP adenomas inevitably results in the development of adenocarcinomas through additional mutations in oncogenes and/or tumor

suppressor genes. Furthermore, activation of the Wnt signaling pathway, including loss-of-function mutations in APC and stabilizing mutations in β-catenin, can induce hyperplastic development and tumor growth in mouse models (Oshima et al., 1997, *Cancer Res.*, 57:1644-9; Harada et al., 1999, *EMBO J.*, 18:5931-42).

[0009] Thus the Wnt pathway has been identified as a target for cancer therapy and treatment. As drug discovery and development advances, especially in the cancer field, the "one drug fits all" approach is shifting to a "personalized medicine" strategy. Personalized medicine strategies may include treatment regimens that are based upon cancer biomarkers, including prognostic markers, pharmacodynamic markers, and predictive markers. In general, predictive biomarkers assess the likelihood that a tumor or cancer will be responsive to or sensitive to a specific therapeutic agent, and may allow for the identification and/or the selection of patients most likely to benefit from the use of that agent.

[0010] The invention provides the identification of predictive biomarkers associated with the use of Wnt pathway inhibitors in the treatment of cancer. Also provided are methods of using the predictive biomarkers for identifying, selecting, and/or classifying tumors and/or patients with cancer as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor. Methods for treating patients with a Wnt inhibitor that are predicted to be responsive to treatment are also provided.

SUMMARY OF THE INVENTION

[0011] Provided are biomarkers for identifying patients likely to respond to treatment with Wnt pathway inhibitors. Additionally provided are methods for identifying tumors and/or patients that are likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor. Further provided are methods of treating cancer in a patient with a Wnt pathway inhibitor, wherein the patient is predicted to be or has been identified as likely to be responsive to the Wnt pathway inhibitor.

[0012] In one aspect, the invention provides a method of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CRBP2, WIF1, and DKK1; and (c) identifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression level of the biomarkers. In some embodiments, a method of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CRBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a

negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor. As used herein, "standardized" and "normalized" may be used interchangeably. In some embodiments, the method comprises identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor in combination with paclitaxel.

[0013] In another aspect, the invention provides a method of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) classifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression level of the biomarkers. In some embodiments, a method of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor. In some embodiments, the method comprises classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor in combination with paclitaxel.

[0014] In another aspect, the invention provides a method of determining the responsiveness (or sensitivity) of a human tumor to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the genes FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) determining the responsiveness of the tumor to treatment based upon the expression level of the biomarkers. In some embodiments, a method of determining the responsiveness or sensitivity of a human tumor to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the genes FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to or sensitive to the Wnt pathway inhibitor. In some embodiments, the method comprises determining the responsiveness or sensitivity of a human tumor to treatment with a Wnt pathway inhibitor in combination with paclitaxel.

[0015] In another aspect, the invention provides a method of identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a

sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers. In some embodiments, a method of identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment with the Wnt pathway inhibitor. In some embodiments, the method comprises identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor in combination with paclitaxel.

[0016] In another aspect, the invention provides a method of selecting a patient with cancer for treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) selecting the patient for treatment based upon the expression level of the biomarkers. In some embodiments, a method of selecting a patient with cancer for treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; and (d) selecting the patient for treatment when their tumor sample has a positive decision value. In some embodiments, the method comprises selecting a patient with cancer for treatment with a Wnt pathway inhibitor in combination with paclitaxel.

[0017] In another aspect, the invention provides a method of treating cancer in a patient, comprising: (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (b) administering to the patient who is likely to response to treatment an effective amount of the Wnt pathway inhibitor. In some embodiments, a method of treating cancer in a patient comprises: (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the

sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that a patient is predicted to respond to treatment; and (b) administering to the patient who is predicted to response to treatment an effective amount of the Wnt pathway inhibitor. In some embodiments, the method comprises identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor in combination with paclitaxel. In some embodiments, the method comprises administering to the patient the Wnt pathway inhibitor in combination with paclitaxel.

[0018] In another aspect, the invention provides a method of treating cancer in a patient, comprising: administering an effective amount of a Wnt pathway inhibitor to the patient; wherein the patient is predicted to respond to treatment with a Wnt inhibitor based upon expression levels of a biomarker signature in a patient tumor sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, a method of treating cancer in a patient comprises: administering an effective amount of a Wnt pathway inhibitor to the patient; wherein the patient is predicted to respond to treatment based upon a positive decision value calculated from the weighted sum of the standardized expression of biomarkers in a biomarker signature in a patient tumor sample, wherein the set of biomarkers comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the patient is predicted to respond to treatment with a Wnt pathway inhibitor in combination with paclitaxel. In some embodiments, the method comprises administering to the patient the Wnt pathway inhibitor in combination with paclitaxel.

[0019] In another aspect, the invention provides a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising: (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (b) administering an effective amount of the Wnt pathway inhibitor to the patient. In some embodiments, a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor comprises: (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates that a patient is predicted to respond to treatment; and (b) administering an effective amount of the Wnt pathway

inhibitor to the patient whose tumor has a positive decision value. In some embodiments, the method comprises identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor in combination with paclitaxel. In some embodiments, the method comprises administering to the patient the Wnt pathway inhibitor in combination with paclitaxel.

[0020] In another aspect, the invention provides a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising: administering an effective amount of a Wnt pathway inhibitor to a patient; wherein the patient is identified as likely to respond to treatment with a Wnt inhibitor based upon expression levels of a biomarker signature in a patient tumor sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor comprises: administering an effective amount of a Wnt pathway inhibitor to a patient; wherein the patient is identified as likely to respond to treatment based upon a positive decision value calculated from the weighted sum of the standardized expression of biomarkers in a biomarker signature in a patient tumor sample, wherein the set of biomarkers comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the patient is identified as likely to respond to treatment with a Wnt pathway inhibitor in combination with paclitaxel. In some embodiments, the method comprises administering to the patient the Wnt pathway inhibitor in combination with paclitaxel.

[0021] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, DKK1, EP300, and CTBP1. In some embodiments, the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, DKK1, EP300, CTBP1, WNT6, WNT3, FZD2, APC, TLE2, DVL2, PITX2, WISP1, GSK3B, WNT9A, FZD7, and LEF1. In some embodiments, the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1, and at least one additional biomarker from Table 2.

[0022] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the Wnt pathway inhibitor is an antibody. In some embodiments, the Wnt pathway inhibitor is an antibody that specifically binds at least one Frizzled (FZD) protein or fragment thereof. In some embodiments, the Wnt pathway inhibitor is an antibody that specifically binds at least one FZD protein selected from the group consisting of: FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In some embodiments, the Wnt pathway inhibitor is an antibody that specifically binds at least one FZD protein selected from the group consisting of: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the Wnt pathway inhibitor is an antibody which comprises: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), and (b) a light chain

CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:6).

[0023] In certain embodiments, the Wnt pathway inhibitor is an antibody which comprises a heavy chain variable region comprising SEQ ID NO:7 and a light chain variable region comprising SEQ ID NO:8. In certain embodiments, the Wnt pathway inhibitor is an antibody which comprises a heavy chain variable region and a light chain variable region encoded by the plasmid deposited with ATCC as PTA-9541. In certain embodiments, the Wnt pathway inhibitor is an antibody which comprises a heavy chain and a light chain encoded by the plasmid deposited with ATCC as PTA-9541. In some embodiments, the Wnt pathway inhibitor is antibody OMP-18R5.

[0024] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the Wnt pathway inhibitor is a soluble receptor. In some embodiments, the Wnt pathway inhibitor comprises the extracellular domain of a FZD receptor protein. In some embodiments, the Wnt pathway inhibitor comprises a Fri domain of a FZD protein. In some embodiments, the Wnt pathway inhibitor comprises the Fri domain of FZD8. In certain embodiments, the Wnt pathway inhibitor comprises the Fri domain of FZD8 and a human Fc domain. In some embodiments, the Wnt pathway inhibitor is the soluble receptor OMP-54F28.

[0025] In some embodiments, the tumor is selected from the group consisting of a breast tumor, lung tumor, a colon tumor, glioma, a gastrointestinal tumor, a renal tumor, an ovarian tumor, a liver tumor, a colorectal tumor, an endometrial tumor, a kidney tumor, a prostate tumor, a thyroid tumor, a neuroblastoma, a pancreatic tumor, a glioblastoma multiforme, a cervical tumor, a stomach tumor, a bladder tumor, a hepatoma, melanoma, and a head and neck tumor. In some embodiments, the tumor is a breast tumor.

[0026] In some embodiments, the cancer is selected from the group consisting of a breast cancer, lung cancer, a colon cancer, glioma, a gastrointestinal cancer, a renal cancer, an ovarian cancer, a liver cancer, a colorectal cancer, an endometrial cancer, a kidney cancer, a prostate cancer, a thyroid cancer, a neuroblastoma, a pancreatic cancer, a glioblastoma multiforme, a cervical cancer, a stomach cancer, a bladder cancer, a hepatoma, melanoma, and a head and neck cancer. In some embodiments, the cancer is breast cancer.

[0027] In some embodiments, the method further comprises administering a second therapeutic agent to the patient. In some embodiments, the second therapeutic agent is a chemotherapeutic agent. In some embodiments, the second therapeutic agent is paclitaxel.

[0028] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the sample includes, but is not limited to, any clinically relevant tissue sample, such as a tumor biopsy, a core biopsy tissue sample, a fine needle aspirate, a hair follicle, or a sample of bodily fluid, such as blood, plasma, serum, lymph, ascitic fluid, cystic fluid, or urine. In some embodiments, the sample is taken from a patient having a tumor or cancer. In some embodiments, the sample is a primary tumor. In some embodiments, the sample is a metastasis.

In some embodiments, the sample is a tissue sample. In some embodiments, the sample is a tumor sample. In some embodiments, the sample is a fresh frozen (FF) tissue sample. In some embodiments, the sample is a formalin-fixed paraffin embedded (FFPE) tissue sample. In some embodiments, the sample is whole blood, plasma, or serum. In some embodiments, the sample is cells. In some embodiments, the sample is circulating tumor cells (CTCs).

[0029] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the expression level of a biomarker is determined using PCR-based methods, such as but not limited to, reverse transcription PCR (RT-PCR), quantitative RT-PCR (qPCR), TaqMan™, or TaqMan™ low density array (TLDA). In some embodiments, the expression level of a biomarker is determined using a microarray.

[0030] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the standardized expression of each biomarker is determined by measuring an expression level for each biomarker and multiplying it by a corresponding weight, wherein the weight for each biomarker is determined by the biomarker expression. In certain embodiments, the decision value is calculated according to the equation: 0.4560427*FBXW2 + 0.3378467*CCND2 - 0.4809354*RHOU + 0.409029*CTBP2 + 0.3291529*WIF1 + 0.2926374*DKK1 + 0.04662682.

[0031] In some embodiments, the expression level of a biomarker is measured or determined by a PCR-based assay. In some embodiments, the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 are measured using polynucleotides selected from the group consisting of SEQ ID NOs:62-79. In some embodiments, the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 are measured using (a) a forward primer of SEQ ID NO:62, a reverse primer of SEQ ID NO:63, and a probe comprising SEQ ID NO:64; (b) a forward primer of SEQ ID NO:65, a reverse primer of SEQ ID NO:66, and a probe comprising SEQ ID NO:67; (c) a forward primer of SEQ ID NO:68, a reverse primer of SEQ ID NO:69, and a probe comprising SEQ ID NO:70; (d) a forward primer of SEQ ID NO:71, a reverse primer of SEQ ID NO:72, and a probe comprising SEQ ID NO:73; (e) a forward primer of SEQ ID NO:74, a reverse primer of SEQ ID NO:75, and a probe comprising SEQ ID NO:76; and (f) a forward primer of SEQ ID NO:77, a reverse primer of SEQ ID NO:78, and a probe comprising SEQ ID NO:79.

[0032] In some embodiments, the expression level of a biomarker is measured or determined by multi-analyte profile testing, radioimmunoassay (RIA), Western blot assay, immunofluorescent assay, enzyme immunoassay, enzyme linked immunosorbent assay (ELISA), immunoprecipitation assay, chemiluminescent assay, immunohistochemical assay, dot blot assay, or slot blot assay. In some embodiments wherein the assay uses an antibody, the antibody is detectably labeled. In some embodiments, the label is selected from the group consisting of an immunofluorescent label, a chemiluminescent label, a phosphorescent label, an enzyme label, a radiolabel, an avidin/biotin label, colloidal gold particles, colored particles, and magnetic particles.

[0033] The invention also provides a kit comprising a container, wherein the container contains at least one reagent for specifically detecting the expression of at least one biomarker of the invention. In certain embodiments, the reagent is an antibody or nucleic acid probe that binds a biomarker of the invention.

[0034] In some embodiments, a kit comprises polynucleotides selected from the group consisting of SEQ ID NOs:62-79. In some embodiments, a kit comprises (a) a forward primer of SEQ ID NO:62, a reverse primer of SEQ ID NO:63, and a probe comprising SEQ ID NO:64; (b) a forward primer of SEQ ID NO:65, a reverse primer of SEQ ID NO:66, and a probe comprising SEQ ID NO:67; (c) a forward primer of SEQ ID NO:68, a reverse primer of SEQ ID NO:69, and a probe comprising SEQ ID NO:70; (d) a forward primer of SEQ ID NO:71, a reverse primer of SEQ ID NO:72, and a probe comprising SEQ ID NO:73; (e) a forward primer of SEQ ID NO:74, a reverse primer of SEQ ID NO:75, and a probe comprising SEQ ID NO:76; and (f) a forward primer of SEQ ID NO:77, a reverse primer of SEQ ID NO:78, and a probe comprising SEQ ID NO:79.

[0035] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but also each member of the group individually and all possible subgroups of the main group, and also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0036] Figures 1A-1H. Classification of responsive or non-responsive breast tumors. Figure 1A. Breast tumor OMP-B34 cells were injected subcutaneously into NOD/SCID mice. Figure 1B. Breast tumor OMP-B39 cells were injected subcutaneously into NOD/SCID mice. Figure 1C. Breast tumor OMP-B44 cells were injected subcutaneously into NOD/SCID mice. Figure 1D. Breast tumor OMP-B59 cells were injected subcutaneously into NOD/SCID mice. Figure 1E. Breast tumor OMP-B60 cells were injected subcutaneously into NOD/SCID mice. Figure 1F. Breast tumor UM-T01 cells were injected subcutaneously into NOD/SCID mice. Figure 1G. Breast tumor UM-T03 cells were injected subcutaneously into NOD/SCID mice. Figure 1H. Breast tumor UM-PE13 cells were injected subcutaneously into NOD/SCID mice. For each experiment, mice were treated with OMP-18R5 antibody (-■-), taxol (-▲-), a combination of OMP-18R5 and taxol (-▼-), or a control antibody (-●-). Data is shown as tumor volume (mm^3) over days post-treatment.

[0037] Figure 2. Performance curve for the top 20 ranked genes.

[0038] Figure 3. PCA plot of 6 selected genes.

[0039] Figure 4. Correlation of the 6-gene biomarker signature with ratio of tumor volume.

[0040] Figure 5. Prediction of tumor responsiveness based upon classification probability analysis. T = tumor used in training set for establishment of 6-gene signature.

[0041] Figures 6A-6F. *In vivo* validation of predictive biomarkers. Figure 6A. Breast tumor OMP-B29 cells were injected subcutaneously into NOD/SCID mice. Figure 6B. Breast tumor OMP-B71 cells were injected subcutaneously into NOD/SCID mice. Figure 6C. Breast tumor OMP-B84 cells were injected subcutaneously into NOD/SCID mice. Figure 6D. Breast tumor OMP-B90 cells were injected subcutaneously into NOD/SCID mice. Figure 6E. Breast tumor UM-T02 cells were injected subcutaneously into NOD/SCID mice. Figure 6F. Breast tumor UM-T06 cells were injected subcutaneously into NOD/SCID mice. For each experiment, mice were treated with OMP-18R5 antibody (-■-), taxol (-▲-), a combination of OMP-18R5 and taxol (-▼-), or a control antibody (-●-). Data is shown as tumor volume (mm^3) over days post-treatment.

[0042] Figure 7. Population prevalence estimation of the 6-gene biomarker signature using three public datasets.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0043] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0044] The term "biomarker" as used herein may include but is not limited to, nucleic acids and proteins, and variants and fragments thereof. A biomarker may include DNA comprising the entire or partial nucleic acid sequence encoding the biomarker, or the complement of such a sequence. Biomarker nucleic acids useful in the invention are considered to include both DNA and RNA comprising the entire or partial sequence of any of the nucleic acid sequences of interest. Biomarker proteins are considered to comprise the entire or partial amino acid sequence of any of the biomarker proteins or polypeptides.

[0045] The term "antibody" as used herein refers to an immunoglobulin molecule that recognizes and specifically binds a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing, through at least one antigen-binding site within the variable region of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, single chain antibodies, antibody fragments (such as Fab, Fab', F(ab')2, and Fv fragments), single chain Fv (scFv) antibodies, multispecific antibodies such as bispecific antibodies, monospecific antibodies, monovalent antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen-binding site of an antibody, and any other modified immunoglobulin molecule comprising an antigen-binding site as long as the antibodies exhibit the desired biological activity. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), based on the identity of their heavy chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have

different and well-known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules, including but not limited to, toxins and radioisotopes.

[0046] The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments. "Antibody fragment" as used herein comprises at least one antigen-binding site or epitope-binding site.

[0047] The term "variable region" of an antibody refers to the variable region of an antibody light chain, or the variable region of an antibody heavy chain, either alone or in combination. The variable region of a heavy chain or a light chain generally consists of four framework regions (FR) connected by three complementarity determining regions (CDRs), also known as "hypervariable regions". The CDRs in each chain are held together in close proximity by the framework regions and contribute to the formation of the antigen-binding site(s) of the antibody. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al., 1991, *Sequences of Proteins of Immunological Interest, 5th Edition*, National Institutes of Health, Bethesda, MD), and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-Lazikani et al., 1997, *J. Mol. Biol.,* 273:927-948). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[0048] The term "monoclonal antibody" as used herein refers to a homogeneous antibody population involved in the highly specific recognition and binding of a single antigenic determinant or epitope. This is in contrast to polyclonal antibodies that typically include a mixture of different antibodies directed against a variety of different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (e.g., Fab, Fab', F(ab')2, Fv), single chain (scFv) antibodies, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen-binding site. Furthermore, "monoclonal antibody" refers to such antibodies made by any number of techniques, including but not limited to, hybridoma production, phage selection, recombinant expression, and transgenic animals.

[0049] The term "humanized antibody" as used herein refers to antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Methods used to generate humanized antibodies are well known in the art.

[0050] The term "human antibody" as used herein refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human. A human antibody may be made using any of the techniques known in the art.

[0051] The term "chimeric antibody" as used herein refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable regions of the light chain and the heavy chain correspond to the variable regions of an antibody derived from one species of mammals (e.g., mouse, rat, rabbit, etc.) with the desired

specificity, affinity, and/or binding capability, while the constant regions correspond to sequences from an antibody derived from another species (usually human).

[0052] The term "affinity-matured antibody" as used herein refers to an antibody with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alterations(s). The definition also includes alterations in non-CDR residues made in conjunction with alterations to CDR residues. Preferred affinity-matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, techniques may include affinity maturation by VH and VL domain shuffling, random mutagenesis of CDR and/or framework residues, and site-directed mutagenesis.

[0053] The terms "epitope" and "antigenic determinant" are used interchangeably herein and refer to that portion of an antigen capable of being recognized and specifically bound by a particular antibody. When the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0054] The terms "selectively binds" or "specifically binds" mean that a binding agent or an antibody reacts or associates more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including unrelated or related proteins. In certain embodiments "specifically binds" means, for instance, that an antibody binds a protein with a K_D of about 0.1mM or less, but more usually less than about 1μM. In certain embodiments, "specifically binds" means that an antibody binds a target at times with a K_D of at least about 0.1μM or less, at other times at least about 0.01μM or less, and at other times at least about 1nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include an antibody that recognizes a protein in more than one species (e.g., human FZD and mouse FZD). Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include an antibody (or other polypeptide or binding agent) that recognizes more than one protein (e.g., human FZD1 and human FZD7). It is understood that, in certain embodiments, an antibody or binding agent that specifically binds a first target may or may not specifically bind a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding, i.e. binding to a single target. Thus, a binding agent may, in certain embodiments, specifically bind more than one target. In certain embodiments, multiple targets may be bound by the same binding site on the agent or antibody. For example, an antibody may, in certain instances, comprise two identical antigen-binding sites, each of which specifically binds the same epitope on two or more proteins. In

certain alternative embodiments, an antibody may be bispecific or multispecific and comprise at least two antigen-binding sites with differing specificities. By way of non-limiting example, a bispecific agent may comprise one binding site that recognizes a target on one protein (e.g., human FZD) and further comprise a second, different binding site that recognizes a different target on a second protein (e.g., a human WNT protein). Generally, but not necessarily, reference to binding means specific binding.

[0055] The terms "polypeptide" and "peptide" and "protein" are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention may be based upon antibodies, in certain embodiments, the polypeptides can occur as single chains or associated chains (e.g., dimers).

[0056] The terms "polynucleotide" and "nucleic acid" are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

[0057] "Conditions of high stringency" may be identified by conditions that: (1) employ low ionic strength and high temperature for washing, for example 15mM sodium chloride/1.5mM sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 in 5x SSC (0.75M NaCl, 75mM sodium citrate) at 42°C; or (3) employ during hybridization 50% formamide in 5x SSC, 50mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2x SSC and 50% formamide, followed by a wash consisting of 0.1x SSC containing EDTA at 55°C.

[0058] The terms "identical" or percent "identity" in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin

Package, and variations thereof. In some embodiments, two nucleic acids or polypeptides of the invention are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 40-60 residues, at least about 60-80 residues in length or any integral value therebetween. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a nucleotide sequence.

[0059] A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Preferably, conservative substitutions in the sequences of the polypeptides and antibodies of the invention do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the polypeptide or antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art.

[0060] The term "vector" as used herein means a construct, which is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

[0061] As used herein the term "soluble receptor" refers to an extracellular domain (or a fragment thereof) of a receptor protein preceding the first transmembrane domain of the receptor that can be secreted from a cell in soluble form. Generally this is the N-terminal portion of the receptor protein.

[0062] As used herein the term "FZD soluble receptor" or "soluble FZD receptor" refers to an N-terminal extracellular fragment of a FZD receptor protein preceding the first transmembrane domain of the receptor that can be secreted from a cell in soluble form. FZD soluble receptors comprising the entire N-terminal extracellular domain (ECD) as well as smaller fragments are encompassed by the term. Thus, FZD soluble receptors comprising a FZD Fri domain are also included in this term.

[0063] A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

[0064] The term "substantially pure" as used herein refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0065] The terms "cancer" and "cancerous" as used herein refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, blastoma, sarcoma, and hematologic cancers such as lymphoma and leukemia.

[0066] The terms "tumor" and "neoplasm" as used herein refer to any mass of tissue that results from excessive cell growth or proliferation, either benign (non-cancerous) or malignant (cancerous) including pre-cancerous lesions.

[0067] The term "metastasis" as used herein refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at a new location. A "metastatic" or "metastasizing" cell is one that loses adhesive contacts with neighboring cells and migrates (e.g., via the bloodstream or lymph) from the primary site of disease to secondary sites.

[0068] The terms "cancer stem cell" and "CSC" and "tumor stem cell" and "tumor initiating cell" are used interchangeably herein and refer to cells from a cancer or tumor that: (1) have extensive proliferative capacity; 2) are capable of asymmetric cell division to generate one or more types of differentiated cell progeny wherein the differentiated cells have reduced and/or limited proliferative or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. These properties confer on the cancer stem cells the ability to form or establish a tumor or cancer upon serial transplantation into an immunocompromised host (e.g., a mouse) compared to the majority of tumor cells that fail to form tumors. Cancer stem cells undergo self-renewal versus differentiation in a chaotic manner to form tumors with abnormal cell types that can change over time as mutations occur.

[0069] The terms "cancer cell" and "tumor cell" refer to the total population of cells derived from a cancer or tumor or pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the cancer cell population, and tumorigenic stem cells (cancer stem cells). As used herein, the terms "cancer cell" or "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

[0070] The term "tumorigenic" as used herein refers to the functional features of a cancer stem cell including the properties of self-renewal (giving rise to additional tumorigenic cancer stem cells) and proliferation to generate all other tumor cells (giving rise to differentiated and thus non-tumorigenic tumor cells).

[0071] The term "tumorigenicity" as used herein refers to the ability of a random sample of cells from the tumor to form palpable tumors upon serial transplantation into immunocompromised hosts (e.g., mice). This definition also includes enriched and/or isolated populations of cancer stem cells that form palpable tumors upon serial transplantation into immunocompromised hosts (e.g., mice).

[0072] The term "patient" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "patient" and "subject" are used interchangeably herein in reference to a human patient.

[0073] The term "pharmaceutically acceptable" refers to a product or compound approved (or approvable) by a regulatory agency of the Federal government or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

[0074] The terms "pharmaceutically acceptable excipient, carrier or adjuvant" or "acceptable pharmaceutical carrier" refer to an excipient, carrier, or adjuvant that can be administered to a subject, together with at least one agent (e.g., an antibody) of the present disclosure, and which does not destroy the activity of the agent. The excipient, carrier, or adjuvant should be non-toxic when administered with an agent in doses sufficient to deliver a therapeutic effect.

[0075] The terms "effective amount" or "therapeutically effective amount" or "therapeutic effect" refer to an amount of a binding agent, an antibody, polypeptide, polynucleotide, small organic molecule, or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of a drug (e.g., an antibody) has a therapeutic effect and as such can reduce the number of cancer cells; decrease tumorigenicity, tumorigenic frequency, or tumorigenic capacity; reduce the number or frequency of cancer stem cells; reduce the tumor size; reduce the cancer cell population; inhibit and/or stop cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibit and/or stop tumor or cancer cell metastasis; inhibit and/or stop tumor or cancer cell growth; relieve to some extent one or more of the symptoms associated with the cancer; reduce morbidity and mortality; improve quality of life; or a combination of such effects. To the extent the agent, for example an antibody, prevents growth and/or kills existing cancer cells, it can be referred to as cytostatic and/or cytotoxic.

[0076] The terms "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already diagnosed with the disorder; those prone to have the disorder; and

those in whom the disorder is to be prevented. In some embodiments, a subject is successfully "treated" according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of and/or complete absence of cancer cells; a reduction in the tumor size; an inhibition of tumor growth; inhibition of and/or an absence of cancer cell infiltration into peripheral organs including the spread of cancer cells into soft tissue and bone; inhibition of and/or an absence of tumor or cancer cell metastasis; inhibition and/or an absence of cancer growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity; reduction in the number or frequency of cancer stem cells; or some combination of such effects.

[0077] As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[0078] It is understood that wherever embodiments are described herein with the language "comprising" otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided. It is also understood that wherever embodiments are described herein with the language "consisting essentially of" otherwise analogous embodiments described in terms of "consisting of" are also provided.

[0079] The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

II. Methods of use of predictive biomarkers

[0080] Provided herein are methods for identifying, classifying, and/or selecting tumors and/or patients with cancer that are likely to be responsive ("sensitive") or non-responsive ("resistant") to treatment with a Wnt pathway inhibitor. In addition, provided are methods for treating patients with cancer who are likely to respond to treatment, are predicted to respond to treatment, and/or have been identified to respond to treatment with a Wnt pathway inhibitor.

[0081] Provided herein is a method of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CRBP2, WIF1, and DKK1; and (c) identifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression level of the biomarkers. In some embodiments, a method of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CRBP2,

WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor.

[0082] Provided herein is a method of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) classifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression level of the biomarkers. In some embodiments, a method of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor.

[0083] Provided herein is a method of determining the responsiveness (or sensitivity) of a human tumor to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the genes FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) determining the responsiveness of the tumor to treatment based upon the expression level of the biomarkers. In some embodiments, a method of determining the responsiveness or sensitivity of a human tumor to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the genes FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor.

[0084] Provided herein is a method of identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers. In some embodiments, a method of

identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment with the Wnt pathway inhibitor. In some embodiments, the method further comprises selecting the patient for treatment when their tumor sample has a positive decision value. In some embodiments, the method further comprises administering a therapeutically effective amount of the Wnt pathway inhibitor to the patient.

[0085] Provided herein is a method of selecting a patient with cancer for treatment with a Wnt pathway inhibitor, the method comprising: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) selecting the patient for treatment based upon the expression level of the biomarkers. In some embodiments, a method of selecting a patient with cancer for treatment with a Wnt pathway inhibitor comprises: (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; and (d) selecting the patient for treatment when their tumor sample has a positive decision value. In some embodiments, the method further comprises administering a therapeutically effective amount of the Wnt pathway inhibitor to the patient.

[0086] Provided herein is a method of treating cancer in a patient, comprising: (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (b) administering to the patient who is likely to response to treatment an effective amount of the Wnt pathway inhibitor. In some embodiments, a method of treating cancer in a patient comprises: (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that the patient is predicted to respond to

treatment; and (b) administering to the patient who is predicted to response to treatment an effective amount of the Wnt pathway inhibitor.

[0087] In another aspect, the invention provides a method of treating cancer in a patient, comprising: administering an effective amount of a Wnt pathway inhibitor to the patient; wherein the patient is predicted to respond to treatment with a Wnt inhibitor based upon expression levels of a biomarker signature in a patient tumor sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, a method of treating cancer in a patient comprises: administering an effective amount of a Wnt pathway inhibitor to the patient; wherein the patient is predicted to respond to treatment based upon a positive decision value calculated from the weighted sum of the standardized expression of biomarkers in a biomarker signature in a patient tumor sample, wherein the set of biomarkers comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

[0088] Provided herein is a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising: (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (b) administering an effective amount of the Wnt pathway inhibitor to the patient. In some embodiments, a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor comprises: (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment; and (b) administering an effective amount of the Wnt pathway inhibitor to the patient whose tumor has a positive decision value.

[0089] In another aspect, the invention provides a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising: administering an effective amount of a Wnt pathway inhibitor to a patient; wherein the patient is identified as likely to respond to treatment with a Wnt inhibitor based upon expression levels of a biomarker signature in a patient tumor sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, a method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor comprises: administering an effective amount of a Wnt pathway inhibitor to a patient; wherein the patient is identified as likely to respond to treatment based

upon a positive decision value calculated from the weighted sum of the standardized expression of biomarkers in a biomarker signature in a patient tumor sample, wherein the set of biomarkers comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the patient is identified as likely to respond to treatment with a Wnt pathway inhibitor in combination with paclitaxel. In some embodiments, the method comprises administering to the patient the Wnt pathway inhibitor in combination with paclitaxel.

[0090] Provided herein is a use for identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, wherein the use comprises (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CRBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor.

[0091] Provided herein is a use for classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, wherein the use comprises (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor.

[0092] Provided herein is a use for determining the sensitivity of a human tumor to treatment with a Wnt pathway inhibitor, wherein the use comprises (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the genes FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor.

[0093] Provided herein is a use for identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor, wherein the use comprises (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates

that the patient is predicted to respond to treatment with the Wnt pathway inhibitor. In some embodiments, the use further comprises selecting the patient for treatment when their tumor sample has a positive decision value. In some embodiments, the use further comprises administering a therapeutically effective amount of the Wnt pathway inhibitor to the patient.

[0094] Provided herein is a use for selecting a patient with cancer for treatment with a Wnt pathway inhibitor, wherein the use comprises (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; and (d) selecting the patient for treatment when their tumor sample has a positive decision value. In some embodiments, the use further comprises administering a therapeutically effective amount of the Wnt pathway inhibitor to the patient.

[0095] Provided herein is a Wnt pathway inhibitor for use in treating cancer in a patient, the use comprising: (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment; and (b) administering to the patient who is predicted to response to treatment an effective amount of the Wnt pathway inhibitor.

[0096] Provided herein is a use for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, the use comprising: (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment; and (b) administering an effective amount of the Wnt pathway inhibitor to the patient whose tumor has a positive decision value.

[0097] Provided herein is a Wnt pathway inhibitor for use in treating cancer in a patient identified to likely to respond to treatment with a Wnt pathway inhibitor wherein the identification of the patient comprises: (i) measuring the expression level of each biomarker of a biomarker signature in the cancer sample obtained from the patient, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (ii) calculating a decision

value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment.

[0098] Provided herein is a Wnt pathway inhibitor for use in treating cancer in a patient, wherein the patient is one for whom a positive decision value is calculated based upon the standardized expression of each biomarker of the biomarker signature in a cancer sample of the patient, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

[0099] In some embodiments of the methods described herein, the biomarker signature comprises two or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises three or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises four or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises five or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature consists of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

[00100] In some embodiments, the biomarker signature comprises one or more additional biomarkers, in addition to at least one of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises one or more additional biomarkers selected from the genes listed in Table 2, in addition to at least one of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises one or more of the biomarkers EP300, CTBP1, WNT6, WNT9A, SNT3, FZD2, FZD7, APC, TLE2, DVL2, PITX2, WISP1, GSK3B, and LEF1, in addition to at least one of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, DKK1, EP300, and CTBP1. In some embodiments, the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, DKK1, EP300, CTBP1, WNT6, WNT3, FZD2, APC, TLE2, DVL2, PITX2, WISP1, GSK3B, WNT9A, FZD7, and LEF1.

[00101] In some embodiments of the methods described herein, the biomarker signature comprises FBXW2. In some embodiments, the biomarker signature comprises CCND2. In some embodiments, the biomarker signature comprises RHOU. In some embodiments, the biomarker signature comprises CTBP2. In some embodiments, the biomarker signature comprises WIF1. In some embodiments, the biomarker signature comprises DKK1.

[00102] In some embodiments of the methods described herein, the biomarker signature comprises FBXW2 and CCND2. In some embodiments, the biomarker signature comprises FBXW2 and RHOU. In some embodiments, the biomarker signature comprises FBXW2 and CTBP2. In some embodiments, the biomarker signature comprises FBXW2 and WIF1. In some embodiments, the

biomarker signature comprises FBXW2 and DKK1. In some embodiments, the biomarker signature comprises CCND2 and RHOU. In some embodiments, the biomarker signature comprises CCND2 and CTBP2. In some embodiments, the biomarker signature comprises CCND2 and WIF1. In some embodiments, the biomarker signature comprises CCND2 and DKK1. In some embodiments, the biomarker signature comprises RHOU and CTBP2. In some embodiments, the biomarker signature comprises RHOU and WIF1. In some embodiments, the biomarker signature comprises RHOU and DKK1. In some embodiments, the biomarker signature comprises CTBP2 and WIF1. In some embodiments, the biomarker signature comprises CTBP2 and DKK1. In some embodiments, the biomarker signature comprises WIF1 and DKK1.

[00103] In some embodiments of the methods described herein, the biomarker signature comprises FBXW2, CCND2, and RHOU. In some embodiments, the biomarker signature comprises FBXW2, CCND2, and CTBP2. In some embodiments, the biomarker signature comprises FBXW2, CCND2, and WIF1. In some embodiments, the biomarker signature comprises FBXW2, CCND2, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, RHOU, and CTBP2. In some embodiments, the biomarker signature comprises FBXW2, RHOU, and WIF1. In some embodiments, the biomarker signature comprises FBXW2, RHOU, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, CTBP2, and WIF1. In some embodiments, the biomarker signature comprises FBXW2, CTBP2, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises CCND2, RHOU, and CTBP2. In some embodiments, the biomarker signature comprises CCND2, RHOU, and WIF1. In some embodiments, the biomarker signature comprises CCND2, RHOU, and DKK1. In some embodiments, the biomarker signature comprises CCND2, CTBP2, and WIF1. In some embodiments, the biomarker signature comprises CCND2, CTBP2, and DKK1. In some embodiments, the biomarker signature comprises CCND2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises RHOU, CTBP2, and WIF1. In some embodiments, the biomarker signature comprises RHOU, CTBP2, and DKK1. In some embodiments, the biomarker signature comprises RHOU, WIF1, and DKK1. In some embodiments, the biomarker signature comprises CTBP2, WIF1, and DKK1.

[00104] In some embodiments of the methods described herein, the biomarker signature comprises FBXW2, CCND2, RHOU, and CTBP2. In some embodiments, the biomarker signature comprises FBXW2, CCND2, RHOU, and WIF1. In some embodiments, the biomarker signature comprises FBXW2, CCND2, RHOU, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, RHOU, CTBP2, and WIF1. In some embodiments, the biomarker signature comprises FBXW2, RHOU, CTBP2, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises CCND2, RHOU, CTBP2, and WIF1. In some embodiments, the biomarker signature comprises CCND2, RHOU, CTBP2, and DKK1. In some embodiments, the biomarker signature comprises

CCND2, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises RHOU, CTBP2, WIF1, and DKK1. In some embodiments, any of these signatures may comprise one or more additional biomarkers.

[00105] In some embodiments of the methods described herein, the biomarker signature comprises FBXW2, CCND2, RHOU, CTBP2, and WIF1. In some embodiments, the biomarker signature comprises FBXW2, CCND2, RHOU, CTBP2, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, CCND2, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, CCND2, RHOU, WIF1, and DKK1. In some embodiments, the biomarker signature comprises FBXW2, RHOU, CTBP2, WIF1, and DKK1. In some embodiments, the biomarker signature comprises CCND2, RHOU, CTBP2, WIF1, and DKK1.

[00106] In some embodiments, the sample includes, but is not limited to, any clinically relevant tissue sample, such as a tumor biopsy, a core biopsy tissue sample, a fine needle aspirate, a hair follicle, or a sample of bodily fluid, such as blood, plasma, serum, lymph, ascitic fluid, cystic fluid, or urine. In some embodiments, the sample is taken from a patient having a tumor or cancer. In some embodiments, the sample is a primary tumor. In some embodiments, the sample is a metastasis. The sample may be taken from a human, or from non-human mammals such as, mice, rats, non-human primates, canines, felines, ruminants, swine, or sheep. In some embodiments, samples are taken from a subject at multiple time points, for example, before treatment, during treatment, and/or after treatment. In some embodiments, samples are taken from different locations in the subject, for example, a sample from a primary tumor and a sample from a metastasis in a distant location.

[00107] In some embodiments, the sample is a paraffin-embedded fixed tissue sample. In some embodiments, the sample is a formalin-fixed paraffin embedded (FFPE) tissue sample. In some embodiments, the sample is a fresh tissue (e.g., tumor) sample. In some embodiments, the sample is a frozen tissue sample. In some embodiments, the sample is a fresh frozen (FF) tissue (e.g., tumor) sample. In some embodiments, the sample is a cell isolated from a fluid. In some embodiments, the sample comprises circulating tumor cells (CTCs). In some embodiments, the sample is an archival tissue sample. In some embodiments, the sample is an archival tissue sample with known diagnosis, treatment, and/or outcome history. In some embodiments, the sample is a block of tissue. In some embodiments, the sample is dispersed cells. In some embodiments, the sample size is from about 1 cell to about 1 x 10^6 cells or more. In some embodiments, the sample size is about 10 cells to about 1 x 10^5 cells. In some embodiments, the sample size is about 10 cells to about 10,000 cells. In some embodiments, the sample size is about 10 cells to about 1,000 cells. In some embodiments, the sample size is about 10 cells to about 100 cells. In some embodiments, the sample size is about 1 cell to about 10 cells. In some embodiments, the sample size is a single cell.

[00108] In some embodiments, the sample is processed to DNA or RNA. In some embodiments, RNA is isolated from the sample. In some embodiments, mRNA is isolated from the sample. In some embodiments, RNA is isolated from cells by procedures that involve cell lysis and denaturation

of the proteins contained therein. In some embodiments, DNase is added to remove DNA. In some embodiments, RNase inhibitors are added to the lysis buffer. In some embodiments, a protein denaturation/digestion step is added to the protocol. Methods for preparing total and mRNA are well known in the art and RNA isolation kits are commercially available (e.g., RNeasy mini kit, Qiagen, USA). In some embodiments, the RNA is amplified by PCR-based techniques.

[00109] Determination of biomarker expression levels may be performed by any suitable method including, but are not limited to, methods based on analyses of polynucleotide expression, sequencing of polynucleotides, and/or analyses of protein expression. For example, determination of biomarker expression levels may be performed by detecting the expression of mRNA expressed from the genes of interest, and/or by detecting the expression of a polypeptide encoded by the genes.

[00110] Commonly used methods for the analysis of polynucleotides, include Southern blot analysis, Northern blot analysis, and in situ hybridization, RNAse protection assays, and polymerase chain reaction (PCR)-based methods, such as reverse transcription polymerase chain reaction (RT-PCR), quantitative PCR (qPCR) as known as real-time PCR, TaqMan™, TaqMan™ low density array (TLDA), anchored PCR, competitive PCR, rapid amplification of cDNA ends (RACE), and microarray analyses. RT-PCR is a quantitative method that can be used to compare mRNA levels in different samples to examine gene expression profiles. A variation of RT-PCR is real time quantitative PCR, which measures PCR product accumulation through a dual-labeled fluorigenic probe (e.g., TaqMan™ probe). There are many other PCR-based techniques known to one of skill in the art, including but not limited to, differential display, amplified fragment length polymorphism, BeadArray™ technology, high coverage expression profiling (HiCEP) and digital PCR. Representative methods for sequencing-based gene expression analyses include Serial Analysis of Gene Expression (SAGE), Massively Parallel Signature Sequencing (MPSS), and NexGen sequencing analysis, including mRNA sequencing.

[00111] In certain embodiments, the biomarker expression is determined using a qPCR assay. For example, total RNA is extracted from a fresh frozen (FF) tissue sample or total RNA is extracted from a macro-dissected formalin-fixed paraffin embedded (FFPE) tissue sample. The quantity and quality of the total RNA is assessed by standard spectrophotometry and/or any other appropriate method (e.g., an Agilent Bioanalyzer). Following RNA extraction, the RNA sample is reverse transcribed using standard methods and/or a commercially available cDNA synthesis kit (e.g., Roche Transcriptor First Strand cDNA synthesis kit). The resultant cDNA is pre-amplified using, for example, an ABI pre-amplification kit. Expression of the biomarker(s) (e.g., FBXW2, CCND2, RHOU, CTBP2, WIF1, and/or DKK1) are assessed on, for example, a Roche Lightcycler 480 system (Roche Diagnostics) using an ABI TaqMan Gene Expression Mastermix. qPCR reactions are performed in triplicate. For each assay a subset of the samples is run without reverse transcription (the RT-neg control), as well as, control samples run without template. A universal human reference RNA sample is included on each plate to act as a positive control. Suitable reference genes are identified from a standard panel of

reference genes. Candidate reference genes are selected with different cellular functions to eliminate risk of co-regulation. The most suitable reference genes are evaluated and selected using specific software and algorithms (e.g., Genex software; GeNorm and Normfinder algorithms). The expression level of each biomarker is normalized using the selected optimum reference genes. In some embodiments, these normalized (or standardized) expression values for each biomarker are used to calculate the decision value of the sample. In some embodiments, these normalized (or standardized) expression values for each biomarker are used to calculate an expression level.

[00112] In some embodiments, biomarker expression is determined using a PCR-based assay comprising specific primers and/or probes for each biomarker (e.g., FBXW2, CCND2, RHOU, CTBP2, WIF1, and/or DKK1). As used herein, the term "probe" refers to any molecule that is capable of selectively binding a specifically intended target biomolecule. Probes can be synthesized by one of skill in the art using known techniques, or derived from biological preparations. Probes may include but are not limited to, RNA, DNA, proteins, peptides, aptamers, antibodies, and organic molecules. The term "primer" or "probe" encompasses oligonucleotides that have a sequence of a specific SEQ ID NO or oligonucleotides that have a sequence complementary to a specific SEQ ID NO. In some embodiments, the probe is modified. In some embodiments, the probe is modified with a quencher. In some embodiments, the probe is labeled. Labels can include, but are not limited to, colorimetric, fluorescent, chemiluminescent, or bioluminescent labels.

[00113] In some embodiments, biomarker expression of each biomarker is determined using a specific primer set and probe. In some embodiments, a specific primer set consists of a forward primer and a reverse primer. In some embodiments, CCND2 expression is determined using a polynucleotide comprising the sequence of GCTGTCTCTGATCCGCAAGC (SEQ ID NO:62), a polynucleotide comprising the sequence of GACGGTGGGTACATGGCAAAC (SEQ ID NO:63), and a polynucleotide comprising the sequence of CCTTCATTGCTCTGTGTGCCACCGAC (SEQ ID NO:64), or complements thereof. In some embodiments, CCND2 expression is determined using a forward primer of sequence GCTGTCTCTGATCCGCAAGC (SEQ ID NO:62) and a reverse primer of sequence GACGGTGGGTACATGGCAAAC (SEQ ID NO:63). In some embodiments, CCND2 expression is determined using a probe of sequence CCTTCATTGCTCTGTGTGCCACCGAC (SEQ ID NO:64).

[00114] In some embodiments, CTBP2 expression is determined using isolated a polynucleotide comprising the sequence of ATCCGTGGGGAGACGCTG (SEQ ID NO:65), a polynucleotide comprising the sequence of CTCGAACTGCAACCGCCTG (SEQ ID NO:66), and a polynucleotide comprising the sequence of CCCGTGCGACCAAAGCCAATGAGG (SEQ ID NO:67), or complements thereof. In some embodiments, CTBP2 expression is determined using a forward primer of sequence ATCCGTGGGGAGACGCTG (SEQ ID NO:65) and a reverse primer of sequence of CTCGAACTGCAACCGCCTG (SEQ ID NO:66). In some embodiments, CTBP2 expression is determined using a probe of sequence CCCGTGCGACCAAAGCCAATGAGG (SEQ ID NO:67).

[00115] In some embodiments, DKK1 expression is determined using isolated a polynucleotide comprising the sequence of GACCATTGACAACTACCAGCCGTA (SEQ ID NO:68), a polynucleotide comprising the sequence of TGGGACTAGCGCAGTACTCATC (SEQ ID NO:69), and a polynucleotide comprising the sequence of TGCCGCACTCCTCGTCCTCTG (SEQ ID NO:70), or complements thereof. In some embodiments, DKK1 expression is determined using a forward primer of sequence GACCATTGACAACTACCAGCCGTA (SEQ ID NO:68) and a reverse primer of sequence of TGGGACTAGCGCAGTACTCATC (SEQ ID NO:69). In some embodiments, DKK1 expression is determined using a probe of sequence TGCCGCACTCCTCGTCCTCTG (SEQ ID NO:70).

[00116] In some embodiments, FBXW2 expression is determined using a polynucleotide comprising the sequence of GCCAGTTATGATATTCTCAGGGTCA (SEQ ID NO:71), a polynucleotide comprising the sequence of AGCAGGGCAAAGATATCTCCAAA (SEQ ID NO:72), and a polynucleotide comprising the sequence of AGACTCCTGAGATAGCAAACTTGGCCT (SEQ ID NO:73), or complements thereof. In some embodiments, FBXW2 expression is determined using a forward primer of sequence GCCAGTTATGATATTCTCAGGGTCA (SEQ ID NO:71) and a reverse primer of sequence AGCAGGGCAAAGATATCTCCAAA (SEQ ID NO:72). In some embodiments, FBXW2 expression is determined using a probe of sequence AGACTCCTGAGATAGCAAACTTGGCCT (SEQ ID NO:73).

[00117] In some embodiments, RHOU1 expression is determined using a polynucleotide comprising the sequence of CCCACCGAGTACATCCCTACTG (SEQ ID NO:74), a polynucleotide comprising the sequence of CAGTGTCACAGAGTTGGAGTCTCA (SEQ ID NO:75), and a polynucleotide comprising the sequence of CGCCCATCCACAGACACCACCG (SEQ ID NO:76), or complements thereof. In some embodiments, RHOU1 expression is determined using a forward primer of sequence CCCACCGAGTACATCCCTACTG (SEQ ID NO:74) and a reverse primer of sequence CAGTGTCACAGAGTTGGAGTCTCA (SEQ ID NO:75). In some embodiments, RHOU1 expression is determined using a probe of sequence CGCCCATCCACAGACACCACCG (SEQ ID NO:76).

[00118] In some embodiments, WIF1 expression is determined using a polynucleotide comprising the sequence of GTTCCAAAGGTTACCAGGGAGAC (SEQ ID NO:77), a polynucleotide comprising the sequence of GTTGGGTTCATGGCAGGTTCC (SEQ ID NO:78), and a polynucleotide comprising the sequence of CCAGGCTCGCAGACAGGCTTTGAAC (SEQ ID NO:79), or complements thereof. In some embodiments, WIF1 expression is determined using a forward primer of sequence GTTCCAAAGGTTACCAGGGAGAC (SEQ ID NO:77) and a reverse primer of sequence GTTGGGTTCATGGCAGGTTCC (SEQ ID NO:78). In some embodiments, WIF1 expression is determined using a probe of sequence CCAGGCTCGCAGACAGGCTTTGAAC (SEQ ID NO:79).

[00119] In some embodiments of any of the methods described herein, the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 are measured using polynucleotides selected from the group consisting of SEQ ID NOs:62-79. In some embodiments of any of the methods described herein, the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 are measured using (a) a forward primer of SEQ ID NO:62, a reverse primer of SEQ ID NO:63, and a probe comprising SEQ ID NO:64; (b) a forward primer of SEQ ID NO:65, a reverse primer of SEQ ID NO:66, and a probe comprising SEQ ID NO:67; (c) a forward primer of SEQ ID NO:68, a reverse primer of SEQ ID NO:69, and a probe comprising SEQ ID NO:70; (d) a forward primer of SEQ ID NO:71, a reverse primer of SEQ ID NO:72, and a probe comprising SEQ ID NO:73; (e) a forward primer of SEQ ID NO:74, a reverse primer of SEQ ID NO:75, and a probe comprising SEQ ID NO:76; and (f) a forward primer of SEQ ID NO:77, a reverse primer of SEQ ID NO:78, and a probe comprising SEQ ID NO:79.

[00120] In some embodiments, the expression level of each biomarker (e.g., FBXW2, CCND2, RHOU, CTBP2, WIF1, and/or DKK1) is determined in a separate assay (e.g., 6 assays). In some embodiments, the reference gene(s) and normalization methods for each assay are the same for all 6 assays. In some embodiments, the expression levels of several biomarkers (e.g., FBXW2, CCND2, RHOU, CTBP2, WIF1, and/or DKK1) are detected in a single multiplex assay.

[00121] Alternatively, biomarker expression levels may be determined by amplifying complementary DNA (cDNA) or complementary RNA (cRNA) produced from mRNA and analyzing it using a microarray. Microarray technology allows for simultaneous analysis of the expression of thousands of genes. A number of different array configurations and methods for their production are known to those skilled in the art. In addition, microarrays are commercially available (e.g., Affymetrix GeneChips) or can be custom-produced. Microarrays currently in wide use include cDNA arrays and oligonucleotide arrays. In general, polynucleotides of interest (e.g., probes or probe sets) are plated, or arrayed, on a microchip substrate. In some embodiments, probes to at least 10, 25, 50, 100, 500, 1000, 5000, 10,000, 20,000, or 25,000 or more genes are immobilized on an array substrate. The substrate may be a porous or nonporous support, such as a glass, plastic or gel surface. The probes can include DNA, RNA, copolymer sequences of DNA and RNA, DNA and/or RNA analogues, or combinations thereof. In some embodiments, a microarray includes a support with an ordered array of binding sites for each individual gene. The microarrays can be addressable arrays or positionally addressable arrays, e.g., each probe of the array is located at a known, predetermined position on the solid support such that the identity of each probe can be determined from its position of the array.

[00122] Each probe on the microarray can be between 10-50,000 nucleotides in length. In some embodiments, the probes of the microarray can consist of nucleotide sequences with lengths of less than about 1,000 nucleotides, less than about 750 nucleotides, less than about 500 nucleotides, less than about 250 nucleotides, less than about 100 nucleotides, or less than about 50 nucleotides in length. Generally, an array includes positive control probes and negative control probes.

[00123] In certain embodiments, the biomarker expression is determined using a microarray. For example, total RNA is extracted from a fresh frozen (FF) tissue sample or total RNA is extracted from a macro-dissected formalin-fixed paraffin embedded (FFPE) tissue sample. The quantity and quality of the total RNA is assessed by standard spectrophotometry and/or any other appropriate technology (e.g., an Agilent Bioanalyzer). Following RNA extraction, the RNA sample is amplified using standard methods and/or a commercially available amplification system (e.g., NuGEN Ovation RNA Amplification System V2). The amplified cDNA is fragmented, labeled, and hybridized to a microarray (e.g., using NuGEN Encore Biotin Module and Affymetrix GeneChip array) following standard procedures. The array is washed, stained, and scanned in accordance with the instructions for the microarray. The microarray data is pre-processed, the probe-level intensity measurements are background corrected, normalized, and summarized as expression measurements using the Robust Multichip algorithm (RMA). The probe level data is summarized to get the expression level of each biomarker (e.g., FBXW2, CCND2, RHOU, CTBP2, WIF1, and/or DKK1). A combination of quality parameter threshold and data reduction techniques (e.g., principal component analysis) is applied to the data set to establish profile quality and identify potential outlying samples. These normalized (or standardized) expression values for each biomarker are used to calculate the decision value of the sample.

[00124] In some embodiments, biomarker expression is analyzed by studying the protein expression of the gene or genes of interest. Commonly used methods for the analysis of protein expression, include but are not limited to, immunohistochemistry (IHC)-based, antibody-based, and mass spectrometry-based methods. Antibodies, generally monoclonal antibodies, may be used to detect expression of a gene product (e.g., protein). In some embodiments, the antibodies can be detected by direct labeling of the antibodies themselves. In other embodiments, an unlabeled primary antibody is used in conjunction with a labeled secondary antibody. Immunohistochemistry methods and/or kits are well known in the art and are commercially available.

[00125] In some embodiments, biomarker expression is determined by an assay known to those of skill in the art, including but not limited to, multi-analyte profile test, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, Western blot assay, immunofluorescent assay, enzyme immunoassay, immunoprecipitation assay, chemiluminescent assay, immunohistochemical assay, dot blot assay or slot blot assay. In some embodiments, wherein an antibody is used in the assay the antibody is detectably labeled. The antibody labels may include, but are not limited to, immunofluorescent label, chemiluminescent label, phosphorescent label, enzyme label, radiolabel, avidin/biotin, colloidal gold particles, colored particles and magnetic particles.

[00126] Other suitable methods for analyzing biomarker expression include proteomics-based methods. Proteomics includes, among other things, study of the global changes of protein expression in a sample. In some embodiments, a proteomic method comprises the following steps: (1) separation of individual proteins in a sample by 2-D electrophoresis (2-D PAGE), (2) identification of individual

proteins recovered from the gel (e.g., by mass spectrometry or N-terminal sequencing), and (3) analysis of the data using bioinformatics. In some embodiments, a proteomic method comprises using a tissue microarray (TMA). Tissue arrays may be constructed according to a variety of techniques known to one of skill in the art. In certain embodiments, a manual tissue arrayer is used to remove a "core" from a paraffin block prepared from a tissue sample. The core is then inserted into a separate paraffin block in a designated location on a grid. Cores from as many as about 400 samples can be inserted into a single recipient block. The resulting tissue array may be processed into thin sections for analysis. In some embodiments, a proteomic method comprises an antibody microarray. In some embodiments, a proteomic method comprises using mass spectrometry, including but not limited to, SELDI, MALDI, electro spray, and surface plasmon resonance methods. In some embodiments, a proteomic method comprises bead-based technology, including but not limited to, antibodies on beads in an array format. In some embodiments, the proteomic method comprises a reverse phase protein microarray (RPPM). In some embodiments, the proteomic method comprises multiplexed protein profiling, including but not limited to, the Global Proteome Survey (GPS) method.

[00127] In some embodiments, the biomarker signature is identified by differential gene expression between two samples. In some embodiments, the biomarker signature is identified by differential gene expression between two samples which comprise genes differentially expressed in cancer cells as compared to normal cells. In some embodiments, the biomarker signature comprises genes differentially expressed in tumorigenic cancer stem cells as compared to non-tumorigenic cancer cells. In some embodiments, the biomarker signature comprises genes differentially expressed in cells from a tumor which is responsive to a specific treatment as compared to cells from a tumor which is non-responsive to the same treatment.

[00128] In some embodiments, expression profiles are determined using microarray analysis. The microarray data identifies gene profiles comprising similarly and differentially expressed genes between two samples. In some embodiments, the expression profiles are refined, filtered, and/or subdivided into biomarker signatures based on fold expression change. In some embodiments, all genes above a certain fold expression change are included in the biomarker signature. The fold expression change may be elevated, reduced or both elevated and reduced. In some embodiments, all genes with a 2-fold or more expression change are included in the biomarker signature. In some embodiments, all genes with a 2.5-fold or more expression change are included in the biomarker signature. In some embodiments, all genes with a 3-fold or more expression change are included in the biomarker signature. In some embodiments, all genes with a 3.5-fold or more expression change are included in the biomarker signature. In some embodiments, all genes with a 4-fold or more expression change are included in the biomarker signature.

[00129] In some embodiments, the gene expression profiles are refined, filtered, and/or subdivided into biomarker signatures based on statistical analyses. The statistical methods may include, but are not limited to, cluster analysis, supported vector machines (SVM) analysis, supported vector machines

- recursive feature elimination (SVM-RFE) analysis, Platt scaling, neural networks, and other algorithms. In some embodiments, the gene expression profiles are analyzed using a t-test analysis. In some embodiments, the gene expression profiles are analyzed using paired-sample empirical Baysian analysis. In some embodiments, a combination of statistical analyses is used. In some embodiments, SVM models are used to obtain decision values based on the training data. In some embodiments, the decision values are calculated by a weighted sum of the standardized expression of a set of biomarkers. In some embodiments, a positive decision value indicates a tumor predicted to be a responder while a negative decision value indicates a tumor predicted to be a non-responder. In some embodiments, classification probabilities for responders and non-responders are obtained using Platt scaling (Platt, 1999, *Advances in Large Margin Classifiers*, pp. 61-74, MIT Press). Platt scaling may comprise fitting a logistic distribution using maximum likelihood to decision values obtained, for example, by SVM models. In some embodiments, tumors associated with probabilities higher than 0.5 would be predicted to be a responder while tumors with probabilities lower than 0.5 would be predicted to be a non-responder.

[00130] In some embodiments of any of the methods or uses described herein, classification probabilities of a tumor (in regard to responder or non-responder status) are obtained based on the decision values. In some embodiments, the probabilities are obtained by fitting a logistic regression on the decision values. In some embodiments, tumors associated with probabilities higher than 0.5 are predicted to be a responder while tumors with probabilities lower than 0.5 are predicted to be a non-responder.

[00131] In some embodiments, a biomarker signature is obtained by a series of analytical steps. For example, expression data from a training set of samples are obtained from microarray analyses. The data are preprocessed to get an expression matrix with specific genes. Genes with near zero variance are removed, as are genes with expression values below a pre-determined level. The remaining genes are ranked using SVM-RFE analysis. Leave-one-out cross-validation (LOOCV) methods are used to identify and select the best predictive genes and also to measure positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity.

[00132] In some embodiments, all genes with elevated expression, reduced expression, or both, with a P value across samples of 0.01 or less are included in the biomarker signature. In some embodiments, all genes with elevated expression, reduced expression or both, with a P value across samples of 0.005 or less are included in the biomarker signature. In some embodiments, all genes with elevated expression, reduced expression or both, with a P value across samples of 0.001 or less are included in the biomarker signature. In some embodiments, all genes with elevated expression, reduced expression or both, with a FDR (False Discovery Rate) of 0.25 or less are included in the biomarker signature. In some embodiments, all genes with elevated expression, reduced expression or both, with a FDR of 0.1 or less, 0.01 or less, or 0.001 or less are included in the biomarker signature.

[00133] In some embodiments, the gene expression profiles and/or biomarker signatures are refined, filtered, and/or subdivided based on statistical models. In some embodiments, the gene expression profiles and/or biomarker signatures are refined, filtered, and/or subdivided based on survival analysis models. These models may include, but are not limited to, Kaplan-Meier survival models, Cox proportional models, Cox proportional hazard models, chi-square analysis, univariate logistic regression models, multivariate competing risk models, linear discriminate analysis models, parametric regression models and correlation analysis models.

[00134] In some embodiments, the gene expression profiles and/or biomarker signatures are refined, filtered, subdivided and/or tested using gene expression array datasets that have associated clinical outcomes. There are several databases that contain datasets that are available to the public, for example, Gene Expression Omnibus (GEO) and ArrayExpress.

[00135] In some embodiments, the gene expression profiles and/or biomarker signatures are refined using biological function parameters, and/or gene sets. For example, in some embodiments, gene expression profiles, and/or biomarker signatures are refined using Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005, *PNAS,* 102: 15545-15550). In some embodiments, the gene expression profiles are refined based on their ability to predict clinical outcome.

[00136] In some of the embodiments of the methods described herein, the Wnt pathway inhibitor is an anti-FZD antibody as described herein. In some of the embodiments of the methods described herein, the Wnt pathway inhibitor is an antibody that specifically binds at least one Frizzled (FZD) protein or portion thereof. In some embodiments, the anti-FZD antibody specifically binds at least one FZD protein selected from the group consisting of: FZD1, FZD2, FZD5, FZD7, and FZD8. In other embodiments, the anti-FZD antibody comprises: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), and (b) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:6). In some embodiments, the anti-FZD antibody comprises a heavy chain variable region comprising the amino acids of SEQ ID NO:7. In some embodiments, the anti-FZD antibody comprises a light chain variable region comprising the amino acids of SEQ ID NO:8. In some embodiments, the anti-FZD antibody comprises a heavy chain variable region comprising the amino acids of SEQ ID NO:7 and a light chain variable region comprising the amino acids of SEQ ID NO:8. In some embodiments, the anti-FZD antibody is antibody OMP-18R5. In some embodiments, the anti-FZD antibody is encoded by the plasmid having ATCC deposit no. PTA-9541. In other embodiments, the anti-FZD antibody competes for specific binding to at least one human FZD protein with an antibody encoded by the plasmid deposited with ATCC having deposit no. PTA-9541.

[00137] In some embodiments of the methods described herein, the tumor is selected from the group consisting of a breast tumor, lung tumor, a colon tumor, glioma, a gastrointestinal tumor, a renal

tumor, an ovarian tumor, a liver tumor, a colorectal tumor, an endometrial tumor, a kidney tumor, a prostate tumor, a thyroid tumor, a neuroblastoma, a pancreatic tumor, a glioblastoma multiforme, a cervical tumor, a stomach tumor, a bladder tumor, a hepatoma, melanoma, and a head and neck tumor. In some embodiments, the tumor is a breast tumor. In some embodiments, the tumor is a HER2-negative breast tumor. In some embodiments, the tumor is a triple negative breast cancer (TNBC) tumor.

[00138] In some embodiments of the methods described herein, the cancer is selected from the group consisting of a breast cancer, lung cancer, a colon cancer, glioma, a gastrointestinal cancer, a renal cancer, an ovarian cancer, a liver cancer, a colorectal cancer, an endometrial cancer, a kidney cancer, a prostate cancer, a thyroid cancer, a neuroblastoma, a pancreatic cancer, a glioblastoma multiforme, a cervical cancer, a stomach cancer, a bladder cancer, a hepatoma, melanoma, and a head and neck cancer. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is a HER2-negative breast cancer. In some embodiments, the cancer is a triple negative breast cancer (TNBC).

[00139] In some of the embodiments of the methods described herein, the method comprises treating a patient with a Wnt pathway inhibitor described herein (e.g., an anti-FZD antibody), particularly after the patient has been identified as being responsive to treatment with the Wnt pathway inhibitor. In some embodiments, the treatment comprises administering at least one additional therapeutic agent in combination with the Wnt pathway inhibitor. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the Wnt pathway inhibitor. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

[00140] Useful classes of therapeutic agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[00141] Therapeutic agents that may be administered in combination with the Wnt pathway inhibitors include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the administration of a Wnt pathway inhibitor of the present invention in combination with a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. Treatment with a Wnt pathway inhibitor (e.g, an anti-FZD antibody) can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or

consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *The Chemotherapy Source Book, 4th Edition*, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

[00142] Chemotherapeutic agents useful in the instant invention include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel (TAXOL) and docetaxel (TAXOTERE); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine

(XELODA); and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain embodiments, the additional therapeutic agent is paclitaxel (taxol).

[00143] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HCl, daunorubicin citrate, mitoxantrone HCl, actinomycin D, etoposide, topotecan HCl, teniposide (VM-26), and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these.

[00144] In certain embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these.

[00145] In certain embodiments, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some embodiments, the agent is a taxane. In certain embodiments, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain embodiments, the agent is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (nab-paclitaxel; ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative embodiments, the antimitotic agent comprises a vinca alkaloid, such as vincristine, binblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some embodiments, the antimitotic agent is an inhibitor of kinesin Eg5 or an inhibitor of a mitotic kinase such as Aurora A or Plk1. In certain embodiments, where the chemotherapeutic agent administered in combination with a Wnt pathway inhibitor is an anti-mitotic agent, the cancer or tumor being treated is breast cancer or a breast tumor. In certain embodiments, the additional therapeutic agent is paclitaxel (taxol) or albumin-bound paclitaxel.

[00146] In some embodiments, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of a Wnt pathway inhibitor of the present invention with a small molecule that acts as an inhibitor against additional tumor-associated antigens including, but not limited to, EGFR, ErbB2, HER2, and/or VEGF. In certain embodiments, the additional therapeutic agent is a small molecule that inhibits a cancer stem

cell pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the BMP pathway.

[00147] Certain embodiments of the present invention comprise a method of identifying a human breast tumor that is likely to be responsive to or non-responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising (a) obtaining a sample of the human breast tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast tumor is predicted to be responsive to treatment with the antibody and a negative decision value indicates the tumor is predicted to be non-responsive to treatment with the antibody. Some embodiments comprise a method of identifying a patient with breast cancer that is likely to be responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the breast cancer; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody. Some embodiments comprise a method of selecting a patient with breast cancer for treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the breast cancer; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody; and selecting the patient for treatment when their tumor sample has a positive decision value.

[00148] Some embodiments of the present invention comprise a method of treating breast cancer in a patient, comprising: (a) identifying if the patient is likely to respond to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, wherein the identification comprises: (i) obtaining a sample of the patient's breast cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the

standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment; and (b) administering to the patient who is predicted to response to treatment an effective amount of the antibody.

[00149] Certain embodiments of the present invention comprise a method of identifying a human breast tumor that is likely to be responsive to or non-responsive to treatment with anti-FZD antibody OMP-18R5 in combination with paclitaxel, the method comprising (a) obtaining a sample of the human breast tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast tumor is predicted to be responsive to treatment and a negative decision value indicates the tumor is predicted to be non-responsive to treatment. Some embodiments comprise a method of identifying a patient with breast cancer that is likely to be responsive to treatment with the anti-FZD antibody OMP-18R5 in combination with paclitaxel, the method comprising: (a) obtaining a sample of the breast cancer; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment. Some embodiments comprise a method of selecting a patient with breast cancer for treatment with the anti-FZD antibody OMP-18R5 in combination with paclitaxel, the method comprising: (a) obtaining a sample of the breast cancer; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment; and selecting the patient for treatment when their tumor sample has a positive decision value.

[00150] Some embodiments of the present invention comprise a method of treating breast cancer in a patient, comprising: (a) identifying if the patient is likely to respond to treatment with the anti-FZD antibody OMP-18R5 in combination with paclitaxel, wherein the identification comprises: (i) obtaining a sample of the patient's breast cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment; and (b) administering to the patient who is predicted to response to treatment an effective amount of the antibody and paclitaxel.

III. Wnt pathway inhibitors

[00151] The present invention provides methods for identifying tumors and/or patients with cancer that are likely to be responsive to or sensitive to treatment with Wnt pathway inhibitors. As used herein "Wnt pathway inhibitor" includes, but is not limited to, Frizzled (FZD) binding agents and Wnt-binding agents. FZD-binding agents may include antibodies that specifically bind to FZD proteins. Wnt-binding agents may include antibodies that specifically bind to Wnt proteins as well as soluble FZD receptors that bind to Wnt proteins.

[00152] In certain embodiments, the Wnt pathway inhibitors are agents that bind one or more human FZD proteins. In some embodiments, the FZD-binding agents specifically bind one, two, three, four, five, six, seven, eight, nine, or ten FZD proteins. In some embodiments, the FZD-binding agent binds one or more FZD proteins selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In some embodiments, FZD-binding agent binds one or more FZD proteins comprising FZD1, FZD2, FZD5, FZD7, and/or FZD8. In certain embodiments, FZD-binding agent binds FZD7. In certain embodiments, FZD-binding agent binds FZD5 and/or FZD8. In certain embodiments, the FZD-binding agent specifically binds FZD1, FZD2, FZD5, FZD7, and FZD8. Non-limiting examples of FZD-binding agents can be found in U.S. Patent No. 7,982,013.

[00153] In certain embodiments, the FZD-binding agent is a FZD antagonist. In certain embodiments, the FZD-binding agent is a Wnt pathway antagonist. In certain embodiments, the FZD-binding agent inhibits Wnt signaling. In some embodiments, the FZD-binding agent inhibits canonical Wnt signaling.

[00154] In some embodiments, the FZD-binding agents are antibodies. In some embodiments, the FZD-binding agents are polypeptides. In certain embodiments, the FZD-binding agent is an antibody or a polypeptide comprising an antigen-binding site. In certain embodiments, an antigen-binding site of a FZD-binding antibody or polypeptide described herein is capable of binding (or binds) one, two, three, four, five, or more human FZD proteins. In certain embodiments, an antigen-binding site of the FZD-binding antibody or polypeptide is capable of specifically binding one, two, three, four, or five human FZD proteins selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9 and FZD10. In some embodiments, when the FZD-binding agent is an antibody that binds more than one FZD protein, it may be referred to as a "pan-FZD antibody".

[00155] In certain embodiments, the FZD-binding agent (e.g., antibody) specifically binds the extracellular domain (ECD) of the one or more human FZD proteins to which it binds. In certain embodiments, the FZD-binding agent specifically binds within the Fri domain (also known as the cysteine-rich domain (CRD)) of the human FZD protein to which it binds. Sequences of the Fri domain of each of the human FZD proteins are known in the art and are provided as SEQ ID NO:13 (FZD1), SEQ ID NO:14 (FZD2), SEQ ID NO:15 (FZD3), SEQ ID NO:16 (FZD4), SEQ ID NO:17

(FZD5), SEQ ID NO:18 (FZD6), SEQ ID NO:19 (FZD7), SEQ ID NO:20 (FZD), SEQ ID NO:21 (FZD9), and SEQ ID NO:22 (FZD10).

[00156] In certain embodiments, the FZD-binding agent binds one, two, three, four, five, or more FZD proteins. In some embodiments, the FZD-binding agent specifically binds one, two, three, four, or five FZD proteins selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In some embodiments, the FZD-binding agent specifically binds at least FZD5 and FZD8.

[00157] In some embodiments, the FZD-binding agent binds at least one human FZD protein with a dissociation constant (K_D) of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some embodiments, a FZD-binding agent binds at least one FZD protein with a K_D of about 10nM or less. In some embodiments, a FZD-binding agent binds at least one FZD protein with a K_D of about 1nM or less. In some embodiments, a FZD-binding agent binds at least one FZD protein with a K_D of about 0.1nM or less. In certain embodiments, a FZD-binding agent binds each of one or more (e.g., 1, 2, 3, 4, or 5) of FZD1, FZD2, FZD5, FZD7, and FZD8 with a K_D of about 40nM or less. In certain embodiments, the FZD-binding agent binds to each of one or more of FZD1, FZD2, FZD5, FZD7, and FZD8 with a K_D of about 10nM or less. In certain embodiments, the FZD-binding agent binds each of FZD1, FZD2, FZD5, FZD7, and FZD8 with a K_D of about 10nM. In some embodiments, the K_D of the binding agent (e.g., an antibody) to a FZD protein is the K_D determined using a FZD-Fc fusion protein comprising at least a portion of the FZD extracellular domain or FZD-Fri domain immobilized on a Biacore chip.

[00158] In certain embodiments, the FZD-binding agent binds one or more (for example, two or more, three or more, or four or more) human FZD proteins with an EC_{50} of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, or about 1nM or less. In certain embodiments, a FZD-binding agent binds to more than one FZD protein with an EC_{50} of about 40nM or less, about 20nM or less, or about 10nM or less. In certain embodiments, the FZD-binding agent has an EC_{50} of about 20nM or less with respect to one or more (e.g., 1, 2, 3, 4, or 5) of the following FZD proteins: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding agent has an EC_{50} of about 10nM or less with respect to one or more (e.g., 1, 2, 3, 4, or 5) of the following FZD proteins: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding agent has an EC_{50} of about 40nM or less or 20nM or less with respect to binding of FZD5 and/or FZD8.

[00159] In certain embodiments, the Wnt pathway inhibitor is a FZD-binding agent which is an antibody. In some embodiments, the antibody is a recombinant antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In certain embodiments, the antibody is an IgG1 antibody. In certain embodiments, the antibody is an IgG2 antibody. In certain embodiments, the antibody is an antibody fragment

comprising an antigen-binding site. In some embodiments, the antibody is monovalent, monospecific, or bivalent. In some embodiments, the antibody is a bispecific antibody or a multispecific antibody. In some embodiments, the antibody is conjugated to a cytotoxic moiety. In some embodiments, the antibody is isolated. In some embodiments, the antibody is substantially pure.

[00160] The FZD-binding agents (e.g., antibodies) of the present invention can be assayed for specific binding by any method known in the art. The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as Biacore analysis, FACS analysis, immunofluorescence, immunocytochemistry, Western blot analysis, radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitation reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well-known in the art (see, e.g., Ausubel et al., Editors, *1994-present, Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, NY).

[00161] In certain embodiments, the invention provides a Wnt pathway inhibitor which is a FZD-binding agent (e.g., an antibody) that comprises a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3). In some embodiments, the FZD-binding agent further comprises a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:6). In some embodiments, the FZD-binding agent comprises a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:6). In certain embodiments, the FZD-binding agent comprises: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), and (b) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:6).

[00162] In certain embodiments, the invention provides a FZD-binding agent (e.g., an antibody) that comprises: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and (f) a light chain CDR3 comprising

QSYANTLSL (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain embodiments, the amino acid substitutions are conservative substitutions.

[00163] In certain embodiments, the invention provides a FZD-binding agent (e.g., an antibody) that comprises a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:7, and/or a light chain variable region having at least 80% sequence identity to SEQ ID NO:8. In certain embodiments, the FZD-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:7. In certain embodiments, the FZD-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:8. In certain embodiments, the FZD-binding agent comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:7, and/or a light chain variable region having at least about 95% sequence identity to SEQ ID NO:8. In certain embodiments, the FZD-binding agent comprises a heavy chain variable region comprising SEQ ID NO:7 and/or a light chain variable region comprising SEQ ID NO:8. In certain embodiments, the FZD-binding agent comprises a heavy chain variable region comprising SEQ ID NO:7 and a light chain variable region comprising SEQ ID NO:8. In certain embodiments, the FZD-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:7 and a light chain variable region consisting essentially of SEQ ID NO:8.

[00164] In certain embodiments, the invention provides a FZD-binding agent (e.g., an antibody) that comprises: (a) a heavy chain having at least 90% sequence identity to SEQ ID NO:9 (with or without the signal sequence) or SEQ ID NO:11; and/or (b) a light chain having at least 90% sequence identity to SEQ ID NO:10 (with or without the signal sequence) or SEQ ID NO:12. In some embodiments, the FZD-binding agent comprises: (a) a heavy chain having at least 95% sequence identity to SEQ ID NO:9 (with or without the signal sequence) or SEQ ID NO:11; and/or (b) a light chain having at least 95% sequence identity to SEQ ID NO:10 (with or without the signal sequence) or SEQ ID NO:12. In some embodiments, the FZD-binding agent comprises a heavy chain comprising SEQ ID NO:9 (with or without the signal sequence) or SEQ ID NO:11, and/or a light chain comprising SEQ ID NO:10 (with or without the signal sequence) or SEQ ID NO:12. In some embodiments, the FZD-binding agent comprises a heavy chain comprising SEQ ID NO:11 and a light chain comprising SEQ ID NO:12. In some embodiments, the FZD-binding agent comprises a heavy chain consisting essentially of amino acids 20-463 of SEQ ID NO:9 and a light chain consisting essentially of amino acids 20-232 of SEQ ID NO:10. In some embodiments, the FZD-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:11 and a light chain consisting essentially of SEQ ID NO:12.

[00165] In certain embodiments, the invention provides a Wnt pathway inhibitor which is a FZD-binding agent (e.g., an antibody) that specifically binds at least one of FZD1, FZD2, FZD5, FZD7, and/or FZD8, wherein the FZD-binding agent (e.g., an antibody) comprises one, two, three, four, five, and/or six of the CDRs of antibody OMP-18R5. Antibody OMP-18R5 (also known as 18R5 and

vantictumab), as well as other FZD-binding agents, has been previously described in U.S. Patent No. 7,982,013. DNA encoding the heavy chain and light chain of the OMP-18R5 IgG2 antibody was deposited with the ATCC, under the conditions of the Budapest Treaty on September 29, 2008, and assigned ATCC deposit designation number PTA-9541. In some embodiments, the FZD-binding agent comprises one or more of the CDRs of OMP-18R5, two or more of the CDRs of OMP-18R5, three or more of the CDRs of OMP-18R5, four or more of the CDRs of OMP-18R5, five or more of the CDRs of OMP-18R5, or all six of the CDRs of OMP-18R5.

[00166] The invention provides polypeptides which are Wnt pathway inhibitors. The polypeptides include, but are not limited to, antibodies that specifically bind human FZD proteins. In some embodiments, a polypeptide binds one or more FZD proteins selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In some embodiments, a polypeptide binds FZD1, FZD2, FZD5, FZD7, and/or FZD8. In some embodiments, a polypeptide binds FZD1, FZD2, FZD5, FZD7, and FZD8.

[00167] In certain embodiments, a polypeptide comprises one, two, three, four, five, and/or six of the CDRs of antibody OMP-18R5. In some embodiments, a polypeptide comprises CDRs with up to four (i.e., 0, 1, 2, 3, or 4) amino acid substitutions per CDR. In certain embodiments, the heavy chain CDR(s) are contained within a heavy chain variable region. In certain embodiments, the light chain CDR(s) are contained within a light chain variable region.

[00168] In some embodiments, the invention provides a polypeptide that specifically binds one or more human FZD proteins, wherein the polypeptide comprises an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:7, and/or an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:8. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:7. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:8. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:7, and/or an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:8. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:7, and/or an amino acid sequence comprising SEQ ID NO:8.

[00169] In some embodiments, a FZD-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

[00170] In certain embodiments, a FZD-binding agent comprises the heavy chain variable region and light chain variable region of the OMP-18R5 antibody. In certain embodiments, a FZD-binding agent comprises the heavy chain and light chain of the OMP-18R5 antibody (with or without the leader sequence).

[00171] In certain embodiments, a FZD-binding agent comprises, consists essentially of, or consists of, the antibody OMP-18R5.

[00172] In certain embodiments, a FZD-binding agent (e.g., antibody) competes for specific binding to one or more human FZD proteins with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:7 and a light chain variable region comprising SEQ ID NO:8. In certain embodiments, a FZD-binding agent (e.g., antibody) competes for specific binding to one or more human FZD proteins with an antibody that comprises a heavy chain comprising SEQ ID NO:9 (with or without the signal sequence) and a light chain comprising SEQ ID NO:10 (with or without the signal sequence). In certain embodiments, a FZD-binding agent (e.g., antibody) competes for specific binding to one or more human FZD proteins with an antibody that comprises a heavy chain comprising SEQ ID NO:11 and a light chain comprising SEQ ID NO:12. In certain embodiments, a FZD-binding agent competes with antibody OMP-18R5 for specific binding to one or more human FZD proteins. In some embodiments, a FZD-binding agent or antibody competes for specific binding to one or more human FZD proteins in an *in vitro* competitive binding assay.

[00173] In certain embodiments, a FZD-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on one or more human FZD proteins as an antibody of the invention. In another embodiment, a FZD-binding agent is an antibody that binds an epitope on one or more human FZD proteins that overlaps with the epitope on a FZD protein bound by an antibody of the invention. In certain embodiments, a FZD-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on one or more FZD proteins as antibody OMP-18R5. In another embodiment, the FZD-binding agent is an antibody that binds an epitope on one or more human FZD proteins that overlaps with the epitope on a FZD protein bound by antibody OMP-18R5.

[00174] In certain embodiments, the Wnt pathway inhibitors are agents that bind one or more human Wnt proteins. In certain embodiments, the agents specifically bind one, two, three, four, five, six, seven, eight, nine, ten, or more Wnt proteins. In some embodiments, the Wnt-binding agents bind one or more human Wnt proteins selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11, and Wnt16. In certain embodiments, a Wnt-binding agent binds one or more (or two or more, three or more, four or more, five or more, etc.) Wnt proteins selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In certain embodiments, the one or more (or two or more, three or more, four or more, five or more, etc.) Wnt proteins are selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, and Wnt10b.

[00175] In certain embodiments, the Wnt-binding agent is a Wnt antagonist. In certain embodiments, the Wnt-binding agent is a Wnt pathway antagonist. In certain embodiments, the Wnt-binding agent inhibits Wnt signaling. In some embodiments, the Wnt-binding agent inhibits canonical Wnt signaling.

[00176] In some embodiments, the Wnt-binding agent is an antibody. In some embodiments, the Wnt-binding agent is a polypeptide. In certain embodiments, the Wnt-binding agent is an antibody or a polypeptide comprising an antigen-binding site. In certain embodiments, an antigen-binding site of a Wnt-binding antibody or polypeptide described herein is capable of binding (or binds) one, two, three, four, five, or more human Wnt proteins. In certain embodiments, an antigen-binding site of the Wnt-binding antibody or polypeptide is capable of specifically binding one, two, three, four, or five human Wnt proteins selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. Non-limiting examples of Wnt-binding agents can be found in International Publication WO 2011/088127.

[00177] In certain embodiments, a Wnt-binding agent binds to the C-terminal cysteine rich domain of one or more human Wnt proteins. In certain embodiments, the Wnt-binding agent binds a domain within the one or more Wnt proteins selected from the group consisting of: SEQ ID NO:46 (Wnt1), SEQ ID NO:47 (Wnt2), SEQ ID NO:48 (Wnt2b), SEQ ID NO:49 (Wnt3), SEQ ID NO:50 (Wnt3a), SEQ ID NO:51 (Wnt7a), SEQ ID NO:52 (Wnt7b), SEQ ID NO:53 (Wnt8a), SEQ ID NO:54 (Wnt8b), SEQ ID NO:55 (Wnt10a), and SEQ ID NO:56 (Wnt10b).

[00178] In certain embodiments, the Wnt-binding agent binds one or more (e.g., two or more, three or more, or four or more) Wnt proteins with a K_D **of about 1μM or less, about 100nM or less, about** 40nM or less, about 20nM or less, or about 10nM or less. For example, in certain embodiments, a Wnt-binding agent described herein that binds more than one Wnt protein, binds those Wnt proteins with a K_D of about 100nM or less, about 20nM or less, or about 10nM or less. In certain embodiments, the Wnt-binding agent binds each of one or more (e.g., 1, 2, 3, 4, or 5) Wnt proteins with a K_D of about 40nM or less, wherein the Wnt proteins are selected from the group consisting of: Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In some embodiments, the K_D of the binding agent (e.g., an antibody) to a Wnt protein is the K_D determined using a Wnt fusion protein comprising at least a portion of the Wnt C-terminal cysteine rich domain immobilized on a Biacore chip.

[00179] In certain embodiments, the Wnt-binding agent binds one or more (for example, two or more, three or more, or four or more) human Wnt proteins with an EC_{50} **of about 1μM or less, about 100nM** or less, about 40nM or less, about 20nM or less, about 10nM or less, or about 1nM or less. In certain embodiments, a Wnt-binding agent binds to more than one Wnt with an EC_{50} of about 40nM or less, about 20nM or less, or about 10nM or less. In certain embodiments, the Wnt-binding agent has an EC_{50} of about 20nM or less with respect to one or more (e.g., 1, 2, 3, 4, or 5) of Wnt proteins Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11, and/or Wnt16. In certain embodiments, the Wnt-binding agent has an EC_{50} of about 10nM or less with respect to one or more (e.g., 1, 2, 3, 4, or 5) of the following Wnt proteins Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, and/or Wnt10b.

[00180] In certain embodiments, the Wnt pathway inhibitor is a Wnt-binding agent which is an antibody. In some embodiments, the antibody is a recombinant antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In certain embodiments, the antibody is an IgG1 antibody. In certain embodiments, the antibody is an IgG2 antibody. In certain embodiments, the antibody is an antibody fragment comprising an antigen-binding site. In some embodiments, the antibody is monovalent, monospecific, or bivalent. In some embodiments, the antibody is a bispecific antibody or a multispecific antibody. In some embodiments, the antibody is conjugated to a cytotoxic moiety. In some embodiments, the antibody is isolated. In some embodiments, the antibody is substantially pure.

[00181] The Wnt-binding agents (e.g., antibodies) of the present invention can be assayed for specific binding by any method known in the art as described herein for FZD-binding agents.

[00182] In certain embodiments, the Wnt-binding agent is a soluble receptor. In certain embodiments, the Wnt-binding agent comprises the extracellular domain of a FZD receptor protein. In some embodiments, the Wnt-binding agent comprises a Fri domain of a FZD protein. In some embodiments, a soluble receptor comprising a FZD Fri domain can demonstrate altered biological activity (e.g., increased protein half-life) compared to a soluble receptor comprising the entire FZD ECD. Protein half-life can be further modified (i.e., increased) by covalent modification with polyethylene glycol (PEG) or polyethylene oxide (PEO). In certain embodiments, the FZD protein is a human FZD protein. In certain embodiments, the human FZD protein is FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, or FZD10. Non-limiting examples of soluble FZD receptors can be found in U.S. Patent Nos. 7,723,477 and 7,947,277 and U.S. Patent Publication No. 2013/0034551.

[00183] The predicted Fri domains for each of the human FZD1-10 proteins are provided as SEQ ID NOs:13-22. The predicted minimal Fri domains for each of the human FZD1-10 proteins are provided as SEQ ID NOs:23-32. Those of skill in the art may differ in their understanding of the exact amino acids corresponding to the various Fri domains. Thus, the N-terminus and/or C-terminus of the domains outlined above and herein may extend or be shortened by 1, 2, 3, 4, 5, 6, 7, 8, 9, or even 10 amino acids.

[00184] In certain embodiments, the Wnt-binding agent comprises a Fri domain of a human FZD protein, or a fragment or variant of the Fri domain that binds one or more human Wnt proteins. In certain embodiments, the human FZD protein is FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, or FZD10. In certain embodiments, the human FZD protein is FZD4. In certain embodiments, the human FZD protein is FZD5. In certain embodiments, the human FZD protein is FZD8. In certain embodiments, the human FZD protein is FZD10. In certain embodiments, the FZD protein is FZD4 and the Wnt-binding agent comprises SEQ ID NO:16. In certain embodiments, the FZD protein is FZD5 and the Wnt-binding agent comprises SEQ ID NO:17. In certain embodiments,

the FZD protein is FZD7 and the Wnt-binding agent comprises SEQ ID NO:19. In certain embodiments, the FZD protein is FZD8 and the Wnt-binding agent comprises SEQ ID NO:20. In certain embodiments, the FZD protein is FZD10 and the Wnt-binding agent comprises SEQ ID NO:22. In certain embodiments, the FZD protein is FZD8 and the Wnt-binding agent comprises SEQ ID NO:33.

[00185] In some embodiments, the Wnt-binding agent comprises a Fri domain comprising the minimal Fri domain of FZD1 (SEQ ID NO:23), the minimal Fri domain of FZD2 (SEQ ID NO:24), the minimal Fri domain of FZD3 (SEQ ID NO:25), the minimal Fri domain of FZD4 (SEQ ID NO:26), the minimal Fri domain of FZD5 (SEQ ID NO:27), the minimal Fri domain of FZD6 (SEQ ID NO:28), the minimal Fri domain of FZD7 (SEQ ID NO:29), the minimal Fri domain of FZD8 (SEQ ID NO:30), the minimal Fri domain of FZD9 (SEQ ID NO:31), or the minimal Fri domain of FZD10 (SEQ ID NO:32). In some embodiments, the Wnt-binding agent comprises a Fri domain comprising the minimal Fri domain of FZD8 (SEQ ID NO:30).

[00186] In some embodiments, the Wnt-binding agent comprises a Fri domain consisting essentially of the Fri domain of FZD1, the Fri domain of FZD2, the Fri domain of FZD3, the Fri domain of FZD4, the Fri domain of FZD5, the Fri domain of FZD6, the Fri domain of FZD7, the Fri domain of FZD8, the Fri domain of FZD9, or the Fri domain of FZD10. In some embodiments, the Wnt-binding agent comprises a Fri domain consisting essentially of the Fri domain of FZD8.

[00187] In some embodiments, the Wnt-binding agent comprises a sequence selected from the group consisting of: SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33. In some embodiments, the Wnt-binding agent comprises a Fri domain consisting essentially of SEQ ID NO:20. In some embodiments, the Wnt-binding agent comprises a Fri domain consisting essentially of SEQ ID NO:33.

[00188] In certain embodiments, the Wnt-binding agent comprises a variant of any one of the aforementioned FZD Fri domain sequences that comprises one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc.) conservative substitutions and is capable of binding Wnt protein(s).

[00189] In certain embodiments, a Wnt-binding agent, such as an agent comprising a Fri domain of a human FZD receptor, further comprises a non-FZD polypeptide. In some embodiments, a FZD soluble receptor may include FZD ECD or Fri domains linked to other non-FZD functional and structural polypeptides including, but not limited to, a human Fc region, protein tags (e.g., myc, FLAG, GST), other endogenous proteins or protein fragments, or any other useful protein sequence including any linker region between a FZD ECD or Fri domain and a second polypeptide. In certain embodiments, the non-FZD polypeptide comprises a human Fc region. The Fc region can be obtained

from any of the classes of immunoglobulin, IgG, IgA, IgM, IgD and IgE. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, the Fc region is a human IgG2 Fc region. In some embodiments, the Fc region is a wild-type Fc region. In some embodiments, the Fc region is a mutated Fc region. In some embodiments, the Fc region is truncated at the N-terminal end by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, (e.g., in the hinge domain). In some embodiments, an amino acid in the hinge domain is changed to hinder undesirable disulfide bond formation. In some embodiments, a cysteine is replaced with a serine to hinder or block undesirable disulfide bond formation. In some embodiments, the Fc region is truncated at the C-terminal end by 1, 2, 3, or more amino acids. In some embodiments, the Fc region is truncated at the C-terminal end by 1 amino acid. In certain embodiments, the non-FZD polypeptide comprises SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In certain embodiments, the non-FZD polypeptide consists essentially of SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In certain embodiments, the non-FZD polypeptide consists essentially of SEQ ID NO:36 or SEQ ID NO:37.

[00190] In certain embodiments, a Wnt-binding agent is a fusion protein comprising at least a minimal Fri domain of a FZD receptor and a Fc region. As used herein, a "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. In some embodiments, the C-terminus of the first polypeptide is linked to the N-terminus of the immunoglobulin Fc region. In some embodiments, the first polypeptide (e.g., a FZD Fri domain) is directly linked to the Fc region (i.e. without an intervening linker). In some embodiments, the first polypeptide is linked to the Fc region via a linker.

[00191] As used herein, the term "linker" refers to a linker inserted between a first polypeptide (e.g., a FZD component) and a second polypeptide (e.g., a Fc region). In some embodiments, the linker is a peptide linker. Linkers should not adversely affect the expression, secretion, or bioactivity of the polypeptide. Linkers should not be antigenic and should not elicit an immune response. Suitable linkers are known to those of skill in the art and often include mixtures of glycine and serine residues and often include amino acids that are sterically unhindered. Other amino acids that can be incorporated into useful linkers include threonine and alanine residues. Linkers can range in length, for example from 1-50 amino acids in length, 1-22 amino acids in length, 1-10 amino acids in length, 1-5 amino acids in length, or 1-3 amino acids in length. Linkers may include, but are not limited to, SerGly, GGSG, GSGS, GGGS, S(GGS)n where n is 1-7, GRA, poly(Gly), poly(Ala), ESGGGGVT (SEQ ID NO:57), LESGGGGVT (SEQ ID NO:58), GRAQVT (SEQ ID NO:59), WRAQVT (SEQ ID NO:60), and ARGRAQVT (SEQ ID NO:61). As used herein, a "linker" is an intervening peptide sequence that does not include amino acid residues from either the C-terminus of the first polypeptide (e.g., a FZD Fri domain) or the N-terminus of the second polypeptide (e.g., the Fc region).

[00192] In some embodiments, the Wnt-binding agent comprises a FZD Fri domain, a Fc region, and a linker connecting the FZD Fri domain to the Fc region. In some embodiments, the FZD Fri domain

comprises SEQ ID NO:20, SEQ ID NO:30, or SEQ ID NO:33. In some embodiments, the linker comprises ESGGGGVT (SEQ ID NO:57) or LESGGGGVT (SEQ ID NO:58).

[00193] In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, or SEQ ID NO:33; and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38, wherein the first polypeptide is directly linked to the second polypeptide. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:20 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:20 and a second polypeptide comprising SEQ ID NO:36 or SEQ ID NO:37. In some embodiments, the Wnt-binding agent comprises a first polypeptide consisting essentially of SEQ ID NO:20 and a second polypeptide consisting essentially of SEQ ID NO:36 or SEQ ID NO:37. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:30 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:30 and a second polypeptide comprising SEQ ID NO:36 or SEQ ID NO:37. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:33 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:33 and a second polypeptide comprising SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:35. In some embodiments, the Wnt-binding agent comprises a first polypeptide consisting essentially of SEQ ID NO:33 and a second polypeptide consisting essentially of SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:35.

[00194] In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, or SEQ ID NO:33; and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38, wherein the first polypeptide is connected to the second polypeptide by a linker. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:20 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:20 and a second polypeptide comprising SEQ ID NO:36 or SEQ ID NO:37. In some embodiments, the Wnt-

binding agent comprises a first polypeptide consisting essentially of SEQ ID NO:20 and a second polypeptide consisting essentially of SEQ ID NO:36 or SEQ ID NO:37. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:30 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:33 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide comprising SEQ ID NO:33 and a second polypeptide comprising SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:35. In some embodiments, the Wnt-binding agent comprises a first polypeptide consisting essentially of SEQ ID NO:33 and a second polypeptide consisting essentially of SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:35.

[00195] In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, or SEQ ID NO:33; and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38, wherein the first polypeptide is directly linked to the second polypeptide. In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:20 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:30 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:33 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38.

[00196] In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, or SEQ ID NO:33; and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38, wherein the first polypeptide is connected to the second polypeptide by a linker. In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:20 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:30 and a second polypeptide

comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38. In some embodiments, the Wnt-binding agent comprises a first polypeptide that is at least 95% identical to SEQ ID NO:33 and a second polypeptide comprising SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:38.

[00197] FZD proteins contain a signal sequence that directs the transport of the proteins. Signal sequences (also referred to as signal peptides or leader sequences) are located at the N-terminus of nascent polypeptides. They target the polypeptide to the endoplasmic reticulum and the proteins are sorted to their destinations, for example, to the inner space of an organelle, to an interior membrane, to the cell outer membrane, or to the cell exterior via secretion. Most signal sequences are cleaved from the protein by a signal peptidase after the proteins are transported to the endoplasmic reticulum. The cleavage of the signal sequence from the polypeptide usually occurs at a specific site in the amino acid sequence and is dependent upon amino acid residues within the signal sequence. Although there is usually one specific cleavage site, more than one cleavage site may be recognized and/or used by a signal peptidase resulting in a non-homogenous N-terminus of the polypeptide. For example, the use of different cleavage sites within a signal sequence can result in a polypeptide expressed with different N-terminal amino acids. Accordingly, in some embodiments, the polypeptides described herein may comprise a mixture of polypeptides with different N-termini. In some embodiments, the N-termini differ in length by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids. In some embodiments, the N-termini differ in length by 1, 2, 3, 4, or 5 amino acids. In some embodiments, the polypeptide is substantially homogeneous, i.e., the polypeptides have the same N-terminus. In some embodiments, the signal sequence of the polypeptide comprises one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc.) amino acid substitutions and/or deletions. In some embodiments, the signal sequence of the polypeptide comprises amino acid substitutions and/or deletions that allow one cleavage site to be dominant, thereby resulting in a substantially homogeneous polypeptide with one N-terminus.

[00198] In some embodiments, the Wnt-binding agent comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45.

[00199] In certain embodiments, the Wnt-binding agent comprises the sequence of SEQ ID NO:39. In certain embodiments, the agent comprises the sequence of SEQ ID NO:39, comprising one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc.) conservative substitutions. In certain embodiments, the agent comprises a sequence having at least about 90%, about 95%, or about 98% sequence identity with SEQ ID NO:39. In certain embodiments, the variants of SEQ ID NO:39 maintain the ability to bind one or more human Wnt proteins.

[00200] In certain embodiments, the Wnt-binding agent comprises the sequence of SEQ ID NO:40. In some embodiments, the Wnt-binding agent is SEQ ID NO:40. In certain alternative embodiments, the agent comprises the sequence of SEQ ID NO:40, comprising one or more (e.g., one, two, three, four,

five, six, seven, eight, nine, ten, etc.) conservative substitutions. In certain embodiments, the agent comprises a sequence having at least about 90%, about 95%, or about 98% sequence identity with SEQ ID NO:40. In certain embodiments, the variants of SEQ ID NO:40 maintain the ability to bind one or more human Wnt proteins.

[00201] In certain embodiments, the Wnt-binding agent comprises the sequence of SEQ ID NO:41. In some embodiments, the Wnt-binding agent is SEQ ID NO:41. In certain alternative embodiments, the agent comprises the sequence of SEQ ID NO:41, comprising one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc.) conservative substitutions. In certain embodiments, the agent comprises a sequence having at least about 90%, about 95%, or about 98% sequence identity with SEQ ID NO:41. In certain embodiments, the variants of SEQ ID NO:41 maintain the ability to bind one or more human Wnt proteins.

[00202] In some embodiments, the Wnt-binding agent is OMP-54F28.

[00203] In certain embodiments, a Wnt-binding agent is a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45. In certain embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:40, and SEQ ID NO:41. In some embodiments, a polypeptide consists essentially of an amino acid sequence selected from the group consisting of: SEQ ID NO:39, SEQ ID NO:40, and SEQ ID NO:41. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:39. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:40. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:41. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:42. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:43. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:44. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:45.

[00204] In some embodiments, the polypeptide is a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:40, and SEQ ID NO:41. In some embodiments, the polypeptide is a substantially purified polypeptide comprising SEQ ID NO:41. In certain embodiments, the substantially purified polypeptide consists of at least 90% of a polypeptide that has an N-terminal sequence of ASA. In some embodiments, the nascent polypeptide comprises a signal sequence that results in a substantially homogeneous polypeptide product with one N-terminal sequence.

[00205] In certain embodiments, a Wnt-binding agent comprises a Fc region of an immunoglobulin. Those skilled in the art will appreciate that some of the binding agents of this invention will comprise fusion proteins in which at least a portion of the Fc region has been deleted or otherwise altered so as to provide desired biochemical characteristics, such as increased cancer cell localization, increased

tumor penetration, reduced serum half-life, or increased serum half-life, when compared with a fusion protein of approximately the same immunogenicity comprising a native or unaltered constant region. Modifications to the Fc region may include additions, deletions, or substitutions of one or more amino acids in one or more domains. The modified fusion proteins disclosed herein may comprise alterations or modifications to one or more of the two heavy chain constant domains (CH2 or CH3) or to the hinge region. In other embodiments, the entire CH2 domain may be removed (ΔCH2 constructs). In some embodiments, the omitted constant region domain is replaced by a short amino acid spacer (e.g., 10 aa residues) that provides some of the molecular flexibility typically imparted by the absent constant region domain.

[00206] In some embodiments, the modified fusion proteins are engineered to link the CH3 domain directly to the hinge region. In other embodiments, a peptide spacer is inserted between the hinge region and the modified CH2 and/or CH3 domains. For example, constructs may be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (modified or unmodified) is joined to the hinge region with a 5-20 amino acid spacer. Such a spacer may be added to ensure that the regulatory elements of the constant domain remain free and accessible or that the hinge region remains flexible. However, it should be noted that amino acid spacers may, in some cases, prove to be immunogenic and elicit an unwanted immune response against the construct. Accordingly, in certain embodiments, any spacer added to the construct will be relatively non-immunogenic so as to maintain the desired biological qualities of the fusion protein.

[00207] In some embodiments, the modified fusion proteins may have only a partial deletion of a constant domain or substitution of a few or even a single amino acid. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase cancer cell localization and/or tumor penetration. Similarly, it may be desirable to simply delete that part of one or more constant region domains that control a specific effector function (e.g., complement C1q binding). Such partial deletions of the constant regions may improve selected characteristics of the binding agent (e.g., serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed fusion proteins may be modified through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (e.g., Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified fusion protein. In certain embodiments, the modified fusion proteins comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as decreasing or increasing effector function, or provide for more cytotoxin or carbohydrate attachment sites.

[00208] It is known in the art that the constant region mediates several effector functions. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the

opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. In addition, the Fc region of an immunoglobulin can bind to a cell expressing a Fc receptor (FcR). There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells, release of inflammatory mediators, placental transfer, and control of immunoglobulin production.

[00209] In some embodiments, the modified fusion proteins provide for altered effector functions that, in turn, affect the biological profile of the administered agent. For example, in some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified agent, thereby increasing cancer cell localization and/or tumor penetration. In other embodiments, the constant region modifications increase or reduce the serum half-life of the agent. In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties.

[00210] In certain embodiments, a modified fusion protein does not have one or more effector functions normally associated with an Fc region. In some embodiments, the agent has no antibody-dependent cell-mediated cytotoxicity (ADCC) activity, and/or no complement-dependent cytotoxicity (CDC) activity. In certain embodiments, the agent does not bind to the Fc receptor and/or complement factors. In certain embodiments, the agent has no effector function.

[00211] In some embodiments, the Wnt-binding agent (e.g., a soluble receptor) described herein is modified to reduce immunogenicity. In general, immune responses against completely normal human proteins are rare when these proteins are used as therapeutics. However, although many fusion proteins comprise polypeptides sequences that are the same as the sequences found in nature, several therapeutic fusion proteins have been shown to be immunogenic in mammals. In some studies, a fusion protein comprising a linker has been found to be more immunogenic than a fusion protein that does not contain a linker. Accordingly, in some embodiments, the polypeptides of the invention are analyzed by computation methods to predict immunogenicity. In some embodiments, the polypeptides are analyzed for the presence of T-cell and/or B-cell epitopes. If any T-cell or B-cell epitopes are identified and/or predicted, modifications to these regions (e.g., amino acid substitutions) may be made to disrupt or destroy the epitopes. Various algorithms and software that can be used to predict T-cell and/or B-cell epitopes are known in the art. For example, the software programs SYFPEITHI, HLA Bind, PEPVAC, RANKPEP, DiscoTope, ElliPro, and Antibody Epitope Prediction are all publicly available.

[00212] In some embodiments, a cell producing any of the Wnt-binding agents (e.g., soluble receptors) or polypeptides described herein is provided. In some embodiments, a composition

comprising any of the Wnt-binding agents (e.g., soluble receptors) or polypeptides described herein is provided. In some embodiments, the composition comprises a polypeptide wherein at least 80%, 90%, 95%, 97%, 98%, or 99% of the polypeptide has an N-terminal sequence of ASA. In some embodiments, the composition comprises a polypeptide wherein 100% of the polypeptide has an N-terminal sequence of ASA. In some embodiments, the composition comprises a polypeptide wherein at least 80% of the polypeptide has an N-terminal sequence of ASA. In some embodiments, the composition comprises a polypeptide wherein at least 90% of the polypeptide has an N-terminal sequence of ASA. In some embodiments, the composition comprises a polypeptide wherein at least 95% of the polypeptide has an N-terminal sequence of ASA.

[00213] The polypeptides described herein can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides. It will be recognized in the art that some amino acid sequences of the invention can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the polypeptides which show substantial activity or which include regions of FZD proteins, such as the protein portions discussed herein. Such mutants include deletions, insertions, inversions, repeats, and type substitutions.

[00214] Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. In certain embodiments, the number of substitutions for any given soluble receptor polypeptide will not be more than 50, 40, 30, 25, 20, 15, 10, 5 or 3.

[00215] Fragments or portions of the polypeptides of the present invention can be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments can be employed as intermediates for producing the full-length polypeptides. These fragments or portion of the polypeptides can also be referred to as “protein fragments” or “polypeptide fragments”.

[00216] A “protein fragment” of this invention is a portion or all of a protein which is capable of binding to one or more human Wnt proteins or one or more human FZD proteins. In some embodiments, the fragment has a high affinity for one or more human Wnt proteins. In some embodiments, the fragment has a high affinity for one or more human FZD proteins. Some fragments of Wnt-binding agents described herein are protein fragments comprising at least part of the extracellular portion of a FZD protein linked to at least part of a constant region of an immunoglobulin (e.g., a Fc region). The binding affinity of the protein fragment can be in the range of about 10^{-11} to 10^{-12}M, although the affinity can vary considerably with fragments of different sizes, ranging from 10^{-7} to 10^{-13}M. In some embodiments, the fragment is about 100 to about 200 amino acids in length and comprises a binding domain linked to at least part of a constant region of an immunoglobulin.

[00217] In some embodiments, the Wnt pathway inhibitors are polyclonal antibodies. Polyclonal antibodies can be prepared by any known method. In some embodiments, polyclonal antibodies are

raised by immunizing an animal (e.g., a rabbit, rat, mouse, goat, donkey) by multiple subcutaneous or intraperitoneal injections of an antigen of interest (e.g., a purified peptide fragment, full-length recombinant protein, or fusion protein). The antigen can be optionally conjugated to a carrier such as keyhole limpet hemocyanin (KLH) or serum albumin. The antigen (with or without a carrier protein) is diluted in sterile saline and usually combined with an adjuvant (e.g., Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. After a sufficient period of time, polyclonal antibodies are recovered from blood and/or ascites of the immunized animal. The polyclonal antibodies can be purified from serum or ascites according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[00218] In some embodiments, the Wnt pathway inhibitors are monoclonal antibodies. Monoclonal antibodies can be prepared using hybridoma methods known to one of skill in the art (see e.g., Kohler and Milstein, 1975, *Nature*, 256:495-497). In some embodiments, using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit from lymphocytes the production of antibodies that will specifically bind the immunizing antigen. In some embodiments, lymphocytes can be immunized *in vitro*. In some embodiments, the immunizing antigen can be a human protein or a portion thereof. In some embodiments, the immunizing antigen can be a mouse protein or a portion thereof.

[00219] Following immunization, lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol, to form hybridoma cells that can then be selected away from unfused lymphocytes and myeloma cells. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen may be identified by a variety of methods including, but not limited to, immunoprecipitation, immunoblotting, and *in vitro* binding assay (e.g., flow cytometry, FACS, ELISA, and radioimmunoassay). The hybridomas can be propagated either in *in vitro* culture using standard methods (J.W. Goding, 1996, *Monoclonal Antibodies: Principles and Practice, 3rd Edition*, Academic Press, San Diego, CA) or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can be purified from the culture medium or ascites fluid according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[00220] In certain embodiments, monoclonal antibodies can be made using recombinant DNA techniques as known to one skilled in the art. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cells, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using conventional techniques. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors which produce the monoclonal antibodies when transfected into host cells such as E. coli, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin proteins.

In other embodiments, recombinant monoclonal antibodies, or fragments thereof, can be isolated from phage display libraries.

[00221] The polynucleotide(s) encoding a monoclonal antibody can further be modified in a number of different manners using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted for those regions of, for example, a human antibody to generate a chimeric antibody, or for a non-immunoglobulin polypeptide to generate a fusion antibody. In some embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

[00222] In some embodiments, the Wnt pathway inhibitor is a humanized antibody. Typically, humanized antibodies are human immunoglobulins in which amino acid residues of the CDRs are replaced by amino acid residues of a CDR from an immunoglobulin of a non-human species (e.g., mouse, rat, rabbit, hamster, etc.) that have the desired specificity, affinity, and/or binding capability using methods known to one skilled in the art. In some embodiments, Fv framework region amino acid residues of a human immunoglobulin are replaced with corresponding amino acid residues from an antibody of a non-human species that has the desired specificity, affinity, and/or binding capability. In some embodiments, the humanized antibody can be further modified by the substitution of additional amino acid residues either in the Fv framework region and/or within the replaced non-human amino acid residues to refine and optimize antibody specificity, affinity, and/or capability. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domain regions containing all, or substantially all, of the CDRs that correspond to the non-human immunoglobulin whereas all, or substantially all, of the framework regions are those of a human immunoglobulin sequence. In some embodiments, the humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. In certain embodiments, such humanized antibodies are used therapeutically because they may reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. Methods used to generate humanized antibodies are well known in the art.

[00223] In certain embodiments, the Wnt pathway inhibitor is a human antibody. Human antibodies can be directly prepared using various techniques known in the art. In some embodiments, immortalized human B lymphocytes immunized *in vitro* or isolated from an immunized individual that produces an antibody directed against a target antigen can be generated. In some embodiments, the human antibody can be selected from a phage library, where that phage library expresses human antibodies. Alternatively, phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable domain gene repertoires from unimmunized donors. Techniques for the generation and use of antibody phage libraries are well-

known in the art. Affinity maturation strategies including, but not limited to, chain shuffling (Marks et al., 1992, *Bio/Technology*, 10:779-783) and site-directed mutagenesis, are known in the art and may be employed to generate high affinity human antibodies.

[00224] In some embodiments, human antibodies can be made in transgenic mice that contain human immunoglobulin loci. These mice are capable, upon immunization, of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016.

[00225] This invention also encompasses bispecific antibodies that specifically recognize at least one human FZD protein or at least one Wnt protein. Bispecific antibodies are capable of specifically recognizing and binding at least two different epitopes. The different epitopes can either be within the same molecule (e.g., two different epitopes on human FZD5) or on different molecules (e.g., one epitope on FZD5 and a different epitope on a second protein). In some embodiments, the bispecific antibodies are monoclonal human or humanized antibodies. In some embodiments, the antibodies can specifically recognize and bind a first antigen target, (e.g., a FZD protein) as well as a second antigen target, such as an effector molecule on a leukocyte (e.g., CD2, CD3, CD28, CD80, or CD86) or a Fc receptor (e.g., CD64, CD32, or CD16) so as to focus cellular defense mechanisms to the cell expressing the first antigen target. In some embodiments, the antibodies can be used to direct cytotoxic agents to cells which express a particular target antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA.

[00226] Bispecific antibodies can be intact antibodies or antibody fragments. Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared (Tutt et al., 1991, *J. Immunol.,* 147:60). Thus, in certain embodiments the antibodies are multispecific. Techniques for making bispecific and multispecific antibodies are known by those skilled in the art.

[00227] In certain embodiments, the antibodies (or other polypeptides) described herein may be monospecific. For example, in certain embodiments, each of the one or more antigen-binding sites that an antibody contains is capable of binding (or binds) a homologous epitope on different proteins. In certain embodiments, an antigen-binding site of a monospecific antibody described herein is capable of binding (or binds), for example, FZD5 and FZD7 (i.e., the same epitope is found on both FZD5 and FZD7 proteins).

[00228] In certain embodiments, the Wnt pathway inhibitor is an antibody fragment comprising an antigen-binding site. Antibody fragments may have different functions or capabilities than intact antibodies; for example, antibody fragments can have increased tumor penetration. Various techniques are known for the production of antibody fragments including, but not limited to, proteolytic digestion of intact antibodies. In some embodiments, antibody fragments include a F(ab')2 fragment produced by pepsin digestion of an antibody molecule. In some embodiments, antibody fragments include a Fab fragment generated by reducing the disulfide bridges of an F(ab')2 fragment.

In other embodiments, antibody fragments include a Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent. In certain embodiments, antibody fragments are produced recombinantly. In some embodiments, antibody fragments include Fv or single chain Fv (scFv) fragments. Fab, Fv, and scFv antibody fragments can be expressed in and secreted from E. coli or other host cells, allowing for the production of large amounts of these fragments. In some embodiments, antibody fragments are isolated from antibody phage libraries as discussed herein. For example, methods can be used for the construction of Fab expression libraries to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a FZD or Wnt protein or derivatives, fragments, analogs or homologs thereof. In some embodiments, antibody fragments are linear antibody fragments. In certain embodiments, antibody fragments are monospecific or bispecific. In certain embodiments, the Wnt pathway inhibitor is a scFv. Various techniques can be used for the production of single-chain antibodies specific to one or more human FZD proteins or one or more human Wnt proteins.

[00229] It can further be desirable, especially in the case of antibody fragments, to modify an antibody in order to increase its serum half-life. This can be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle (e.g., by DNA or peptide synthesis). In some embodiments, an antibody is modified to decrease its serum half-life.

[00230] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells. It is also contemplated that the heteroconjugate antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[00231] For the purposes of the present invention, it should be appreciated that modified antibodies can comprise any type of variable region that provides for the association of the antibody with the target (i.e., a human FZD protein or a human Wnt protein). In this regard, the variable region may comprise or be derived from any type of mammal that can be induced to mount a humoral response and generate immunoglobulins against the desired tumor-associated antigen. As such, the variable region of the modified antibodies can be, for example, of human, murine, non-human primate (e.g. cynomolgus monkeys, macaques, etc.) or rabbit origin. In some embodiments, both the variable and constant regions of the modified immunoglobulins are human. In other embodiments, the variable regions of compatible antibodies (usually derived from a non-human source) can be engineered or specifically tailored to improve the binding properties or reduce the immunogenicity of the molecule.

In this respect, variable regions useful in the present invention can be humanized or otherwise altered through the inclusion of imported amino acid sequences.

[00232] In certain embodiments, the variable domains in both the heavy and light chains are altered by at least partial replacement of one or more CDRs and, if necessary, by partial framework region replacement and sequence modification and/or alteration. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived preferably from an antibody from a different species. It may not be necessary to replace all of the CDRs with all of the CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the antigen-binding site.

[00233] Alterations to the variable region notwithstanding, those skilled in the art will appreciate that the modified antibodies of this invention will comprise antibodies (e.g., full-length antibodies or immunoreactive fragments thereof) in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as increased tumor localization and/or increased serum half-life when compared with an antibody of approximately the same immunogenicity comprising a native or unaltered constant region. In some embodiments, the constant region of the modified antibodies will comprise a human constant region. Modifications to the constant region compatible with this invention comprise additions, deletions or substitutions of one or more amino acids in one or more domains. The modified antibodies disclosed herein may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant domain (CL). In some embodiments, one or more domains are partially or entirely deleted from the constant regions of the modified antibodies. In some embodiments, the modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed (ΔCH2 constructs). In some embodiments, the omitted constant region domain is replaced by a short amino acid spacer (e.g., 10 amino acid residues) that provides some of the molecular flexibility typically imparted by the absent constant region.

[00234] In some embodiments, the modified antibodies are engineered to fuse the CH3 domain directly to the hinge region of the antibody. In other embodiments, a peptide spacer is inserted between the hinge region and the modified CH2 and/or CH3 domains. For example, constructs may be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (modified or unmodified) is joined to the hinge region with a 5-20 amino acid spacer. Such a spacer may be added to ensure that the regulatory elements of the constant domain remain free and accessible or that the hinge region remains flexible. However, it should be noted that amino acid spacers may, in some cases, prove to be immunogenic and elicit an unwanted immune response against the construct.

Accordingly, in certain embodiments, any spacer added to the construct will be relatively non-immunogenic so as to maintain the desired biological qualities of the modified antibodies.

[00235] In some embodiments, the modified antibodies may have only a partial deletion of a constant domain or substitution of a few or even a single amino acid. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase cancer cell localization and/or tumor penetration. Similarly, it may be desirable to simply delete the part of one or more constant region domains that control a specific effector function (e.g. complement C1q binding). Such partial deletions of the constant regions may improve selected characteristics of the antibody (serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies may be modified through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (e.g., Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. In certain embodiments, the modified antibodies comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as decreasing or increasing effector function or provide for more cytotoxin or carbohydrate attachment sites.

[00236] It is known in the art that the constant region mediates several effector functions. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. In addition, the Fc region of an antibody can bind a cell expressing a Fc receptor (FcR). There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells, release of inflammatory mediators, placental transfer, and control of immunoglobulin production.

[00237] In certain embodiments, the Wnt pathway inhibitors are antibodies that provide for altered effector functions. These altered effector functions may affect the biological profile of the administered antibody. For example, in some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody (e.g., anti-FZD antibody) thereby increasing cancer cell localization and/or tumor penetration. In other embodiments, the constant region modifications increase or reduce the serum half-life of the antibody. In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. Modifications to the constant region in

accordance with this invention may easily be made using well known biochemical or molecular engineering techniques well within the purview of the skilled artisan.

[00238] In certain embodiments, a Wnt pathway inhibitor is an antibody does not have one or more effector functions. For instance, in some embodiments, the antibody has no ADCC activity, and/or no CDC activity. In certain embodiments, the antibody does not bind an Fc receptor, and/or complement factors. In certain embodiments, the antibody has no effector function.

[00239] The present invention further embraces variants and equivalents which are substantially homologous to the chimeric, humanized, and human antibodies, or antibody fragments thereof, set forth herein. These can contain, for example, conservative substitution mutations, i.e. the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art and described herein.

[00240] In certain embodiments, the antibodies described herein are isolated. In certain embodiments, the antibodies described herein are substantially pure.

[00241] In some embodiments of the present invention, the Wnt pathway inhibitors are polypeptides. The polypeptides can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides comprising an antibody, or fragment thereof, that bind at least one human FZD protein or at least one Wnt protein. It will be recognized in the art that some amino acid sequences of the invention can be varied without significant effect on the structure or function of the protein. Thus, the invention further includes variations of the polypeptides which show substantial activity or which include regions of an antibody, or fragment thereof, against a human FZD protein or a Wnt protein. In some embodiments, amino acid sequence variations of FZD-binding polypeptides or Wnt-binding polypeptides include deletions, insertions, inversions, repeats, and/or other types of substitutions.

[00242] The polypeptides, analogs and variants thereof, can be further modified to contain additional chemical moieties not normally part of the polypeptide. The derivatized moieties can improve the solubility, the biological half-life, and/or absorption of the polypeptide. The moieties can also reduce or eliminate any undesirable side effects of the polypeptides and variants. An overview for chemical moieties can be found in *Remington: The Science and Practice of Pharmacy, 22st Edition*, 2012, Pharmaceutical Press, London.

[00243] The isolated polypeptides that can be used in the methods described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof.

[00244] In some embodiments, a DNA sequence encoding a polypeptide of interest may be constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[00245] Once assembled (by synthesis, site-directed mutagenesis, or another method), the polynucleotide sequences encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00246] In certain embodiments, recombinant expression vectors are used to amplify and express DNA encoding binding agents (e.g., antibodies or soluble receptors), or fragments thereof, against a human FZD protein or a Wnt protein. For example, recombinant expression vectors can be replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of a FZD-binding agent, a Wnt-binding agent, an anti-FZD antibody or fragment thereof, an anti-Wnt antibody or fragment thereof, or a FZD-Fc soluble receptor operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are "operatively linked" when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments, structural elements intended for use

in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In other embodiments, where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[00247] The choice of an expression control sequence and an expression vector depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from E. coli, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages.

[00248] Suitable host cells for expression of a FZD-binding or Wnt-binding agent (or a protein to use as an antigen) include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example E. coli or Bacillus. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are well-known in the art. Additional information regarding methods of protein production, including antibody production, can be found, e.g., in U.S. Patent Publication No. 2008/0187954, U.S. Patent Nos. 6,413,746 and 6,660,501, and International Patent Publication No. WO 2004/009823.

[00249] Various mammalian culture systems are used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be preferred because such proteins are generally correctly folded, appropriately modified, and biologically functional. Examples of suitable mammalian host cell lines include COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[00250] Expression of recombinant proteins in insect cell culture systems (e.g., baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art (see, e.g., Luckow and Summers, 1988, *Bio/Technology*, 6:47).

[00251] Thus, the present invention provides cells comprising the FZD-binding agents or the Wnt-binding agents described herein. In some embodiments, the cells produce the binding agents (e.g.,

antibodies or soluble receptors) described herein. In certain embodiments, the cells produce an antibody. In certain embodiments, the cells produce antibody OMP-18R5. In some embodiments, the cells produce a soluble receptor. In some embodiments, the cells produce a FZD-Fc soluble receptor. In some embodiments, the cells produce a FZD8-Fc soluble receptor. In some embodiments, the cells produce FZD8-Fc soluble receptor 54F28.

[00252] The proteins produced by a transformed host can be purified according to any suitable method. Standard methods include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine, maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, mass spectrometry (MS), nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), and x-ray crystallography.

[00253] In some embodiments, supernatants from expression systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some embodiments, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some embodiments, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some embodiments, a hydroxyapatite media can be employed, including but not limited to, ceramic hydroxyapatite (CHT). In certain embodiments, one or more reverse-phase HPLC steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a binding agent. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

[00254] In some embodiments, recombinant protein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange, or size exclusion chromatography steps. HPLC can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

[00255] Methods known in the art for purifying antibodies and other proteins also include, for example, those described in U.S. Patent Publication Nos. 2008/0312425, 2008/0177048, and 2009/0187005.

[00256] In certain embodiments, the Wnt-binding agent or the FZD-binding agent is a polypeptide that is not an antibody. A variety of methods for identifying and producing non-antibody polypeptides that bind with high affinity to a protein target are known in the art. See, e.g., Skerra, 2007, *Curr. Opin. Biotechnol.*, 18:295-304; Hosse et al., 2006, *Protein Science*, 15:14-27; Gill et al., 2006, *Curr. Opin. Biotechnol.*, 17:653-658; Nygren, 2008, *FEBS J.*, 275:2668-76; and Skerra, 2008, *FEBS J.*, 275:2677-83. In certain embodiments, phage display technology may be used to produce and/or identify a FZD-binding or Wnt-binding polypeptide. In certain embodiments, the polypeptide comprises a protein scaffold of a type selected from the group consisting of protein A, protein G, a lipocalin, a fibronectin domain, an ankyrin consensus repeat domain, and thioredoxin.

[00257] In certain embodiments, the binding agents can be used in any one of a number of conjugated (i.e. an immunoconjugate or radioconjugate) or non-conjugated forms. In certain embodiments, antibodies can be used in a non-conjugated form to harness the subject's natural defense mechanisms including complement-dependent cytotoxicity and antibody dependent cellular toxicity to eliminate the malignant or cancer cells.

[00258] In some embodiments, the binding agent is conjugated to a cytotoxic agent. In some embodiments, the cytotoxic agent is a chemotherapeutic agent including, but not limited to, methotrexate, adriamicin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents. In some embodiments, the cytotoxic agent is an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, Sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. In some embodiments, the cytotoxic agent is a radioisotope to produce a radioconjugate or a radioconjugated antibody. A variety of radionuclides are available for the production of radioconjugated antibodies including, but not limited to, ^{90}Y, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{131}In, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re and ^{212}Bi. In some embodiments, conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, can be produced. In certain embodiments, conjugates of an antibody and a cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyidithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

[00259] In certain embodiments, the Wnt pathway inhibitor (e.g., antibody or soluble receptor) is an antagonist of at least one Wnt protein (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Wnt proteins). In certain embodiments, the Wnt pathway inhibitor inhibits activity of the Wnt protein(s) to which it binds. In certain embodiments, the Wnt pathway inhibitor inhibits at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100% of the activity of the human Wnt protein(s) to which it binds.

[00260] In certain embodiments, the Wnt pathway inhibitor (e.g., antibody or soluble receptor) inhibits binding of at least one human Wnt to an appropriate receptor. In certain embodiments, the Wnt pathway inhibitor inhibits binding of at least one human Wnt protein to one or more human FZD proteins. In some embodiments, the at least one Wnt protein is selected from the group consisting of: Wnt1, Wnt2, Wnt2b/13, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11, and Wnt16. In some embodiments, the one or more human FZD proteins are selected from the group consisting of: FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In certain embodiments, the Wnt pathway inhibitor inhibits binding of one or more Wnt proteins to FZD1, FZD2, FZD4, FZD5, FZD7, and/or FZD8. In certain embodiments, the Wnt pathway inhibitor inhibits binding of one or more Wnt proteins to FZD8. In certain embodiments, the inhibition of binding of a particular Wnt to a FZD protein by a Wnt pathway inhibitor is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, an agent that inhibits binding of a Wnt to a FZD protein, also inhibits Wnt pathway signaling. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt pathway signaling is an antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt pathway signaling is a FZD-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt pathway signaling is a FZD8-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt pathway signaling is soluble receptor 54F28.

[00261] In certain embodiments, the Wnt pathway inhibitors (e.g., antibody or soluble receptor) described herein are antagonists of at least one human Wnt protein and inhibit Wnt activity. In certain embodiments, the Wnt pathway inhibitor inhibits Wnt activity by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In some embodiments, the Wnt pathway inhibitor inhibits activity of one, two, three, four, five or more Wnt proteins. In some embodiments, the Wnt pathway inhibitor inhibits activity of at least one human Wnt protein selected from the group consisting of: Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11, and Wnt16. In some embodiments, the Wnt-binding agent binds at least one Wnt protein selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In certain embodiments, the at least one Wnt protein is selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In certain

embodiments, a Wnt pathway inhibitor that inhibits human Wnt activity is an antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt activity is a FZD-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt activity is a FZD8-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits human Wnt activity is soluble receptor 54F28.

[00262] In certain embodiments, the Wnt pathway inhibitor described herein is an antagonist of at least one human FZD protein and inhibits FZD activity. In certain embodiments, the Wnt pathway inhibitor inhibits FZD activity by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In some embodiments, the Wnt pathway inhibitor inhibits activity of one, two, three, four, five or more FZD proteins. In some embodiments, the Wnt pathway inhibitor inhibits activity of at least one human FZD protein selected from the group consisting of: FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In certain embodiments, the Wnt pathway inhibitor inhibits activity of FZD1, FZD2, FZD4, FZD5, FZD7, and/or FZD8. In certain embodiments, the Wnt pathway inhibitor inhibits activity of FZD8. In some embodiments, the Wnt pathway inhibitor is an anti-FZD antibody. In certain embodiments, the Wnt pathway inhibitor is anti-FZD antibody OMP-18R5.

[00263] In certain embodiments, the Wnt pathway inhibitor described herein is an antagonist of at least one human Wnt protein and inhibits Wnt signaling. In certain embodiments, the Wnt pathway inhibitor inhibits Wnt signaling by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In some embodiments, the Wnt pathway inhibitor inhibits signaling by one, two, three, four, five or more Wnt proteins. In some embodiments, the Wnt pathway inhibitor inhibits signaling of at least one Wnt protein selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In certain embodiments, a Wnt pathway inhibitor that inhibits Wnt signaling is an antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits Wnt signaling is a soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits Wnt signaling is a FZD-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits Wnt signaling is a FZD8-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits Wnt signaling is soluble receptor 54F28.

[00264] In certain embodiments, a Wnt pathway inhibitor described herein is an antagonist of β-catenin signaling. In certain embodiments, the Wnt pathway inhibitor inhibits β-catenin signaling by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In certain embodiments, a Wnt pathway inhibitor that inhibits β-catenin signaling is an antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits β-catenin signaling is an anti-FZD antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits β-catenin signaling is antibody OMP-18R5. In certain embodiments, a Wnt pathway inhibitor that inhibits β-catenin signaling is a soluble receptor. In certain embodiments, a Wnt pathway

inhibitor that inhibits β-catenin signaling is a FZD-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits β-catenin signaling is a FZD8-Fc soluble receptor.

[00265] In certain embodiments, the Wnt pathway inhibitor described herein inhibits binding of at least one Wnt protein to a receptor. In certain embodiments, the Wnt pathway inhibitor inhibits binding of at least one human Wnt protein to one or more of its receptors. In some embodiments, the Wnt pathway inhibitor inhibits binding of at least one Wnt protein to at least one FZD protein. In some embodiments, the Wnt-binding agent inhibits binding of at least one Wnt protein to FZD1, FZD2, FZD3, FZD4, FDZ5, FDZ6, FDZ7, FDZ8, FDZ9, and/or FZD10. In certain embodiments, the inhibition of binding of at least one Wnt to at least one FZD protein is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one Wnt to at least one FZD protein further inhibits Wnt pathway signaling and/or β-catenin signaling. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is an antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is an anti-FZD antibody. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is antibody OMP-18R5. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is a soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is a FZD-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is a FZD8-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that inhibits binding of at least one human Wnt to at least one FZD protein is FZD8-Fc soluble receptor 54F28.

[00266] In certain embodiments, the Wnt pathway inhibitor described herein blocks binding of at least one Wnt to a receptor. In certain embodiments, the Wnt pathway inhibitor blocks binding of at least one human Wnt protein to one or more of its receptors. In some embodiments, the Wnt pathway inhibitor blocks binding of at least one Wnt to at least one FZD protein. In some embodiments, the Wnt pathway inhibitor blocks binding of at least one Wnt protein to FZD1, FZD2, FZD3, FZD4, FDZ5, FDZ6, FDZ7, FDZ8, FDZ9, and/or FZD10. In certain embodiments, the blocking of binding of at least one Wnt to at least one FZD protein is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one Wnt protein to at least one FZD protein further inhibits Wnt pathway signaling and/or β-catenin signaling. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is an antibody. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is an anti-FZD antibody. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is antibody OMP-18R5. In

certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is a soluble receptor. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is a FZD-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is a FZD8-Fc soluble receptor. In certain embodiments, a Wnt pathway inhibitor that blocks binding of at least one human Wnt to at least one FZD protein is soluble receptor 54F28.

[00267] In certain embodiments, the Wnt pathway inhibitor described herein inhibits Wnt pathway signaling. It is understood that a Wnt pathway inhibitor that inhibits Wnt pathway signaling may, in certain embodiments, inhibit signaling by one or more receptors in the Wnt signaling pathway but not necessarily inhibit signaling by all receptors. In certain alternative embodiments, Wnt pathway signaling by all human receptors may be inhibited. In certain embodiments, Wnt pathway signaling by one or more receptors selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FDZ5, FDZ6, FDZ7, FDZ8, FDZ9, and FZD10 is inhibited. In certain embodiments, the inhibition of Wnt pathway signaling by a Wnt pathway inhibitor is a reduction in the level of Wnt pathway signaling of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is an antibody. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is an anti-FZD antibody. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is antibody OMP-18R5. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is a soluble receptor. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is a FZD-Fc soluble receptor. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is a FZD8-Fc soluble receptor. In some embodiments, a Wnt pathway inhibitor that inhibits Wnt pathway signaling is soluble receptor 54F28.

[00268] In certain embodiments, the Wnt pathway inhibitor described herein inhibits activation of β-catenin. It is understood that a Wnt pathway inhibitor that inhibits activation of β-catenin may, in certain embodiments, inhibit activation of β-catenin by one or more receptors, but not necessarily inhibit activation of β-catenin by all receptors. In certain alternative embodiments, activation of β-catenin by all human receptors may be inhibited. In certain embodiments, activation of β-catenin by one or more receptors selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FDZ5, FDZ6, FDZ7, FDZ8, FDZ9, and FZD10 is inhibited. In certain embodiments, the inhibition of activation of β-catenin by a Wnt-binding agent is a reduction in the level of activation of β-catenin of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In some embodiments, a Wnt pathway inhibitor that inhibits activation of β-catenin is an antibody. In some embodiments, a Wnt pathway inhibitor that inhibits activation of β-catenin is an anti-FZD antibody. In some embodiments, a Wnt pathway inhibitor that inhibits activation of β-catenin is antibody OMP-18R5. In some embodiments, a Wnt pathway inhibitor that

inhibits activation of β-catenin is a soluble receptor. In some embodiments, a Wnt pathway inhibitor that inhibits activation of β-catenin is a FZD-Fc soluble receptor. In some embodiments, a Wnt pathway inhibitor that inhibits activation of β-catenin is a FZD8-Fc soluble receptor. In some embodiments, a Wnt pathway inhibitor that inhibits activation of β-catenin is soluble receptor 54F28.

[00269] *In vivo* and *in vitro* assays for determining whether a Wnt pathway inhibitor inhibits β-catenin signaling are known in the art. For example, cell-based, luciferase reporter assays utilizing a TCF/Luc reporter vector containing multiple copies of the TCF-binding domain upstream of a firefly luciferase reporter gene may be used to measure β-catenin signaling levels *in vitro* (Gazit et al., 1999, *Oncogene*, 18; 5959-66; TOPflash, Millipore, Billerica MA). The level of β-catenin signaling in the presence of one or more Wnt proteins (e.g., Wnt(s) expressed by transfected cells or provided by Wnt-conditioned media) in the presence of a binding agent is compared to the level of signaling without the binding agent present. In addition to the TCF/Luc reporter assay, the effect of a binding agent (or candidate agent) on β-catenin signaling may be measured *in vitro* or *in vivo* by measuring the effect of the agent on the level of expression of β-catenin-regulated genes, such as c-myc (He et al., 1998, *Science*, 281:1509-12), cyclin D1 (Tetsu et al., 1999, *Nature*, 398:422-6), and/or fibronectin (Gradl et al. 1999, *Mol. Cell Biol.*, 19:5576-87). In certain embodiments, the effect of a binding agent on β-catenin signaling may also be assessed by measuring the effect of the agent on the phosphorylation state of Dishevelled-1, Dishevelled-2, Dishevelled-3, LRP5, LRP6, and/or β-catenin.

[00270] In certain embodiments, a Wnt pathway inhibitor has one or more of the following effects: inhibit proliferation of tumor cells, inhibit tumor growth, reduce the frequency of cancer stem cells in a tumor, reduce the tumorigenicity of a tumor, reduce the tumorigenicity of a tumor by reducing the frequency of cancer stem cells in the tumor, trigger cell death of tumor cells, induce cells in a tumor to differentiate, differentiate tumorigenic cells to a non-tumorigenic state, induce expression of differentiation markers in the tumor cells, prevent metastasis of tumor cells, or decrease survival of tumor cells.

[00271] In certain embodiments, a Wnt pathway inhibitor is capable of inhibiting tumor growth. In certain embodiments, a Wnt pathway inhibitor is capable of inhibiting tumor growth *in vivo* (e.g., in a xenograft mouse model, and/or in a human having cancer). In some embodiments, the tumor is a tumor selected from the group consisting of colorectal tumor, colon tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor. In certain embodiments, the tumor is melanoma. In certain embodiments, the tumor is a colorectal tumor. In certain embodiments, the tumor is a pancreatic tumor. In certain embodiments, the tumor is a breast tumor. In certain embodiments, the tumor is a Wnt-dependent tumor.

[00272] In certain embodiments, a Wnt pathway inhibitor is capable of reducing the tumorigenicity of a tumor. In certain embodiments, a Wnt pathway inhibitor is capable of reducing the tumorigenicity of a tumor comprising cancer stem cells in an animal model, such as a mouse xenograft model. In

certain embodiments, the number or frequency of cancer stem cells in a tumor is reduced by at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold. In certain embodiments, the reduction in the number or frequency of cancer stem cells is determined by limiting dilution assay using an animal model. Additional examples and guidance regarding the use of limiting dilution assays to determine a reduction in the number or frequency of cancer stem cells in a tumor can be found, e.g., in International Publication No. WO 2008/042236, and U.S. Patent Publication Nos. 2008/0064049 and 2008/0178305.

[00273] In certain embodiments, the Wnt pathway inhibitors described herein are active *in vivo* for at least 1 hour, at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the Wnt pathway inhibitor is an IgG (e.g., IgG1 or IgG2) antibody that is active *in vivo* for at least 1 hour, at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the Wnt pathway inhibitor is a fusion protein that is active *in vivo* for at least 1 hour, at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 1 week, or at least about 2 weeks.

[00274] In certain embodiments, the Wnt pathway inhibitors described herein have a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the Wnt pathway inhibitor is an IgG (e.g., IgG1 or IgG2) antibody that has a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the Wnt pathway inhibitor is a fusion protein that has a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 1 week, or at least about 2 weeks. Methods of increasing (or decreasing) the half-life of agents such as polypeptides and antibodies are known in the art. For example, known methods of increasing the circulating half-life of IgG antibodies include the introduction of mutations in the Fc region which increase the pH-dependent binding of the antibody to the neonatal Fc receptor (FcRn) at pH 6.0 (see, e.g., U.S. Patent Publication Nos. 2005/0276799, 2007/0148164, and 2007/0122403). Known methods of increasing the circulating half-life of antibody fragments lacking the Fc region include such techniques as PEGylation.

IV. Kits

[00275] Kits for practicing the methods of the invention are further provided. By "kit" is intended any manufacture (e.g., a package or a container) comprising at least one reagent, e.g., an antibody, a nucleic acid probe, etc. for specifically detecting the expression of at least one biomarker of the

invention. The kit may be promoted, distributed, and/or sold as a unit for performing the methods of the present invention. Additionally, the kits may contain a package insert describing the kit and including instructional material for its use.

[00276] In some embodiments, a kit comprises reagents for practicing the methods of the invention using microarray technology. In some embodiments, a kit comprises reagents for practicing the methods of the invention using qPCR assays. Positive and/or negative controls may be included in the kits to validate the activity and correct usage of reagents employed in accordance with the invention. Controls may include samples known to be either positive or negative for the presence of the biomarker of interest, or other samples comprising the biomarkers of interest. The design and use of controls is standard and well within the routine capabilities of those in the art.

[00277] In some embodiments, a kit comprises polynucleotides selected from the group consisting of SEQ ID NOs:62-79. In some embodiments, a kit comprises (a) a forward primer of SEQ ID NO:62, a reverse primer of SEQ ID NO:63, and a probe comprising SEQ ID NO:64; (b) a forward primer of SEQ ID NO:65, a reverse primer of SEQ ID NO:66, and a probe comprising SEQ ID NO:67; (c) a forward primer of SEQ ID NO:68, a reverse primer of SEQ ID NO:69, and a probe comprising SEQ ID NO:70; (d) a forward primer of SEQ ID NO:71, a reverse primer of SEQ ID NO:72, and a probe comprising SEQ ID NO:73; (e) a forward primer of SEQ ID NO:74, a reverse primer of SEQ ID NO:75, and a probe comprising SEQ ID NO:76; and (f) a forward primer of SEQ ID NO:77, a reverse primer of SEQ ID NO:78, and a probe comprising SEQ ID NO:79.

[00278] It will be further appreciated that any or all steps in the methods of the invention could be implemented by personnel or, alternatively, performed in an automated fashion. Thus, the steps of sample preparation, detection of biomarker expression, etc. may be automated.

[00279] Embodiments of the present disclosure can be further defined by reference to the following non-limiting examples, which describe in detail preparation of certain antibodies of the present disclosure and methods for using antibodies of the present disclosure. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the present disclosure.

EXAMPLES

Example 1

Identification of tumors responsive to treatment with a combination of OMP-18R5 and taxol

[00280] The breast tumor xenograft models OMP-B34, OMP-B39, OMP-B44, OMP-B59, OMP-B60, UM-T01, UM-T03, and UM-PE13 were established at OncoMed Pharmaceuticals or the University of Michigan from minimally passaged, patient-derived tumor specimens. Six- to 8-week-old NOD/SCID mice were subcutaneously injected with 2-4 x 10^4 cells of OMP-B34, OMP-B39, OMP-B44, OMP-B59, OMP-B60, UM-T01, UM-T03, or UM-PE13 tumors. Tumors were allowed to grow

until they reached an average volume of 100 to 150mm^3. Tumor-bearing mice were randomized into four groups (n = 10 per group) and treated with control antibody 1B711 (15mg/kg), anti-FZD antibody OMP-18R5 (15mg/kg), taxol (10mg/kg), or OMP-18R5 (15mg/kg) in combination with taxol (10mg/kg). Treatment with antibodies and/or taxol was administered on a weekly basis. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean ± S.E.M.

[00281] To determine if a tumor was responsive to anti-FZD antibody OMP-18R5, single agent tumor volume data was compared with the control while combination treatment with OMP-18R5 and taxol was compared with taxol as a single agent. For this study a "responder" tumor was defined as a tumor showing significantly greater tumor growth inhibition with the combination of OMP-18R5 and taxol as compared to tumor growth inhibition with taxol as single agent.

[00282] The results for each xenograft model are shown in Figures 1A-H. T-tests were conducted at each time point. Multiple comparisons used 2-way repeated measurement ANOVA followed by Bonferroni corrections. The t-tests and 2-way repeated measurement ANOVA were performed using GraphPad Prism5 (GraphPad Software Inc.). The tumors OMP-B59, OMP-B60, UM-T03, and UM-PE13 were shown to be responders, while tumors OMP-B34, OMP-B39, OMP-B44, and UM-T01 were shown to be non-responders. The results are summarized in Table 1.

Table 1

Tumor	Tumor Subtype	Classification
OMP-B34	TNBC	Non-Responder
OMP-B39	TNBC	Non-Responder
OMP-B44	TNBC	Non-Responder
OMP-B59	TNBC	Responder
OMP-B60	TNBC	Responder
UM-T01	TNBC	Non-Responder
UM-T03	ER+PR+HER2+	Responder
UM-PE13	TNBC	Responder

Example 2

Identification of predictive biomarkers

[00283] Microarray analyses were performed on untreated breast tumors OMP-B34, OMP-B39, OMP-B44 which did not respond to treatment with a combination of OMP-18R5 and taxol, ("non-responders"), and UM-T01 and untreated tumors OMP-B59, OMP-B60, UM-T03, and UM-PE13 which did respond to treatment with a combination of OMP-18R5 and taxol ("responders"). RNA was isolated from each tumor using a RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia CA) with DNAse treatment following the manufacturer's instructions. Samples were stored at -80ºC. RNA

was visualized on an Agilent 2100 Bioanalyzer and integrity was confirmed by the presence of intact 28S and 18S ribosomal peaks. All RNA samples had 260/280 ratios > 1.8. Total RNA isolated from each tumor was amplified using the Ovation RNA Amplification System V2 (NuGEN, San Carlos, CA). Amplified, anti-sense single stranded-cDNA was fragmented and biotinylated using the FL-Ovation cDNA Biotin Module V2 (NuGEN). The quality of the cDNA and the fragmented cDNA was assessed by a spectrophotometer and a Bioanalyzer before hybridization to the array. The processed RNA was hybridized to Affymetrix HG-U133 plus 2.0 microarrays (Affymetrix, Santa Clara, CA) as outlined in the manufacturer's technical manuals. After hybridization, the microarrays were washed, scanned, and analyzed. Microarray data were processed to probe set level data by using GeneChip-RMA (Wu et al., 2004, *J. Amer. Stat. Assn.*, 99:909-917). Probe sets that were likely to cross-hybridize with murine markers were removed. To summarize the data to gene level and make sure the probe set with the strongest signals were chosen, maximum expression was used across all probe sets mapping to one gene. Genes with low expression (< 5 on log2 scale) or near-zero variance (< 0.01) were removed. Genes were standardized to N(0,1) by subtracting the log2 scale expression from the mean and dividing by the standard deviation of each gene.

[00284] Analyses were performed using genes from several signaling pathways including canonical, planar cell polarity, Wnt/Ca+2, Wnt signaling negative regulation, cell fate, tissue polarity, cell growth and proliferation, cell migration, cell cycle, and cellular homeostasis (see Table 2).

Table 2

Gene Symbol	Protein Name
AES	Amino-terminal enhancer of split
APC	Adenomatous polyposis coli protein
AXIN1	Axin-1
BCL9	B-cell CLL/lymphoma 9 protein
BTRC	F-box/WD repeat-containing protein 1A
CCND1	G1/S-specific cyclin-D1
CCND2	G1/S-specific cyclin-D2
CCND3	G1/S-specific cyclin-D3
CSNK1A1	Casein kinase I isoform alpha
CSNK1D	Casein kinase I isoform delta
CSNK1G1	Casein kinase I isoform gamma-1
CSNK2A1	Casein kinase II subunit alpha
CTBP1	C-terminal-binding protein 1
CTBP2	C-terminal-binding protein 2
CTNNB1	Catenin beta-1
CTNNBIP1	Beta-catenin-interacting protein 1
CXXC4	CXXC-type zinc finger protein 4
DAAM1	Disheveled-associated activator of morphogenesis 1
DIXDC1	Dixin
DKK1	Dickkopf-related protein 1
DVL1	Segment polarity protein disheveled homolog DVL-1
DVL2	Segment polarity protein disheveled homolog DVL-2
EP300	Histone acetyltransferase p300
FBXW11	F-box/WD repeat-containing protein 11

FBXW2	F-box/WD repeat-containing protein 2
FBXW4	F-box/WD repeat-containing protein 4
FGF4	Fibroblast growth factor 4
FOSL1	Fos-related antigen 1
FOXN1	Forkhead box protein N1
FRAT1	Proto-oncogene FRAT1
FRZB	Secreted frizzled-related protein 3
FSHB	Follitropin subunit beta
FZD1	Frizzled-1
FZD2	Frizzled-2
FZD3	Frizzled-3
FZD4	Frizzled-4
FZD5	Frizzled-5
FZD6	Frizzled-6
FZD7	Frizzled-7
FZD8	Frizzled-8
GSK3A	Glycogen synthase kinase-3 alpha
GSK3B	Glycogen synthase kinase-4 alpha
JUN	Transcription factor AP-1
KREMEN1	Kremen protein 1
LEF1	Lymphoid enhancer-binding factor 1
LRP5	Low-density lipoprotein receptor-related protein 5
LRP6	Low-density lipoprotein receptor-related protein 6
MYC	Myc proto-oncogene protein
NKD1	Protein naked cuticle homolog
NLK	Serine/threonine-protein kinase NLK
PITX2	Pituitary homeobox 2
PORCN	Protein-cysteine N-palmitoyl transferase porcupine
PPP2CA	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform
PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
PYGO1	Pygopus homolog 1
RHOU	Rho-related GTP-binding protein RhoU
SENP2	Sentrin-specific protease 2
SFRP1	Secreted frizzled-related protein 1
SFRP4	Secreted frizzled-related protein 4
SLC9A3R1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1
SOX17	Transcription factor SOX-17
T	Brachyury protein
TCF7	Transcription factor 7
TCF7L1	Transcription factor 7-like 1
TLE1	Transducin-like enhancer protein 1
TLE2	Transducin-like enhancer protein 2
WIF1	Wnt inhibitory factor 1
WISP1	WNT1-inducible signaling pathway protein 1
WNT1	Proto-oncogene Wnt-1
WNT2	Protein Wnt-2
WNT2B	Protein Wnt-2B
WNT3	Protein Wnt-3
WNT3A	Protein Wnt-3a
WNT4	Protein Wnt-4
WNT5A	Protein Wnt-5a
WNT5B	Protein Wnt-5b

WNT6	Protein Wnt-6
WNT7A	Protein Wnt-7a
WNT7B	Protein Wnt-7b
WNT8A	Protein Wnt-8a
WNT9A	Protein Wnt-9a
WNT10A	Protein Wnt-10a
WNT11	Protein Wnt-11
WNT16	Protein Wnt-16

[00285] Support Vector Machines - Recursive Feature Elimination (SVM-RFE) methods (Guyon et al, 2002, *Machine Learning*, 46:389-422) were used to identify genes that could distinguish between the responder and non-responder tumors and Support Vector Machine (SVM) methods (Cortes and Vapnik, 1995, *Machine Learning*, 20:273-297) were used for classification. A leave-one-out cross-validation (LOOCV) method was used to select the number of genes and also to measure positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of the models. A biomarker signature comprising FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 achieved the best performance with PPV=NPV=sensitivity=specificity=100% using the 8 breast tumors (see Figure 2). As shown in Figure 3, principal component analysis (PCA) illustrated that the 6-gene biomarker signature resulted in a near perfect separation of the 8 breast tumors. In addition, strong correlation was observed between the 6-gene biomarker signature and the ratio of tumor volume (RTV) from the *in vivo* experiments described in Example 1 (correlation = 0.95, p-value = 0.0003; cross-validated correlation = 0.89, p-value = 0.00027; Figure 4).

[00286] Decision values were determined from the SVM model based on the training data. For the 6-gene biomarker signature, decision values can be calculated by a weighted sum of the standardized expression of the 6 genes: 0.4560427*FBXW2 + 0.3378467*CCND2 – 0.4809354*RHOU + 0.409029*CTBP2 + 0.3291529*WIF1 + 02926374*DKK1 + 0.04662682. A positive decision value indicated a tumor predicted to be a responder while a negative decision value indicated a tumor predicted to be a non-responder. In addition, classification probabilities can be obtained by fitting a logistic regression on the decision values. Tumors associated with probabilities higher than 0.5 would be predicted to be a responder while tumors with probabilities lower than 0.5 would be predicted to be a non-responder.

Example 3

In vivo validation of predictive biomarkers

[00287] Six additional breast cancer tumors were selected from the OncoMed Tumor Bank and microarray analyses were performed as described in Example 1. The six breast cancer tumors were OMP-B29, OMP-B71, OMP-B84, OMP-B90, UM-T02, and UM-T06. As described herein, classification probability analysis was used with the 6-gene biomarker signature to predict the response of each of these tumors to treatment with anti-FZD antibody OMP-18R5 in combination

with taxol (see Figure 5). In parallel the six tumors were evaluated in *in vivo* xenograft models as described in Example 1 (see Figures 6A-F). As described in Example 1 a "responder" in the in vivo models is a tumor showing significantly greater tumor growth inhibition with the combination of OMP-18R5 and taxol as compared to tumor growth inhibition with taxol as single agent. The predictions based on classification probabilities were compared to the results of the *in vivo* xenograft models. The results are summarized in Table 3.

Table 3

Tumor	Tumor subtype	Classification Probability	Decision Value	Prediction	*In vivo* Response
OMP-B29	ER+PR+HER2-	0.3344	-0.5928	Non-responder	Non-responder
OMP-B71	ER+PR+HER2-	0.9897	1.6789	Responder	Responder
OMP-B84	ER+PR+HER2-	0.4324	-0.4002	Non-responder	Non-responder
OMP-B90	TNBC	0.8152	0.492	Responder	Responder
UM-T02	TNBC	0.4387	-0.3972	Non-responder	Non-responder
UM-T06	ER+PR+HER2-	0.1385	-1.0778	Non-responder	Non-responder

[00288] As shown in Table 3, the response of each of the six breast cancer tumors was accurately predicted by the 6-gene biomarker signature using the decision values and the classification probabilities.

Example 4

Prevalence Estimation of the 6-gene biomarker signature

[00289] Prevalence of a biomarker signature can be defined as the proportion of a population predicted to be a responder based upon the biomarker signature. The prevalence of the 6-gene biomarker signature in HER2 negative (HER2-) and triple negative breast cancer (TNBC) populations was estimated by applying the 6-gene biomarker signature to three publicly available breast cancer microarray data sets. The Cremoux2001 dataset was compiled from Affymetrix U133plus2 microarrays with 226 patients, including 145 HER2- and 81 HER2+, where 51 TNBC were included within the HER2- group. The Wang2011 dataset was compiled from Affymetrix U133plus2 microarrays with 115 patients, including 79 HER2- and 36 HER2+, where 28 TNBC were included within the HER2- group. The Prat2010 dataset was compiled from Agilent Human 1A microarrays with 333 patients, including 215 HER2- and 118 HER2+, where 57 TNBC were included within the HER2- group. Pre-processing of the public data included downloading the data, extracting the probe

sets mapping to the six genes, and collapsing the probe sets to the six genes. Gene level expression data was further standardized by subtracting the mean and dividing by the standard deviation of each gene in the public data. The SVM model built upon the training data was used to classify the public data. Classification probabilities were obtained and the proportion of predicted responders (probability > 0.5) was calculated based on the 6-gene biomarker signature.

[00290] As shown in Figure 7, the predicted prevalence of the 6-gene biomarker signature within the 3 datasets was very similar (approximately 60%). This prediction would suggest that there is a large population of breast cancer patients that would be responsive to therapy with the anti-FZD antibody OMP-18R5 in combination with taxol.

Example 5

qPCR assays for 6-gene biomarker signature

[00291] qPCR assays were developed to determine the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 in a tumor sample. Primers and probes were designed using publicly available mRNA sequences. The primers and probes were generated and used in optimization and validation tests using human fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) human tissue samples. The specific primers and probes are listed in Table 4 (all sequences in 5' to 3' direction). Four reference genes were used for normalization including TOP1 (topoisomerase 1), GUSB (beta-glucuronidase), SDHA (succinate dehydrogenase), and PUM1 (pumilio homolog 1).

Table 4

Gene	Primer/Probe	Sequence	SEQ ID NO
CCND2	Forward Primer	GCTGTCTCTGATCCGCAAGC	SEQ ID NO:62
	Reverse Primer	GACGGTGGGTACATGGCAAAC	SEQ ID NO:63
	Probe	CCTTCATTGCTCTGTGTGCCACCGAC	SEQ ID NO:64
CTBP2	Forward Primer	ATCCGTGGGGAGACGCTG	SEQ ID NO:65
	Reverse Primer	CTCGAACTGCAACCGCCTG	SEQ ID NO:66
	Probe	CCCGTGCGACCAAAGCCAATGAGG	SEQ ID NO:67
DKK1	Forward Primer	GACCATTGACAACTACCAGCCGTA	SEQ ID NO:68
	Reverse Primer	TGGGACTAGCGCAGTACTCATC	SEQ ID NO:69
	Probe	TGCCGCACTCCTCGTCCTCTG	SEQ ID NO:70
FBXW2	Forward Primer	GCCAGTTATGATATTCTCAGGGTCA	SEQ ID NO:71
	Reverse Primer	AGCAGGGCAAAGATATCTCCAAA	SEQ ID NO:72
	Probe	AGACTCCTGAGATAGCAAACTTGGCCT	SEQ ID NO:73
RHOU1	Forward Primer	CCCACCGAGTACATCCCTACTG	SEQ ID NO:74
	Reverse Primer	CAGTGTCACAGAGTTGGAGTCTCA	SEQ ID NO:75
	Probe	CGCCCATCCACAGACACCACCG	SEQ ID NO:76
WIF1	Forward Primer	GTTCCAAAGGTTACCAGGGAGAC	SEQ ID NO:77

	Reverse Primer	GTTGGGTTCATGGCAGGTTCC	SEQ ID NO:78
	Probe	CCAGGCTCGCAGACAGGCTTTGAAC	SEQ ID NO:79

[00292] qPCR was performed on total RNA obtained from 18 xenograft breast tumors. Tumor specimens were harvested and immediately snap frozen and stored at -80°C prior to RNA isolation. Total RNA was extracted using the RNeasy Fibrous Mini Kit (Qiagen, Valencia CA, PN#74704) with TissueLyzer homogenization and DNase I treatment according to the manufacturer's protocol. RNAs were visualized on a Bioanalyzer 2100 (Agilent, Santa Clara, CA) and verified to be intact with RIN values > 6.0. All RNAs had A260/A280 ratios > 1.8.

[00293] qPCR was performed in a two-step manner. First, cDNA was synthesized from total RNA using random hexamers as described in Applied Biosystems User Bulletin 2. TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA. Cat # 4304437 and 4326708) was used in subsequent qPCR reactions according to the manufacturer's protocol. Quantities of gene expression were determined using a Ct (cycle threshold) method from triplicate reactions. Cycle threshold is generally considered to be the number of cycles required for a signal to cross the detection threshold. Ct levels are inversely proportional to the amount of target nucleic acid in a sample. Ct of the six genes are normalized using the Ct levels of the four reference genes. Normalized Ct of the 6-gene signature for the 18 xenograft samples is shown in Table 5.

Table 5

	FBXW2	CCND2	RHOU	CTBP2	WIF1	DKK1
OMP-B84	0.8425	12.5125	4.6775	1.0775	16.4025	5.2575
OMP-B71	0.98375	14.52375	6.46875	0.08875	4.56875	1.14375
OMP-B59	0.83875	2.67375	6.43875	-0.6012	4.90375	10.7888
OMP-B86	2.4725	11.5125	2.5425	1.3275	-0.8825	1.1125
OMP-B39	1.03	12.54	1.44	2.225	2.045	6.365
OMP-B90 p1	1.175	6.955	6.87	1.535	17.535	10.87
OMP-B94	1.67375	1.52875	5.95375	1.56875	9.34875	4.37875
OMP-B40	1.03	16.455	6.775	0.73	16.455	14.985
OMP-B29	1.445	13.63	6.425	0.695	13.63	4.185
OMP-B60	1.6725	14.7775	6.9825	0.4775	-0.8025	8.6775
OMP-B90 p2	0.75875	14.18375	5.78875	0.13875	15.54375	10.7388
UM-T06	1.19875	11.51875	4.27875	1.34375	8.87375	6.26375
OMP-B44	1.61	11.765	4.755	0.505	7.225	9.61
UM-T02	2.255	13.215	4.195	1.075	16.125	4.225
UM-T 3	1.67625	12.58625	5.83625	0.20125	16.21625	4.17125
OMP-B34	0.08625	0.58625	6.21125	0.53125	9.02125	0.06125

UM-PE13	0.925	15.185	4.055	-0.7	15.185	6.62
UM-T01	2.20375	15.11375	6.44375	-0.1062	15.11375	15.1138

[00294] Decision values can be calculated by a weighted sum of the normalized expression of the 6 genes from data generated from the qPCR assays. These decision values are different than the decision values generated from the analysis based on microarray data, however the predictive capabilities of the two models are very similar.

[00295] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

[00296] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

[00297] Following are the sequences disclosed in the application:

OMP-18R5 Heavy chain CDR1 (SEQ ID NO:1)
GFTFSHYTLS

OMP-18R5 Heavy chain CDR2 (SEQ ID NO:2)
VISGDGSYTYYADSVKG

OMP-18R5 Heavy chain CDR3 (SEQ ID NO:3)
NFIKYVFAN

OMP-18R5 Light chain CDR1 (SEQ ID NO:4)
SGDNIGSFYVH

OMP-18R5 Light chain CDR2 (SEQ ID NO:5)
DKSNRPSG

OMP-18R5 Light chain CDR3 (SEQ ID NO:6)
QSYANTLSL

OMP-18R5 Heavy chain variable region amino acid sequence (SEQ ID NO:7)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYY
ADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSS

OMP-18R5 Light chain variable region amino acid sequence (SEQ ID NO:8)
DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPER
FSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG

OMP-18R5 Heavy chain amino acid sequence with predicted signal sequence underlined (SEQ ID NO:9)
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAP
GKGLEWVSVISGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFI
KYVFANWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS

GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC
VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

OMP-18R5 Light chain amino acid sequence with predicted signal sequence underlined (SEQ ID NO:10)
MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGG
TKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVE
TTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

OMP-18R5 Heavy chain amino acid sequence without predicted signal sequence (SEQ ID NO:11)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYY
ADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSAS
TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

OMP-18R5 Light chain amino acid sequence without predicted signal sequence (SEQ ID NO:12)
DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPER
FSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFP
PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLS
LTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Human FZD1 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:13)
QQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDA
GLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFG
FQWPDTLKCEKFPVHGAGELCVGQNTSDKGT

Human FZD2 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:14)
QFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQ
CSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPR
HGAEQICVGQNHSEDG

Human FZD3 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:15)
HSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDF
RPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPY
PRLVDL

Human FZD4 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:16)
FGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQF
FLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNH
MCMEGPGDEEV

Human FZD5 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:17)
ASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFL
CSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVL
CMDYNRSEATT

Human FZD6 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:18)

HSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLC
KAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFD
PHTEFLG

Human FZD7 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:19)
QPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKV
QCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFP
VHGAGEICVGQNTSDGSG

Human FZD8 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:20)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFF
LCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTT

Human FZD9 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:21)
LEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQY
GCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARL
PTRNDPHALCMEAPENA

Human FZD10 Fri domain amino acid sequence without predicted signal sequence (SEQ ID NO:22)
ISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCH
GHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNK
NDPNYLCMEAPNNG

Human FZD1 amino acids 116-227 (SEQ ID NO:23)
CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAP
VCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELC

Human FZD2 amino acids 39-150 (SEQ ID NO:24)
CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAP
VCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQIC

Human FZD3 amino acids 28-133 (SEQ ID NO:25)
CEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAP
ICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDC

Human FZD4 amino acids 48-161 (SEQ ID NO:26)
CDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVP
MCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMC

Human FZD5 amino acids 33-147 (SEQ ID NO:27)
CQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTP
ICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLC

Human FZD6 amino acids 24-129 (SEQ ID NO:28)
CEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVP
TCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYC

Human FZD7 amino acids 49-160 (SEQ ID NO:28)
CQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAP
VCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEIC

Human FZD8 amino acids 35-148 (SEQ ID NO:30)
CQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTP
ICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLC

Human FZD9 amino acids 39-152 (SEQ ID NO:31)
CQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAP
MCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALC

Human FZD10 amino acids 34-147 (SEQ ID NO:32)
CQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAP
MCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLC

Human FZD8 Fri domain amino acid sequence without predicted signal sequence (variant) (SEQ ID NO:33)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFF
LCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDL

Human IgG_1 Fc region (SEQ ID NO:34)
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG_1 Fc region (variant) (SEQ ID NO:35)
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG_1 Fc region (SEQ ID NO:36)
KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG_1 Fc region (SEQ ID NO:37)
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG_2 Fc region (SEQ ID NO:38)
CVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQP
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F03 amino acid sequence (without predicted signal sequence) (SEQ ID NO:39)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFF
LCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTGRADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

FZD8-Fc variant 54F16, 54F17, 54F18, 54F23, 54F25, 54F27, 54F29, 54F31, and 54F34 amino acid sequence (without predicted signal sequence) (SEQ ID NO:40)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFF
LCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

FZD8-Fc variant 54F19, 54F20, 54F24, 54F26, 54F28, 54F30, 54F32, 54F34 and 54F35 amino acid sequence (without predicted signal sequence) (SEQ ID NO:41)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFF
LCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

FZD8-Fc variant 54F03 amino acid sequence with signal sequence (SEQ ID NO:42)
MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGRADKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F16 amino acid sequence with signal sequence (SEQ ID NO:43)
MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F26 with signal sequence (SEQ ID NO:44)
MEWGYLLEVTSLLAALFLLQRSPIVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F28 with signal sequence (SEQ ID NO:45)
MEWGYLLEVTSLLAALLLLQRSPFVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human Wnt1 C-terminal cysteine rich domain (aa 288-370) (SEQ ID NO:46)
DLVYFEKSPNFCTYSGRLGTAGTAGRACNSSSPALDGCELLCCGRGHRTRTQRVTERCNC
TFHWCCHVSCRNCTHTRVLHECL

Human Wnt2 C-terminal cysteine rich domain (aa 267-360) (SEQ ID NO:47)
DLVYFENSPDYCIRDREAGSLGTAGRVCNLTSRGMDSCEVMCCGRGYDTSHVTRMTKCGC
KFHWCCAVRCQDCLEALDVHTCKAPKNADWTTAT

Human Wnt2b C-terminal cysteine rich domain (aa 298-391) (SEQ ID NO:48)
DLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSKGTDGCEIMCCGRGYDTTRVTRVTQCEC
KFHWCCAVRCKECRNTVDVHTCKAPKKAEWLDQT

Human Wnt3 C-terminal cysteine rich domain (aa 273-355) (SEQ ID NO:49)
DLVYYENSPNFCEPNPETGSFGTRDRTCNVTSHGIDGCDLLCCGRGHNTRTEKRKEKCHC
IFHWCCYVSCQECIRIYDVHTCK

Human Wnt3a C-terminal cysteine rich domain (aa 270-352) (SEQ ID NO:50)
DLVYYEASPNFCEPNPETGSFGTRDRTCNVSSHGIDGCDLLCCGRGHNARAERRREKCRC
VFHWCCYVSCQECTRVYDVHTCK

Human Wnt7a C-terminal cysteine rich domain (aa 267-359) (SEQ ID NO:51)
DLVYIEKSPNYCEEDPVTGSVGTQGRACNKTAPQASGCDLMCCGRGYNTHQYARVWQCNC
KFHWCCYVKCNTCSERTEMYTCK

Human Wnt7b C-terminal cysteine rich domain (aa 267-349) (SEQ ID NO:52)
DLVYIEKSPNYCEEDAATGSVGTQGRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNC
KFHWCCFVKCNTCSERTEVFTCK

Human Wnt8a C-terminal cysteine rich domain (aa 248-355) (SEQ ID NO:53)
ELIFLEESPDYCTCNSSLGIYGTEGRECLQNSHNTSRWERRSCGRLCTECGLQVEERKTE
VISSCNCKFQWCCTVKCDQCRHVVSKYYCARSPGSAQSLGRVWFGVYI

Human Wnt8b C-terminal cysteine rich domain (aa 245-351) (SEQ ID NO:54)
ELVHLEDSPDYCLENKTLGLLGTEGRECLRRGRALGRWELRSCRRLCGDCGLAVEERRAE
TVSSCNCKFHWCCAVRCEQCRRRVTKYFCSRAERPRGGAAHKPGRKP

Human Wnt10a C-terminal cysteine rich domain (aa 335-417) (SEQ ID NO:55)
DLVYFEKSPDFCEREPRLDSAGTVGRLCNKSSAGSDGCGSMCCGRGHNILRQTRSERCHC
RFHWCCFVVCEECRITEWVSVCK

Human Wnt10b C-terminal cysteine rich domain (aa 307-389) (SEQ ID NO:56)
ELVYFEKSPDFCERDPTMGSPGTRGRACNKTSRLLDGCGSLCCGRGHNVLRQTRVERCHC
RFHWCCYVLCDECKVTEWVNVCK

Linker (SEQ ID NO:57)
ESGGGGVT

Linker (SEQ ID NO:58)
LESGGGGVT

Linker (SEQ ID NO:59)
GRAQVT

Linker (SEQ ID NO:60)
WRAQVT

Linker (SEQ ID NO:61)
ARGRAQVT

CCND2 Forward Primer (SEQ ID NO:62)
GCTGTCTCTGATCCGCAAGC

CCND2 Reverse Primer (SEQ ID NO:63)
GACGGTGGGTACATGGCAAAC

CCND2 Probe (SEQ ID NO:64)
CCTTCATTGCTCTGTGTGCCACCGAC

CTBP2 Forward Primer (SEQ ID NO:65)
ATCCGTGGGGAGACGCTG

CTBP2 Reverse Primer (SEQ ID NO:66)
CTCGAACTGCAACCGCCTG

CTBP2 Probe (SEQ ID NO:67)
CCCGTGCGACCAAAGCCAATGAGG

DKK1 Forward Primer (SEQ ID NO:68)
GACCATTGACAACTACCAGCCGTA

DKK1 Reverse Primer (SEQ ID NO:69)
TGGGACTAGCGCAGTACTCATC

DKK1 Probe (SEQ ID NO:70)
TGCCGCACTCCTCGTCCTCTG

FBXW2 Forward Primer (SEQ ID NO:71)
GCCAGTTATGATATTCTCAGGGTCA

FBXW2 Reverse Primer (SEQ ID NO:72)
AGCAGGGCAAAGATATCTCCAAA

FBXW2 Probe (SEQ ID NO:73)
AGACTCCTGAGATAGCAAACTTGGCCT

RHOU1 Forward Primer (SEQ ID NO:74)
CCCACCGAGTACATCCCTACTG

RHOU1 Reverse Primer (SEQ ID NO:75)
CAGTGTCACAGAGTTGGAGTCTCA

RHOU1 Probe (SEQ ID NO:76)
CGCCCATCCACAGACACCACCG

WIF1 Forward Primer (SEQ ID NO:77)
GTTCCAAAGGTTACCAGGGAGAC

WIF1 Reverse Primer (SEQ ID NO:78)

GTTGGGTTCATGGCAGGTTCC

WIF1 Probe (SEQ ID NO:79)
CCAGGCTCGCAGACAGGCTTTGAAC

WHAT IS CLAIMED IS:

1. A method of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the human tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) identifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression level of the biomarkers.

2. A method of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the human tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) calculating a decision value based upon the standardized expression of the biomarkers in the signature;

wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive to the Wnt pathway inhibitor.

3. A method of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the human tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) classifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression of the biomarkers.

4. A method of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the human tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) calculating a decision value based upon the standardized expression of the biomarkers in the signature;

wherein a positive decision value indicates the tumor is predicted to be responsive the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be non-responsive the Wnt pathway inhibitor.

5. A method of determining the responsiveness of a human tumor to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the human tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) determining the responsiveness of the tumor to treatment based upon the expression of the biomarkers.

6. A method of determining the responsiveness of a human tumor to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the human tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) calculating a decision value based upon the standardized expression of the biomarkers in the signature;

wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor.

7. A method of identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the patient's tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers.

8. A method of identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the patient's tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) calculating a decision value based upon the standardized expression of the biomarkers in the signature;

wherein a positive decision value indicates that the patient is predicted to respond to treatment with the Wnt pathway inhibitor.

9. A method of selecting a patient with cancer for treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the patient's tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) selecting the patient for treatment based upon the expression level of the biomarkers.

10. A method of selecting a patient with cancer for treatment with a Wnt pathway inhibitor, the method comprising:

(a) obtaining a sample of the patient's tumor;

(b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1;

(c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; and

(d) selecting the patient for treatment when their tumor sample has a positive decision value.

11. A method of treating cancer in a patient, comprising:

(a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises:

(i) obtaining a sample of the patient's tumor;

(ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and

(b) administering an effective amount of a Wnt pathway inhibitor to the patient who is likely to response to treatment.

12. A method of treating cancer in a patient, comprising:

(a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises:

(i) obtaining a sample of the patient's tumor;

(ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature;

wherein a positive decision value indicates that a patient is predicted to respond to treatment with the Wnt pathway inhibitor; and

(b) administering an effective amount of a Wnt pathway inhibitor to the patient who is predicted to response to treatment.

13. A method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising:

(a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises:

(i) obtaining a sample of the patient's cancer;

(ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and

(b) administering an effective amount of the Wnt pathway inhibitor to the patient who is likely to respond to treatment.

14. A method for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising:

(a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises:

(i) obtaining a sample of the patient's cancer;

(ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature;

wherein a positive decision value indicates that a patient is predicted to respond to treatment with the Wnt pathway inhibitor; and

(b) administering an effective amount of the WNT pathway inhibitor to the patient whose tumor has a positive decision value.

15. The method according to any one of claims 1-14, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, DKK1, EP300, CTBP1, WNT6, WNT3, FZD2, APC, TLE2, DVL2, PITX2, WISP1, GSK3B, WNT9A, FZD7, and LEF1.

16. The method according to any one of claims 1-15, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, DKK1, EP300, and CTBP1.

17. The method according to any one of claims 1-16, wherein the biomarker signature comprises two or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

18. The method according to any one of claims 1-16, wherein the biomarker signature comprises three or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

19. The method according to any one of claims 1-16, wherein the biomarker signature comprises four or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

20. The method according to any one of claims 1-16, wherein the biomarker signature comprises five of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

21. The method according to any one of claims 1-16, wherein the biomarker signature comprises the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

22. The method according to any one of claims 1-16, wherein the biomarker signature consists of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

23. The method according to any one of claims 1-22, wherein the expression of each biomarker is measured by a PCR-based assay.

24. The method according to any one of claims 1-23, wherein the expression of each biomarker is measured by a qPCR assay.

25. The method according to any one of claims 1-22, wherein the expression of each biomarker is measured by a microarray.

26. The method according to any one of claims 1-25, wherein the standardized expression of each biomarker is determined by measuring an expression level for each biomarker and multiplying it by a corresponding weight, wherein the weight for each biomarker is determined by the expression signature.

27. The method according to any one of claims 1-26, wherein the decision value is calculated according to the equation: 0.4560427*FBXW2 + 0.3378467*CCND2 - 0.4809354*RHOU + 0.409029*CTBP2 + 0.3291529*WIF1 + 0.2926374*DKK1 + 0.04662682.

28. The method according to any one of claims 1-25, wherein the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 are measured using polynucleotides selected from the group consisting of SEQ ID NOs:62-79.

29. The method of claim 28, wherein the expression levels of FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 are measured using:

(a) a forward primer of SEQ ID NO:62, a reverse primer of SEQ ID NO:63, and a probe comprising SEQ ID NO:64;

(b) a forward primer of SEQ ID NO:65, a reverse primer of SEQ ID NO:66, and a probe comprising SEQ ID NO:67;

(c) a forward primer of SEQ ID NO:68, a reverse primer of SEQ ID NO:69, and a probe comprising SEQ ID NO:70;

(d) a forward primer of SEQ ID NO:71, a reverse primer of SEQ ID NO:72, and a probe comprising SEQ ID NO:73;

(e) a forward primer of SEQ ID NO:74, a reverse primer of SEQ ID NO:75, and a probe comprising SEQ ID NO:76; and

(f) a forward primer of SEQ ID NO:77, a reverse primer of SEQ ID NO:78, and a probe comprising SEQ ID NO:79.

30. The method according to any one of claims 1-29, wherein the Wnt pathway inhibitor is an antibody.

31. The method according to any one of claims 1-30, wherein the Wnt pathway inhibitor is an antibody that specifically binds at least one Frizzled (FZD) protein or portion thereof.

32. The method of claim 30 or claim 31, wherein the antibody specifically binds at least one FZD protein selected from the group consisting of: FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10.

33. The method of claim 30 or claim 31, wherein the antibody specifically binds at least one FZD protein selected from the group consisting of: FZD1, FZD2, FZD5, FZD7, and FZD8.

34. The method according to any one of claims 1-33, wherein the Wnt pathway inhibitor is an antibody comprising:
(a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), and
(b) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:4), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:5), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:6).

35. The method according to any one of claims 1-34, wherein the Wnt pathway inhibitor is an antibody comprising a heavy chain variable region comprising SEQ ID NO:7 and a light chain variable region comprising SEQ ID NO:8.

36. The method according to any one of claims 1-34, wherein the Wnt pathway inhibitor is an antibody comprising a heavy chain variable region and a light chain variable region encoded by the plasmid deposited with ATCC as PTA-9541.

37. The method according to any one of claims 30-36, wherein the antibody is a monoclonal antibody, a recombinant antibody, a chimeric antibody, a bispecific antibody, a humanized antibody, a human antibody, or a antibody fragment comprising an antigen-binding site.

38. The method according to any one of claims 1-35, wherein the Wnt pathway inhibitor is antibody OMP-18R5.

39. The method according to any one of claims 1-29, wherein the Wnt pathway inhibitor is a soluble receptor.

40. The method of claim 39, wherein the soluble receptor comprises a Fri domain of a human FZD protein.

41. The method of claim 38, wherein the Fri domain of the human FZD protein is selected from the group consisting of: the Fri domain of FZD1, the Fri domain of FZD2, the Fri domain of FZD3, the Fri domain of FZD4, the Fri domain of FZD5, the Fri domain of FZD6, the Fri domain of FZD7, the Fri domain of FZD8, the Fri domain of FZD9, or the Fri domain of FZD10.

42. The method of claim 40, wherein the Fri domain of the human FZD protein comprises the Fri domain of FZD8.

43. The method according to any one of claims 39-42, wherein the soluble receptor further comprises a non-FZD polypeptide.

44. The method of claim 43, wherein the non-FZD polypeptide comprises a human Fc region.

45. The method according to any one of claims 39-44, wherein the Wnt pathway inhibitor is FZD8-Fc soluble receptor OMP-54F28.

46. The method according to any one of claims 1-45, wherein the tumor is selected from the group consisting of: a breast tumor, a lung tumor, a colon tumor, a colorectal tumor, a melanoma, a pancreatic tumor, a gastrointestinal tumor, a renal tumor, an ovarian tumor, a neuroendocrine tumor, a liver tumor, an endometrial tumor, a kidney tumor, a prostate tumor, a thyroid tumor, a neuroblastoma, a glioma, a glioblastoma multiforme, a cervical tumor, a stomach tumor, a bladder tumor, a hepatoma, and a head and neck tumor.

47. The method of according to any one of claims 1-45, wherein the tumor is a breast tumor.

48. The method of claim 47, wherein the breast tumor is a HER2-negative breast tumor.

49. The method of claim 47, wherein the breast tumor is a triple negative breast cancer (TNBC) tumor.

50. The method according to any one of claims 1-49, wherein the treatment with a Wnt pathway inhibitor is in combination with one or more additional therapeutic agents.

51. The method of claim 50, wherein the additional therapeutic agent is a chemotherapeutic agent.

52. The method of claim 50, wherein the additional therapeutic agent is paclitaxel.

53. The method of claim 50, wherein the additional therapeutic agent is nab-bound paclitaxel (ABRAXANE).

54. The method according to any one of claims 1-53, wherein the sample is a tissue sample or a tumor biopsy.

55. The method according to any one of claims 1-53, wherein the sample is a formalin-fixed paraffin embedded (FFPE) sample.

56. A method of identifying a human breast tumor that is likely to be responsive to or non-responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising:

(a) obtaining a sample of the human breast tumor;

(b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature;

wherein a positive decision value indicates the breast tumor is predicted to be responsive to treatment with the antibody and a negative decision value indicates the tumor is predicted to be non-responsive to treatment with the antibody.

57. A method of identifying a patient with breast cancer that is likely to be responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising:

(a) obtaining a sample of the breast tumor;

(b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and

(c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature;

wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody.

58. A method of selecting a patient with breast cancer that is likely to be responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising:

(a) obtaining a sample of the breast tumor;

(b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1;

(c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature;

wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody; and

(d) selecting the patient for treatment when their tumor sample has a positive decision value.

59. The method of claim 56 or claim 57, further comprising:

(d) selecting a patient for treatment when the breast cancer is predicted to be responsive to treatment with the antibody.

60. The method according to any one of claims 56-59, further comprising administering an effective therapeutic amount of the antibody to the patient.

61. The method of claim 60, wherein the antibody is OMP-18R5.

62. The method of claim 56-61, wherein the treatment comprises the antibody in combination with paclitaxel.

63. A method of treating cancer in a patient, comprising: administering an effective amount of a Wnt pathway inhibitor to the patient, wherein the patient is predicted to respond to treatment with the Wnt pathway inhibitor based upon expression levels of a biomarker signature in a patient tumor sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1.

64. A kit for detecting FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 in a sample, wherein the kit comprises polynucleotides selected from the group consisting of SEQ ID NOs:62-79.

65. The kit of claim 64, which comprises:

(a) a forward primer of SEQ ID NO:62, a reverse primer of SEQ ID NO:63, and a probe comprising SEQ ID NO:64;

(b) a forward primer of SEQ ID NO:65, a reverse primer of SEQ ID NO:66, and a probe comprising SEQ ID NO:67;

(c) a forward primer of SEQ ID NO:68, a reverse primer of SEQ ID NO:69, and a probe comprising SEQ ID NO:70;

(d) a forward primer of SEQ ID NO:71, a reverse primer of SEQ ID NO:72, and a probe comprising SEQ ID NO:73;

(e) a forward primer of SEQ ID NO:74, a reverse primer of SEQ ID NO:75, and a probe comprising SEQ ID NO:76; and

(f) a forward primer of SEQ ID NO:77, a reverse primer of SEQ ID NO:78, and a probe comprising SEQ ID NO:79.

Fig. 1A

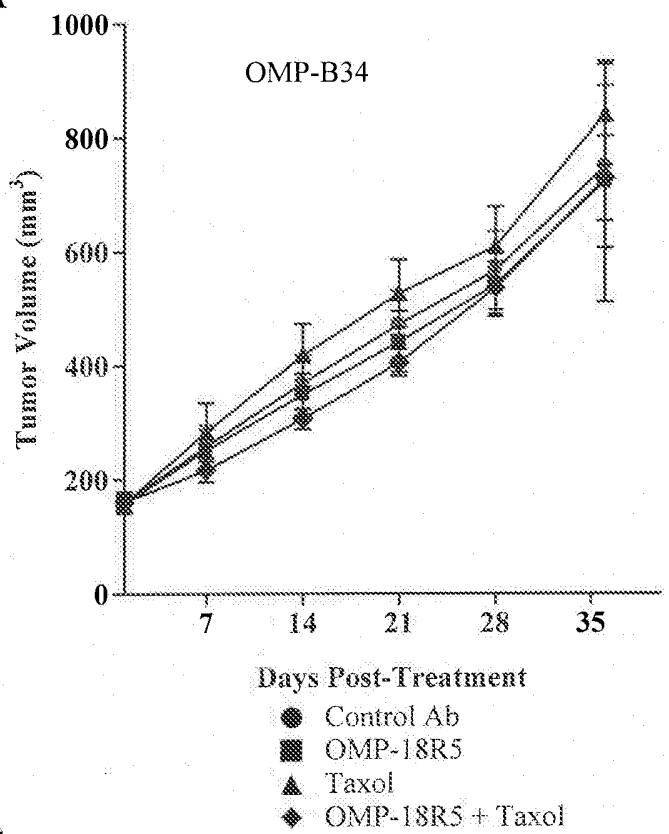

OMP-B34
Tumor Volume (mm³)
1000
800
600
400
200
0
7
14
21
28
35
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1B

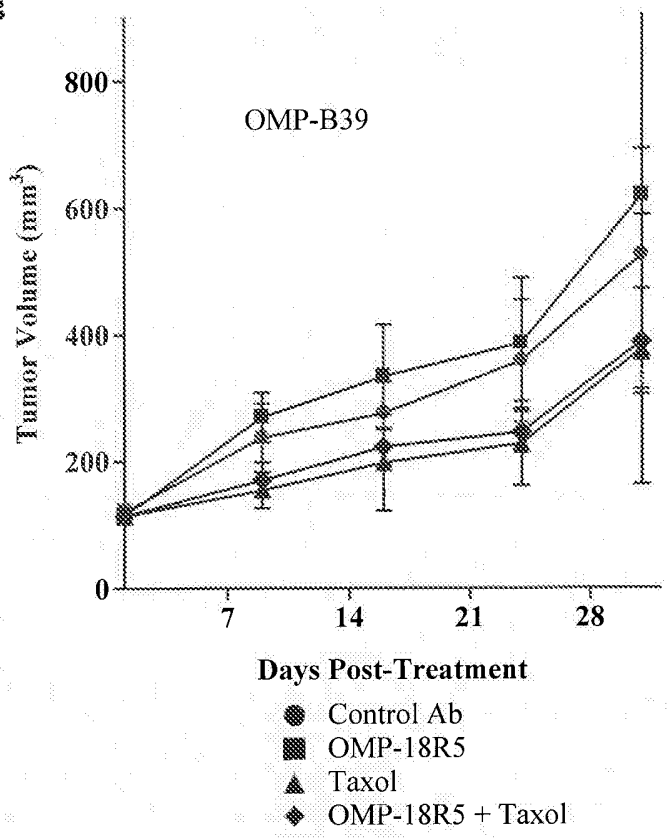

OMP-B39
Tumor Volume (mm³)
800
600
400
200
0
7
14
21
28
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1C

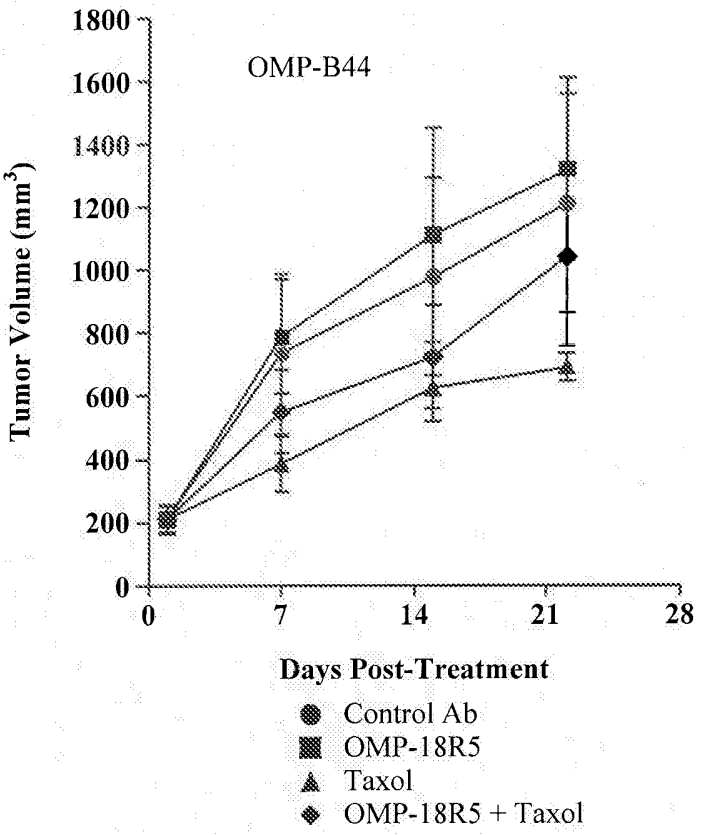

OMP-B44
Tumor Volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1D

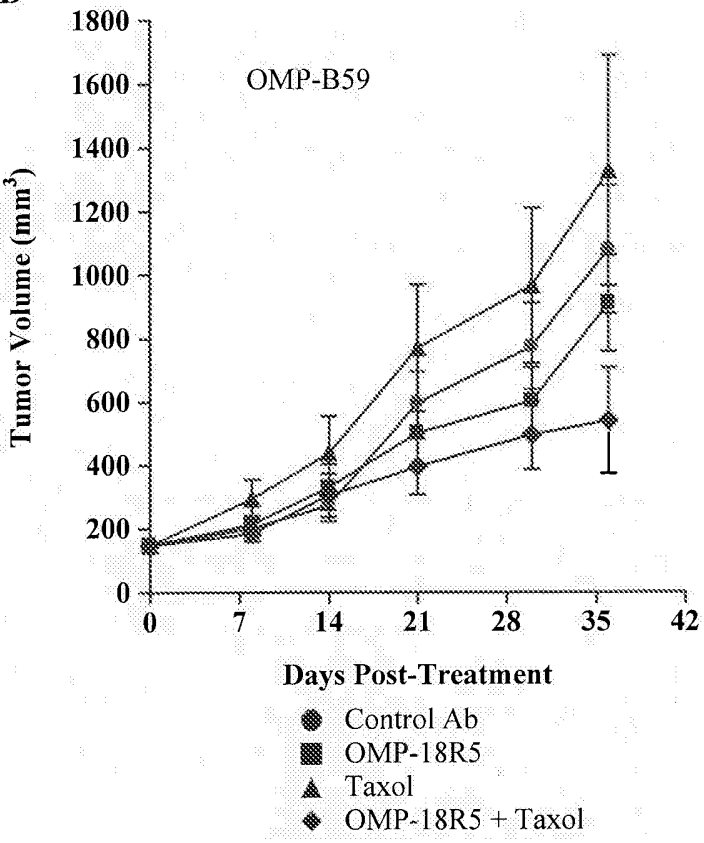

OMP-B59
Tumor Volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
35
42
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1E

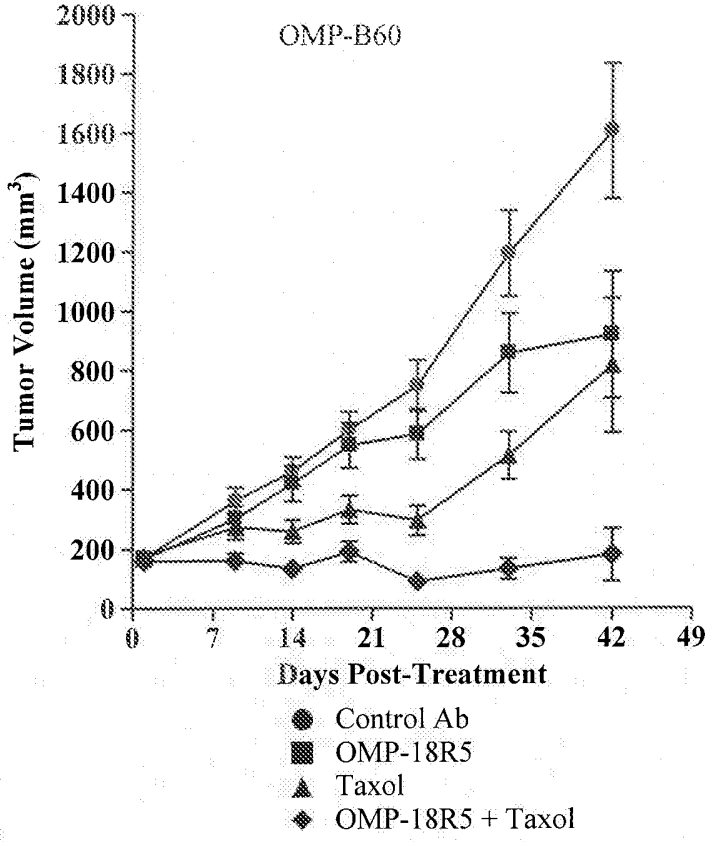

OMP-B60
Tumor Volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
35
42
49
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1F

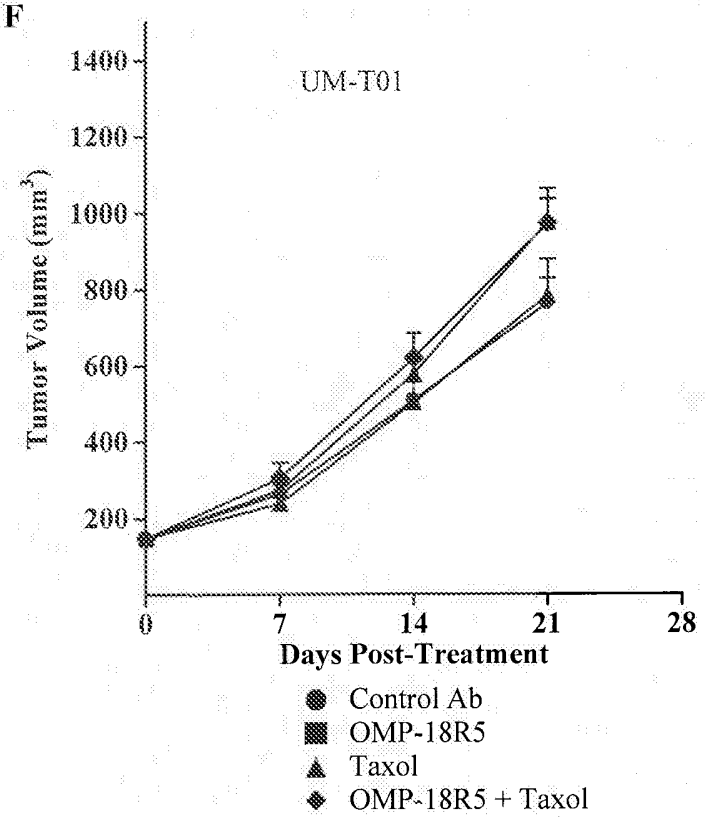

UM-T01
Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
7
14
21
28
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1G

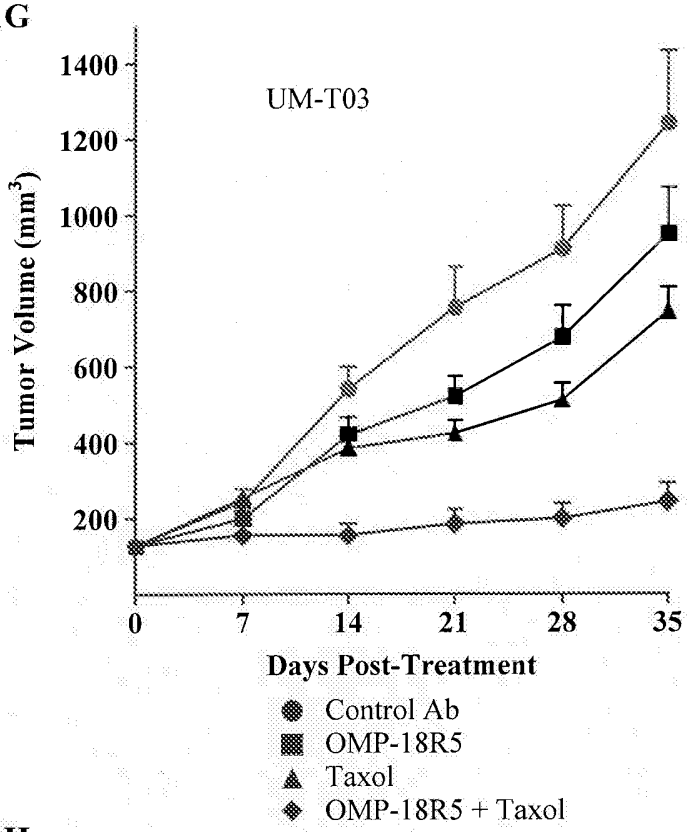
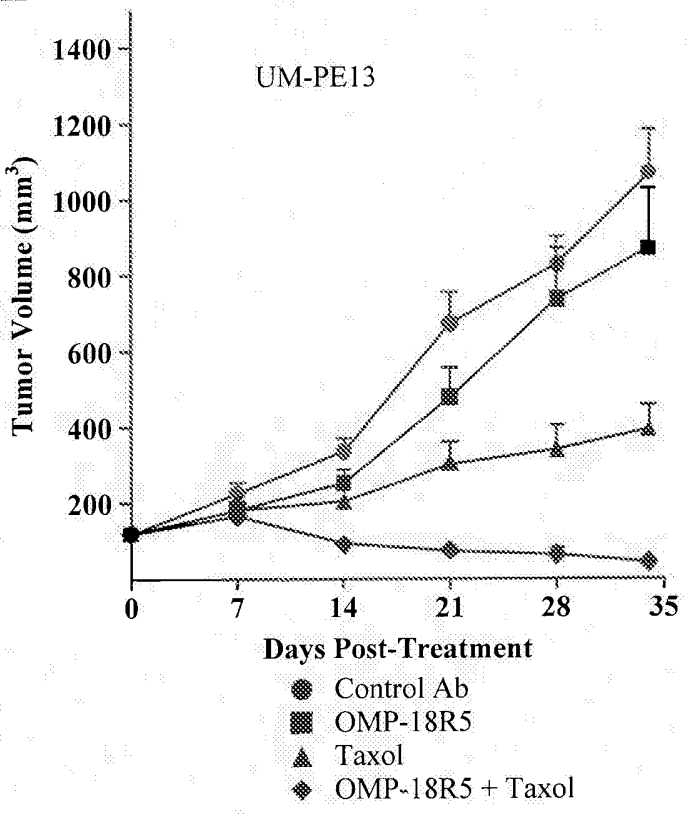

UM-T03
Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 1H


UM-PE13
Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

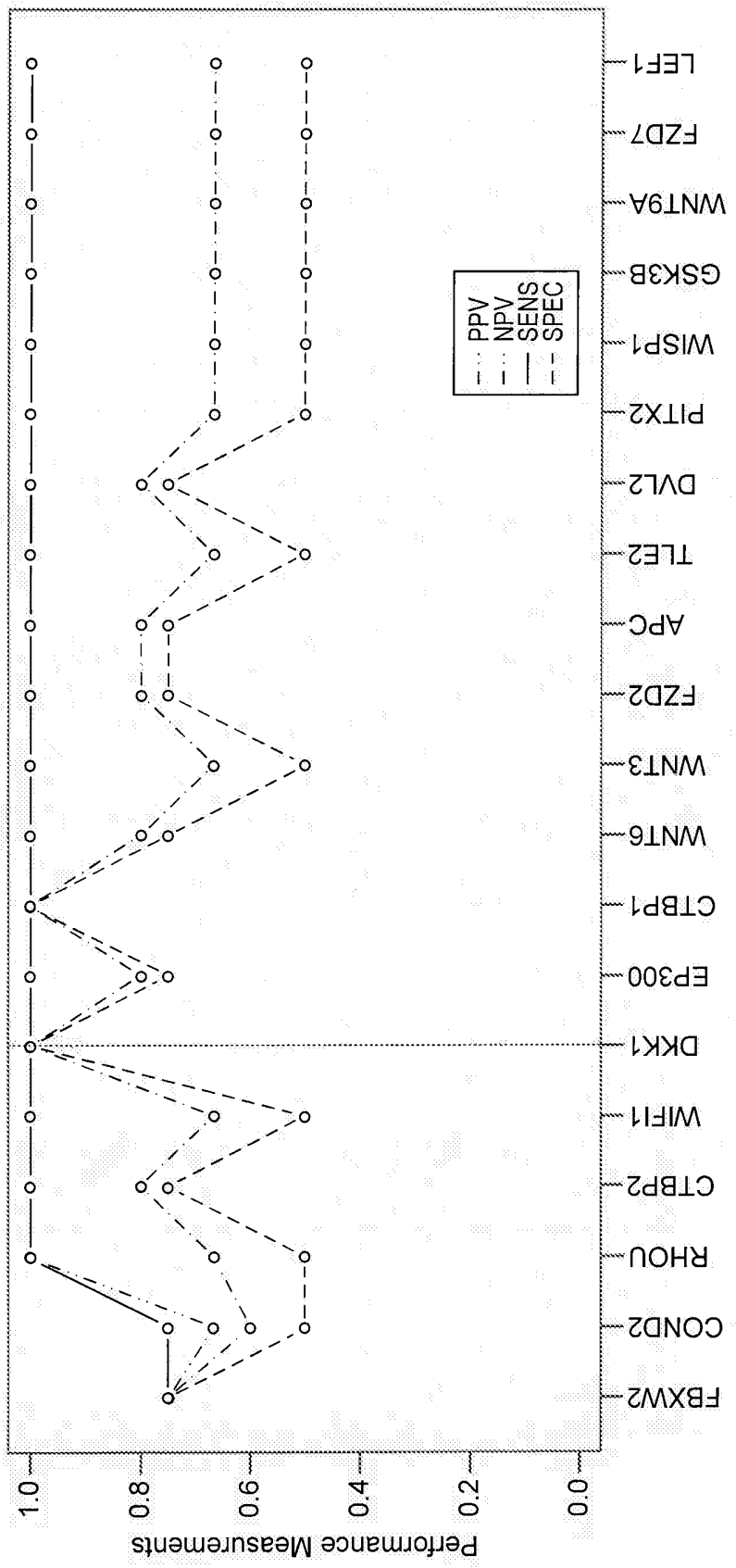

Performance Measurements
1.0
0.8
0.6
0.4
0.2
0.0
PPV
NPV
SENS
SPEC
FBXW2
CONDD2
RHOU
CTBP2
WIF11
DKK1
EP300
CTBP1
WNT6
WNT3
FZD2
APC
TLE2
DVL2
PITX2
WISP1
GSK3B
WNT9A
FZD7
LEF1

Fig. 2

Fig. 3

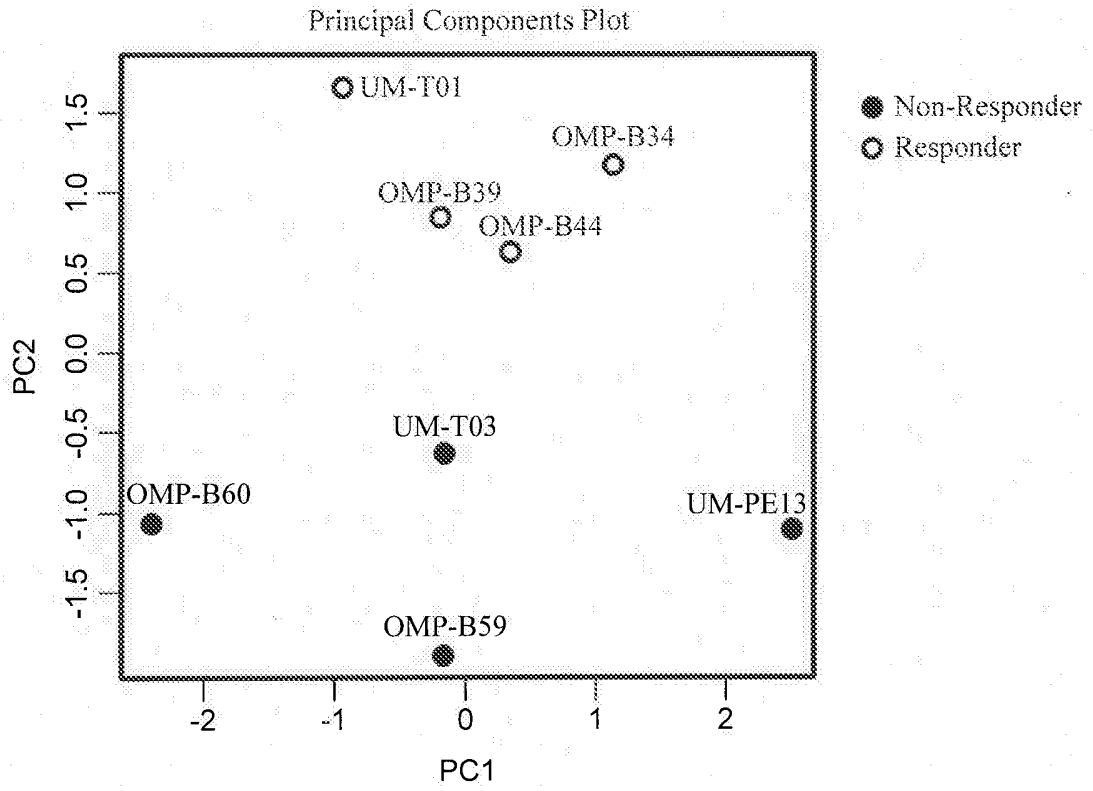
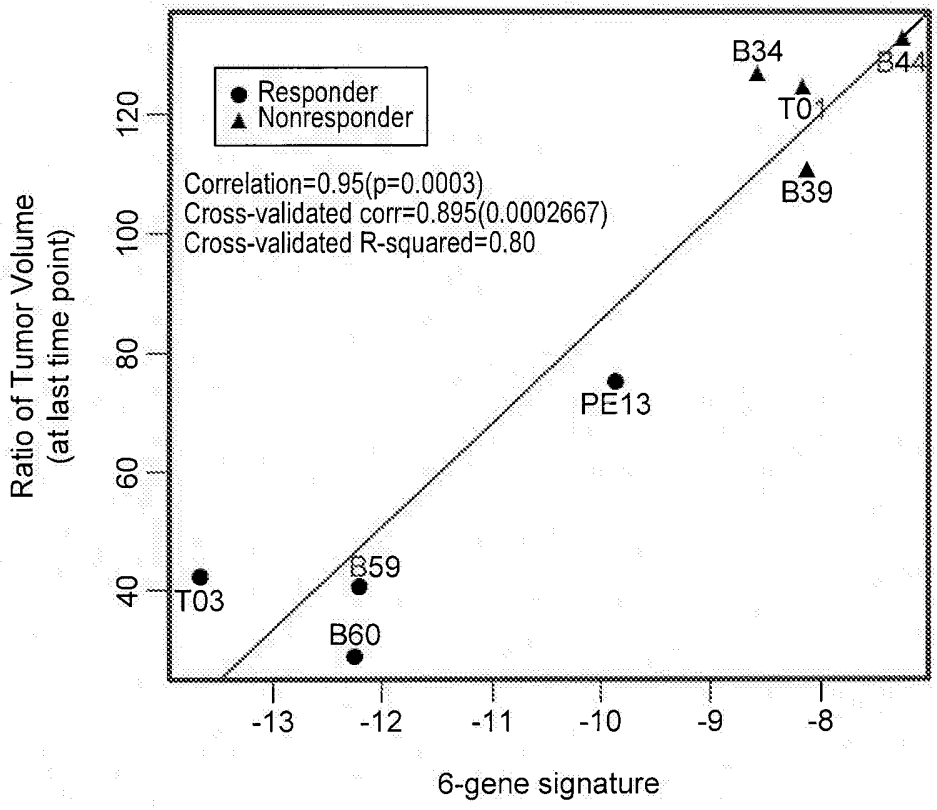

Principal Components Plot
UM-T01
OMP-B34
OMP-B39
OMP-B44
UM-T03
OMP-B60
UM-PE13
OMP-B59
Non-Responder
Responder
PC2
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2
-1
0
1
2
PC1

Fig. 4


Responder
Nonresponder
Correlation=0.95(p=0.0003)
Cross-validated corr=0.895(0.0002667)
Cross-validated R-squared=0.80
Ratio of Tumor Volume
(at last time point)
120
100
80
60
40
-13
-12
-11
-10
-9
-8
6-gene signature
B34
B44
T01
B39
PE13
B59
T03
B60

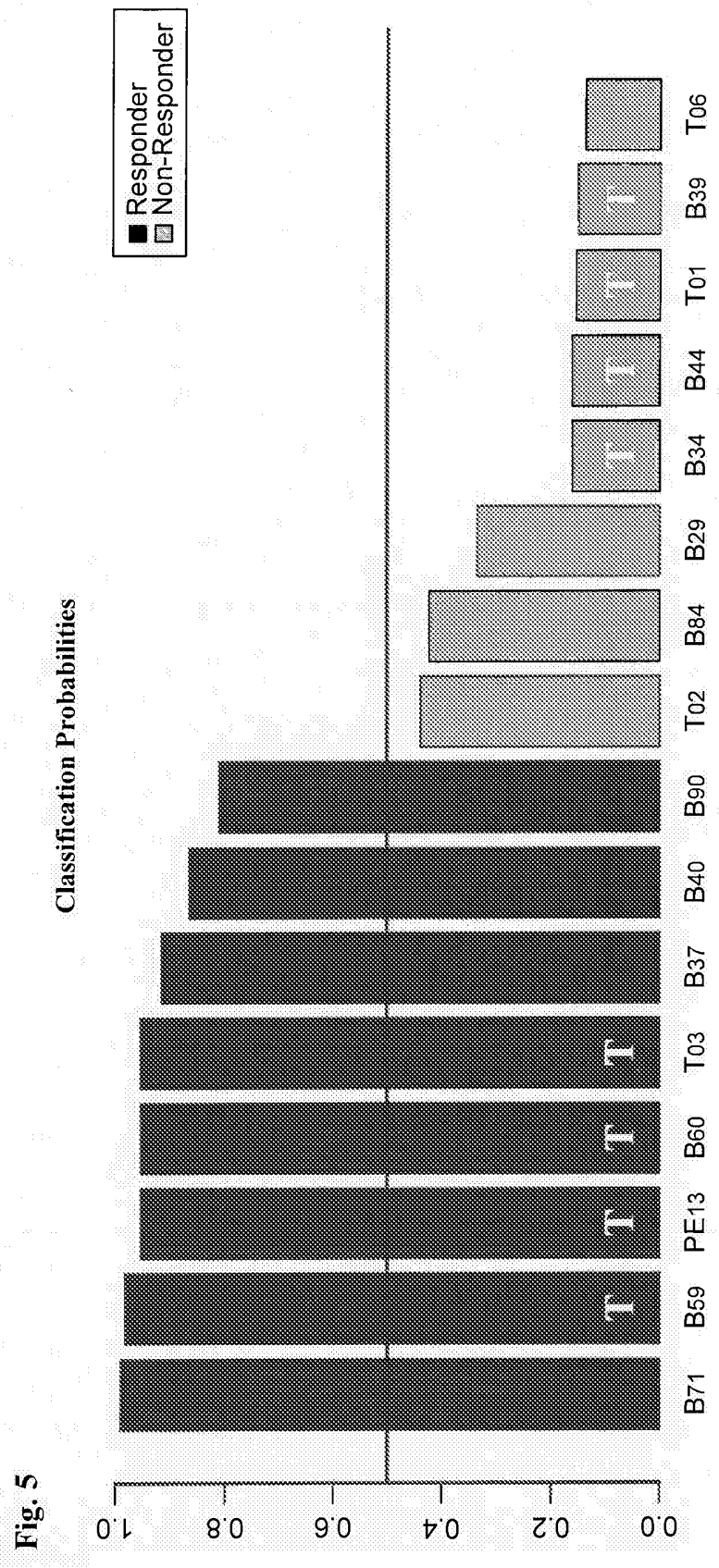

Responder
Non-Responder
Classification Probabilities
0.0
0.2
0.4
0.6
0.8
1.0
B71
B59
PE13
B60
T03
B37
B40
B90
T02
B84
B29
B34
B44
T01
B39
T06

Fig. 5

Fig. 6A

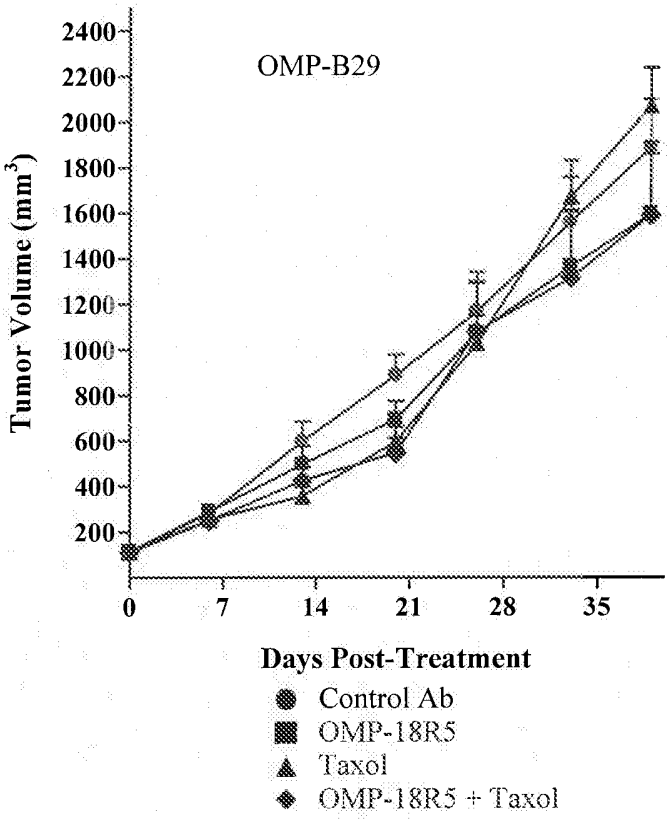

OMP-B29
Tumor Volume (mm3)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 6B

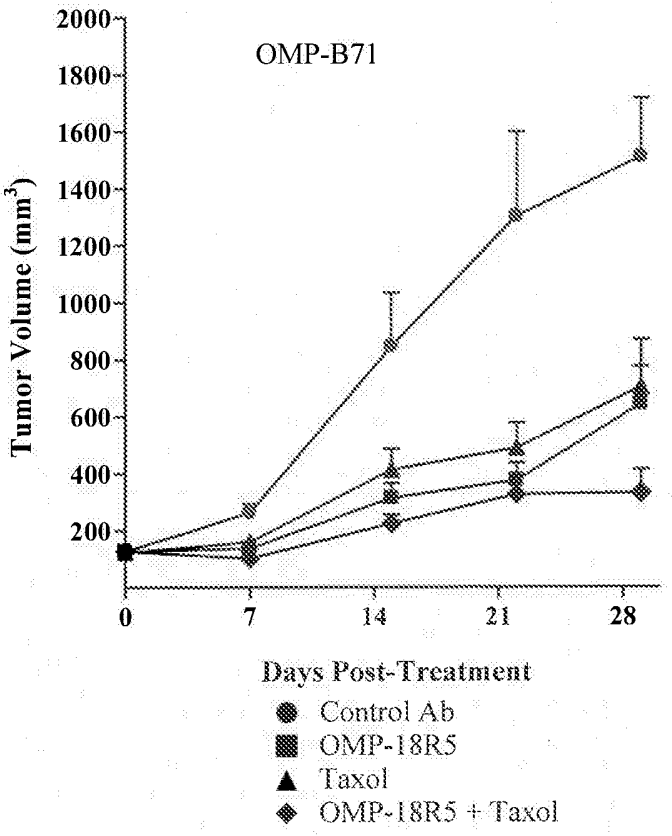

OMP-B71
Tumor Volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7
14
21
28
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 6C

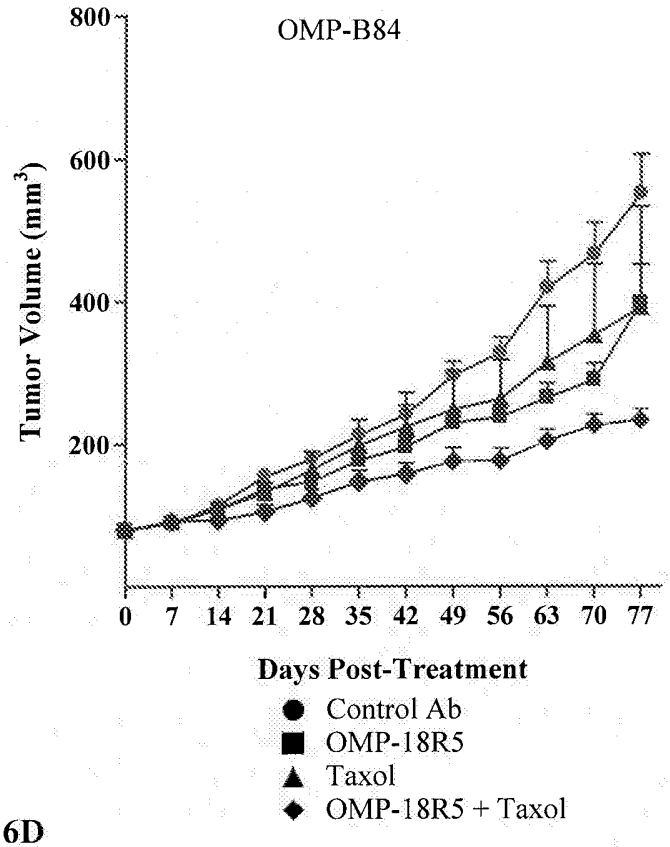

OMP-B84
Tumor Volume (mm3)
800
600
400
200
0 7 14 21 28 35 42 49 56 63 70 77
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 6D

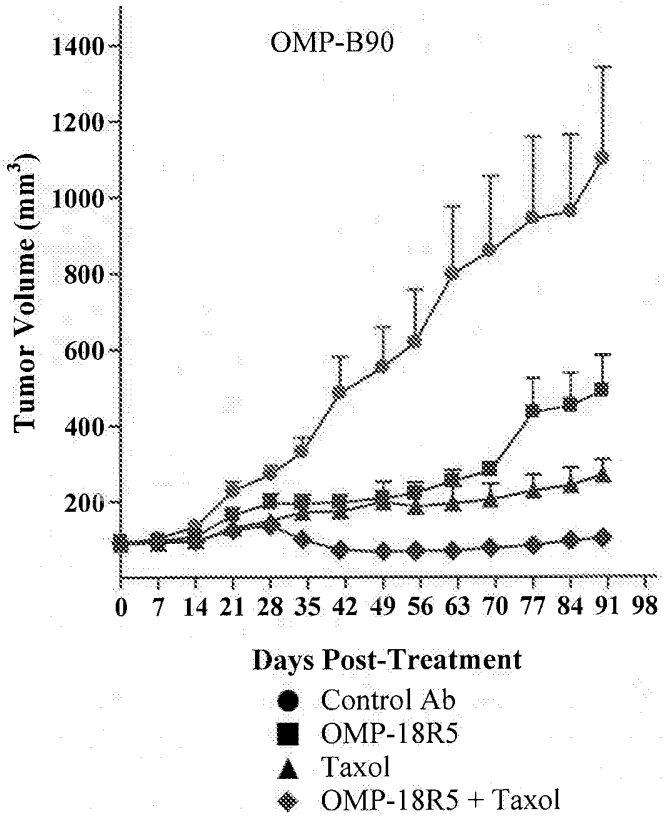

OMP-B90
Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 6E

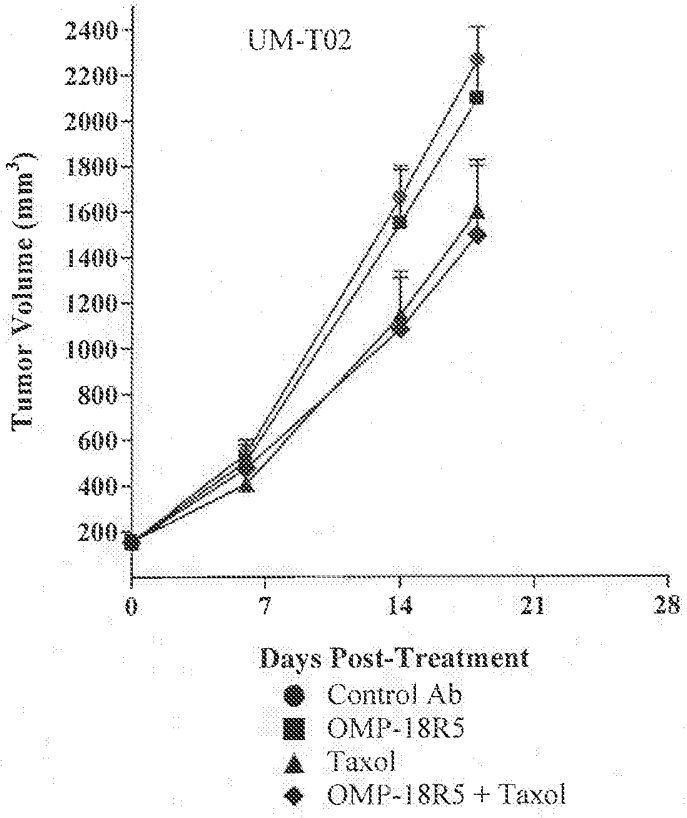

UM-T02
Tumor Volume (mm3)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7
14
21
28
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 6F

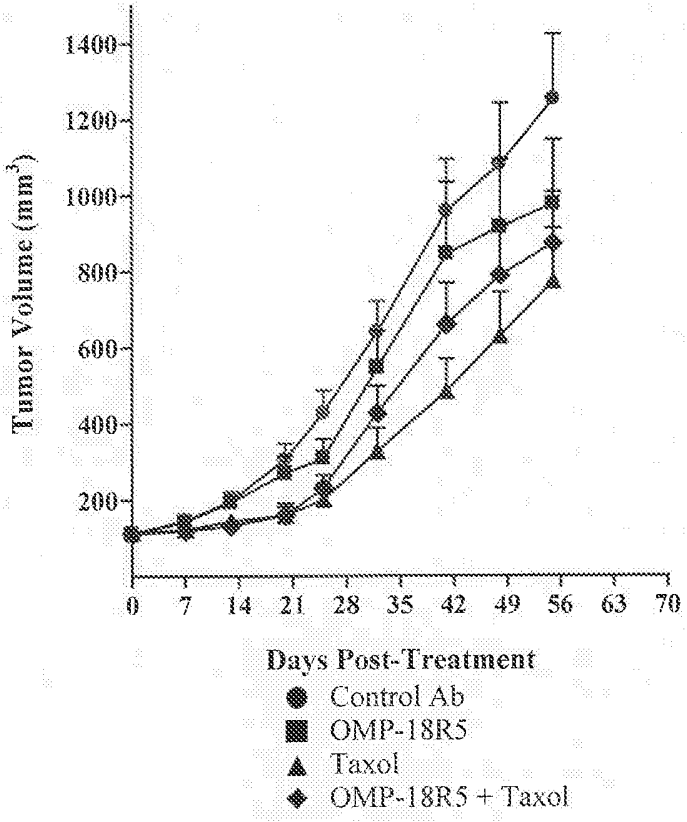
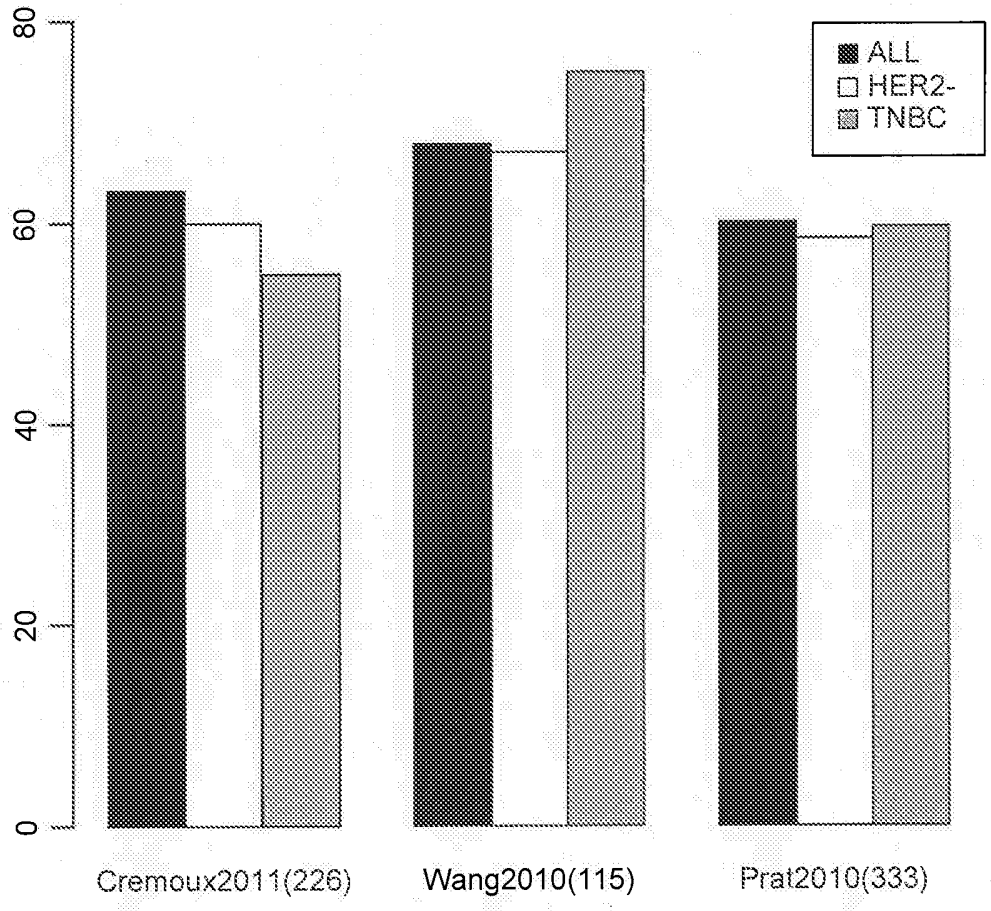

Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
42
49
56
63
70
Days Post-Treatment
Control Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

Fig. 7


80
60
40
20
0
ALL
HER2-
TNBC
Cremoux2011(226)
Wang2010(115)
Prat2010(333)

INTERNATIONAL SEARCH REPORT

PCT/US2014/068097 30.04.2015

International application No.
PCT/US14/68097

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68; A61K 38/00 (2015.01)

CPC - C12Q 1/6886, 1/6883; A61K 38/00

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)

IPC(8): C12Q 1/68; A61K 38/00 (2015.01)
CPC: C12Q 1/6886, 1/6883; A61K 38/00; USPC: 435/6.14, 6.1, 4; 702/19

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)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; Google; PubMed; ScienceDirect; 'Wnt signaling,' frizzled, 'FBXW2,' cancer, antibody, 'predict response'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0169025 A1 (ARTHUR, WT et al.) July 1, 2010; paragraphs [0010], [0018], [0038], [0043], [0098]	63
---		-----
Y		1-15, 56-59
---		----
A		64, 65
Y	KABACIK, S et al. Gene Expression Following Ionising Radiation: Identification Of Biomarkers For Dose Estimation And Prediction Of Individual Response. International Journal of Radiation Biology. February 2011; Vol. 87, No. 2; pages 115-129; page 3, right column, second paragraph; page 6, Table 3; page 13, right column, second paragraph.	1-15, 56-59
Y	US 2004/0247593 A1 (HE, B et al.) December 9, 2004; paragraphs [0008]-[0010]	56-59
A	WO 2008/039071 A2 (AGENDIA B.V.) April 3, 2008; probe sequence 32	64, 65
A	WO 2001/02568 A2 (CHIRON CORPORATION, et al.) January 11, 2001; SEQ ID NOs 402, 3331	64, 65

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 2015 (16.04.2015)	30 APR 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

International application No.
PCT/US14/68097

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Groups I+: Claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 56 (in-part), 65 (in-part) + SEQ ID NOs: 70-72

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/US14/68097

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-15, 56-59 and 63-65 are directed toward methods of identifying or classifying a human tumor or identifying or selecting a human patient with a tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor; determining the responsiveness of a human tumor to treatment with a Wnt pathway inhibitor; a method of treating cancer in a patient, comprising: (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, and administering an effective amount of a Wnt pathway inhibitor to the patient who is predicted to response to treatment; wherein the tumor is a human breast cancer tumor, and the treatment is antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8; as well as a kit comprising polynucleotides selected from the group consisting of SEQ ID NOs: 62-79.

The methods of identifying, classifying, selecting, determining the responsiveness of, and treating a cancer will be searched to the extent that the marker encompasses FBXW2, and the polynucleotide encompasses the FBXW2-associated polynucleotides, SEQ ID NOs: 70-72 (FBXW2-associated polynucleotides). It is believed that Claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 56 (in-part), 57 (in-part), 58 (in-part), 59 (in-part), 63 (in-part), 64 (in-part) and 65 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass this marker and SEQ ID NOs: 70-72 (FBXW2-associated polynucleotides). Additional marker(s) and associated sequences will searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected marker(s) and associated sequences. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: a marker encompassing CCND2, with associated SEQ ID NOs: 62-64 (CCND2-assocated polynucleotides).

Groups I+ share the technical features including: methods of identifying a human tumor that is likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the methods comprising the following methods: (1): (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) identifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression level of the biomarkers; and (2): (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be nonresponsive to the Wnt pathway inhibitor; methods of classifying a human tumor as likely to be responsive or non-responsive to treatment with a Wnt pathway inhibitor, the methods comprising the following methods: (I): (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) classifying the tumor as likely to be responsive or non-responsive to treatment based upon the expression of the biomarkers; (II): (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates the tumor is predicted to be responsive the Wnt pathway inhibitor and a negative decision value indicates the tumor is predicted to be nonresponsive the Wnt pathway inhibitor; methods of determining the responsiveness of a human tumor to treatment with a Wnt pathway inhibitor, the methods comprising the following methods: (i) (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) determining the responsiveness of the tumor to treatment based upon the expression of the biomarkers; and (ii): (a) obtaining a sample of the human tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor; methods of identifying a patient with cancer who is likely to respond to treatment with a Wnt pathway inhibitor, the method comprising the following methods: (1): (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (2): (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that the patient is predicted to respond to treatment with the Wnt pathway inhibitor; methods of selecting a patient with cancer for treatment with a Wnt pathway inhibitor, the method comprising: the following methods: ... Continued on Next Supplemental Page ...

-***-Continued on Next Supplemental Page-***-

Box No. V:
-Continued from Citations and Explanations:

... Continued from Previous Supplemental Page ... (1): (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) selecting the patient for treatment based upon the expression level of the biomarkers; and (II): (a) obtaining a sample of the patient's tumor; (b) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; and (d) selecting the patient for treatment when their tumor sample has a positive decision value; methods of treating cancer in a patient, comprising the following methods: (i): (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's tumor; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (b) administering an effective amount of a Wnt pathway inhibitor to the patient who is likely to response to treatment; and (ii): (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's tumor; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that a patient is predicted to respond to treatment with the Wnt pathway inhibitor; and (b) administering an effective amount of a Wnt pathway inhibitor to the patient who is predicted to response to treatment; methods for increasing the likelihood of effective treatment with a Wnt pathway inhibitor, comprising the following methods: (1): (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) identifying the patient who is likely to respond to treatment based upon the expression level of the biomarkers; and (b) administering an effective amount of the Wnt pathway inhibitor to the patient who is likely to respond to treatment; and (2): (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor, wherein the identification comprises: (i) obtaining a sample of the patient's cancer; (ii) measuring the expression level of each biomarker of a biomarker signature in the sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature; wherein a positive decision value indicates that a patient is predicted to respond to treatment with the Wnt pathway inhibitor; and (b) administering an effective amount of the WNT pathway inhibitor to the patient whose tumor has a positive decision value; a method of treating cancer in a patient, comprising: administering an effective amount of a Wnt pathway inhibitor to the patient, wherein the patient is predicted to respond to treatment with the Wnt pathway inhibitor based upon expression levels of a biomarker signature in a patient tumor sample, wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; a kit for detecting FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 in a sample, wherein the kit comprises polynucleotides selected from the group consisting of SEQ ID NOs: 62-79; a method of identifying a human breast tumor that is likely to be responsive to or non-responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the human breast tumor; (b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast tumor is predicted to be responsive to treatment with the antibody and a negative decision value indicates the tumor is predicted to be non-responsive to treatment with the antibody and a method of selecting a patient with breast cancer that is likely to be responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the breast tumor; (b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody; and (d) selecting the patient for treatment when their tumor sample has a positive decision value.

However, these shared technical features are previously disclosed by US 2010/0169025 A1 to Arthur, et al. (hereinafter 'Arthur') in view of US 2004/0247593 A1 to He, et al. (hereinafter 'He').

Arthur discloses methods of identifying (paragraph [0010]) a human tumor (patients tumors; paragraph [0010]) that is likely to be responsive or non-responsive to treatment (to prospectively identify patients with pathway targeting inhibitors; paragraph [0010]) with a Wnt pathway inhibitor (to a pathway targeting inhibitor, including a Wnt pathway inhibitor; paragraphs [0010], [0018]), the methods comprising the following methods: (1): (a) obtaining a sample of the human tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); and (c) identifying the tumor as likely to be responsive or non-responsive to treatment (using the pathway regulation status as an indicator of the likelihood that a subject will respond to therapies, including inhibitors of the Wnt pathway (paragraph [0018]) based upon the expression level of the biomarkers (paragraphs [0010], [0018]); and (2): (a) obtaining a sample of the human tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018])); (b) measuring the expression level of each biomarker of a biomarker signature in the sample (determining (measuring) the expression of biomarkers of a biomarker signature in the sample; paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); and ... Continued on Next Supplemental Page ...

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

... Continued from Previous Supplemental Page ... (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score; paragraph [0087]); wherein a positive decision value (wherein a signature score above a threshold value; paragraph [0087]) indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor (indicates the tumor has unregulated Wnt signaling and may be responsive to treatment with a Wnt signaling inhibitor; paragraphs [0018], [0087]) and a negative decision value indicates the tumor is predicted to be nonresponsive to the Wnt pathway inhibitor (paragraph [0091]); a method of classifying (paragraph [0010]) a human tumor (patients tumors; paragraph [0010]) as likely to be responsive or non-responsive to treatment (to prospectively identify patients with pathway targeting inhibitors; paragraph [0010]) with a Wnt pathway inhibitor (to a pathway targeting inhibitor, including a Wnt pathway inhibitor; paragraphs [0010], [0018]), the methods comprising the following methods: (1): (a) obtaining a sample of the human tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); and (c) classifying the tumor as likely to be responsive or non-responsive to treatment (using the pathway regulation status as an indicator of the likelihood that a subject will respond to therapies, including inhibitors of the Wnt pathway; paragraph [0018]) based upon the expression level of the biomarkers (paragraphs [0010], [0018]); and (2): (a) obtaining a sample of the human tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018])); (b) measuring the expression level of each biomarker of a biomarker signature in the sample (determining (measuring) the expression of biomarkers of a biomarker signature in the sample; paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score; paragraph [0087]); wherein a positive decision value (wherein a signature score above a threshold value; paragraph [0087]) indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor (indicates the tumor has unregulated Wnt signaling and may be responsive to treatment with a Wnt signaling inhibitor; paragraphs [0018], [0087]) and a negative decision value indicates the tumor is predicted to be nonresponsive to the Wnt pathway inhibitor (paragraph [0091]); methods of determining the responsiveness of (methods of determining the likelihood of a tumor to respond; paragraph [0018]) a human tumor (patients tumors (a human tumor); paragraph [0010]) for treatment with a Wnt pathway inhibitor (to therapy (treatment) with a Wnt pathway inhibitor; paragraph [0018]), the methods comprising the following methods: (i) (a) obtaining a sample of the human tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1); paragraph [0033], Table 1); and (c) determining the responsiveness of the tumor to treatment (determining the likelihood of the tumor to respond to therapy; paragraph [0018]) based upon the expression of the biomarkers (based upon a signature score which depends on the expression of the biomarkers; paragraphs [0018], [0087]); and (ii): (a) obtaining a sample of the human tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (wherein the signature comprises one or more biomarkers; paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1); paragraph [0033], Table 1); and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score (calculating a decision value based upon the standardized expression of the biomarkers in the signature); paragraph [0087]); wherein a positive decision value (wherein a signature score above a threshold value; paragraph [0087]) indicates the tumor is predicted to be responsive to the Wnt pathway inhibitor (indicates the tumor has unregulated Wnt signaling and may be responsive to treatment with a Wnt signaling inhibitor, paragraphs [0018], [0087]); methods of identifying a patient with cancer (paragraphs [0010], [0018]) who is likely to respond to treatment with a Wnt pathway inhibitor (likely to respond to therapy (treatment) with a Wnt pathway inhibitor; paragraph [0018]), the method comprising the following methods: (1): (a) obtaining a sample of the patient's tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); and (c) identifying the patient who is likely to respond to treatment (using the pathway regulation status as an indicator of the likelihood that a subject will respond to therapies including inhibitors of the Wnt pathway; paragraph [0018]) based upon the expression level of the biomarkers (paragraphs [0010], [0018]); and (2): (a) obtaining a sample of the patient's tumor (obtaining a cell sample of a subject, including a tumor cell sample (paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1) paragraph [0033]; Table 1); and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score; paragraph [0087]); ... Continued on Next Supplemental Page ...

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

... Continued from Previous Supplemental Page ... wherein a positive decision value (wherein a signature score above a threshold value; paragraph [0087]) indicates that the patient is predicted to respond to treatment with the Wnt pathway inhibitor (indicates the tumor in the subject has unregulated Wnt signaling and may be responsive to treatment with a Wnt signaling inhibitor; paragraphs [0018], [0087]); methods of selecting (paragraph [0043]) a patient with cancer for treatment (a treatment for a patient with cancer; paragraph [0043]) with a Wnt pathway inhibitor (paragraph [0043]), the method comprising: the following methods: (1): (a) obtaining a sample of the patient's tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); and (c) selecting the patient for treatment based upon the expression level of the biomarkers (selecting the treatment for the patient (selecting the patient for treatment) based upon the expression levels of the biomarkers, as determined by a signature score; paragraphs [0018], [0043], [0087]); and (II): (a) obtaining a sample of the patient's tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1) paragraph [0033]; Table 1); and (c) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score; paragraph [0087]); and (d) selecting the patient for treatment (selecting a treatment for the patient; paragraph [0043]) when their tumor sample has a positive decision value (when the tumor sample has a signature score above a threshold; paragraphs [0043], [0087]); methods of treating cancer in a patient (methods of treating cancer in subject (patient); paragraphs [0010], [0018]), comprising the following methods: (i): (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor (identifying if the tumor in the subject is likely to respond to therapy with a Wnt pathway inhibitor; paragraph [0018]), wherein the identification comprises: (i) obtaining a sample of the patient's tumor (paragraphs [0010], [0018]); (b) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1) paragraph [0033], Table 1); and (iii) identifying the patient who is likely to respond to treatment (identifying if the tumor in the subject is likely to respond to therapy; paragraph [0018]) based upon the expression level of the biomarkers (paragraphs [0010], [0018]); and (b) administering an effective amount (providing effective treatment of cancer; paragraphs [0010], [0038]) of a Wnt pathway inhibitor to the patient who is likely to respond to treatment (of a Wnt pathway inhibitor to the patient who is likely to respond to treatment; paragraphs [0010], [0018]); and (ii): (a) identifying if the patient is likely to respond to treatment with a Wnt pathway inhibitor (identifying if the tumor in the subject is likely to respond to therapy with a Wnt pathway inhibitor; paragraph [0018]), wherein the identification comprises: (i) obtaining a sample of the patient's tumor (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (ii) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1) paragraph [0033]; Table 1); and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score (calculating a decision value based upon the standardized expression of the biomarkers in the signature); paragraph [0087]); wherein a positive decision value (wherein a signature score above a threshold value; paragraph [0087]) indicates that a patient is predicted to respond to treatment with the Wnt pathway inhibitor (indicates the tumor in the subect has unregulated Wnt signaling and may be responsive to treatment with a Wnt signaling inhibitor; paragraphs [0018], [0087])); and (b) administering an effective amount (providing effective treatment of cancer; paragraphs [0010], [0038]) of a Wnt pathway inhibitor to the patient who is predicted to respond to treatment (of a Wnt pathway inhibitor to the patient who is likely (predicted) to respond to treatment; paragraphs [0010], [0018]); methods for increasing the likelihood of effective treatment with a Wnt pathway inhibitor (methods for identifying patients having tumors likely to respond to therapy with a Wnt pathway inhibitor to provide effective treatment of cancer; paragraphs [0018], [0038]), comprising the following methods: (1): (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor (identifying if a tumor in a subject is likely to respond to therapy with a Wnt pathway inhibitor; paragraph [0018]), wherein the identification comprises: (i) obtaining a sample of the patient's cancer (obtaining a sample of the patient's tumor (cancer); paragraphs [0010], [0018]); (ii) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signature (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1) paragraph [0033], Table 1) and (iii) identifying the patient who is likely to respond to treatment (identifying if the tumor in the subject is likely to respond to therapy (identifying the patient who is likely to respond to treatment); paragraph [0018]) based upon the expression level of the biomarkers (paragraphs [0010], [0018]); and (b) administering an effective amount (providing effective treatment of cancer; paragraphs [0010], [0038]) of a Wnt pathway inhibitor to the patient who is likely to respond to treatment (paragraphs [0010], [0018]); and (2): (a) identifying if a patient has a tumor that is likely to respond to treatment with a Wnt pathway inhibitor (identifying if a tumor in a subject is likely to respond to therapy with a Wnt pathway inhibitor; paragraph [0018]), wherein the identification comprises: (i) obtaining a sample of the patient's cancer (obtaining a cell sample of a subject, including a tumor cell sample; paragraphs [0010], [0018]); (ii) measuring the expression level of each biomarker (assessing pathway activation status by measuring gene expression signatures for pathway activation; paragraphs [0010], [0018]) of a biomarker signatuture (paragraphs [0010], [0018]) in the sample (paragraphs [0010], [0018]), wherein the signature comprises one or more of the biomarkers (paragraphs [0010], [0018]) FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1 (FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1) paragraph [0033]; Table 1)); .. Continued on Next Supplemental Page ...

-***-Continued on Next Supplemental Page-***-

International application No.
PCT/US14/68097

-***-Continued from Previous Supplemental Page:

... Continued from Previous Supplemental Page ... and (iii) calculating a decision value based upon the standardized expression of the biomarkers in the signature (calculating a signature score based on the gene expression, determining if the score is significantly different from a mean score and determining that the sample has Wnt pathway degregulation based on the score; paragraph [0087]); wherein a positive decision value (wherein a signature score above a threshold value; paragraph [0087]) indicates that a patient is predicted to respond to treatment with the Wnt pathway inhibitor (indicates the tumor in the subect has unregulated Wnt signaling and may be responsive to treatment with a Wnt signaling inhibitor; paragraphs [0018], [0087])); and (b) administering an effective amount (providing effective treatment of cancer; paragraphs [0010], [0038]) of the WNT pathway inhibitor to the patient whose tumor has a positive decision value (of a Wnt pathway inhibitor to the patient whose tumor has a signature score above a threshold; paragraphs [0010], [0018], [0087]); a method of treating cancer in a patient (a method of providing effective treatment of cancer in a patient; paragraphs [0010], [0038]), comprising: administering an effective amount (providing effective treatment of cancer; paragraphs [0010], [0038]) of a Wnt pathway inhibitor to the patient (paragraph [0018]), wherein the patient is predicted to respond to treatment with the Wnt pathway inhibitor (wherein the tumor in the subject is likely to respond to therapy with the Wnt pathway inhibitor; paragraph [0018]) based upon expression levels of a biomarker signature (paragraphs [0018], [0087]) in a patient tumor sample (in a subject (patient) tumor sample; paragraph [0018]), wherein the signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; paragraph [0033], Table 1); a kit (paragraph [0248]), wherein the kit comprises polynucleotides (wherein the kit comprises a microarray ready for hybridization to polynucleotide molecules; paragraph [0248]); and wherein Wnt pathway activation is common in metaplastic carcinomas of the breast (paragraph [0009]), the use of antibodies to affect the Wnt signaling pathway (paragraph [0101]), and wherein the sequence of DKK1 is disclosed in SEQ ID NO: 91, which includes SEQ ID NO: 67 of the instant PCT application (the sequence of DKK1 is disclosed in SEQ ID NO: 91 of the Arthur reerence, including nt 379-402 therof; Table 1 and sequence listing; wherein nt 379-402 of SEQ ID NO: 91 of the Arthur reference is 100% identical to SEQ ID NO: 67 of the instant PCT application).

Arthur does not disclose: selected from the group consisting of SEQ ID NOs: 62-79; a method of identifying a human breast tumor that is likely to be responsive to or non-responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the human breast tumor; (b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast tumor is predicted to be responsive to treatment with the antibody and a negative decision value indicates the tumor is predicted to be non-responsive to treatment with the antibody and a method of selecting a patient with breast cancer that is likely to be responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the breast tumor; (b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody; and (d) selecting the patient for treatment when their tumor sample has a positive decision value.

He discloses methods of inhibiting the growth of cancer cells that overexpress a Wnt protein (paragraph [0008]); including the use of an agent including an antibody to any of FZD1, FZD2, FZD5, FZD7 or FZD8 (including the use of an agent including an antibody to any of FZD1, FZD2, FZD5, FZD7 or FZD8; paragraphs [0008], [0009]), including wherein the cancer is breast cancer (paragraph [0011]); and wherein over-expression of DKK induces apoptosis in cancer cells (paragraphs [0063], [0204]).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Arthur, for integrating the treatment for breast cancer using an anti-frizzled antibody, such as an anti-FZD1, FZD2, FZD5, FZD7 or FZD8 antibody, as disclosed by He, for providing an effective treatment for breast cancer determined to have altered Wnt pathway activity, as previously disclosed by Arthur, including alterations in expression of key apoptosis-inducing Wnt pathway proteins, such as DKK1, as disclosed by He, thereby producing a method of identifying a human breast tumor that is likely to be responsive to or non-responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the human breast tumor; (b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; and (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast tumor is predicted to be responsive to treatment with the antibody and a negative decision value indicates the tumor is predicted to be non-responsive to treatment with the antibody and a method of selecting a patient with breast cancer that is likely to be responsive to treatment with an antibody that specifically binds at least one human frizzled (FZD) selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, the method comprising: (a) obtaining a sample of the breast tumor; (b) measuring the biomarker expression level of each biomarker of a biomarker signature in the sample, wherein the biomarker signature comprises one or more of the biomarkers FBXW2, CCND2, RHOU, CTBP2, WIF1, and DKK1; (c) calculating a decision value based upon the standardized expression of the biomarkers in the biomarker signature; wherein a positive decision value indicates the breast cancer is predicted to be responsive to treatment with the antibody; and (d) selecting the patient for treatment when their tumor sample has a positive decision value. Additionally, it would have been obvious to a person of ordinary skill in the art, at the time the invention, to have recognized that the antibodies for treatment of breast cancer would have been useful inhibitors of the Wnt pathway for treatment in cancers having unregulated Wnt pathway activity, as previously disclosed by Arthur. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Arthur, for implementing determining appropriate sequences within the target genes for the production of probes for a kit, or for accompanying primers, where the primer sequences would have been selected to have sufficient separation between the two to produce an identifiable PCR product in the amplification of cellular RNA into DNA as a part of performing the determination of the expression of target genes, while having minimal self-complementarity, or cross-primer complementarity, for selecting sequences, such as nt 379-402 of SEQ ID NO: 91, as disclosed by Arthur, as an appropriate primer or probe sequence for a kit for the effective determination of the expression of the target genes in a tumor sample, as disclosed by Arthur.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Arthur and He references, unity of invention is lacking.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105829547 A
(43)申请公布日 2016.08.03

(21)申请号 201480065718.7

(22)申请日 2014.12.02

(30)优先权数据
61/910,663 2013.12.02 US
61/975,339 2014.04.04 US

(85)PCT国际申请进入国家阶段日
2016.06.01

(86)PCT国际申请的申请数据
PCT/US2014/068097 2014.12.02

(87)PCT国际申请的公布数据
WO2015/084808 EN 2015.06.11

(83)生物保藏信息
PTA-9541 2008.09.29

(71)申请人 昂科梅德制药有限公司
地址 美国加利福尼亚州

(72)发明人 安·M·卡保恩 张春

(74)专利代理机构 上海胜康律师事务所 31263
代理人 樊英如 邱晓敏

(51)Int.Cl.
C12Q 1/68(2006.01)
A61K 38/00(2006.01)

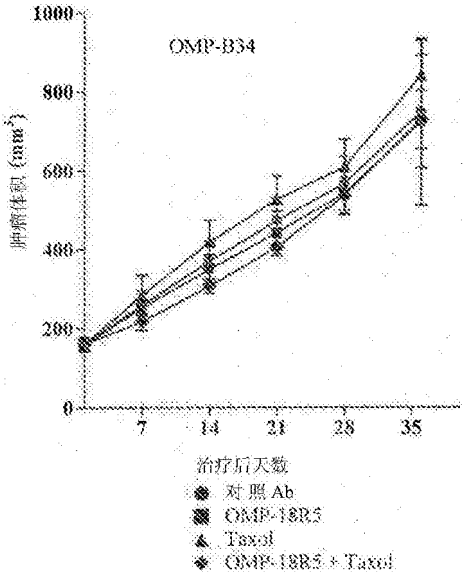
权利要求书6页 说明书69页
序列表43页 附图19页
按照条约第19条修改的权利要求书3页

(54)发明名称

与Wnt途径抑制剂有关的预测性生物标记物的鉴别

(57)摘要

本发明提供用于鉴别可能对Wnt途径抑制剂的治疗有反应的肿瘤的生物标记物。本发明亦提供用于鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的肿瘤及/或病患的方法。本发明提供用于治疗癌症病患的方法，其中该癌症系经预测为对Wnt途径抑制剂有反应。



CN 105829547 A

1.一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的方法，该方法包含：

(a)获得该人肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据所述生物标记物的表达量，鉴别该肿瘤为可能对治疗有反应或无反应。

2.一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的方法，该方法包含：

(a)获得该人肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据该标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应，且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。

3.一种分类人肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法，该方法包含：

(a)获得该人肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据所述生物标记物的表达，分类该肿瘤为可能对治疗有反应或无反应。

4.一种分类人肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法，该方法包含：

(a)获得该人肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据该标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该肿瘤系经预测为对Wnt途径抑制剂有反应，且阴性判定值显示该肿瘤系经预测为对Wnt途径抑制剂无反应。

5.一种测定人肿瘤对Wnt途径抑制剂的治疗的反应性的方法，该方法包含：

(a)获得该人肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据所述生物标记物的表达，判定该肿瘤对治疗的反应性。

6.一种测定人肿瘤对Wnt途径抑制剂的治疗的反应性的方法，该方法包含：

(a)获得该人肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据该标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应。

7.一种鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的方法，该方法包含：

(a)获得该病患肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据所述生物标记物的表达量，鉴别可能对治疗有反应的病患。

8.一种鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的方法，该方法包含：

(a)获得该病患肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据该标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该病患系经预测为对Wnt途径抑制剂的治疗有反应。

9.一种选择癌症病患以接受Wnt途径抑制剂的治疗的方法，该方法包含：

(a)获得该病患肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据所述生物标记物的表达量，选择该将接受治疗的病患。

10.一种选择癌症病患以接受Wnt途径抑制剂的治疗的方法，该方法包含：

(a)获得该病患肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；

(c)根据该生物标记物标签中的所述生物标记物的标准化表达，计算判定值；以及

(d)当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。

11.一种治疗病患的癌症的方法，其包含：

(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含：

(i)获得该病患肿瘤的样本；

(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(iii)根据所述生物标记物的表达量，鉴别可能对治疗有反应的病患；以及

(b)对该可能对治疗有反应的病患投予有效量的Wnt途径抑制剂。

12.一种治疗病患的癌症的方法，其包含：

(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含：

(i)获得该病患肿瘤的样本；

(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(iii)根据该标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示病患系经预测为对Wnt途径抑制剂的治疗有反应；以及

(b)对该经预测为对治疗有反应的病患投予有效量的Wnt途径抑制剂。

13.一种增加Wnt途径抑制剂的有效治疗的可能性的方法，其包含：

(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤，其中该鉴别包含：

(i)获得该病患癌症的样本;

(ii)测量该样本中的生物标记物标签的各生物标记物的表达量,其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及

(iii)根据所述生物标记物的表达量,鉴别可能对治疗有反应的病患;以及

(b)对该可能对治疗有反应的病患投予有效量的该Wnt途径抑制剂。

14.一种增加Wnt途径抑制剂的有效治疗的可能性的方法,其包含:

(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤,其中该鉴别包含:

(i)获得该病患癌症的样本;

(ii)测量该样本中的生物标记物标签的各生物标记物的表达量,其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及

(iii)根据该标签中的所述生物标记物的标准化表达,计算判定值;

其中阳性判定值显示病患系经预测为对Wnt途径抑制剂的治疗有反应;以及

(b)对肿瘤具有阳性判定值的病患投予有效量的该Wnt途径抑制剂。

15.如权利要求1至14中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1、EP300、CTBP1、WNT6、WNT3、FZD2、APC、TLE2、DVL2、PITX2、WISP1、GSK3B、WNT9A、FZD7及LEF1中的一或多种。

16.如权利要求1至15中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1、EP300及CTBP1中的一或多种。

17.如权利要求1至16中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的两种或两种以上。

18.如权利要求1至16中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的三种或三种以上。

19.如权利要求1至16中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的四种或四种以上。

20.如权利要求1至16中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的五种或五种以上。

21.如权利要求1至16中任一项的方法,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1。

22.如权利要求1至16中任一项的方法,其中该生物标记物标签由生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1组成。

23.如权利要求1至22中任一项的方法,其中各生物标记物的表达系由PCR为基础的测试测量。

24.如权利要求1至23中任一项的方法,其中各生物标记物的表达系由qPCR测试测量。

25.如权利要求1至22中任一项的方法,其中各生物标记物的表达系由微阵列测量。

26.如权利要求1至25中任一项的方法,其中各生物标记物的标准化表达系藉由测量各生物标记物的表达量且将其乘以对应重量决定,其中各生物标记物的重量系由该表达标签决定。

27.如权利要求1至26中任一项的方法,其中该判定值系根据下述方程式计算:0.4560427*FBXW2+0.3378467*CCND2-0.4809354*RHOU+0.409029*CTBP2+0.3291529*WIF1+

0.2926374*DKK1+0.04662682。

28.如权利要求1至25中任一项的方法，其中FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量系利用选自SEQ ID NO:62至79的多核苷酸测量。

29.如权利要求28的方法，其中FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量系利用下列测量：

(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物及包含SEQ ID NO:64的探针；

(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物及包含SEQ ID NO:67的探针；

(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物及包含SEQ ID NO:70的探针；

(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物及包含SEQ ID NO:73的探针；

(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物及包含SEQ ID NO:76的探针；以及

(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物及包含SEQ ID NO:79的探针。

30.如权利要求1至29中任一项的方法，其中该Wnt途径抑制剂系抗体。

31.如权利要求第1至30中任一项的方法，其中该Wnt途径抑制剂系与至少一种卷曲(FZD)蛋白或其的一部分特异性结合的抗体。

32.如权利要求30或31的方法，其中该抗体与选自下列的至少一种FZD蛋白特异性结合：FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9及FZD10。

33.如权利要求30或31的方法，其中该抗体与选自下列的至少一种FZD蛋白特异性结合：FZD1、FZD2、FZD5、FZD7及FZD8。

34.如权利要求1至33中任一项的方法，其中该Wnt途径抑制剂系抗体，其包含：

(a)重链CDR1、重链CDR2及重链CDR3，该重链CDR1包含GFTFSHYTLS(SEQ ID NO:1)，该重链CDR2包含VISGDGSYTYYADSVKG(SEQ ID NO:2)且该重链CDR3包含NFIKYVFAN(SEQ ID NO:3)，以及

(b)轻链CDR1、轻链CDR2及轻链CDR3，该轻链CDR1包含SGDNIGSFYVH(SEQ ID NO:4)，该轻链CDR2包含DKSNRPSG(SEQ ID NO:5)且该轻链CDR3包含QSYANTLSL(SEQ ID NO:6)。

35.如权利要求1至34中任一项的方法，其中该Wnt途径抑制剂是包含重链可变区及轻链可变区的抗体，该重链可变区包含SEQ ID NO:7且该轻链可变区包含SEQ ID NO:8。

36.如权利要求1至34中任一项的方法，其中该Wnt途径抑制剂是包含重链可变区及轻链可变区的抗体，该抗体系由保藏于美国菌种保存中心(ATCC)编号为PTA-9541的质粒编码。

37.如权利要求30至36中任一项的方法，其中该抗体系单克隆抗体、重组抗体、嵌合抗体、双特异性抗体、人化抗体、人抗体或包含抗原结合部位的抗体片段。

38.如权利要求1至35中任一项的方法，其中该Wnt途径抑制剂是抗体OMP-18R5。

39.如权利要求1至29中任一项的方法，其中该Wnt途径抑制剂是可溶性受体。

40.如权利要求39的方法，其中该可溶性受体包含人FZD蛋白的Fri结构域。

41.如权利要求38的方法，其中该人FZD蛋白的Fri结构域系选自下列：FZD1的Fri结构域、FZD2的Fri结构域、FZD3的Fri结构域、FZD4的Fri结构域、FZD5的Fri结构域、FZD6的Fri结构域、FZD7的Fri结构域、FZD8的Fri结构域、FZD9的Fri结构域、或FZD10的Fri结构域。

42.如权利要求40的方法，其中该人FZD蛋白的Fri结构域包含FZD8的Fri结构域。

43.如权利要求39至42中任一项的方法，其中该可溶性受体另包含非FZD多肽。

44.如权利要求43的方法，其中该非FZD多肽包含人Fc区。

45.如权利要求39至44中任一项的方法，其中该Wnt途径抑制剂是FZD8-Fc可溶性受体OMP-54F28。

46.如权利要求1至45中任一项的方法，其中该肿瘤系选自乳房肿瘤、肺肿瘤、结肠肿瘤、结直肠肿瘤、黑色素瘤、胰脏肿瘤、胃肠道肿瘤、肾肿瘤、卵巢肿瘤、神经内分泌肿瘤、肝脏肿瘤、子宫内膜肿瘤、肾脏肿瘤、前列腺肿瘤、甲状腺肿瘤、神经胚细胞瘤、神经胶质瘤、多形性神经胶质母细胞瘤、子宫颈肿瘤、胃肿瘤、膀胱肿瘤、肝肿瘤以及头颈肿瘤。

47.如权利要求1至45中任一项的方法，其中该肿瘤是乳房肿瘤。

48.如权利要求47的方法，其中该乳房肿瘤是HER2阴性乳房肿瘤。

49.如权利要求47的方法，其中该乳房肿瘤是三阴性乳癌(TNBC)肿瘤。

50.如权利要求1至49中任一项的方法，其中该Wnt途径抑制剂的治疗系与一或多种额外治疗剂组合。

51.如权利要求50的方法，其中该额外治疗剂是化学治疗剂。

52.如权利要求50的方法，其中该额外治疗剂是太平洋紫杉醇(paclitaxel)。

53.如权利要求50的方法，其中该额外治疗剂系经nab结合的太平洋紫杉醇(ABRAXANE)。

54.如权利要求1至53中任一项的方法，其中该样本系组织样本或肿瘤活体样本。

55.如权利要求1至53中任一项的方法，其中该样本系经福尔马林固定的石蜡包埋(FFPE)样本。

56.一种鉴别可能对抗体治疗有反应或无反应的人乳房肿瘤的方法，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，该方法包含：

(a)获得该人乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的生物标记物表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据该生物标记物标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该乳房肿瘤系经预测为对该抗体治疗有反应，且阴性判定值显示该肿瘤系经预测为对该抗体治疗无反应。

57.一种鉴别可能对抗体治疗有反应的乳癌病患的方法，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，该方法包含：

(a)获得该乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的生物标记物表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及

(c)根据该生物标记物标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该乳癌系经预测为对该抗体治疗有反应。

58.一种选择可能对抗体治疗有反应的乳癌病患的方法，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，该方法包含：

(a)获得该乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的生物标记物表达量，其中该生

物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；

(c)根据该生物标记物标签中的所述生物标记物的标准化表达，计算判定值；

其中阳性判定值显示该乳癌系经预测为对该抗体治疗有反应；以及

(d)当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。

59.如权利要求56或57的方法，其另包含：

(d)当该乳癌系经预测为对该抗体治疗有反应，选择该病患以接受治疗。

60.如权利要求56至59中任一项的方法，其另包含对该病患投予有效治疗量的该抗体。

61.如权利要求60的方法，其中该抗体是OMP-18R5。

62.如权利要求56至61的方法，其中该治疗包含该抗体与太平洋紫杉醇的组合。

63.一种治疗病患的癌症的方法，其包含：对该病患投予有效量的Wnt途径抑制剂，其中根据病患肿瘤样本中生物标记物标签的表达量，该病患系经预测为对该Wnt途径抑制剂的治疗有反应，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。

64.一种用于检测样本中的FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的试剂盒，其中该试剂盒包含选自SEQ ID NO:62至79的多核苷酸。

65.如权利要求64的试剂盒，其包含：

(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物及包含SEQ ID NO:64的探针；

(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物及包含SEQ ID NO:67的探针；

(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物及包含SEQ ID NO:70的探针；

(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物及包含SEQ ID NO:73的探针；

(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物及包含SEQ ID NO:76的探针；以及

(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物及包含SEQ ID NO:79的探针。

与Wnt途径抑制剂有关的预测性生物标记物的鉴别

相关申请案的交互参照

[0001] 本申请案主张于2013年12月2日提出的美国临时申请案第61/910,663号及2014年4月4日提出的美国临时申请案第61/975,339号的优先权，各案以参照方式整体纳入此处。

技术领域

[0002] 本发明关于癌症治疗的领域。更特别地，本发明提供用于鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的肿瘤的方法。此外，本发明提供用于鉴别、选择及/或治疗可能对单独使用或与其他治疗剂组合的Wnt途径抑制剂的治疗有反应的癌症病患的方法。

背景技术

[0003] 癌症是已开发国家的主要死因之一，光是在美国每年就有大约160万人被诊断出癌症而且有超过550,000例死亡。整体来说，预期每3人中超过1人会在有生之年发展出某些形式的癌。有超过200种不同的癌症，其中四种：乳癌、肺癌、结直肠癌及前列腺癌，占美国所有新病例的几乎一半(Siegel et al.,2012,CA:A Cancer J.for Clin.,62:10-29)。

[0004] 信号传导途径通常连接细胞外信号至细胞核，导致直接或间接控制细胞生长、分化、存活、及死亡的基因表达。然而，在许多种类的癌症中，信号传导途径失调且可能与肿瘤起始及/或进展有关。与人癌症发生有关的信号传导途径包括但不限于Wnt途径、Ras-Raf-MEK-ERK或MAPK途径、PI3K-AKT途径、CDKN2A/CDK4途径、Bcl-2/TP53途径、及缺口(NOTCH)途径。

[0005] Wnt信号传导途径是胚胎模式形成、后胚胎组织维持及干细胞生物学的重要调节因子之一。更特别地，Wnt信号传导在细胞极性的产生和细胞命运决定包括干细胞族群自我更新中扮演重要角色。未受调节的Wnt途径活化与许多人癌症有关，一般相信该活化可改变细胞的发育命运。咸信活化Wnt途径可能使肿瘤细胞维持在未分化状态及/或导致不受控制的增生。这可使得癌的发生藉由破坏控制正常发育及组织修复的恒定机转进行(于Reya&Clevers,2005,Nature,434:843-50;Beachy et al.,2004,Nature,432:324-31中回顾)。

[0006] Wnt信号传导途径首先在果蝇发育突变无翅(wg)以及小鼠原致癌基因int-1(现称Wnt1)中阐述(Nusse&Varmus,1982,Cell,31:99-109;Van Ooyen&Nusse,1984,Cell,39:233-40;Cabrera et al.,1987,Cell,50:659-63;Rijsewijk et al.,1987,Cell,50:649-57)。Wnt基因编码经脂质修饰的分泌糖蛋白，在哺乳动物中已识别出19种不同的Wnt蛋白。这些分泌型配体活化由卷曲(FZD)受体家族成员及低密度脂蛋白(LDL)受体相关蛋白5或6(LRP5/6)组成的受体复合体。FZD受体是G蛋白偶合受体(GPCR)超家族的成员，包含七个跨膜结构域以及一个大型胞外N端配体结合结构域。N端配体结合结构域包含10个保守性半胱氨酸，被称为多半胱氨酸区(CRD)或“Fri结构域”。有十种人FZD受体，FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9及FZD10。不同的FZD CRD对特定Wnt蛋白有不同的结合亲和性(Wu&Nusse,2002,J.Biol.Chem.,277:41762-9)。此外，FZD受体可被分成活化典型β-连环蛋白途径者及活化非典型途径者(Miller et al.,1999,Oncogene,18:7860-72)。

[0007] Wnt信号传导于癌症的角色，首先是因为识别Wnt1(原名int1)为藉由邻近插入小鼠病毒而转化的乳房肿瘤的致癌基因而被发现(Nusse&Varmus,1982,Cell,31:99-109)。从这些早期发现以来，Wnt信号传导在乳癌中的角色的其他证据已持续累积。举例来说，β-连环蛋白于转基因小鼠乳腺中的过度表达导致增生及腺癌(Imbert et al.,2001,J.Cell Biol.,153:555-68;Michaelson&Leder,2001,Oncogene,20:5093-9)，然而丧失Wnt信号传导扰乱正常乳腺发育(Tepera et al.,2003,J.Cell Sci.,116:1137-49;Hatsell et al.,2003,J.Mammary Gland Biol.Neoplasia,8:145-58)。在人乳癌中，β-连环蛋白累积表示超过50％的癌中有经活化的Wnt信号传导，虽然特定突变尚未被识别，但已观察到卷曲受体表达上调(Brennan&Brown,2004,J.Mammary Gland Biol.Neoplasia,9:119-31;Malovanovic et al.,2004,Int.J.Oncol.,25:1337-42)。

[0008] Wnt途径的活化亦与大肠直肠癌、肺癌、胰癌及黑色素瘤有关。大约5至10％的所有大肠直肠癌为遗传性，其中主要癌症类型之一为家族性腺瘤息肉症(FAP)。FAP是一种体染色体显性疾病，其中大约80％的受影响个体包含大肠腺瘤息肉(APC)基因的种系突变。另外也在其他Wnt途径成分包括Axin及β-连环蛋白发现突变。个别腺瘤为包含第二失活等位基因的上皮细胞的种系过度生长，大量FAP腺瘤无可避免地导致腺癌经由致癌基因及/或抑瘤基因的额外突变发生。另外，Wnt信号传导途径的活化，包括APC的功能丧失突变及β-连环蛋白的稳定突变，可诱导小鼠模型中的增生发育及肿瘤生长(Oshima et al.,1997,Cancer Res.,57:1644-9;Harada et al.,1999,EMBO J.,18:5931-42)。

[0009] 因此，Wnt途径已被识别为癌症疗法及癌症治疗的目标。随着药物研究发展的进步，特别是在癌症领域中，“以一种药物治疗所有癌症”的方法转变成“个人化医药”策略。个人化医药策略可包括根据癌症生物标记物的治疗方案，该等生物标记物包括预后性标记、药物药效学标记及预测性标记。一般而言，预测性生物标记物评估肿瘤或癌症将对特定治疗剂有反应或敏感性的可能性，且可能能够鉴别及/或选择最可能得益于使用该剂的病患。

[0010] 本发明提供与使用Wnt途径抑制剂治疗癌症有关的预测性生物标记物的鉴别。本发明亦提供使用该预测性生物标记物以鉴别、选择及/或分类可能对Wnt途径抑制剂的治疗有反应或无反应的肿瘤及/或癌症病患的方法。本发明亦提供以Wnt抑制剂治疗病患的方法，该病患系经预测为对治疗有反应。

发明内容

[0011] 本发明提供用于鉴别可能对Wnt途径抑制剂的治疗有反应的病患的生物标记物。本发明另提供用于鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的肿瘤及/或病患的方法。本发明进一步提供以Wnt途径抑制剂治疗病患癌症的方法，其中该病患系经预测为或已经鉴别为可能对该Wnt途径抑制剂有反应。

[0012] 在一方面中，本发明提供一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的方法，该方法包含：(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签(biomarker signature)的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CRBP2、WIF1及DKK1中的一或多种；以及(c)根据该等生物标记物的表达量，鉴别该肿瘤为可能对治疗有反应或无反应。在一些实施方式中，一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的方法包含：(a)获得该人肿瘤的样本；(b)

测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CRBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应，且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。如本文中所使用，“标准化”及“正常化”可互相交换使用。在一些实施方式中，该方法包含鉴别可能对Wnt途径抑制剂与太平洋紫杉醇组合的治疗有反应或无反应的人肿瘤。

[0013] 在另一方面中，本发明提供一种分类人肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法，该方法包含：(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该等生物标记物的表达量，分类该肿瘤为可能对治疗有反应或无反应。在一些实施方式中，一种分类人肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法包含：(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应，且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。在一些实施方式中，该方法包含分类人肿瘤为可能对Wnt途径抑制剂与太平洋紫杉醇组合的治疗有反应或无反应。

[0014] 在另一方面中，本发明提供一种测定人肿瘤对Wnt途径抑制剂的治疗的反应性(或敏感性)的方法，该方法包含：(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含基因FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该等生物标记物的表达量，测定该肿瘤对治疗的反应性。在一些实施方式中，一种测定人肿瘤对Wnt途径抑制剂的治疗的反应性或敏感性的方法包含：(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含基因FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应或敏感。在一些实施方式中，该方法包含测定人肿瘤对Wnt途径抑制剂与太平洋紫杉醇的组合治疗的反应性或敏感性。

[0015] 在另一方面中，本发明提供一种鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的方法，该方法包含：(a)获得该病患肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该等生物标记物的表达量，鉴别可能对治疗有反应的病患。在一些实施方式中，一种鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的方法包含：(a)获得该病患肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对该Wnt途径抑制剂的治疗有反应。在一些实施方式中，该方法包含鉴别对Wnt途径抑制剂与太平洋紫杉醇的组合治疗可能有反

应的癌症病患。

[0016] 在另一方面中，本发明提供一种选择癌症病患以接受Wnt途径抑制剂的治疗的方法，该方法包含：(a)获得该病患肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；(c)根据该等生物标记物的表达量，选择该将接受治疗的病患。在一些实施方式中，一种选择癌症病患以接受Wnt途径抑制剂的治疗的方法包含：(a)获得该病患肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；以及(d)当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。在一些实施方式中，该方法包含选择癌症病患以接受Wnt途径抑制剂与太平洋紫杉醇的组合治疗。

[0017] 在另一方面中，本发明提供一种治疗病患的癌症的方法，其包含：(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该等生物标记物的表达量，鉴别可能对治疗有反应的病患；以及(b)对该可能对治疗有反应的病患投予有效量的Wnt途径抑制剂。在一些实施方式中，一种治疗病患的癌症的方法包含：(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对该经预测为对治疗有反应的病患投予有效量的Wnt途径抑制剂。在一些实施方式中，该方法包含鉴别该病患是否可能对Wnt途径抑制剂与太平洋紫杉醇的组合治疗有反应。在一些实施方式中，该方法包含对该病患投予该Wnt途径抑制剂与太平洋紫杉醇的组合。

[0018] 在另一方面中，本发明提供一种治疗病患的癌症的方法，其包含：对该病患投予有效量的Wnt途径抑制剂，其中根据病患肿瘤样本中生物标记物标签的表达量，该病患系经预测为对该Wnt抑制剂治疗有反应，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，一种治疗病患的癌症的方法包含：对该病患投予有效量的Wnt途径抑制剂；其中根据由病患肿瘤样本中生物标记物标签的生物标记物的标准化表达的加权总和所计算的阳性判定值，该病患系经预测为对治疗有反应，其中该生物标记物组包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，该病患系经预测为对Wnt途径抑制剂与太平洋紫杉醇的组合治疗有反应。在一些实施方式中，该方法包含对该病患投予该Wnt途径抑制剂与太平洋紫杉醇的组合。

[0019] 在另一方面中，本发明提供一种增加Wnt途径抑制剂的有效治疗的可能性的方法，其包含：(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该等生物标记物的表达量，鉴别可能对治疗有反应的病患；以及

(b)对该病患投予有效量的Wnt途径抑制剂。在一些实施方式中，一种增加Wnt途径抑制剂的有效治疗的可能性的方法包含：(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对肿瘤具有阳性判定值的病患投予有效量的该Wnt途径抑制剂。在一些实施方式中，该方法包含鉴别病患是否具有可能对Wnt途径抑制剂与太平洋紫杉醇的组合治疗有反应的肿瘤。在一些实施方式中，该方法包含对该病患投予该Wnt途径抑制剂与太平洋紫杉醇的组合。

[0020] 在另一方面中，本发明提供一种增加Wnt途径抑制剂的有效治疗的可能性的方法，其包含：对病患投予有效量的Wnt途径抑制剂；其中根据病患肿瘤样本中生物标记物标签的表达量，该病患系经鉴别为可能对该Wnt抑制剂治疗有反应，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，一种增加Wnt途径抑制剂的有效治疗的可能性的方法包含：对病患投予有效量的Wnt途径抑制剂；其中根据由病患肿瘤样本中生物标记物标签的生物标记物的标准化表达的加权总和所计算的阳性判定值，该病患系经鉴别为可能对治疗有反应，其中该生物标记物组包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，该病患系经鉴别为可能对Wnt途径抑制剂与太平洋紫杉醇的组合治疗有反应。在一些实施方式中，该方法包含对该病患投予该Wnt途径抑制剂与太平洋紫杉醇的组合。

[0021] 在前述各个方面的某些实施方式中，以及本文他处所述的其他方面及/或实施方式中，生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1、EP300及CTBP1中的一或多种。在一些实施方式中，该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1、EP300、CTBP1、WNT6、WNT3、FZD2、APC、TLE2、DVL2、PITX2、WISP1、GSK3B、WNT9A、FZD7及LEF1中的一或多种。在一些实施方式中，该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种，以及来自表2的至少一种额外生物标记物。

[0022] 在前述各种方面的某些实施方式，以及本文他处所述的其他方面及/或实施方式中，该Wnt途径抑制剂系抗体。在一些实施方式中，该Wnt途径抑制剂系与至少一种卷曲(FZD)蛋白或其片段特异性结合的抗体。在一些实施方式中，该Wnt途径抑制剂系与选自下列的至少一种FZD蛋白特异性结合的抗体：FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9及FZD10。在一些实施方式中，该Wnt途径抑制剂系与选自下列的至少一种FZD蛋白特异性结合的抗体：FZD1、FZD2、FZD5、FZD7及FZD8。在某些实施方式中，该Wnt途径抑制剂系包含下列的抗体：(a)包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1、包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2、及包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，及(b)包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1、包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2、及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3。

[0023] 在某些实施方式中，该Wnt途径抑制剂系包含下列的抗体：包含SEQ ID NO:7的重链可变区及包含SEQ ID NO:8的轻链可变区。在某些实施方式中，该Wnt途径抑制剂系包含重链可变区及轻链可变区的抗体，该抗体系由保藏于美国菌种保存中心(ATCC)编号为PTA-

9541的质粒编码。在某些实施方式中，该Wnt途径抑制剂系包含重链及轻链的抗体，该抗体系由保藏于美国菌种保存中心(ATCC)编号为PTA-9541的质粒编码。在一些实施方式中，该Wnt途径抑制剂系抗体OMP-18R5。

[0024] 在前述各种方面的某些实施方式，以及本文他处所述的其他方面及/或实施方式中，该Wnt途径抑制剂系可溶性受体。在一些实施方式中，该Wnt途径抑制剂包含FZD受体蛋白的胞外结构域。在一些实施方式中，该Wnt途径抑制剂包含FZD蛋白的Fri结构域。在一些实施方式中，该Wnt途径抑制剂包含FZD8的Fri结构域。在一些实施方式中，该Wnt途径抑制剂包含FZD8的Fri结构域及人Fc结构域。在一些实施方式中，该Wnt途径抑制剂是可溶性受体OMP-54F28。

[0025] 在一些实施方式中，该肿瘤系选自乳房肿瘤、肺肿瘤、结肠肿瘤、神经胶质瘤、胃肠道肿瘤、肾肿瘤、卵巢肿瘤、肝脏肿瘤、结直肠肿瘤、子宫内膜肿瘤、肾脏肿瘤、前列腺肿瘤、甲状腺肿瘤、神经胚细胞瘤、胰脏肿瘤、多形性神经胶质母细胞瘤、子宫颈肿瘤、胃肿瘤、膀胱肿瘤、肝肿瘤、黑色素瘤以及头颈肿瘤。在一些实施方式中，该肿瘤系乳房肿瘤。

[0026] 在一些实施方式中，该癌症系选自乳癌、肺癌、结肠癌、神经胶质瘤、胃肠道癌、肾癌、卵巢癌、肝脏癌、结直肠癌、子宫内膜癌、肾脏癌、前列腺癌、甲状腺癌、神经胚细胞瘤、胰脏癌、多形性神经胶质母细胞瘤、子宫颈癌、胃癌、膀胱癌、肝肿瘤、黑色素瘤以及头颈癌。在一些实施方式中，该癌系乳癌。

[0027] 在一些实施方式中，本方法另包含对该病患投予第二治疗剂。在一些实施方式中，该第二治疗剂系化学治疗剂。在一些实施方式中，该第二治疗剂系太平洋紫杉醇(paclitaxel)。

[0028] 在前述各种方面的某些实施方式，以及本文他处所述的其他方面及/或实施方式中，该样本包括但不限于任何临床上重要的组织样本，诸如肿瘤活体样本、核心活体组织样本、细针抽吸样本、毛囊或体液样本如血液、血浆、血清、淋巴液、腹水、囊液或尿液。在一些实施方式中，该样本系取自具有肿瘤或癌的病患。在一些实施方式中，该样本系原发性肿瘤。在一些实施方式中，该样本系转移性肿瘤。在一些实施方式中，该样本系组织样本。在一些实施方式中，该样本系肿瘤样本。在一些实施方式中，该样本系新鲜冷冻(FF)组织样本。在一些实施方式中，该样本系经福尔马林固定的石蜡包埋(FFPE)组织样本。在一些实施方式中，该样本系全血、血浆或血清。在一些实施方式中，该样本系细胞。在一些实施方式中，该样本系循环肿瘤细胞(CTC)。

[0029] 在前述各种方面的某些实施方式，以及本文他处所述的其他方面及/或实施方式中，生物标记物的表达量系利用基于PCR的方法测定，诸如但不限于逆转录PCR(RT-PCR)、定量RT-PCR(qPCR)、TaqMan™或TaqMan™低密度阵列(TLDA)。在一些实施方式中，该生物标记物的表达量系利用微阵列测定。

[0030] 在前述各种方面的某些实施方式，以及本文他处所述的其他方面及/或实施方式中，各生物标记物的标准化表达系藉由测量各生物标记物的表达量且将其乘以对应重量决定，其中各生物标记物的重量系由该生物标记物表达决定。在某些实施方式中，该判定值系根据下列方程式计算：0.4560427*FBXW2+0.3378467*CCND2-0.4809354*RHOU+0.409029*CTBP2+0.3291529*WIF1+0.2926374*DKK1+0.04662682。

[0031] 在一些实施方式中，该生物标记物的表达量系利用基于PCR的方法测量或测定。在

一些实施方式中，FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量系利用选自SEQ ID NO:62至79的多核苷酸测量。在一些实施方式中，FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量系利用下列测量：(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物、及包含SEQ ID NO:64的探针；(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物、及包含SEQ ID NO:67的探针；(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物、及包含SEQ ID NO:70的探针；(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物、及包含SEQ ID NO:73的探针；(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物、及包含SEQ ID NO:76的探针；以及(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物、及包含SEQ ID NO:79的探针。

[0032] 在一些实施方式中，生物标记物的表达量系藉由下列测量或测定：多分析物特性测定、放射性免疫测定(RIA)、西方印迹分析试验、免疫荧光分析、酵素免疫分析、酵素连接免疫吸附试验(ELISA)、免疫沉降试验、化学发光试验、免疫组织化学试验、斑点印迹法或狭缝印迹法。在一些其中该等分析试验使用抗体的实施方式中，该抗体系经可检测地标记。在一些实施方式中，该标记系选自免疫荧光标记、化学发光标记、磷光标记、酵素标记、放射标记、抗生物素蛋白/生物素标记、胶体金粒子、彩色粒子及磁性粒子。

[0033] 本发明亦提供一种包含容器的试剂盒，其中该容器包含至少一种用于特异性检测本发明的至少一种生物标记物的表达的试剂。在某些实施方式中，该试剂系与本发明的生物标记物结合的抗体或核酸探针。

[0034] 在一些实施方式中，试剂盒包含选自SEQ ID NO:62至79的多核苷酸。在一些实施方式中，试剂盒包含：(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物、及包含SEQ ID NO:64的探针；(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物、及包含SEQ ID NO:67的探针；(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物、及包含SEQ ID NO:70的探针；(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物、及包含SEQ ID NO:73的探针；(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物、及包含SEQ ID NO:76的探针；以及(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物、及包含SEQ ID NO:79的探针。

[0035] 本发明的方式或实施方式系以马库什群组或其他选择性形式的群组描述，本发明不仅包含被标示为整体的整个群组，但亦包含该群组的个别成员及该主要群组中所有可能的亚群，且亦包含不含其中一或多个群组成员的主要群组。本发明亦设想明确排除该申请专利的发明中任何群组成员的一或多种。

附图简要说明

[0036] 图1A至1H：反应性或非反应性乳房肿瘤的分类。图1A：乳房肿瘤OMP-B34细胞系经皮下注射至NOD/SCID小鼠。图1B：乳房肿瘤OMP-B39细胞系经皮下注射至NOD/SCID小鼠。图1C：乳房肿瘤OMP-B44细胞系经皮下注射至NOD/SCID小鼠。图1D：乳房肿瘤OMP-B59细胞系经皮下注射至NOD/SCID小鼠。图1E：乳房肿瘤OMP-B60细胞系经皮下注射至NOD/SCID小鼠。图1F：乳房肿瘤UM-T01细胞系经皮下注射至NOD/SCID小鼠。图1G：乳房肿瘤UM-T03细胞系经皮下注射至NOD/SCID小鼠。图1H：乳房肿瘤UM-PE13细胞系经皮下注射至NOD/SCID小鼠。在每个实验中，小鼠系经OMP-18R5抗体(-■-)、紫杉醇(taxol)(-▲-)、OMP-18R5与taxol的组合(-▼-)或对照抗体(-●-)治疗。数据以治疗后天数的肿瘤体积(mm^3)显示。

[0037] 图2:排名前20的基因的表现曲线。

[0038] 图3:6种经选择的基因的PCA图。

[0039] 图4:6基因生物标记物标签与肿瘤体积比例的相关性。

[0040] 图5:根据分类机率分析预测肿瘤反应性。T＝用于训练组中以建立6基因标签的肿瘤。

[0041] 图6A至6F:预测性生物标记物的活体内验证。图6A:乳房肿瘤OMP-B29细胞系经皮下注射至NOD/SCID小鼠。图6B:乳房肿瘤OMP-B71细胞系经皮下注射至NOD/SCID小鼠。图6C:乳房肿瘤OMP-B84细胞系经皮下注射至NOD/SCID小鼠。图6D:乳房肿瘤OMP-B90细胞系经皮下注射至NOD/SCID小鼠。图6E:乳房肿瘤UM-T02细胞系经皮下注射至NOD/SCID小鼠。图6F:乳房肿瘤UM-T06细胞系经皮下注射至NOD/SCID小鼠。在每个实验中,小鼠系经OMP-18R5抗体(-■-)、紫杉醇(taxol)(-▲-)、OMP-18R5与taxol的组合(-▼-)或对照抗体(-●-)治疗。数据以治疗后天数的肿瘤体积(mm^3)显示。

[0042] 图7:使用三个公众数据库进行6基因生物标记物标签的族群盛行率预测。

具体实施方式

本发明的详细说明

I.定义

[0043] 为了促进对本发明的了解,以下定义一些用语及用词。

[0044] 如本文中所使用的用语“生物标记物”可包括但不限于核酸及蛋白以及其变体及片段。生物标记物可包括DNA,其包含编码该生物标记物的整个或部分核酸序列或该序列的补体序列。可用于本发明的生物标记物核酸被认为是包括DNA及RNA二者,该RNA包含受到关注的任何核酸序列的整个或部分序列。生物标记物蛋白被认为是包含该生物标记物蛋白或多肽的任一者的整个或部分氨基酸序列。

[0045] 本文所使用的用语“抗体”系指免疫球蛋白分子,该免疫球蛋白分子藉由其可变区内的至少一个抗原结合部位,识别标靶(例如蛋白、多肽、肽、碳水化合物、多核苷酸、脂质、或前述的组合)且与的特异性结合。如本文所使用,该用语包含完整多克隆抗体、完整单克隆抗体、单链抗体、抗体片段(诸如Fab、Fab’、F(ab’)2、及Fv片段)、单链Fv(scFv)抗体、多特异性抗体诸如双特异性抗体、单特异性抗体、单价抗体、嵌合抗体、人化抗体、人抗体、包含抗体的抗原结合部位的融合蛋白,及任何其他包含抗原结合部位的经修饰的免疫球蛋白分子,只要该等抗体展现所欲的生物活性。抗体可为下列五种主要免疫球蛋白类型中的任一者:IgA、IgD、IgE、IgG及IgM或彼等的亚型(同型)(例如IgG1、IgG2、IgG3、IgG4、IgA1及IgA2),此系根据彼等分别被称为α、δ、ε、γ、及μ的重链恒定结构域命名。不同类型的免疫球蛋白具有不同且广为周知的次单位结构及三维构型。抗体可为未经修饰(naked)或与其他分子缀合(conjugated),该等其他分子包括但不限于毒素及放射性同位素。

[0046] 用语“抗体片段”系指完整抗体的部分且系指完整抗体的抗原性决定可变区。抗原片段的实例包括但不限于Fab、Fab’、F(ab’)2及Fv片段、线性抗体、单链抗体及自抗体片段形成的多特异性抗体。此处所使用的“抗体片段”包含至少一个抗原结合部位或表位结合部位。

[0047] 抗体的“可变区”用语系指抗体轻链的可变区或抗体重链的可变区(不论单独或组

合指称)。重链或轻链的可变区通常由四个框架区(FR)及连接该四个框架区的三个互补决定区(CDR)组成,该三个CDR又名"超变异区"。各链中的CDR被框架区拉近,导致形成该抗体的抗原结合部位。至少有两种技术用于测定CDR:(1)基于跨种序列变异性的方法(即Kabat et al.,1991,Sequences of Proteins of Immunological Interest,5th Edition,National Institutes of Health,Bethesda,MD);及(2)基于抗原-抗体复合物的结晶学研究的方法(Al-Lazikani et al.,1997,J.Mol.Biol.,273:927-948)。此外,有时该领域组合使用这两种技术以测定CDR。

[0048] 此处所使用的用语"单克隆抗体"系指同源性抗体群,其高度特异性识别及结合单一抗原性决定簇或表位。此与多克隆抗体相反,多克隆抗体通常包括多种以不同抗原决定簇为目标的不同抗体的混合。用语"单克隆抗体"包含完整及全长单克隆抗体,也包含抗体片段(例如Fab、Fab'、F(ab')2、Fv)、单链(scFv)抗体、包含抗体部分的融合蛋白质、及任何其他包含抗原结合部位的经修饰的免疫球蛋白分子。另外,"单克隆抗体"系指由许多技术制备的该等抗体,该等技术包括但不限于杂交瘤产制、噬菌体选择、重组表达及转基因动物。

[0049] 此处所使用的用语"人化抗体"系指包含极少化非人序列的系为特定免疫球蛋白链、嵌合性免疫球蛋白或其片段的抗体。用于制备人化抗体的方法系该领域所广为周知。

[0050] 如本文所使用的用语"人抗体"系指由人体产制的抗体或具有对应由人体产制的抗体的氨基酸序列的抗体。人抗体可利用任何该领域已知的技术制备。

[0051] 此处所使用的用语"嵌合抗体"系指其中该免疫球蛋白分子的氨基酸序列系源自二或更多个物种的抗体。通常,轻链及重链的可变区皆对应源自一哺乳动物物种(例如小鼠、大鼠、兔等)的具有所欲特异性、亲和性及/或结合能力的抗体的可变区,然而该等恒定区对应源自另一物种(通常是人)的抗体中的序列。

[0052] 此处所使用的用语"亲和性成熟抗体"系指在其一或多个CDR中具有一或多个改变的抗体,该改变导致该抗体相较于不具有该等改变的亲代抗体对抗原的亲和性增加。该定义亦包括与CDR残基改变一起发生的非CDR残基的改变。较佳的亲和性成熟抗体将具有纳摩尔或甚至皮摩尔程度的对标靶抗原的亲和性。亲和性成熟抗体系藉由该领域已知的方法产制。例如,包括藉由VH及VL结构域替换、CDR及/或架构残基的随机突变形成、及定点突变形成的亲和性成熟技术。

[0053] 用语"表位"及"抗原决定簇"在此处可交换使用,系指可被特定抗体辨识且特异性结合的抗原部分。当抗原系多肽时,表位可自连续氨基酸或藉由蛋白质的三级折叠并列的非连续氨基酸形成。自连续氨基酸形成的表位(又称为线性表位)通常在蛋白质变性时仍被保留,然而藉由三级折叠形成的表位(又称为构象表位)通常在蛋白质变性时丧失。表位通常包括至少3个及更通常地至少5或8至10个呈独特空间构象的氨基酸。

[0054] 用语"选择性结合"或"特异性结合"系指结合剂或抗体以更频繁、更快速、更长时间、更高亲和性或上述条件的某些组合与表位、蛋白质或标靶分子反应或结合,相较于可供选择的物质包括非相关或相关蛋白。在某些实施方式中,"特异性结合"系指例如抗体以大约0.1mM或更低,但通常低于大约1μM的K_D与蛋白质结合。在某些实施方式中,"特异性结合"系指抗体有时以至少约0.1μM或更低,有时以至少约0.01μM或更低,且有时以至少约1nM或更低的K_D与标靶结合。由于不同物种的同源性蛋白质之间具有序列一致性,因此特异性结

合可包括辨识超过一个物种的蛋白质(例如人FZD及小鼠FZD)的抗体。同样地,由于不同蛋白的多肽序列的某些区域内具有同源性,因此特异性结合可包括辨识超过一种蛋白(例如人FZD1及人FZD7)的抗体(或其他多肽或结合剂)。应了解的是,在某些实施方式中,与第一标靶特异性结合的抗体或结合剂可能或可能不与第二标靶特异性结合。因此,"特异性结合"不一定需要(虽然可包括)排他性结合(即与单一标靶结合)。因此,在某些实施方式中,结合剂与一种以上的标靶特异性结合。在某些实施方式中,多重标靶可能由该剂或抗体上的相同的结合部位结合。举例来说,在某些情况下,抗体可能包含二个完全相同的抗原结合部位,该二个抗原结合部位各自与二或多个蛋白上的相同表位特异性结合。在某些可供选择的实施方式中,抗体可能为双特异性或多特异性,且包含至少二个具有不同特异性的抗原结合部位。以非限制性实例而言,双特异性剂可包含一个辨识一个蛋白(例如人FZD)上的标靶的结合部位,且另包含第二个辨识第二蛋白(例如人WNT蛋白)上的不同标靶的不同结合部位。一般来说(但不必然),所谓的结合系指特异性结合。

[0055] 用语"多肽"和"肽"以及"蛋白"在此处可交换使用,这些用语系指任何长度的氨基酸的聚合物。该聚合物可为线性或分支,其可能包含经修饰的氨基酸,且其可能被非氨基酸中断。该等用语亦包含经天然或人为干预修饰的氨基酸聚合物;例如双硫键形成、糖基化、脂化、乙酰化、磷酸化或任何其他操纵或修饰,诸如与标记成份缀合。该定义亦包括例如包含一或多个氨基酸类似物(包括例如非天然氨基酸)的多肽,以及包含本领域公知的其他修饰的多肽。应了解的是,由于本发明的多肽可能以抗体为主,因此在某些实施方式中,该多肽可能为单链或相连的链(例如二聚体)。

[0056] 用语"多核苷酸"及"核酸"在此处可交换使用,系指任何长度的核苷酸的聚合物,包括DNA及RNA。该核苷酸可为脱氧核糖核苷酸、核糖核苷酸、经修饰的核苷酸或碱基及/或彼等的类似物,或任何可藉由DNA或RNA聚合酶被纳入聚合物中的底物。

[0057] "高严谨度的条件"可识别为下列条件:(1)采用低离子张力及高温清洗,例如于50℃的15mM氯化钠/1.5mM柠檬酸钠/0.1%十二基硫酸钠;(2)在杂交期间采用变性剂,诸如甲酰胺,例如42℃的具有0.1%牛血清白蛋白/0.1%Ficoll/0.1%聚乙烯基吡咯烷酮/50mM磷酸钠缓冲液的于pH 6.5的5x SSC(0.75M NaCl,75mM柠檬酸钠)中的50%(体积/体积)甲酰胺;或(3)于杂交期间采用42℃于5x SSC中的50%甲酰胺、50mM磷酸钠(pH 6.8)、0.1%焦磷酸钠、5x丹哈德(Denhardt's)溶液、经超声波处理的鲑鱼精子DNA(50μg/ml)、0.1%SDS及10%硫酸葡聚糖,于42℃以0.2x SSC及50%甲酰胺清洗,之后于55℃以含有EDTA的0.1x SSC进行清洗。

[0058] 在提及二或多个核酸或多肽时,用语"一致"或百分比"一致性"系指当二或多个序列或子序列经比较及比对(需要时导入间格)以达最高对应性且不把任何保守性氨基酸取代当作序列一致性的部分时,该二或多个序列或子序列系相同或具有相同的特定百分比的核苷酸或氨基酸残基。该百分比一致性可利用序列比较软件或算法测量,或藉由目视检查测量。多种可被用于取得氨基酸或核苷酸序列比对的算法及软件系该领域所广为周知。该等算法及软件包括但不限于BLAST、ALIGN、Megalign、BestFit、GCG Wisconsin软件包及彼等的变化性产品。在一些实施方式中,本发明的二个核酸或多肽系实质上一致,表示当彼等经比较或比对以达最高对应性时,利用序列比较算法或目视检查得知彼等具有至少70%、至少75%、至少80%、至少85%、至少90%且在一些实施方式中至少95%、96%、97%、98%、

99％的核苷酸或氨基酸残基一致性。在一些实施方式中，一致性存在于至少约10、至少约20、至少约40至60残基、至少约60至80残基长度或介于之间的任何整数长度的序列区域。在一些实施方式中，一致性存在于60至80残基以上的更长区域，诸如至少约80至100残基，且在一些实施方式中该等序列系与经比较的全长序列诸如核苷酸序列的编码区域实质上一致。

[0059] “保守性氨基酸取代”系指其中一个氨基酸残基被另一个具有类似侧链的氨基酸残基取代的取代。具有类似侧链的氨基酸残基群系于该领域中定义，包括碱性侧链(例如赖氨酸、精氨酸、组氨酸)、酸性侧链(例如天冬氨酸、谷氨酸)、不带电极性侧链(例如甘氨酸、天冬酰胺、谷氨酰胺、丝氨酸、苏氨酸、酪氨酸、半胱氨酸)、非极性侧链(例如丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、甲硫氨酸、色氨酸)、β-分支侧链(例如苏氨酸、缬氨酸、异亮氨酸)及芳香族侧链(例如酪氨酸、苯丙氨酸、色氨酸、组氨酸)。举例来说，以苯丙氨酸取代酪氨酸系保守性取代。较佳地，本发明的多肽及抗体的序列中的保守性取代不废除含有该氨基酸序列的多肽或抗体与该多肽或抗体原本所结合的抗原的结合。识别不消除抗原结合性的核苷酸及氨基酸保守性取代的方法系该领域所广为周知。

[0060] 此处所使用的用语“载体”系指建构物，该建构物能在宿主细胞中递送及通常表达一或多种感兴趣的基因或序列。载体的实例包括但不限于病毒性载体、裸DNA或RNA表达载体、质粒载体、粘粒载体、噬菌体载体、与阳离子缩合剂有关的DNA或RNA表达载体，及包封于脂质粒中的DNA或RNA表达载体。

[0061] 此处使用的用语“可溶性受体”系指受体蛋白在该受体的第一跨膜结构域之前的胞外结构域(或其片段)，其可以可溶形式自细胞分泌。通常此为该受体蛋白的N端部分。

[0062] 此处使用的用语“FZD可溶性受体”或“可溶性FZD受体”系指FZD受体蛋白在该受体的第一跨膜结构域之前的N端胞外片段，其可以可溶形式自细胞分泌。包含整个N端胞外域(ECD)的FZD可溶性受体以及较小片段系由该用语涵盖。因此，包含FZD Fri结构域的FZD可溶性受体亦包括于此用语中。

[0063] 经“分离”的多肽、抗体、多核苷酸、载体、细胞或组成物系呈现未见于天然中的形式的多肽、抗体、多核苷酸、载体、细胞或组成物。经分离的多肽、抗体、多核苷酸、载体、细胞或组成物包括该些经纯化至一定程度而使彼等不再以见于天然中的形式存在者。在一些实施方式中，经纯化的多肽、抗体、多核苷酸、载体、细胞或组成物系实质上纯的。

[0064] 此处所使用的用语“实质上纯的”系指其为至少50％纯的(即不含污染物)、至少90％纯的、至少95％纯的、至少98％纯的或至少99％纯的物质。

[0065] 此处所使用的用语“癌”及“癌性”系指称或描述哺乳动物的生理状况，其中细胞群具有未受调节的细胞生长的特征。癌的实例包括但不限于癌(carcinoma)、胚细胞瘤、肉瘤及血液性癌诸如淋巴瘤及白血病。

[0066] 此处所使用的用语“肿瘤”及“瘤(neoplasm)”系指任何由过度细胞生长或增生所导致的组织团块，不论是良性(非癌性)或包括癌前性病灶的恶性(癌性)。

[0067] 此处所使用的用语“转移”系指癌藉以自原发部位扩散或转移至身体其他区域且在新位置发展类似癌性病灶的过程。“转移”或“转移性”细胞系指与邻近细胞丧失黏着接触且(例如经由血流或淋巴)自疾病的原发部位移动至继发性部位的细胞。

[0068] 用语“癌干细胞”、“CSC”、“肿瘤干细胞”及“肿瘤起始细胞”在此处可交换使用，系

指源自癌或肿瘤且具有下列特性的细胞：(1)具有广泛增生能力，(2)能进行不对称细胞分裂以产生一或多种类型的经分化的细胞后代，其中该经分化的细胞具有减少及/或受限的增生或发育能力，及(3)能进行对称细胞分裂以自我更新或自我维持。这些特性授予癌干细胞在连续移植至免疫不全宿主(例如小鼠)时能形成或建立肿瘤或癌的能力，大部分肿瘤细胞无法形成肿瘤。癌干细胞以混乱方式进行自我更新及分化，以形成具有异常细胞类型的肿瘤，该细胞类型可在将来突变发生时改变。

[0069] 用语“癌细胞”及“肿瘤细胞”系指源自癌或肿瘤或癌前病灶的整体细胞族群，包括非肿瘤发生性细胞(其包含大部分的肿瘤细胞族群)及肿瘤发生性干细胞(癌干细胞)。此处使用的用语“癌细胞”或“肿瘤细胞”当仅用于指称该些缺乏更新及分化能力的细胞时，将由用语“非肿瘤发生性”修饰以区别该些肿瘤细胞与癌干细胞。

[0070] 此处所使用的用语“肿瘤发生性”系指癌干细胞的功能特性，包括自我更新(导致额外的肿瘤发生性癌干细胞)及增生以产生所有其他肿瘤细胞(导致经分化及因此非肿瘤发生性肿瘤细胞)的特性。

[0071] 此处所使用的用语“肿瘤发生性”系指源自肿瘤的随机细胞样本在连续移植至免疫不全宿主(例如小鼠)时形成明显肿瘤的能力。此定义亦包括当连续移植至免疫不全宿主(例如小鼠)时形成明显肿瘤的经富集及/或经分离的癌干细胞族群。

[0072] 用语“病患”系指任何动物(例如哺乳动物)，包括但不限于人、非人灵长动物、犬、猫、囓齿动物及该类似动物，该动物将成为特定治疗的接受者。通常，关于人病患的用语“病患”及“个体”在此处可交换使用。

[0073] 用语“医药上可接受”系指经美国联邦政府的管理机关或州政府核准(或可核准)或经明列于美国药典或其他普遍公认的药典中以用于动物(包括人)的产品或化合物。

[0074] 用语“医药上可接受的赋形剂、载体或佐剂”或“可接受的医药载体”系指可与本发明的至少一种剂(例如抗体)一起投予至个体的赋形剂、载体或佐剂，且该赋形剂、载体或佐剂不破坏该剂的活性。该赋形剂、载体或佐剂当与足以达到治疗效应的剂量的剂一起投予时应不具毒性。

[0075] 用语“有效量”或“治疗有效量”或“治疗效应”系指有效“治疗”个体或哺乳动物的疾病或疾患的结合剂、抗体、多肽、多核苷酸、小型有机分子或其他药物的量。以癌为例，药物(例如抗体)的治疗有效量具有治疗效应且因此可减少癌细胞的数量；降低肿瘤发生性、肿瘤发生频率、或肿瘤发生能力；减少癌干细胞的数量或频率；减少肿瘤大小；减少癌细胞群；抑制及/或停止癌细胞浸润至外围器官包括例如癌扩散至软组织及骨；抑制及/或停止肿瘤或癌细胞转移；抑制及/或停止肿瘤或癌细胞生长；缓解一或多种与癌相关的症状的严重程度；减少发病率及死亡率；促进生活质量；或该等效应的组合。以该剂举例来说抗体预防现存癌细胞生长及/或杀死现存癌细胞的方面而言，其可被称为细胞静止性及/或细胞毒性。

[0076] 用语“治疗”或“缓和”系指1)治疗性措施，该措施治愈、减缓、减轻经诊断的病理状况或疾患的症状及/或停止该经诊断的病理状况或疾患的进展及2)预防性或防范性措施，该措施预防或减缓标靶病理状况或疾患的发展。因此该些需要治疗者包括该些已经诊断为罹患该疾患者、该些易于罹患该疾患者，以及该些欲预防该疾患者。在一些实施方式中，个体系经本发明的方法成功“治疗”，若该病患显示下列一或多项：癌细胞的数量减少及/或完

全消失;肿瘤大小减少;肿瘤生长的抑制;抑制及/或缺乏癌细胞浸润至外围器官包括癌细胞扩散至软组织及骨;抑制及/或缺乏肿瘤或癌细胞转移;抑制及/或缺乏癌生长;缓解一或多种与该特定癌相关的症状;减少发病率及死亡率;改善生活质量;减少肿瘤发生性;减少癌干细胞的数量或频率;或该等效应的一些组合。

[0077] 如本揭示内容及权利要求书中所使用者,单数形式的"一"(a,an)及"该"(the)包含复数形式除非上下文另外清楚地说明。

[0078] 应了解的是,只要此处的实施方式系以用语"包含"描述,其亦提供其他以"由...组成"及/或"实质上由...组成"的用语所描述的类似实施方式。也应了解的是,只要此处的实施方式系以用语"实质上由...组成"描述,其亦提供其他以"由...组成"的用语所描述的类似实施方式。

[0079] 当使用于此处诸如"A及/或B"的词组中,用语"及/或"系意图包括A及B两者、A或B、A(单独)及B(单独)。同样地,使用于词组诸如"A、B及/或C"中的用语"及/或"系意图包含下列实施方式中的各者:A、B及C;A、B或C;A或C;A或B;B或C;A及C;A及B;B及C;A(单独);B(单独);及C(单独)。

II.使用预测性生物标记物的方法

[0080] 本文中提供用于鉴别、分类及/或选择可能对Wnt途径抑制剂的治疗有反应("敏感性")或无反应("抗性")的肿瘤及/或癌症病患的方法。此外,提供用于治疗可能对治疗有反应、经预测为对治疗有反应及/或已经鉴别为对Wnt途径抑制剂的治疗有反应的癌症病患的方法。

[0081] 本文中提供一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的方法,该方法包含:(a)获得该人肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CRBP2、WIF1及DKK1中的一或多种;以及(c)根据该等生物标记物的表达量,鉴别该肿瘤为可能对治疗有反应或无反应。在一些实施方式中,一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的方法包含:(a)获得该人肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CRBP2、WIF1及DKK1中的一或多种;以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达,计算判定值;其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应,且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。

[0082] 本文中提供一种分类人肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法,该方法包含:(a)获得该人肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(c)根据该等生物标记物的表达量,分类该肿瘤为可能对治疗有反应或无反应。在一些实施方式中,一种分类人肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法包含:(a)获得该人肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(c)根据该生物标记物标签中的生物标记物的标准化表达,计算判定值;其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应,且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。

[0083] 本文中提供一种测定人肿瘤对Wnt途径抑制剂的治疗的反应性(敏感性)的方法，该方法包含:(a)获得该人肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含基因FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(c)根据该等生物标记物的表达量，测定该肿瘤对治疗的反应性。在一些实施方式中，一种测定人肿瘤对Wnt途径抑制剂的治疗的反应性或敏感性的方法包含:(a)获得该人肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含基因FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值;其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应。

[0084] 本文中提供一种鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的方法，该方法包含:(a)获得该病患肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(c)根据该等生物标记物的表达量，鉴别可能对治疗有反应的病患。在一些实施方式中，一种鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的方法包含:(a)获得该病患肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值;其中阳性判定值显示该病患系经预测为对该Wnt途径抑制剂的治疗有反应。在一些实施方式中，该方法另包含当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。在一些实施方式中，本方法另包含对该病患投予治疗有效量的Wnt途径抑制剂。

[0085] 本文中提供一种选择癌症病患以接受Wnt途径抑制剂的治疗的方法，该方法包含:(a)获得该病患肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;(c)根据该等生物标记物的表达量，选择该将接受治疗的病患。在一些实施方式中，一种选择癌症病患以接受Wnt途径抑制剂的治疗的方法包含:(a)获得该病患肿瘤的样本;(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值;以及(d)当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。在一些实施方式中，本方法另包含对该病患投予治疗有效量的Wnt途径抑制剂。

[0086] 本文中提供一种治疗病患的癌症的方法，其包含:(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含:(i)获得该病患癌症的样本;(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(iii)根据该等生物标记物的表达量，鉴别可能对治疗有反应的病患;以及(b)对该可能对治疗有反应的病患投予有效量的Wnt途径抑制剂。在一些实施方式中，一种治疗病患的癌症的方法包含:(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含:(i)获得该病患癌症的样本;(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(iii)根据该标签中的生

物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对该经预测为对治疗有反应的病患投予有效量的Wnt途径抑制剂。

[0087] 在另一方面中，本发明提供一种治疗病患的癌症的方法，其包含：对该病患投予有效量的Wnt途径抑制剂，其中根据病患肿瘤样本中生物标记物标签的表达量，该病患系经预测为对该Wnt抑制剂治疗有反应，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，一种治疗病患的癌症的方法包含：对该病患投予有效量的Wnt途径抑制剂；其中根据由病患肿瘤样本中生物标记物标签的生物标记物的标准化表达的加权总和所计算的阳性判定值，该病患系经预测为对治疗有反应，其中该生物标记物组包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。

[0088] 本文中提供一种增加Wnt途径抑制剂的有效治疗的可能性的方法，其包含：(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该等生物标记物的表达量，鉴别可能对治疗有反应的病患；以及(b)对该病患投予有效量的Wnt途径抑制剂。在一些实施方式中，一种增加Wnt途径抑制剂的有效治疗的可能性的方法包含：(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对肿瘤具有阳性判定值的病患投予有效量的该Wnt途径抑制剂。

[0089] 在另一方面中，本发明提供一种增加Wnt途径抑制剂的有效治疗的可能性的方法，其包含：对病患投予有效量的Wnt途径抑制剂；其中根据病患肿瘤样本中生物标记物标签的表达量，该病患系经鉴别为可能对该Wnt抑制剂治疗有反应，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，一种增加Wnt途径抑制剂的有效治疗的可能性的方法包含：对病患投予有效量的Wnt途径抑制剂；其中根据由病患肿瘤样本中生物标记物标签的生物标记物的标准化表达的加权总和所计算的阳性判定值，该病患系经鉴别为可能对治疗有反应，其中该生物标记物组包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。在一些实施方式中，该病患系经鉴别为可能对Wnt途径抑制剂与太平洋紫杉醇的组合治疗有反应。在一些实施方式中，该方法包含对该病患投予该Wnt途径抑制剂与太平洋紫杉醇的组合。

[0090] 本文中提供一种用于鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的用途，其中该用途包含(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CRBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应，且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。

[0091] 本文中提供一种用于分类可能对Wnt途径抑制剂的治疗有反应或无反应的人肿瘤的用途，其中该用途包含(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的

各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应，且阴性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂无反应。

[0092] 本文中提供一种用于测定人肿瘤对Wnt途径抑制剂的治疗的敏感性的用途，其中该用途包含(a)获得该人肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含基因FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该肿瘤系经预测为对该Wnt途径抑制剂有反应。

[0093] 本文中提供一种用于鉴别对Wnt途径抑制剂的治疗可能有反应的癌症病患的用途，其中该用途包含(a)获得该病患肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对该Wnt途径抑制剂的治疗有反应。在一些实施方式中，该用途另包含当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。在一些实施方式中，该用途另包含对该病患投予治疗有效量的Wnt途径抑制剂。

[0094] 本文中提供一种用于选择癌症病患以接受Wnt途径抑制剂的治疗的用途，其中该用途包含(a)获得该病患肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；以及(d)当该病患的肿瘤样本具有阳性判定值，选择该病患以接受治疗。在一些实施方式中，该用途另包含对该病患投予治疗有效量的Wnt途径抑制剂。

[0095] 本文中提供一种用于治疗病患的癌症的用途中的Wnt途径抑制剂，该用途包含：(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对该经预测为对治疗有反应的病患投予有效量的Wnt途径抑制剂。

[0096] 本文中提供一种增加Wnt途径抑制剂的有效治疗的可能性的用途，该用途包含：(a)鉴别病患是否具有可能对Wnt途径抑制剂的治疗有反应的肿瘤，其中该鉴别包含：(i)获得该病患癌症的样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对肿瘤具有阳性判定值的病患投予有效量的该Wnt途径抑制剂。

[0097] 本文中提供一种用于治疗经鉴别为对Wnt途径抑制剂的治疗可能有反应的病患的癌症的用途的Wnt途径抑制剂，其中鉴别该病患包含：(i)测量自该病患获得的癌症样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物

FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种;以及(ii)根据该特征中的该等生物标记物的标准化表达,计算判定值;其中阳性判定值显示该病患系经预测为对该治疗有反应。

[0098] 本文中提供一种用于治疗病患的癌症的Wnt途径抑制剂,其中该病患系经计算为具有阳性判定值者,该判定值系根据该病患的癌症样本中的生物标记物标签的各生物标记物的标准化表达计算,其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种。

[0099] 在本文所述的方法的一些实施方式中,该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的两种或两种以上。在一些实施方式中,该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的三种或三种以上。在一些实施方式中,该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的四种或四种以上。在一些实施方式中,该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的五种或五种以上。在一些实施方式中,该生物标记物标签包含FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1。在一些实施方式中,该生物标记物标签系由FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1组成。

[0100] 在一些实施方式中,该生物标记物标签除了生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的至少一者之外,还包含一或多种额外的生物标记物。在一些实施方式中,该生物标记物标签除了生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的至少一者之外,还包含一或多种额外的选自表2所列的基因的生物标记物。在一些实施方式中,该生物标记物标签除了生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的至少一者之外,还包含生物标记物EP300、CTBP1、WNT6、WNT9A、SNT3、FZD2、FZD7、APC、TLE2、DVL2、PITX2、WISP1、GSK3B及LEF1中的一或多种。在一些实施方式中,该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1、EP300及CTBP1中的一或多种。在一些实施方式中,该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1、EP300、CTBP1、WNT6、WNT3、FZD2、APC、TLE2、DVL2、PITX2、WISP1、GSK3B、WNT9A、FZD7及LEF1中的一或多种。

[0101] 在本文所述的方法的一些实施方式中,该生物标记物标签包含FBXW2。在一些实施方式中,该生物标记物标签包含CCND2。在一些实施方式中,该生物标记物标签包含RHOU。在一些实施方式中,该生物标记物标签包含CTBP2。在一些实施方式中,该生物标记物标签包含WIF1。在一些实施方式中,该生物标记物标签包含DKK1。

[0102] 在本文所述的方法的一些实施方式中,该生物标记物标签包含FBXW2及CCND2。在一些实施方式中,该生物标记物标签包含FBXW2及RHOU。在一些实施方式中,该生物标记物标签包含FBXW2及CTBP2。在一些实施方式中,该生物标记物标签包含FBXW2及WIF1。在一些实施方式中,该生物标记物标签包含FBXW2及DKK1。在一些实施方式中,该生物标记物标签包含CCND2及RHOU。在一些实施方式中,该生物标记物标签包含CCND2及CTBP2。在一些实施方式中,该生物标记物标签包含CCND2及WIF1。在一些实施方式中,该生物标记物标签包含CCND2及DKK1。在一些实施方式中,该生物标记物标签包含RHOU及CTBP2。在一些实施方式中,该生物标记物标签包含RHOU及WIF1。在一些实施方式中,该生物标记物标签包含RHOU及DKK1。在一些实施方式中,该生物标记物标签包含CTBP2及WIF1。在一些实施方式中,该生物标记物标签包含CTBP2及DKK1。在一些实施方式中,该生物标记物标签包含WIF1及DKK1。

[0103] 在本文所述的方法的一些实施方式中，该生物标记物标签包含FBXW2、CCND2及RHOU。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2及CTBP2。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2及WIF1。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、RHOU及CTBP2。在一些实施方式中，该生物标记物标签包含FBXW2、RHOU及WIF1。在一些实施方式中，该生物标记物标签包含FBXW2、RHOU及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、CTBP2及WIF1。在一些实施方式中，该生物标记物标签包含FBXW2、CTBP2及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含CCND2、RHOU及CTBP2。在一些实施方式中，该生物标记物标签包含CCND2、FBXW2、RHOU及WIF1。在一些实施方式中，该生物标记物标签包含CCND2、RHOU及DKK1。在一些实施方式中，该生物标记物标签包含CCND2、CTBP2及WIF1。在一些实施方式中，该生物标记物标签包含CCND2、CTBP2及DKK1。在一些实施方式中，该生物标记物标签包含CCND2、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含RHOU、CTBP2及WIF1。在一些实施方式中，该生物标记物标签包含RHOU、CTBP2及DKK1。在一些实施方式中，该生物标记物标签包含RHOU、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含CTBP2、WIF1及DKK1。

[0104] 在本文所述的方法的一些实施方式中，该生物标记物标签包含FBXW2、CCND2、RHOU及CTBP2。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2、FBXW2、RHOU及WIF1。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2、RHOU及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、RHOU、CTBP2及WIF1。在一些实施方式中，该生物标记物标签包含FBXW2、RHOU、CTBP2及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、CTBP2、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含CCND2、RHOU、CTBP2及WIF1。在一些实施方式中，该生物标记物标签包含CCND2、RHOU、CTBP2及DKK1。在一些实施方式中，该生物标记物标签包含CCND2、CTBP2、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含RHOU、CTBP2、WIF1及DKK1。在一些实施方式中，该些生物标记物标签中的任一者可包含一或多种额外的生物标记物。

[0105] 在本文所述的方法的一些实施方式中，该生物标记物标签包含FBXW2、CCND2、RHOU、CTBP2及WIF1。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2、RHOU、CTBP2及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2、CTBP2、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、CCND2、RHOU、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含FBXW2、RHOU、CTBP2、WIF1及DKK1。在一些实施方式中，该生物标记物标签包含CCND2、RHOU、CTBP2、WIF1及DKK1。

[0106] 在一些实施方式中，该样本包括但不限于任何临床上重要的组织样本，诸如肿瘤活体样本、核心活体组织样本、细针抽吸样本、毛囊或体液样本如血液、血浆、血清、淋巴液、腹水、囊液或尿液。在一些实施方式中，该样本系取自具有肿瘤或癌的病患。在一些实施方式中，该样本系原发性肿瘤。在一些实施方式中，该样本系转移性肿瘤。该样本可能采集自人或非人哺乳动物，诸如小鼠、大鼠、非人灵长动物、犬、猫、反刍动物、猪或绵羊。在一些实施方式中，样本系在多重时间点自个体采集，例如治疗前、治疗期间及/或治疗后。在一些实施方式中，样本系自个体的不同部位采集，例如来自原发性肿瘤的样本及来自远处位置的转移的样本。

[0107] 在一些实施方式中，该样本系经石蜡包埋固定的组织样本。在一些实施方式中，该样本系经福尔马林固定的石蜡包埋(FFPE)组织样本。在一些实施方式中，该样本系新鲜组织(例如肿瘤)样本。在一些实施方式中，该样本系冷冻组织样本。在一些实施方式中，该样本系新鲜冷冻(FF)组织(例如肿瘤)样本。在一些实施方式中，该样本系自流体分离的细胞。在一些实施方式中，该样本包含循环肿瘤细胞(CTC)。在一些实施方式中，该样本系档案组织样本。在一些实施方式中，该样本系具有已知诊断、治疗及/或结果病史的档案组织样本。在一些实施方式中，该样本系组织块(block oftissue)。在一些实施方式中，该样本系分散细胞。在一些实施方式中，该样本大小系约1个细胞至约1×10^6个细胞或更多。在一些实施方式中，该样本大小系约10个细胞至约1×10^5个细胞。在一些实施方式中，该样本大小系约10个细胞至约10,000个细胞。在一些实施方式中，该样本大小系约10个细胞至约1,000个细胞。在一些实施方式中，该样本大小系约10个细胞至约100个细胞。在一些实施方式中，该样本大小系约1个细胞至约10个细胞。在一些实施方式中，该样本大小系单一细胞。

[0108] 在一些实施方式中，该样本系处理成DNA或RNA。在一些实施方式中，RNA系自该样本分离。在一些实施方式中，mRNA系自该样本分离。在一些实施方式中，RNA系藉由涉及细胞溶解及使其中所含的蛋白变性的程序自细胞分离。在一些实施方式中，添加DNA酶以移除DNA。在一些实施方式中，添加RNA酶抑制剂至溶解缓冲液。在一些实施方式中，在该程序中加入蛋白变性/分解的步骤。用于制备全RNA及mRNA的方法系该领域所广为周知，RNA分离试剂盒可自商业途径获得(例如RNeasy迷你试剂盒，Qiagen，USA)。在一些实施方式中，RNA系由PCR为基础的技术扩增。

[0109] 生物标记物表达量的测定可由任何适当方法进行，包括但不限于基于分析多核苷酸表达、测序多核苷酸及/或分析蛋白表达的方法。举例来说，生物标记物表达量的测定可藉由检测由受到关注的基因所表达的mRNA的表达及/或藉由检测由该等基因所编码的多肽的表达进行。

[0110] 经常用于分析多核苷酸的方法包括南方墨点分析、北方墨点分析及原位杂交、RNA酶保护分析、及聚合酶连锁反应(PCR)为基础的方法，诸如逆转录聚合酶连锁反应(RT-PCR)、定量PCR(qPCR)(亦称为实时PCR)、TaqMan™、TaqMan™低密度阵列(TLDA)、锚定PCR、竞争PCR、cDNA末端快速扩增(RACE)、及微阵列分析。RT-PCR是一种可用来比较不同样本中的mRNA量，以检测基因表达特性的定量方法。RT-PCR的变型是实时定量PCR，其透过双标记产荧光探针(例如TaqMan™探针)测量PCR产物累积。有许多其他熟悉此项技术者已知的以PCR为基础的技术，包括但不限于差异性展示、扩增片段长度多型性、BeadArray™技术、高涵盖度表达分析(HiCEP)及数字PCR。用于以测序为基础的基因表达分析的代表性方法包括基因表达连续分析(SAGE)、大规模平行标签测序(MPSS)、及NexGen测序分析，包括mRNA测序。

[0111] 在某些实施方式中，生物标记物表达系使用qPCR测试测定。例如，自新鲜冷冻(FF)组织样本萃取总RNA或自巨观解剖的经福尔马林固定的石蜡包埋(FFPE)组织样本萃取总RNA。总RNA的定量及定性系藉由标准分光亮度法及/或其他适当方法(例如Agilent Bioanalyzer)进行。在RNA萃取之后，RNA样本系利用标准方法及/或市售cDNA合成试剂盒(例如Roche Transcriptor First Strand cDNA合成试剂盒)进行逆转录。所形成的cDNA利用例如ABI预扩增试剂盒进行预扩增。生物标记物(例如FBXW2、CCND2、RHOU、CTBP2、WIF1及/或DKK1)的表达系于例如Roche Lightcycler 480系统(Roche Diagnostics)上使用ABI

TaqMan Gene Expression Mastermix检测。qPCR反应重复进行三次。每次分析时，在未经逆转录下分析样本亚群(RT阴性对照)以及在无模板下分析对照样本。每个板上包括通用人参考RNA样本以作为阳性对照。自标准参考基因组识别适当的参考基因。选择具有不同细胞功能的候选参考基因以消除共调节的风险。最适当的参考基因系使用特定软件及算法评估及选择(例如Genex软件;GeNorm及Normfinder算法)。各生物标记物的表达量利用该经选择的最佳参考基因加以正常化。在一些实施方式中，各生物标记物的这些经正常化(或标准化)的表达值被用来计算该样本的判定值。在一些实施方式中，各生物标记物的这些经正常化(或标准化)的表达值被用来计算表达量。

[0112] 在一些实施方式中，生物标记物表达系利用以PCR为基础的分析测定，该分析包含各生物标记物(例如FBXW2、CCND2、RHOU、CTBP2、WIF1及/或DKK1)的特定引物及/或探针。如本文中所使用，用语“探针”系指能与特定所欲的标靶生物分子选择性结合的任何分子。探针可由熟悉此项技术者使用已知技术合成，或可衍生自生物制剂。探针可包括但不限于RNA、DNA、蛋白质、肽、适体、抗体及有机分子。用语“引物”或“探针”包含具有特定SEQ ID NO序列的寡核苷酸或具有与特定SEQ ID NO互补的序列的寡核苷酸。在一些实施方式中，该探针系经修饰。在一些实施方式中，该探针系经淬灭剂修饰。在一些实施方式中，该探针系经标记。标记包括但不限于比色标记、荧光标记、化学发光标记或生物发光标记。

[0113] 在一些实施方式中，各生物标记物的生物标记物表达系利用特定引物组及探针测定。在一些实施方式中，特定引物组系由正向引物及反向引物组成。在一些实施方式中，CCND2表达系利用包含序列GCTGTCTCTGATCCGCAAGC(SEQ ID NO:62)的多核苷酸、包含序列GACGGTGGGTACATGGCAAAC(SEQ ID NO:63)的多核苷酸及包含序列CCTTCATTGCTCTGTGTGCCACCGAC(SEQ ID NO:64)的多核苷酸或彼等的互补物测定。在一些实施方式中，CCND2表达系利用序列GCTGTCTCTGATCCGCAAGC(SEQ ID NO:62)的正向引物及序列GACGGTGGGTACATGGCAAAC(SEQ ID NO:63)的反向引物测定。在一些实施方式中，CCND2表达系利用序列CCTTCATTGCTCTGTGTGCCACCGAC(SEQ ID NO:64)的探针测定。

[0114] 在一些实施方式中，CTBP2表达系利用经分离的包含序列ATCCGTGGGGAGACGCTG(SEQ ID NO:65)的多核苷酸、包含序列CTCGAACTGCAACCGCCTG(SEQ ID NO:66)的多核苷酸及包含序列CCCGTGCGACCAAAGCCAATGAGG(SEQ ID NO:67)的多核苷酸或彼等的互补物测定。在一些实施方式中，CTBP2表达系利用序列ATCCGTGGGGAGACGCTG(SEQ ID NO:65)的正向引物及序列CTCGAACTGCAACCGCCTG(SEQ ID NO:66)的反向引物测定。在一些实施方式中，CTBP2表达系利用序列CCCGTGCGACCAAAGCCAATGAGG(SEQ ID NO:67)的探针测定。

[0115] 在一些实施方式中，DKK1表达系利用经分离的包含序列GACCATTGACAACTACCAGCCGTA(SEQ ID NO:68)的多核苷酸、包含序列TGGGACTAGCGCAGTACTCATC(SEQ ID NO:69)的多核苷酸及包含序列TGCCGCACTCCTCGTCCTCTG(SEQ ID NO:70)的多核苷酸或彼等的互补物测定。在一些实施方式中，DKK1表达系利用序列GACCATTGACAACTACCAGCCGTA(SEQ ID NO:68)的正向引物及序列TGGGACTAGCGCAGTACTCATC(SEQ ID NO:69)的反向引物测定。在一些实施方式中，DKK1表达系利用序列TGCCGCACTCCTCGTCCTCTG(SEQ ID NO:70)的探针测定。

[0116] 在一些实施方式中，FBXW2表达系利用包含序列GCCAGTTATGATATTCTCAGGGTCA(SEQ ID NO:71)的多核苷酸、包含序列AGCAGGGCAAAGATATCTCCAAA(SEQ ID NO:72)的多核苷酸及

包含序列AGACTCCTGAGATAGCAAACTTGGCCT(SEQ ID NO:73)的多核苷酸或彼等的互补物测定。在一些实施方式中,FBXW2表达系利用序列GCCAGTTATGATATTCTCAGGGTCA(SEQ ID NO:71)的正向引物及序列AGCAGGGCAAAGATATCTCCAAA(SEQ ID NO:72)的反向引物测定。在一些实施方式中,FBXW2表达系利用序列AGACTCCTGAGATAGCAAACTTGGCCT(SEQ ID NO:73)的探针测定。

[0117] 在一些实施方式中,RHOU1表达系利用包含序列CCCACCGAGTACATCCCTACTG(SEQ ID NO:74)的多核苷酸、包含序列CAGTGTCACAGAGTTGGAGTCTCA(SEQ ID NO:75)的多核苷酸及包含序列CGCCCATCCACAGACACCACCG(SEQ ID NO:76)的多核苷酸或彼等的互补物测定。在一些实施方式中,RHOU1表达系利用序列CCCACCGAGTACATCCCTACTG(SEQ ID NO:74)的正向引物及序列CAGTGTCACAGAGTTGGAGTCTCA(SEQ ID NO:75)的反向引物测定。在一些实施方式中,RHOU1表达系利用序列CGCCCATCCACAGACACCACCG(SEQ ID NO:76)的探针测定。

[0118] 在一些实施方式中,WIF1表达系利用包含序列GTTCCAAAGGTTACCAGGGAGAC(SEQ ID NO:77)的多核苷酸、包含序列GTTGGGTTCATGGCAGGTTCC(SEQ ID NO:78)的多核苷酸及包含序列CCAGGCTCGCAGACAGGCTTTGAAC(SEQ ID NO:79)的多核苷酸或彼等的互补物测定。在一些实施方式中,WIF1表达系利用序列GTTCCAAAGGTTACCAGGGAGAC(SEQ ID NO:77)的正向引物及序列GTTGGGTTCATGGCAGGTTCC(SEQ ID NO:78)的反向引物测定。在一些实施方式中,WIF1表达系利用序列CCAGGCTCGCAGACAGGCTTTGAAC(SEQ ID NO:79)的探针测定。

[0119] 在本文所述的任何方法的一些实施方式中,FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量系利用选自SEQ ID NO:62至79的多核苷酸测量。在本文所述的任何方法的一些实施方式中,FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量系利用下列测量:(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物、及包含SEQ ID NO:64的探针;(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物、及包含SEQ ID NO:67的探针;(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物、及包含SEQ ID NO:70的探针;(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物、及包含SEQ ID NO:73的探针;(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物、及包含SEQ ID NO:76的探针;以及(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物、及包含SEQ ID NO:79的探针。

[0120] 在一些实施方式中,各生物标记物(例如FBXW2、CCND2、RHOU、CTBP2、WIF1及/或DKK1)的表达量系于分开分析(例如6个分析)中测定。在一些实施方式中,所有6个分析中的各分析的参考基因及标准化方法系为相同。在一些实施方式中,数种生物标记物(例如FBXW2、CCND2、RHOU、CTBP2、WIF1及/或DKK1)的表达量系于单一多重分析中检测。

[0121] 或者,生物标记物表达量可藉由扩增产自mRNA的互补性DNA(cDNA)或互补性RNA(cRNA)及使用微阵列分析其加以测定。微阵列技术能够同步分析数千基因的表达。一些不同的阵列构造及其产制方法系本领域技术人员所知。此外,微阵列可自商业途径获得(例如Affymetrix基因芯片)或可定制化产制。目前广为使用的微阵列包括cDNA阵列及寡核苷酸阵列。通常,受到关注的多核苷酸(例如探针或探针组)系经涂布(plated)或阵列化(arrayed)于微芯片基材上。在一些实施方式中,至少10、25、50、100、500、1000、5000、10,000、20,000或25,000个或更多基因的探针系经固定于阵列基材上。该基材可为多孔或无孔性支撑物,诸如玻璃、塑料或胶体表面。探针可包括DNA、RNA、DNA及RNA的共聚物序列、DNA及/或RNA类似物,或彼等的组合。在一些实施方式中,微阵列包括支撑物以及各个别基因的

结合位点的有序阵列。该等微阵列可为可寻址(addressable)的阵列或位置可寻址的阵列，例如阵列的各探针系位于固体支撑物上已知、预先决定的位置，以使各探针的识别可自其阵列位置决定。

[0122] 在微阵列上的各探针可具有介于10至50,000个核苷酸长度。在一些实施方式中，微阵列的探针可由长度小于约1,000个核苷酸、小于约750个核苷酸、小于约500个核苷酸、小于约250个核苷酸、小于约100个核苷酸、或小于约50个核苷酸的核苷酸序列组成。通常，阵列包括阳性对照探针及阴性对照探针。

[0123] 在某些实施方式中，生物标记物表达系使用微阵列测定。例如，自新鲜冷冻(FF)组织样本萃取总RNA或自巨观解剖的经福尔马林固定的石蜡包埋(FFPE)组织样本萃取总RNA。总RNA的定量及定性系藉由标准分光亮度法及/或其他适当技术(例如Agilent Bioanalyzer)进行。在RNA萃取之后，该RNA样本系利用标准方法及/或市售扩增系统(例如NuGEN OvationRNA扩增系统V2)扩增。该经扩增的cDNA系依照标准程序经切段(fragmented)、标记及杂交至微阵列(例如使用NuGEN Encore生物素模块及Affymetrix基因芯片阵列)。该阵列系根据微阵列说明经清洗、染色及扫描。微阵列数据系经预先处理，探针级强度测量值系经背景校正、正常化及使用Robust多芯片算法(RMA)概述为表达测量值。探针量数据系经概述以取得各生物标记物的表达量(例如FBXW2、CCND2、RHOU、CTBP2、WIF1及/或DKK1)。性质参数限值及数据减除技术(例如主成分分析)的组合系应用至数据组以建立定性数据及识别可能的异常样本。各生物标记物的这些经正常化(或标准化)的表达值被用来计算该样本的判定值。

[0124] 在一些实施方式中，生物标记物表达系藉由研究受到关注的基因的蛋白表达加以分析。常用的分析蛋白表达的方法包括但不限于以免疫组织化学(IHC)为基础、以抗体为基础及以质谱仪为基础的方法。抗体(通常为单克隆抗体)可被用于检测基因产物(例如蛋白质)的表达。在一些实施方式中，抗体可藉由直接标示抗体本身加以检测。在其他实施方式中，未经标示的一级抗体系与经标示的二级抗体组合使用。免疫组织化学方法及/或试剂盒系该领域所广为周知且为市售可得者。

[0125] 在一些实施方式中，生物标记物表达系藉由本领域技术人员已知的试验测定，包括但不限于多分析物检测试验、酶连接免疫吸附测定(ELISA)、放射性免疫测定、西方印迹分析试验、免疫荧光分析、酶免疫分析、免疫沉降试验、化学发光试验、免疫组织化学试验、斑点印迹法或狭缝印迹法。在一些实施方式中，当抗体系用于分析中时，该抗体系经可检测地标记。该等抗体标记可包括但不限于免疫荧光标记、化学发光标记、磷光标记、酶标记、放射标记、抗生物素蛋白/生物素、胶体金粒子、彩色粒子及磁性粒子。

[0126] 其他用于分析生物标记物表达的适当方法包括以蛋白质粒为基础的方法。蛋白质粒学包括(除其他者外)样本中蛋白表达的整体改变的研究。在一些实施方式中，蛋白质粒方法包含下列步骤：(1)藉由2D电泳(2-D PAGE)分离样本中的个别蛋白、(2)识别自胶体回收的个别蛋白(例如藉由质谱分析或N端测序)、及(3)利用生物信息学分析数据。在一些实施方式中，蛋白质粒方法包含使用组织微阵列(TMA)。组织阵列可根据各种本领域技术人员所知的技术建构。在某些实施方式中，手动组织阵列器被用于自由组织样本制备的石蜡块移除“核心”。该核心接着被插入另一石蜡块在网格上的指定位置。来自多达约400个样本的核心可被插入单一接受块中。所形成的组织阵列可经处理成为薄切片以供分析。在一些实

施方式中，蛋白质粒方法包含抗体微阵列。在一些实施方式中，蛋白质粒方法包含使用质谱仪，包括但不限于SELDI、MALDI、电喷洒及表面电浆共振方法。在一些实施方式中，蛋白质粒方法包含以珠为基础的技术，包括但不限于呈阵列形式的在珠上的抗体。在一些实施方式中，蛋白质粒方法包含反相蛋白微阵列(RPPM)。在一些实施方式中，蛋白质粒方法包含多重蛋白分析，包括但不限于全蛋白质粒分析(GPS)方法。

[0127] 在一些实施方式中，生物标记物标签系藉由二个样本之间的不同基因表达鉴别。在一些实施方式中，生物标记物标签系藉由包含在癌细胞与正常细胞中表达不同的基因的二个样本之间的不同基因表达鉴别。在一些实施方式中，生物标记物标签包含在肿瘤发生性癌干细胞与非肿瘤发生性癌细胞之间表达不同的基因。在一些实施方式中，生物标记物标签包含在对特定治疗有反应的肿瘤细胞与对相同治疗无反应的肿瘤细胞之间表达不同的基因。

[0128] 在一些实施方式中，表达特性系利用微阵列分析测定。微阵列数据鉴别包含二个样本之间类似地及差异地表达的基因的基因特性。在一些实施方式中，表达特性系根据倍数表达变化加以改良、过滤及/或细分成生物标记物标签。在一些实施方式中，所有在特定倍数表达变化以上的基因皆被包括于生物标记物标签中。倍数表达变化可为增加、减少或同时增加及减少。在一些实施方式中，所有具有2倍或以上的表达变化的基因皆被包括于生物标记物标签中。在一些实施方式中，所有具有2.5倍或以上的表达变化的基因皆被包括于生物标记物标签中。在一些实施方式中，所有具有3倍或以上的表达变化的基因皆被包括于生物标记物标签中。在一些实施方式中，所有具有3.5倍或以上的表达变化的基因皆被包括于生物标记物标签中。在一些实施方式中，所有具有4倍或以上的表达变化的基因皆被包括于生物标记物标签中。

[0129] 在一些实施方式中，基因表达特性系根据统计分析加以改良、过滤及/或细分成生物标记物标签。统计方法可包括但不限于聚类分析、支持向量机(SVM)分析、支持向量机递归特性消除(SVM-RFE)分析、普拉图定标(Platt scaling)、类神经网络及其他算法。在一些实施方式中，基因表达特性系利用t检验分析加以分析。在一些实施方式中，基因表达特性系利用成对样本经验贝氏(Baysian)分析加以分析。在一些实施方式中，使用统计分析的组合。在一些实施方式中，SVM模型被用于根据训练数据获得判定值。在一些实施方式中，判定值系藉由生物标记物组的标准化表达的加权总和加以计算。在一些实施方式中，阳性判定值显示肿瘤经预测为有反应者，而阴性判定值显示肿瘤经预测为无反应者。在一些实施方式中，有反应者及无反应者的分类机率系利用普拉图定标获得(Platt,1999,Advances in Large Margin Classifiers,pp.61-74,MIT Press)。普拉图定标可包含使用最大可能性适配逻辑分布至藉由例如SVM模型获得的判定值。在一些实施方式中，具有高于0.5的机率的肿瘤将被预测为有反应者，而具有低于0.5的机率的肿瘤将被预测为无反应者。

[0130] 在本文所述的任何方法或用途的一些实施方式中，肿瘤的分类机率(关于有反应或无反应的状态)系根据判定值获得。在一些实施方式中，该等机率系藉由适配逻辑回归至判定值上获得。在一些实施方式中，具有高于0.5的机率的肿瘤系经预测为有反应者，而具有低于0.5的机率的肿瘤系经预测为无反应者。

[0131] 在一些实施方式中，生物标记物标签系藉由一系列分析步骤获得。举例来说，来自训练组样本的表达数据系得自微阵列分析。数据系经前处理以取得具有特定基因的表达矩

阵。移除具有近零变异的基因，也移除表达值低于预定量的基因。剩余基因利用SVM-RFE分析排序。留一交叉验证(LOOCV)方法被用于鉴别及选择最佳预测基因且也用于测量阳性预测值(PPV)、阴性预测值(NPV)、敏感性及特异性。

[0132] 在一些实施方式中，在样本之间P值为0.01或更小的所有增加表达、减少表达或增加及减少表达两者的基因被包括于生物标记物标签中。在一些实施方式中，在样本之间P值为0.005或更小的所有增加表达、减少表达或增加及减少表达两者的基因被包括于生物标记物标签中。在一些实施方式中，在样本之间P值为0.001或更小的所有增加表达、减少表达或增加及减少表达两者的基因被包括于生物标记物标签中。在一些实施方式中，错误拒绝率(FDR)为0.25或更小的所有增加表达、减少表达或增加及减少表达两者的基因被包括于生物标记物标签中。在一些实施方式中，FDR为0.1或更小、0.01或更小或0.001或更小的所有增加表达、减少表达或增加及减少表达两者的基因被包括于生物标记物标签中。

[0133] 在一些实施方式中，基因表达特性及/或生物标记物标签系根据统计模型加以改良、过滤及/或细分。在一些实施方式中，基因表达特性及/或生物标记物标签系根据存活分析模型加以改良、过滤及/或细分。这些模型可包括但不限于卡普兰-迈尔(Kaplan-Meier)存活模型、Cox比例模型、Cox比例风险模型、卡方分析、单变量逻辑式回归模型、多变量竞争风险模型、线性区别分析模型、参数回归模型及相关分析模型。

[0134] 在一些实施方式中，基因表达特性及/或生物标记物标签系利用具有相关临床结果的基因表达阵列数据组加以改良、过滤、细分及/或测试。有数个数据库例如Gene Expression Omnibus(GEO)及ArrayExpress包含公众可用的数据组。

[0135] 在一些实施方式中，基因表达特性及/或生物标记物标签系利用生物功能参数及/或基因组加以改良。例如，在一些实施方式中，基因表达特性及/或生物标记物标签系利用基因组富集分析(GSEA)加以改良(Subramanian et al.,2005,PNAS,102:15545-15550)。在一些实施方式中，基因表达特性系根据它们预测临床结果的能力加以改良。

[0136] 在本文所述的方法的一些实施方式中，该Wnt途径抑制剂系如本文所述的抗FZD抗体。在本文所述的方法的一些实施方式中，该Wnt途径抑制剂系与至少一种卷曲(FZD)蛋白或其的一部分特异性结合的抗体。在一些实施方式中，该抗FZD抗体与选自下列的至少一种FZD蛋白特异性结合：FZD1、FZD2、FZD5、FZD7及FZD8。在其他实施方式中，该抗FZD抗体包含：(a)包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1、包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2、及包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，及(b)包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1、包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2、及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3。在一些实施方式中，该抗FZD抗体包含重链可变区，该重链可变区含有SEQ ID NO:7的氨基酸。在一些实施方式中，该抗FZD抗体包含轻链可变区，该轻链可变区含有SEQ ID NO:8的氨基酸。在一些实施方式中，该抗FZD抗体包含重链可变区及轻链可变区，该重链可变区含有SEQ ID NO:7的氨基酸，该轻链可变区含有SEQ ID NO:8的氨基酸。在一些实施方式中，该抗FZD抗体系抗体OMP-18R5。在一些实施方式中，该抗FZD抗体系藉由具有美国菌种保存中心(ATCC)保藏编号PTA-9541的质粒编码。在其他实施方式中，该抗FZD抗体与保藏于ATCC编号为PTA-9541的质粒所编码的抗体竞争与至少一种人FZD蛋白的特异性结合。

[0137] 在本文所述的方法的一些实施方式中，该肿瘤系选自乳房肿瘤、肺肿瘤、结肠肿

瘤、神经胶质瘤、胃肠道肿瘤、肾肿瘤、卵巢肿瘤、肝脏肿瘤、结直肠肿瘤、子宫内膜肿瘤、肾脏肿瘤、前列腺肿瘤、甲状腺肿瘤、神经胚细胞瘤、胰脏肿瘤、多形性神经胶质母细胞瘤、子宫颈肿瘤、胃肿瘤、膀胱肿瘤、肝肿瘤、黑色素瘤以及头颈肿瘤。在一些实施方式中，该肿瘤系乳房肿瘤。在一些实施方式中，该肿瘤系HER2阴性乳房肿瘤。在一些实施方式中，该肿瘤系三阴性乳癌(TNBC)肿瘤。

[0138] 在本文所述的方法的一些实施方式中，该癌症系选自乳癌、肺癌、结肠癌、神经胶质瘤、胃肠道癌、肾癌、卵巢癌、肝脏癌、结直肠癌、子宫内膜癌、肾脏癌、前列腺癌、甲状腺癌、神经胚细胞瘤、胰脏癌、多形性神经胶质母细胞瘤、子宫颈癌、胃癌、膀胱癌、肝肿瘤、黑色素瘤以及头颈癌。在一些实施方式中，该癌系乳癌。在一些实施方式中，该癌系HER2阴性乳癌。在一些实施方式中，该癌系三阴性乳癌(TNBC)。

[0139] 在本文所述的方法的一些实施方式中，该方法包含以本文所述的Wnt途径抑制剂(例如抗FZD抗体)治疗病患，特别是在病患已被鉴别为对Wnt途径抑制剂的治疗有反应之后。在一些实施方式中，该治疗包含投予至少一种额外治疗剂与Wnt途径抑制剂的组合。额外的治疗剂可于投予该Wnt途径抑制剂之前、的同时及/或之后投予。在一些实施方式中，该至少一种额外治疗剂包含1、2、3或更多种额外的治疗剂。

[0140] 有用类别的治疗剂包括例如抗微管蛋白剂、耳抑素、DNA次要凹槽结合剂、DNA复制抑制剂、烷化剂(例如铂复合物诸如顺铂(cisplatin)、单(铂)、二(铂)及三核铂复合物及卡铂(carboplatin))、蒽环类(anthracycline)、抗生素、抗叶酸剂、抗代谢物、化学疗法致敏剂、双联霉素(duocarmycin)、依托泊苷(etoposide)、氟化嘧啶、离子载体、莱克西托素(lexitropsin)、亚硝基尿素、普拉汀诺(platinol)、嘌呤抗代谢物、嘌呤霉素(puromycin)、放射线致敏剂、类固醇、紫杉烷、拓扑异构酶抑制剂、长春花生物碱或该类似物。在某些实施方式中，第二治疗剂系烷化剂、抗代谢物、抗有丝分裂剂、拓扑异构酶抑制剂或血管生成抑制剂。

[0141] 可与该Wnt途径抑制剂组合投予的治疗剂包括化学治疗剂。因此，在一些实施方式中，该方法或治疗牵涉投予本发明的Wnt途径抑制剂与化学治疗剂或多种不同化学治疗剂的鸡尾酒组合的组合。Wnt途径抑制剂(例如抗FZD抗体)的治疗可发生于投予化学治疗之前、的同时或之后。组合投予可包括于单一医药制剂中共投或利用分开的制剂共投，或以任何顺序连续投予但通常在一段期间内以使所有活性剂可同步展现彼等的生物活性。该等化学治疗剂的准备及投予计划可根据制造商的说明使用或由经验丰富的医生凭经验决定。该等化学治疗的准备及投予计划亦描述于The Chemotherapy Source Book,4th Edition,2008,M.C.Perry,Editor,Lippincott,Williams&Wilkins,Philadelphia,PA。

[0142] 可用于本发明的化学治疗剂包括但不限于：烷化剂诸如噻替派(thiotepa)及环磷酰胺(cyclophosphamide)(CYTOXAN)；烷基磺酸盐诸如白消安(busulfan)、英丙舒凡(improsulfan)及哌泊舒凡(piposulfan)；氮丙啶诸如苯多巴(benzodopa)、卡波醌(carboquone)、甲基优瑞多巴(meturedopa)及优瑞多巴(uredopa)；伸乙亚胺(ethylenimines)及甲基三聚氰胺(methylmelamines)包括阿草特胺(altretamine)、三亚乙基三聚氰胺(triethylenemelamine)、三乙烯磷酰胺(trietylenephosphoramide)、三乙烯硫磷酰胺(triethylenethiophosphoramide)及三羟甲基三聚氰胺(trimethylolmelamime)；氮芥子气诸如氯芥苯丁酸(chlorambucil)、萘氮芥

(chlornaphazine)、氯磷酰胺(cholophosphamide)、雌二醇氮芥(estramustine)、异环磷酸胺(ifosfamide)、双氯乙基甲胺(mechlorethamine)、盐酸氧氮芥(mechlorethamine oxide hydrochloride)、霉法兰(melphalan)、新氮芥(novembichin)、胆甾醇苯乙酸氮芥(phenesterine)、松龙苯芥(prednimustine)、氯乙环磷酰胺(trofosfamide)、尿嘧啶芥(uracil mustard);亚硝基脲(nitrosourea)诸如卡氮芥(carmustine)、吡葡亚硝脲(chlorozotocin)、福莫司汀(fotemustine)、罗氮芥(lomustine)、尼氮芥(nimustine)、雷诺氮芥(ranimustine);抗生素诸如阿克拉霉素(aclacinomycin)、放线菌素(actinomycin)、安曲霉素(anthramycin)、氮丝氨酸(azaserine)、博来霉素(bleomycin)、放线菌素C(cactinomycin)、卡利奇霉素(calicheamicin)、卡拉比辛(carabicin)、洋红霉素(carminomycin)、嗜癌素(carzinophilin)、色霉素(chromomycin)、达克霉素(dactinomycin)、正定霉素(daunorubicin)、地托比星(detorubicin)、6-重氮-5-羰基-L-正亮氨酸、多柔比星(doxorubicin)、表阿霉素(epirubicin)、依索比星(esorubicin)、伊达比星(idarubicin)、麻西罗霉素(marcellomycin)、丝裂霉素(mitomycins)、霉酚酸、诺加霉素(nogalamycin)、橄榄霉素(olivomycin)、培洛霉素(peplomycin)、波弗霉素(porfiromycin)、嘌呤霉素(puromycin)、三铁阿霉素(quelamycin)、罗多比星(rodorubicin)、链霉黑素(streptonigrin)、链脲佐菌素(streptozocin)、杀结核菌素(tubercidin)、乌苯美司(ubenimex)、新制癌菌素(zinostatin)、佐柔比星(zorubicin);抗代谢剂诸如甲胺喋呤(methotrexate)及5-氟尿嘧啶(5-FU);叶酸类似物诸如二甲叶酸(denopterin)、甲胺喋呤、蝶罗呤(pteropterin)、三甲蝶呤(trimetrexate);嘌呤类似物诸如氟达拉滨(fludarabine)、6-巯基嘌呤(6-mercaptopurine)、硫咪嘌呤(thiamiprine)、硫鸟嘌呤;嘧啶类似物诸如安西他滨(ancitabine)、阿扎胞苷(azacitidine)、6-硫唑脲嘧啶(6-azauridine)、卡莫氟(carmofur)、胞嘧啶阿拉伯糖苷、二脱氧尿苷、脱氧氟尿苷(doxifluridine)、依诺他滨(enocitabine)、氟尿苷(floxuridine)、5-FU;雄性素诸如卡鲁睾酮(calusterone)、丙酸屈他雄酮(dromostanolone propionate)、硫雄甾醇(epitiostanol)、美雄烷(mepitiostane)、睾内酮(testolactone);抗肾上腺剂诸如胺鲁米特(aminoglutethimide)、米托坦(mitotane)、曲洛司坦(trilostane);叶酸补充剂诸如亚叶酸;醋葡醛内酯(aceglatone);醛磷酰胺糖苷(aldophosphamide glycoside);胺基酮戊酸(aminolevulinic acid);安吖啶(amsacrine);贝斯特氮芥(bestrabucil);比生群(bisantrene);依达曲沙(edatrexate);地弗胺(defofamine);秋水仙胺(demecolcine);地吖醌(diaziquone);艾弗米素(elformithine);依利醋铵(elliptinium acetate);依托格鲁(etoglucid);硝酸镓(gallium nitrate);羟基脲;香菇糖(lentinan);氯尼达明(lonidamine);米托胍腙(mitoguazone);米托蒽醌(mitoxantrone);莫哌达醇(mopidamol);二胺硝吖啶(nitracrine);喷司他丁(pentostatin);蛋胺氮芥(phenamet);吡柔比星(pirarubicin);鬼臼酸(podophyllinic acid);2-乙基酰肼(2-ethylhydrazide);丙卡巴肼(procarbazine);PSK;雷佐生(razoxane);西佐喃(sizofuran);锗螺胺(spirogermanium);细交链孢菌酮酸(tenuazonic acid);三亚胺醌(triaziquone);2,2',2"-三氯三乙胺(2,2',2"-trichlorotriethylamine);乌拉坦(urethan);长春地辛(vindesine);达卡巴嗪(dacarbazine);甘露莫司汀(mannomustine);二溴甘露醇(mitobronitol);二溴卫矛醇(mitolactol);哌泊溴烷(pipobroman);加塞拖素

(gacytosine);阿拉伯糖苷(Ara-C);类紫杉醇(taxoids)例如太平洋紫杉醇(TAXOL)及多西紫杉醇(TAXOTERE);苯丁酸氮芥(chlorambucil);吉西他滨(gemcitabine);6-硫鸟嘌呤;巯嘌呤(mercaptopurine);铂类似物诸如顺铂(cisplatin)及卡铂(carboplatin);长春碱(vinblastine);铂(platinum);依托泊苷(etoposide)(VP-16);异环磷酰胺(ifosfamide);丝裂霉素C;米托蒽醌(mitoxantrone);长春新碱(vincristine);长春瑞滨(vinorelbine);温诺平(navelbine);米托蒽醌(novantrone);替尼泊苷(teniposide);道诺霉素(daunomycin);胺喋呤(aminopterin);伊班膦酸盐(ibandronate);CPT11;拓扑异构酶抑制剂RFS 2000;二氟甲基鸟氨酸(DMFO);视黄酸;埃斯培拉霉素(esperamicin);卡培他滨(capecitabine)(XELODA)及上述任一剂的医药上可接受的盐、酸或衍生物。化学治疗剂亦包括用来调节或抑制荷尔蒙对肿瘤的作用的抗荷尔蒙剂,诸如抗雌激素剂包括例如它莫西芬(tamoxifen)、雷洛昔芬(raloxifene)、芳香酶抑制剂4(5)-咪唑、4-羟基它莫西芬、曲沃昔芬(trioxifene)、雷洛昔芬(keoxifene)、LY117018、奥那司酮(onapristone)及托瑞米芬(toremifene)(FARESTON);及抗雄性素剂诸如氟他胺(flutamide)、尼鲁米特(nilutamide)、比卡鲁胺(bicalutamide)、柳普林(leuprolide)及戈舍瑞林(goserelin);及上述任一剂的医药上可接受的盐、酸或衍生物。在某些实施方式中,该额外治疗剂系太平洋紫杉醇(紫杉醇(taxol))。

[0143] 在某些实施方式中,该化学治疗剂系拓扑异构酶抑制剂。拓扑异构酶抑制剂系干扰拓扑异构酶(例如拓扑异构酶I或II)的活性的化学治疗剂。拓扑异构酶抑制剂包括但不限于盐酸多柔比星(doxorubicin HCl)、柠檬酸正定霉素(daunorubicin citrate)、盐酸米托蒽醌(mitoxantrone HCl)、放线菌素D、依托泊苷(etoposide)、盐酸拓扑替康(topotecan HCl)、替尼泊苷(teniposide)(VM-26)及伊立替康(irinotecan),以及这些任一剂的医药上可接受的盐、酸或衍生物。

[0144] 在某些实施方式中,该化学治疗剂系抗代谢剂。抗代谢剂系一化学物质,其结构类似正常生化反应所需的代谢物,但仍有足够的不同处以干扰一或多种细胞正常功能,诸如细胞分裂。抗代谢剂包括但不限于吉西他滨(gemcitabine)、氟尿嘧啶(fluorouracil)、卡培他滨(capecitabine)、甲胺喋呤钠、雷替曲塞(ralitrexed)、培美曲塞(pemetrexed)、替加氟(tegafur)、胞嘧啶阿拉伯糖苷(cytosine arabinoside)、硫鸟嘌呤、5-氮杂胞苷、6-巯基嘌呤、硫唑嘌呤、6-硫鸟嘌呤、喷司他丁(pentostatin)、磷酸氟达拉滨(fludarabine phosphate)及克拉屈滨(cladribine),以及这些任一剂的医药上可接受的盐、酸或衍生物。

[0145] 在某些实施方式中,该化学治疗剂系抗有丝分裂剂,包括但不限于与微管蛋白结合的剂。在一些实施方式中,该剂系紫杉烷。在某些实施方式中,该剂系太平洋紫杉醇(paclitaxel)或多西紫杉醇(docetaxel),或太平洋紫杉醇或多西紫杉醇的医药上可接受的盐、酸或衍生物。在某些实施方式中,该剂系太平洋紫杉醇(TAXOL)、多西紫杉醇(TAXOTERE)、白蛋白结合型太平洋紫杉醇(nab-paclitaxel;ABRAXANE)、DHA-太平洋紫杉醇或PG-太平洋紫杉醇。在某些替代性实施方式中,该抗有丝分裂剂包含长春花生物碱,诸如长春新碱(vincristine)、长春碱(vinblastine)、长春瑞滨(vinorelbine)或长春地辛(vindesine)或彼等的医药上可接受的盐、酸或衍生物。在一些实施方式中,该抗有丝分裂剂系驱动蛋白(kinesin)Eg5的抑制剂或有丝分裂激酶诸如Aurora A或Plk1的抑制剂。在某些实施方式中,当该与Wnt途径抑制剂组合投予的化学治疗剂系抗有丝分裂剂时,该经治疗

的癌或肿瘤系乳癌或乳房肿瘤。在某些实施方式中，该额外治疗剂系太平洋紫杉醇(taxol)或与白蛋白结合的太平洋紫杉醇。

[0146] 在一些实施方式中，额外治疗剂包含诸如小分子的剂。举例来说，治疗可涉及组合投予本发明的Wnt途径抑制剂与作为其他肿瘤相关抗原的抑制剂的小分子，其他肿瘤相关性蛋白质包括但不限于EGFR、ErbB2、HER2及/或VEGF。在某些实施方式中，该额外治疗剂系抑制癌干细胞途径的小分子。在一些实施方式中，该额外治疗剂系缺口途径的抑制剂。在一些实施方式中，该额外治疗剂系Wnt途径的抑制剂。在一些实施方式中，该额外治疗剂系BMP途径的抑制剂。

[0147] 本发明的某些实施方式包含一种鉴别可能对抗体的治疗有反应或无反应的人乳房肿瘤的方法，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，其中该方法包含(a)获得该人乳房肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该乳房肿瘤系经预测为对该抗体的治疗有反应，且阴性判定值显示该肿瘤系经预测为对该抗体的治疗无反应。某些实施方式包含一种鉴别可能对抗体治疗有反应的乳癌病患的方法，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，该方法包含：(a)获得该乳癌的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该乳癌系经预测为对该抗体的治疗有反应。某些实施方式包含一种选择乳癌病患以接受抗体治疗的方法，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，该方法包含：(a)获得该乳癌的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值，其中阳性判定值显示该乳癌系经预测为对该抗体的治疗有反应，以及；当病患的肿瘤样本具有阳性判定值时，选择该病患以接受治疗。

[0148] 本发明的某些实施方式包含一种治疗病患的乳癌的方法，其包含：(a)鉴别可能对抗体治疗有反应的病患，该抗体与至少一种选自FZD1、FZD2、FZD5、FZD7及FZD8的人卷曲蛋白(FZD)特异性结合，其中该鉴别包含：(i)获得该病患的乳癌样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1中的一或多种；以及(iii)根据该标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对该经预测为对治疗有反应的病患投予有效量的抗体。

[0149] 本发明的某些实施方式包含一种鉴别可能对抗FZD抗体OMP-18R5与太平洋紫杉醇的组合治疗有反应或无反应的人乳房肿瘤的方法，该方法包含(a)获得该人乳房肿瘤的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及(c)根据该生物标记物标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该乳房肿瘤系经预测为对

该治疗有反应，且阴性判定值显示该肿瘤系经预测为对该治疗无反应。某些实施方式包含一种鉴别可能对抗FZD抗体OMP-18R5与太平洋紫杉醇的组合治疗有反应的乳癌病患的方法，该方包含：(a)获得该乳癌的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值；其中阳性判定值显示该乳癌系经预测为对该治疗有反应。某些实施方式包含一种选择乳癌病患以接受抗FZD抗体OMP-18R5与太平洋紫杉醇的组合治疗的方法，该方包含：(a)获得该乳癌的样本；(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；(c)根据该生物标记物标签中的该等生物标记物的标准化表达，计算判定值，其中阳性判定值显示该乳癌系经预测为对该治疗有反应，以及；当病患的肿瘤样本具有阳性判定值时，选择该病患以接受治疗。

[0150] 本发明的某些实施方式包含一种治疗病患的乳癌的方法，其包含：(a)鉴别该病患是否可能对抗FZD抗体OMP-18R5与太平洋紫杉醇的组合治疗有反应，其中该鉴别包含：(i)获得该病患的乳癌样本；(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及(iii)根据该标签中的生物标记物的标准化表达，计算判定值；其中阳性判定值显示该病患系经预测为对治疗有反应；以及(b)对该经预测为对治疗有反应的病患投予有效量的抗体与太平洋紫杉醇。

III.Wnt途径抑制剂

[0151] 本发明提供用于鉴别可能对Wnt途径抑制剂的治疗有反应或敏感的肿瘤及/或癌症病患的方法。如本文中所使用，“Wnt途径抑制剂”包括但不限于卷曲(FZD)结合剂及Wnt结合剂。FZD结合剂可包括与FZD蛋白特异性结合的抗体。Wnt结合剂可包括与Wnt蛋白特异性结合的抗体以及与Wnt蛋白结合的可溶性FZD受体。

[0152] 在某些实施方式中，该Wnt途径抑制剂系与一或多种人FZD蛋白结合的剂。在一些实施方式中，该FZD结合剂与一、二、三、四、五、六、七、八、九、或十种FZD蛋白特异性结合。在一些实施方式中，该FZD结合剂与一或多种选自下列的FZD蛋白结合：FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9、及FZD10。在一些实施方式中，FZD结合剂与一或多种FZD蛋白结合，该一或多种FZD蛋白包含FZD1、FZD2、FZD5、FZD7、及/或FZD8。在某些实施方式中，FZD结合剂与FZD7结合。在某些实施方式中，FZD结合剂与FZD5及/或FZD8结合。在某些实施方式中，该FZD结合剂与FZD1、FZD2、FZD5、FZD7、及FZD8特异性结合。FZD结合剂的非限制性实例可见于美国专利第7,982,013号。

[0153] 在某些实施方式中，该FZD结合剂系FZD拮抗剂。在某些实施方式中，该FZD结合剂系Wnt途径拮抗剂。在某些实施方式中，该FZD结合剂抑制Wnt信号传导。在一些实施方式中，该FZD结合剂抑制典型Wnt信号传导。

[0154] 在一些实施方式中，该FZD结合剂系抗体。在一些实施方式中，该FZD结合剂系多肽。在某些实施方式中，该FZD结合剂系包含抗原结合部位的抗体或多肽。在某些实施方式中，本文所述的FZD结合抗体或多肽的抗原结合部位能与一、二、三、四、五、或更多种人FZD蛋白结合。在某些实施方式中，该FZD结合抗体或多肽的抗原结合部位能与选自FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9及FZD10的一、二、三、四、或五种人FZD蛋白特异性

结合。在一些实施方式中，当该FZD结合剂系与超过一种FZD蛋白结合的抗体时，其可能被称为“泛FZD抗体”。

[0155] 在某些实施方式中，该FZD结合剂(例如抗体)与其所结合的一或多种人FZD蛋白的胞外域(ECD)特异性结合。在某些实施方式中，该FZD结合剂在其所结合的人FZD蛋白的Fri结构域(亦称为多半胱氨酸区(CRD))内特异性结合。各种人FZD蛋白的Fri结构域的序列系该领域已知，且提供于此处为SEQ ID NO:13(FZD1)、SEQ ID NO:14(FZD2)、SEQ ID NO:15(FZD3)、SEQ ID NO:16(FZD4)、SEQ ID NO:17(FZD5)、SEQ ID NO:18(FZD6)、SEQ ID NO:19(FZD7)、SEQ ID NO:20(FZD)、SEQ ID NO:21(FZD9)、及SEQ ID NO:22(FZD10)。

[0156] 在某些实施方式中，该FZD结合剂与一、二、三、四、五、或多种FZD蛋白结合。在一些实施方式中，该FZD结合剂与选自FZD1、FZD2、FZD5、FZD7、及FZD8中的一、二、三、四、或五种FZD蛋白特异性结合。在一些实施方式中，该FZD结合剂与至少FZD5及FZD8特异性结合。

[0157] 在一些实施方式中，该FZD结合剂以大约1μM或更低、大约100nM或更低、大约40nM或更低、大约20nM或更低、大约10nM或更低、大约1nM或更低、或大约0.1nM或更低的解离常数(K_D)与至少一种人FZD蛋白结合。在一些实施方式中，FZD结合剂以大约10nM或更低的K_D与至少一种FZD蛋白结合。在一些实施方式中，FZD结合剂以大约1nM或更低的K_D与至少一种FZD蛋白结合。在一些实施方式中，FZD结合剂以大约0.1nM或更低的K_D与至少一种FZD蛋白结合。在某些实施方式中，FZD结合剂以约40nM或更低的K_D与一或多种(例如1、2、3、4、或5种)FZD1、FZD2、FZD5、FZD7、及FZD8的各者结合。在某些实施方式中，该FZD结合剂以约10nM或更低的K_D与一或多种FZD1、FZD2、FZD5、FZD7、及FZD8的各者结合。在某些实施方式中，该FZD结合剂以约10nM的K_D与FZD1、FZD2、FZD5、FZD7、及FZD8的各者结合。在一些实施方式中，该结合剂(例如抗体)与FZD蛋白的K_D系利用固定于Biacore芯片上的FZD-Fc融合蛋白测得的K_D，该FZD-Fc融合蛋白包含至少部分的FZD胞外域或FZD-Fri结构域。

[0158] 在某些实施方式中，该FZD结合剂以约1μM或更低、约100nM或更低、约40nM或更低、约20nM或更低、约10nM或更低、或约1nM或更低的EC_{50}与一或多种(例如二或多种、三或多种、或四或多种)人FZD蛋白结合。在某些实施方式中，FZD结合剂以约40nM或更低、约20nM或更低、或约10nM或更低的EC_{50}与超过一种FZD蛋白结合。在某些实施方式中，该FZD结合剂对下列一或多种(例如1、2、3、4、或5种)FZD蛋白具有约20nM或更低的EC_{50}：FZD1、FZD2、FZD5、FZD7及FZD8。在某些实施方式中，该FZD结合剂对下列一或多种(例如1、2、3、4、或5种)FZD蛋白具有约10nM或更低的EC_{50}：FZD1、FZD2、FZD5、FZD7及FZD8。在某些实施方式中，该FZD结合剂在与FZD5及/或FZD8结合方面具有约40nM或更低或20nM或更低的EC_{50}。

[0159] 在某些实施方式中，该Wnt途径抑制剂系FZD结合剂，且该FZD结合剂系抗体。在一些实施方式中，该抗体系重组抗体。在一些实施方式中，该抗体系单克隆抗体。在一些实施方式中，该抗体系嵌合抗体。在一些实施方式中，该抗体系人化抗体。在一些实施方式中，该抗体系人抗体。在某些实施方式中，该抗体系IgG1抗体。在某些实施方式中，该抗体系IgG2抗体。在某些实施方式中，该抗体系包含抗原结合部位的抗体片段。在一些实施方式中，该抗体系单价、单特异性、或双价。在一些实施方式中，该抗体系双特异性抗体或多特异性抗体。在一些实施方式中，该抗体系与细胞毒性部分缀合。在一些实施方式中，该抗体系经分离。在一些实施方式中，该抗体系实质上纯的。

[0160] 本发明的FZD结合剂(例如抗体)的特异性结合可使用该领域已知的任何方法检

测。可使用的免疫检测包括但不限于竞争性及非竞争性检测系统，该等系统利用诸如Biacore分析、FACS分析、免疫荧光、免疫细胞化学、西方墨点分析、放射性免疫测定、ELISA、“三明治式”免疫测定、免疫沉淀分析、沉淀反应、胶体扩散沉淀反应、免疫扩散分析、凝集测定、补体固定测定、免疫放射分析、荧光免疫分析及蛋白质A免疫分析的技术。该等检测系例行性检测且为该领域中广为周知(见例如Ausubel et al.,Editors,1994-present,Current Protocols in Molecular Biology,John Wiley&Sons,Inc.,New York,NY)。

[0161] 在某些实施方式中，本发明提供Wnt途径抑制剂，其系包含重链CDR1、重链CDR2、及重链CDR3的FZD结合剂(例如抗体)，该重链CDR1包含GFTFSHYTLS(SEQ ID NO:1)，该重链CDR2包含VISGDGSYTYYADSVKG(SEQ ID NO:2)，且该重链CDR3包含NFIKYVFAN(SEQ ID NO:3)。在一些实施方式中，该FZD结合剂另包含轻链CDR1、轻链CDR2、及轻链CDR3，该轻链CDR1包含SGDNIGSFYVH(SEQ ID NO:4)，该轻链CDR2包含DKSNRPSG(SEQ ID NO:5)，且该轻链CDR3包含QSYANTLSL(SEQ ID NO:6)。在一些实施方式中，该FZD结合剂包含：包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1、包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2、及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3。在某些实施方式中，该FZD结合剂包含：(a)包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1、包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2、及包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，及(b)包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1、包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2、及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3。

[0162] 在某些实施方式中，本发明提供包含下列的FZD结合剂(例如抗体)：(a)包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，或其的包含1、2、3、或4个氨基酸取代的变异体；(b)包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，或其的包含1、2、3、或4个氨基酸取代的变异体；(c)包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，或其的包含1、2、3、或4个氨基酸取代的变异体；(d)包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，或其的包含1、2、3、或4个氨基酸取代的变异体；(e)包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，或其的包含1、2、3、或4个氨基酸取代的变异体；及(f)包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3，或其的包含1、2、3、或4个氨基酸取代的变异体。在某些实施方式中，该氨基酸取代系保守性取代。

[0163] 在某些实施方式中，本发明提供包含重链可变区及/或轻链可变区的FZD结合剂(例如抗体)，该重链可变区与SEQ ID NO:7具有至少约80％序列一致性，该轻链可变区与SEQ ID NO:8具有至少80％序列一致性。在某些实施方式中，该FZD结合剂包含与SEQ ID NO:7具有至少约85％、至少约90％、至少约95％、至少约97％、或至少约99％序列一致性的重链可变区。在某些实施方式中，该FZD结合剂包含与SEQ ID NO:8具有至少约85％、至少约90％、至少约95％、至少约97％、或至少约99％序列一致性的轻链可变区。在某些实施方式中，该FZD结合剂包含与SEQ ID NO:7具有至少约95％序列一致性的重链可变区，及/或与SEQ ID NO:8具有至少约95％序列一致性的轻链可变区。在某些实施方式中，该FZD结合剂包含：包含SEQ ID NO:7的重链可变区，及/或包含SEQ ID NO:8的轻链可变区。在某些实施方式中，该FZD结合剂包含：包含SEQ ID NO:7的重链可变区及包含SEQ ID NO:8的轻链可变区。在某些实施方式中，该FZD结合剂包含：实质上由SEQ ID NO:7组成的重链可变区及实质上由SEQ ID NO:8组成的轻链可变区。

[0164] 在某些实施方式中，本发明提供包含下列的FZD结合剂(例如抗体)：(a)与SEQ ID NO:9(有或无信号序列)或SEQ ID NO:11具有至少90％序列一致性的重链；及/或(b)与SEQ

ID NO:10(有或无信号序列)或SEQ ID NO:12具有至少90％序列一致性的轻链。在一些实施方式中,该FZD结合剂包含:(a)与SEQ ID NO:9(有或无信号序列)或SEQ ID NO:11具有至少95％序列一致性的重链;及/或(b)与SEQ ID NO:10(有或无信号序列)或SEQ ID NO:12具有至少95％序列一致性的轻链。在一些实施方式中,该FZD结合剂包含:包含SEQ ID NO:9(有或无信号序列)或SEQ ID NO:11的重链,及/或包含SEQ ID NO:10(有或无信号序列)或SEQ ID NO:12的轻链。在一些实施方式中,该FZD结合剂包含:包含SEQ ID NO:11的重链及包含SEQ ID NO:12的轻链。在一些实施方式中,该FZD结合剂包含:实质上由SEQ ID NO:9的氨基酸20至463所组成的重链及实质上由SEQ ID NO:10的氨基酸20至232所组成的轻链。在一些实施方式中,该FZD结合剂包含:实质上由SEQ ID NO:11组成的重链及实质上由SEQ ID NO:12组成的轻链。

[0165] 在某些实施方式中,本发明提供Wnt途径抑制剂,其系与FZD1、FZD2、FZD5、FZD7及/或FZD8中的至少一者特异性结合的FZD结合剂(例如抗体),其中该FZD结合剂(例如抗体)包含抗体OMP-18R5的一、二、三、四、五、及/或六个CDR。抗体OMP-18R5(亦称为18R5及凡地吐单抗(vantictumab)),以及其他FZD结合剂,已于美国专利第7,982,013号中先行描述。编码该OMP-18R5IgG2抗体的重链及轻链的DNA,依照布达佩斯条约的规定,于2008年9月29日以ATCC编号PTA-9541保藏于美国菌种保存中心。在一些实施方式中,该FZD结合剂包含OMP-18R5的1或多个CDR、OMP-18R5的2或多个CDR、OMP-18R5的3或多个CDR、OMP-18R5的4或多个CDR、OMP-18R5的5或多个CDR、或OMP-18R5的所有6个CDR。

[0166] 本发明提供其系Wnt途径抑制剂的多肽。该等多肽包括但不限于与人FZD蛋白特异性结合的抗体。在一些实施方式中,多肽与一或多种选自下列的FZD蛋白结合:FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9、及FZD10。在一些实施方式中,多肽与FZD1、FZD2、FZD5、FZD7、及/或FZD8结合。在一些实施方式中,多肽与FZD1、FZD2、FZD5、FZD7、及FZD8结合。

[0167] 在某些实施方式中,多肽包含抗体OMP-18R5的一、二、三、四、五、及/或六个CDR。在一些实施方式中,多肽包含其中每个CDR有最多有四个(即0、1、2、3、或4个)氨基酸取代的CDR。在某些实施方式中,该重链CDR被包含于重链可变区之内。在某些实施方式中,该轻链CDR被包含于轻链可变区之内。

[0168] 在一些实施方式中,本发明提供与一或多种人FZD蛋白特异性结合的多肽,其中该多肽包含与SEQ ID NO:7具有至少约80％序列一致性的氨基酸序列,及/或与SEQ ID NO:8具有至少约80％序列一致性的氨基酸序列。在某些实施方式中,该多肽包含与SEQ ID NO:7具有至少约85％、至少约90％、至少约95％、至少约97％、或至少约99％序列一致性的氨基酸序列。在某些实施方式中,该多肽包含与SEQ ID NO:8具有至少约85％、至少约90％、至少约95％、至少约97％、或至少约99％序列一致性的氨基酸序列。在某些实施方式中,该多肽包含与SEQ ID NO:7具有至少约95％序列一致性的氨基酸序列,及/或与SEQ ID NO:8具有至少约95％序列一致性的氨基酸序列。在某些实施方式中,该多肽包含:包含SEQ ID NO:7的氨基酸序列,及/或包含SEQ ID NO:8的氨基酸序列。

[0169] 在一些实施方式中,FZD结合剂包含多肽,该多肽包含选自下列的序列:SEQ ID NO:7、SEQ ID NO:8、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、及SEQ ID NO:12。

[0170] 在某些实施方式中,FZD结合剂包含OMP-18R5抗体的重链可变区及轻链可变区。在

某些实施方式中，FZD结合剂包含(有或无前导序列的)OMP-18R5抗体的重链及轻链。

[0171] 在某些实施方式中，FZD结合剂包含抗体OMP-18R5、实质上由抗体OMP-18R5组成、或由抗体OMP-18R5组成。

[0172] 在某些实施方式中，FZD结合剂(例如抗体)与包含下列的抗体竞争与一或多种人FZD蛋白的特异性结合：包含SEQ ID NO:7的重链可变区及包含SEQ ID NO:8的轻链可变区。在某些实施方式中，FZD结合剂(例如抗体)与包含下列的抗体竞争与一或多种人FZD蛋白的特异性结合：包含SEQ ID NO:9(有或无信号序列)的重链及包含SEQ ID NO:10(有或无信号序列)的轻链。在某些实施方式中，FZD结合剂(例如抗体)与包含下列的抗体竞争与一或多种人FZD蛋白的特异性结合：包含SEQ ID NO:11的重链及包含SEQ ID NO:12的轻链。在某些实施方式中，FZD结合剂与抗体OMP-18R5竞争与一或多种人FZD蛋白的特异性结合。在一些实施方式中，FZD结合剂或抗体于试管内竞争性结合测定中竞争与一或多种人FZD蛋白的特异性结合。

[0173] 在某些实施方式中，FZD结合剂(例如抗体)与一或多种人FZD蛋白上由本发明的抗体所结合的相同表位或实质上相同的表位结合。在另一实施方式中，FZD结合剂系与一或多种人FZD蛋白上的表位结合的抗体，该表位与由本发明的抗体所结合的FZD蛋白上的表位重叠。在某些实施方式中，FZD结合剂(例如抗体)与一或多种FZD蛋白上由抗体OMP-18R5所结合的相同表位或实质上相同的表位结合。在另一实施方式中，该FZD结合剂系与一或多种人FZD蛋白上的表位结合的抗体，该表位与由抗体OMP-18R5所结合的FZD蛋白上的表位重叠。

[0174] 在某些实施方式中，该Wnt途径抑制剂系与一或多种人Wnt蛋白结合的剂。在某些实施方式中，该等剂与一、二、三、四、五、六、七、八、九、十、或更多种Wnt蛋白特异性结合。在一些实施方式中，该Wnt结合剂与一或多种选自下列的人Wnt蛋白结合：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt4、Wnt5a、Wnt5b、Wnt6、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt9a、Wnt9b、Wnt10a、Wnt10b、Wnt11、及Wnt16。在某些实施方式中，Wnt结合剂与一或多种(或二或更多种、三或更多种、四或更多种、五或更多种、等)选自下列的Wnt蛋白结合：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a、及Wnt10b。在某些实施方式中，该一或多种(或二或更多种、三或更多种、四或更多种、五或更多种、等)Wnt蛋白系选自下列：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt8a、Wnt8b、Wnt10a、及Wnt10b。

[0175] 在某些实施方式中，该Wnt结合剂系Wnt拮抗剂。在某些实施方式中，该Wnt结合剂系Wnt途径拮抗剂。在某些实施方式中，该Wnt结合剂抑制Wnt信号传导。在一些实施方式中，该Wnt结合剂抑制典型Wnt信号传导。

[0176] 在一些实施方式中，该Wnt结合剂系抗体。在一些实施方式中，该Wnt结合剂系多肽。在某些实施方式中，该Wnt结合剂系包含抗原结合部位的抗体或多肽。在某些实施方式中，本文所述的Wnt结合抗体或多肽的抗原结合部位能与一、二、三、四、五、或更多种人Wnt蛋白结合。在某些实施方式中，该Wnt结合抗体或多肽的抗原结合部位能与选自Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a及Wnt10b的一、二、三、四或五种人Wnt蛋白特异性结合。Wnt结合剂的非限制性实例可见于国际专利公开案WO 2011/088127。

[0177] 在某些实施方式中，Wnt结合剂与一或多种人Wnt蛋白的C端多半胱氨酸区结合。在某些实施方式中，该Wnt结合剂系与选自下列的一或多种Wnt蛋白内的结构域结合：SEQ ID NO:46(Wnt1)、SEQ ID NO:47(Wnt2)、SEQ ID NO:48(Wnt2b)、SEQ ID NO:49(Wnt3)、SEQ ID

NO:50(Wnt3a)、SEQ ID NO:51(Wnt7a)、SEQ ID NO:52(Wnt7b)、SEQ ID NO:53(Wnt8a)、SEQ ID NO:54(Wnt8b)、SEQ ID NO:55(Wnt10a)、及SEQ ID NO:56(Wnt10b)。

[0178] 在某些实施方式中，该Wnt结合剂以约1μM或更低、约100nM或更低、约40nM或更低、约20nM或更低、或约10nM或更低的K_D与一或多种(例如二或多种、三或多种、或四或多种)Wnt蛋白结合。举例来说，在某些实施方式中，本文所述的与超过一种Wnt蛋白结合的Wnt结合剂，以约100nM或更低、约20nM或更低、或约10nM或更低的K_D与该些Wnt蛋白结合。在某些实施方式中，该Wnt结合剂以约40nM或更低的K_D与一或多种(例如1、2、3、4、或5种)Wnt蛋白中的各者结合，其中该等Wnt蛋白系选自下列：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a及Wnt10b。在一些实施方式中，该结合剂(例如抗体)与Wnt蛋白的K_D系利用固定于Biacore芯片上的包含至少部分的Wnt C端多半胱氨酸区的Wnt融合蛋白测得的K_D。

[0179] 在某些实施方式中，该Wnt结合剂以约1μM或更低、约100nM或更低、约40nM或更低、约20nM或更低、约10nM或更低、或约1nM或更低的EC_{50}与一或多种(例如二或多种、三或多种、或四或多种)人Wnt蛋白结合。在某些实施方式中，Wnt结合剂以约40nM或更低、约20nM或更低、或约10nM或更低的EC_{50}与超过一种Wnt结合。在某些实施方式中，该Wnt结合剂相对于一或多种(例如1、2、3、4、或5种)Wnt蛋白Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt4、Wnt5a、Wnt5b、Wnt6、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt9a、Wnt9b、Wnt10a、Wnt10b、Wnt11、及/或Wnt16具有约20nM或更低的EC_{50}。在某些实施方式中，该Wnt结合剂相对于一或多种(例如1、2、3、4、或5种)下列Wnt蛋白具有约10nM或更低的EC_{50}：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt8a、Wnt8b、Wnt10a、及/或Wnt10b。

[0180] 在某些实施方式中，该Wnt途径抑制剂系Wnt结合剂，且该Wnt结合剂系抗体。在一些实施方式中，该抗体系重组抗体。在一些实施方式中，该抗体系单克隆抗体。在一些实施方式中，该抗体系嵌合抗体。在一些实施方式中，该抗体系人化抗体。在一些实施方式中，该抗体系人抗体。在某些实施方式中，该抗体系IgG1抗体。在某些实施方式中，该抗体系IgG2抗体。在某些实施方式中，该抗体系包含抗原结合部位的抗体片段。在一些实施方式中，该抗体系单价、单特异性、或双价。在一些实施方式中，该抗体系双特异性抗体或多特异性抗体。在一些实施方式中，该抗体系与细胞毒性部分缀合。在一些实施方式中，该抗体系经分离。在一些实施方式中，该抗体系实质上纯的。

[0181] 本发明的Wnt结合剂(例如抗体)的特异性结合可使用如本文所述的用于FZD结合剂的该领域已知的任何方法检测。

[0182] 在某些实施方式中，该Wnt结合剂系可溶性受体。在某些实施方式中，该Wnt结合剂包含FZD受体蛋白的胞外结构域。在一些实施方式中，该Wnt结合剂包含FZD蛋白的Fri结构域。在一些实施方式中，包含FZDFri结构域的可溶性受体相较于包含该完整FZD ECD的可溶性受体可显示改变的生物活性(例如增加蛋白半衰期)。蛋白半衰期可进一步藉由聚乙二醇(PEG)或聚氧乙烯(PEO)的共价修饰加以修饰(例如延长)。在某些实施方式中，该FZD蛋白系人FZD蛋白。在某些实施方式中，该人FZD蛋白系FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9或FZD10。可溶性FZD受体的非限制性实例可见于美国专利第7,723,477及7,947,277号及美国专利公开号2013/0034551中。

[0183] 人FZD1至10蛋白中各者的预测Fri结构域系提供为SEQ ID NO:13至22。人FZD1至

10蛋白中各者的预测最小Fri结构域系提供为SEQ ID NO:23至32。本领域技术人员对于对应各种Fri结构域的确切氨基酸的了解可能互异。因此，上述及本文所述的结构域的N端及/或C端可延长或缩短1、2、3、4、5、6、7、8、9、或甚至10个氨基酸。

[0184] 在某些实施方式中，该Wnt结合剂包含与一或多种人Wnt蛋白结合的人FZD蛋白的Fri结构域，或该Fri结构域的片段或变异体。在某些实施方式中，该人FZD蛋白系FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9、或FZD10。在某些实施方式中，该人FZD蛋白系FZD4。在某些实施方式中，该人FZD蛋白系FZD5。在某些实施方式中，该人FZD蛋白系FZD8。在某些实施方式中，该人FZD蛋白系FZD10。在某些实施方式中，该FZD蛋白系FZD4且该Wnt结合剂包含SEQ ID NO:16。在某些实施方式中，该FZD蛋白系FZD5且该Wnt结合剂包含SEQ ID NO:17。在某些实施方式中，该FZD蛋白系FZD7且该Wnt结合剂包含SEQ ID NO:19。在某些实施方式中，该FZD蛋白系FZD8且该Wnt结合剂包含SEQ ID NO:20。在某些实施方式中，该FZD蛋白系FZD10且该Wnt结合剂包含SEQ ID NO:22。在某些实施方式中，该FZD蛋白系FZD8且该Wnt结合剂包含SEQ ID NO:33。

[0185] 在一些实施方式中，该Wnt结合剂包含Fri结构域，该Fri结构域包含FZD1(SEQ ID NO:23)的最小Fri结构域、FZD2(SEQ ID NO:24)的最小Fri结构域、FZD3(SEQ ID NO:25)的最小Fri结构域、FZD4(SEQ ID NO:26)的最小Fri结构域、FZD5(SEQ ID NO:27)的最小Fri结构域、FZD6(SEQ ID NO:28)的最小Fri结构域、FZD7(SEQ ID NO:29)的最小Fri结构域、FZD8(SEQ ID NO:30)的最小Fri结构域、FZD9(SEQ ID NO:31)的最小Fri结构域、或FZD10(SEQ ID NO:32)的最小Fri结构域。在一些实施方式中，该Wnt结合剂包含Fri结构域，该Fri结构域包含FZD8(SEQ ID NO:30)的最小Fri结构域。

[0186] 在一些实施方式中，该Wnt结合剂包含Fri结构域，该Fri结构域实质上由FZD1的Fri结构域、FZD2的Fri结构域、FZD3的Fri结构域、FZD4的Fri结构域、FZD5的Fri结构域、FZD6的Fri结构域、FZD7的Fri结构域、FZD8的Fri结构域、FZD9的Fri结构域、或FZD10的Fri结构域组成。在一些实施方式中，该Wnt结合剂包含实质上由FZD8的Fri结构域组成的Fri结构域。

[0187] 在一些实施方式中，该Wnt结合剂包含选自下列的序列：SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32及SEQ ID NO:33。在一些实施方式中，该Wnt结合剂包含实质上由SEQ ID NO:20组成的Fri结构域。在一些实施方式中，该Wnt结合剂包含实质上由SEQ ID NO:33组成的Fri结构域。

[0188] 在某些实施方式中，该Wnt结合剂包含前述FZD Fri结构域序列的任一者的变异体，其包含一或多个(例如一、二、三、四、五、六、七、八、九、十个、等)保守性取代且能与Wnt蛋白结合。

[0189] 在某些实施方式中，Wnt结合剂(例如包含人FZD受体的Fri结构域的剂)另包含非FZD多肽。在一些实施方式中，FZD可溶性受体可包括与其他非FZD功能性及结构性多肽连接的FZD ECD或Fri结构域，该等非FZD功能性及结构性多肽包括但不限于人Fc区、蛋白标签(例如myc、FLAG、GST)、其他内源性蛋白或蛋白片段，或任何其他可用的蛋白序列包括在FZD

ECD或Fri结构域与第二多肽之间的任何接头区。在某些实施方式中，该非FZD多肽包含人Fc区。该Fc区可自任一类型的免疫球蛋白如IgG、IgA、IgM、IgD及IgE获得。在一些实施方式中，该Fc区系人IgG1 Fc区。在一些实施方式中，该Fc区系人IgG2Fc区。在一些实施方式中，该Fc区系野生型Fc区。在一些实施方式中，该Fc区系成熟型Fc区。在一些实施方式中，该Fc区的N端系经截短1、2、3、4、5、6、7、8、9或10个氨基酸(例如在铰链结构域)。在一些实施方式中，在铰链结构域的氨基酸系经改变以阻止非所欲的双硫键形成。在一些实施方式中，半胱氨酸系经丝氨酸取代以阻碍或阻止非所欲的双硫键形成。在一些实施方式中，该Fc区的C端系经截短1、2、3、或更多个氨基酸。在一些实施方式中，该Fc区的C端系经截短1个氨基酸。在某些实施方式中，该非FZD多肽包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37或SEQ ID NO:38。在某些实施方式中，该非FZD多肽实质上由SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37或SEQ ID NO:38组成。在某些实施方式中，该非FZD多肽实质上由SEQ ID NO:36或SEQ ID NO:37组成。

[0190] 在某些实施方式中，Wnt结合剂系包含FZD受体的至少一最小Fri结构域与一Fc区的融合蛋白。此处所使用的“融合蛋白”系由包含至少二种基因的核苷酸序列的核酸分子表达的杂合蛋白。在一些实施方式中，该第一多肽的C端系与该免疫球蛋白Fc区的N端连接。在一些实施方式中，该第一多肽(例如FZD Fri结构域)系与该Fc区直接相连(即不含中介肽接头)。在一些实施方式中，该第一多肽系与该Fc区经由接头相连。

[0191] 此处使用的用语“接头”系指插入第一多肽(例如FZD成分)与第二多肽(例如Fc区)之间的接头。在一些实施方式中，该接头系肽接头。接头不应不良影响该多肽的表达、分泌或生物活性。接头应不具抗原性且不应诱发免疫反应。适当的接头系本领域技术人员所知，通常包括甘氨酸及丝氨酸残基的混合物，且通常包括无空间位阻的氨基酸。其他可被纳入于可用接头的氨基酸包括苏氨酸及丙氨酸残基。接头的长度范围广泛，例如1至50个氨基酸长度、1至22个氨基酸长度、1至10个氨基酸长度、1至5个氨基酸长度或1至3个氨基酸长度。接头可能包括但不限于SerGly、GGSG、GSGS、GGGS、S(GGS)n其中n系1至7、GRA、聚(Gly)、聚(Ala)、ESGGGGVT(SEQ ID NO:57)、LESGGGGVT(SEQ ID NO:58)、GRAQVT(SEQ ID NO:59)、WRAQVT(SEQ ID NO:60)及ARGRAQVT(SEQ ID NO:61)。本文所使用的接头系不包括来自该第一多肽(例如FZD Fri结构域)的C端或该第二多肽(例如Fc区)的N端的氨基酸残基的中介肽序列。

[0192] 在一些实施方式中，该Wnt结合剂包含FZD Fri结构域、Fc区及连接该FZD Fri结构域与该Fc区的接头。在一些实施方式中，该FZD Fri结构域包含SEQ ID NO:20、SEQ ID NO:30、或SEQ ID NO:33。在一些实施方式中，该接头包含ESGGGGVT(SEQ ID NO:57)或LESGGGGVT(SEQ ID NO:58)。

[0193] 在一些实施方式中，该Wnt结合剂包含第一多肽及第二多肽，该第一多肽包含SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、或SEQ ID NO:33，该第二多肽包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38，其中该第一多肽系与该第二多肽直接连接。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:20的第一多肽及包含SEQ ID

NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:20的第一多肽及包含SEQ ID NO:36或SEQ ID NO:37的第二多肽。在一些实施方式中，该Wnt结合剂包含：实质上由SEQ ID NO:20组成的第一多肽及实质上由SEQ ID NO:36或SEQ ID NO:37组成的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:30的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:30的第一多肽及包含SEQ ID NO:36或SEQ ID NO:37的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:33的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:33的第一多肽及包含SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:35的第二多肽。在一些实施方式中，该Wnt结合剂包含：实质上由SEQ ID NO:33组成的第一多肽及实质上由SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:35组成的第二多肽。

[0194] 在一些实施方式中，该Wnt结合剂包含第一多肽及第二多肽，该第一多肽包含SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、或SEQ ID NO:33，该第二多肽包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38，其中该第一多肽系藉由接头与该第二多肽连接。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:20的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:20的第一多肽及包含SEQ ID NO:36或SEQ ID NO:37的第二多肽。在一些实施方式中，该Wnt结合剂包含：实质上由SEQ ID NO:20组成的第一多肽及实质上由SEQ ID NO:36或SEQ ID NO:37组成的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:30的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:33的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：包含SEQ ID NO:33的第一多肽及包含SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:35的第二多肽。在一些实施方式中，该Wnt结合剂包含：实质上由SEQ ID NO:33组成的第一多肽及实质上由SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:35组成的第二多肽。

[0195] 在一些实施方式中，该Wnt结合剂包含第一多肽及第二多肽，该第一多肽与SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、或SEQ ID NO:33具有至少95％一致性，该第二多肽包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38，其中该第一多肽系与该第二多肽直接连接。在一些实施方式中，该Wnt结合剂包含：与SEQ ID NO:20具有至少95％一致性的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或

SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：与SEQ ID NO:30具有至少95％一致性的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：与SEQ ID NO:33具有至少95％一致性的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。

[0196] 在一些实施方式中，该Wnt结合剂包含第一多肽及第二多肽，该第一多肽与SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、或SEQ ID NO:33具有至少95％一致性，该第二多肽包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38，其中该第一多肽系经由接头与该第二多肽连接。在一些实施方式中，该Wnt结合剂包含：与SEQ ID NO:20具有至少95％一致性的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：与SEQ ID NO:30具有至少95％一致性的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。在一些实施方式中，该Wnt结合剂包含：与SEQ ID NO:33具有至少95％一致性的第一多肽及包含SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37、或SEQ ID NO:38的第二多肽。

[0197] FZD蛋白包含引导该蛋白运输的信号序列。信号序列(又称信号肽或前导序列)位于新生多肽的N端。它们引导该多肽至内质网且该等蛋白被分类至彼等应该去的地方，例如胞器的内部空间、细胞内部的膜、细胞的外膜或经分泌至细胞外部。大部分信号序列在蛋白被运送至内质网后，藉由信号肽酶与该蛋白切割。自该多肽切割该信号序列通常发生于氨基酸序列的特定位点，且取决于该信号序列内的氨基酸残基。虽然通常有一个特定的切割位点，但信号肽酶可能识别及/或使用一个以上的切割位点，导致该多肽不相同的N端。举例来说，使用信号序列内的不同的切割位点可导致具有不同N端氨基酸的多肽的表达。因此，在一些实施方式中，本文所述的多肽可能包含具有不同N端的多肽的混合物。在一些实施方式中，该N端的长度相差1、2、3、4、5、6、7、8、9、10或更多个氨基酸。在一些实施方式中，该N端的长度相差1、2、3、4或5个氨基酸。在一些实施方式中，该多肽为实质上相同，即该多肽具有相同的N端。在一些实施方式中，该多肽的信号序列包含一或多个(例如一、二、三、四、五、六、七、八、九、十、等等)氨基酸取代及/或删除。在一些实施方式中，该多肽的信号序列包含让一个切割位点变成主要切割位点的氨基酸取代及/或删除，藉此导致具有一种N端的实质上相同的多肽。

[0198] 在一些实施方式中，该Wnt结合剂包含选自下列的氨基酸序列：SEQ ID NO:39、SEQ ID NO:40、SEQ ID NO:41、SEQ ID NO:42、SEQ ID NO:43、SEQ ID NO:44、及SEQ ID NO:45。

[0199] 在某些实施方式中，该Wnt结合剂包含SEQ ID NO:39的序列。在某些实施方式中，该剂包含SEQ ID NO:39的序列，该序列包含一或多个(例如一、二、三、四、五、六、七、八、九、十、等等)保守性取代。在某些实施方式中，该剂包含与SEQ ID NO:39具有至少约90％、约95％、或约98％序列一致性的序列。在某些实施方式中，SEQ ID NO:39的变异体维持其与一或多种人Wnt蛋白结合的能力。

[0200] 在某些实施方式中，该Wnt结合剂包含SEQ ID NO:40的序列。在一些实施方式中，该Wnt结合剂系SEQ ID NO:40。在某些替代性实施方式中，该剂包含SEQ ID NO:40的序列，该序列包含一或多个(例如一、二、三、四、五、六、七、八、九、十、等等)保守性取代。在某些实施方式中，该剂包含与SEQ ID NO:40具有至少约90％、约95％、或约98％序列一致性的序列。在某些实施方式中，SEQ ID NO:40的变异体维持其与一或多种人Wnt蛋白结合的能力。

[0201] 在某些实施方式中，该Wnt结合剂包含SEQ ID NO:41的序列。在一些实施方式中，该Wnt结合剂系SEQ ID NO:41。在某些替代性实施方式中，该剂包含SEQ ID NO:41的序列，该序列包含一或多个(例如一、二、三、四、五、六、七、八、九、十、等等)保守性取代。在某些实施方式中，该剂包含与SEQ ID NO:41具有至少约90％、约95％、或约98％序列一致性的序列。在某些实施方式中，SEQ ID NO:41的变异体维持其与一或多种人Wnt蛋白结合的能力。

[0202] 在一些实施方式中，Wnt结合剂系OMP-54F28。

[0203] 在某些实施方式中，Wnt结合剂系包含选自下列的氨基酸序列的多肽：SEQ ID NO:39、SEQ ID NO:40、SEQ ID NO:41、SEQ ID NO:42、SEQ ID NO:43、SEQ ID NO:44、及SEQ ID NO:45。在某些实施方式中，该多肽包含选自SEQ ID NO:39、SEQ ID NO:40、及SEQ ID NO:41的氨基酸序列。在一些实施方式中，多肽实质上由选自下列的氨基酸序列组成：SEQ ID NO:39、SEQ ID NO:40及SEQ ID NO:41。在某些实施方式中，该多肽包含SEQ ID NO:39的氨基酸序列。在一些实施方式中，该多肽包含SEQ ID NO:40的氨基酸序列。在某些实施方式中，该多肽包含SEQ ID NO:41的氨基酸序列。在某些实施方式中，该多肽包含SEQ ID NO:42的氨基酸序列。在某些实施方式中，该多肽包含SEQ ID NO:43的氨基酸序列。在某些实施方式中，该多肽包含SEQ ID NO:44的氨基酸序列。在某些实施方式中，该多肽包含SEQ ID NO:45的氨基酸序列。

[0204] 在一些实施方式中，该多肽系实质上经纯化的包含选自SEQ ID NO:39、SEQ ID NO:40、及SEQ ID NO:41的氨基酸序列的多肽。在一些实施方式中，该多肽系实质上经纯化的包含SEQ ID NO:41的多肽。在某些实施方式中，该实质上经纯化的多肽系由至少90％的具有ASA的N端序列的多肽所组成。在一些实施方式中，该新生多肽包含导致实质上具有一N端序列的均质多肽产物的信号序列。

[0205] 在某些实施方式中，Wnt结合剂包含免疫球蛋白的Fc区。本领域技术人员将了解，本发明的某些结合剂将包含融合蛋白，相较于具有大约相同免疫原性的包含天然或未经改变的恒定区的融合蛋白，其中至少部分的Fc区系经删除或以其他方式改变，以提供所欲的生化特征，例如增加癌细胞定位、增加肿瘤穿透、减少血清半衰期、或增加血清半衰期。对Fc区的修饰可能包括添加、删除或取代一或多个结构域中的一或多个氨基酸。此处所揭示的经修饰的融合蛋白可能包含对二个重链恒定结构域的一或多种(CH2或CH3)或对铰链区的改变或修饰。在其他实施方式中，整个CH2结构域可被移除(ΔCH2建构体)。在一些实施方式中，该遗漏的恒定区结构域系由短氨基酸间隔子(例如10个aa残基)取代，以提供通常由该遗漏恒定区结构域所授予的一些分子柔韧性。

[0206] 在一些实施方式中，该经修饰的融合蛋白系经建构以直接连接CH3结构域与该铰链区。在其他实施方式中，肽间隔子被插入铰链区与经修饰的CH2及/或CH3结构域之间。举例来说，其中CH2结构域被删除且剩余的CH3结构域(经修饰或未经修饰)系以5至20个氨基酸间隔子与铰链区连接的建构体可被表达。该间隔子可被添加以确保恒定区的调节组件维

持自由及可接近，或该铰链区维持可弯折。然而，应注意氨基酸间隔子在一些情况中可能证实具有免疫原性，且诱发拮抗该建构体的非所欲免疫反应。因此，在某些实施方式中，任何添加至建构体的间隔子将为相对非免疫原性，以维持该融合蛋白的所欲生物性质。

[0207] 在一些实施方式中，该经修饰的融合蛋白可能仅具有恒定结构域的部分删除或取代少数或甚至单一个氨基酸。举例来说，在CH2结构域的选择区域中的单一氨基酸突变可能足以实质上减少Fc结合，因此增加癌细胞定位及/或肿瘤穿透。类似地，所欲的是单纯删除该一或多个恒定区结构域中控制特定效应功能(例如补体C1q结合)的部分。该恒定区的部分删除可增进该结合剂的选择特性(例如血清半衰期)，同时保留其他与该主题恒定区结构域完整有关的所欲功能。另外，如上所述，该揭示融合蛋白的恒定区可经由一或多个氨基酸的突变或取代改质以增进该形成建构物的特性。在这方面可能扰乱由保守性结合位点所提供的活性(例如Fc结合)，同时实质上维持该经修饰的融合蛋白的构造及免疫原性特性。在某些实施方式中，该经修饰的融合蛋白包含添加一或多个氨基酸至恒定区以增进所欲特征诸如减少或增加效应功能或提供更多细胞毒素或碳水化合物连接位点。

[0208] 该领域已知的是恒定区媒介数种效应功能。举例来说，补体的C1成分与(结合至抗原的)IgG或IgM抗体的Fc区结合活化该补体系统。补体活化于细胞病原体的调理作用及溶解中至为重要。补体活化亦刺激发炎反应，且亦与自体免疫超敏性有关。此外，免疫球蛋白的Fc区可与表达Fc受体(FcR)的细胞结合。有一些Fc受体对不同类型的抗体具有特异性，包括IgG(γ受体)、IgE(ε受体)、IgA(α受体)及IgM(μ受体)。抗体与细胞表面上的Fc受体结合引发多种重要且多变的生物反应，包括吞噬及破坏抗体包覆颗粒、清空免疫复合物、藉由杀手细胞溶解经抗体包覆的标靶细胞、释放发炎介质、胚胎转移及控制免疫球蛋白的产制。

[0209] 在一些实施方式中，该经修饰的融合蛋白提供经改变的效应功能，因而影响该投予剂的生物特性。举例来说，在一些实施方式中，删除或不活化(经由点突变或其他方法)恒定区结构域可能减少循环中经修饰的剂与Fc受体结合，因此增加癌细胞定位及/或肿瘤穿透。在其他实施方式中，恒定区修饰增加或减少剂的血清半衰期。在一些实施方式中，恒定区系经修饰以消除双硫键或寡糖基团。

[0210] 在某些实施方式中，经修饰的融合蛋白不具有一或多种通常与Fc区有关的效应功能。在一些实施方式中，该剂不具抗体依赖性细胞媒介性细胞毒性(ADCC)活性及/或不具补体依赖性细胞毒性(CDC)活性。在某些实施方式中，该剂不与该Fc受体及/或补体因子结合。在某些实施方式中，该剂不具效应功能。

[0211] 在一些实施方式中，本文所述的Wnt结合剂(例如可溶性受体)系经修饰以减少免疫原性。通常，当这些蛋白用来作为治疗剂时，拮抗完全正常人蛋白的免疫反应很少发生。然而，虽然许多融合蛋白包含与天然中发现的序列相同的多肽序列，数种治疗性融合蛋白已显示在哺乳动物中具有免疫原性。在一些试验中，包含接头的融合蛋白已被发现比不包含接头的融合蛋白更具免疫原性。因此，在一些实施方式中，本发明的多肽系藉由运算方法分析以预测免疫原性。在一些实施方式中，分析该等多肽中T细胞及/或B细胞表位的存在。若任何T细胞或B细胞表位系经辨识及/或预测，可对这些区域进行修饰(例如氨基酸取代)以扰乱或破坏该等表位。各种可用于预测T细胞及/或B细胞表位的算法及软件系为该领域所知。例如，软件程序SYFPEITHI、HLA Bind、PEPVAC、RANKPEP、DiscoTope、ElliPro、及抗体表位预测(Antibody Epitope Prediction)皆为公众可取得。

[0212] 在一些实施方式中，本发明提供一种产生如本文中所述的任何Wnt结合剂(例如可溶性受体)或多肽的细胞。在一些实施方式中，本发明提供包含如本文中所述的任何Wnt结合剂(例如可溶性受体)或多肽的组成物。在一些实施方式中，该组成物包含多肽，其中至少80％、90％、95％、97％、98％、或99％的该多肽具有ASA的N端序列。在一些实施方式中，该组成物包含多肽，其中100％的该多肽具有ASA的N端序列。在一些实施方式中，该组成物包含多肽，其中至少80％的该多肽具有ASA的N端序列。在一些实施方式中，该组成物包含多肽，其中至少90％的该多肽具有ASA的N端序列。在一些实施方式中，该组成物包含多肽，其中至少95％的该多肽具有ASA的N端序列。

[0213] 本文中所述的多肽可为重组多肽、天然多肽、或合成多肽。在本领域中咸信本发明的一些氨基酸序列可变化而不会对该蛋白的结构或功能造成显著影响。若考虑该等序列上的差异，应记住在蛋白质上将有决定活性的重要区域。因此，本发明另包括多肽的变异体，该变异体显示实质活性或包括FZD蛋白的区域，例如如本文所讨论的蛋白部分。该等突变包括删除、插入、倒位、重复、及类型取代。

[0214] 当然，技艺人士会采用的氨基酸取代的数量取决于许多因素，包括该些于上述者。在某些实施方式中，用于任何给定的可溶性受体多肽中的取代的数量不会超过50、40、30、25、20、15、10、5或3个。

[0215] 本发明的多肽的片段或部分可被采用来藉由肽合成用于产制该对应的全长多肽；因此，该等片段可被采用为用于产制全长多肽的中间物。该等多肽的片段或部分亦可被称为“蛋白片段”或“多肽片段”。

[0216] 本发明的“蛋白片段”系能与一或多种人Wnt蛋白或一或多种人FZD蛋白结合的蛋白的一部分或整体。在一些实施方式中，该片段对一或多种人Wnt蛋白具有高亲和性。在一些实施方式中，该片段对一或多种人FZD蛋白具有高亲和性。此处所描述的Wnt结合剂的一些片段系包含与免疫球蛋白的恒定区(例如Fc区)的至少部分连接的FZD蛋白的胞外部分的至少部分的蛋白片段。该蛋白片段的结合亲和性可为约10^{-11}至10^{-12}M的范围，虽然亲和性可因片段的不同大小而具有从10^{-7}至10^{-13}M的大幅差异。在一些实施方式中，该片段系约100至约200个氨基酸长度，且包含与免疫球蛋白的恒定区的至少部分连接的结合结构域。

[0217] 在一些实施方式中，该Wnt途径抑制剂系多克隆抗体。多克隆抗体可利用任何已知的方法制备。在一些实施方式中，多克隆抗体系藉由以感兴趣的抗原(例如经纯化的肽片段、全长重组蛋白或融合蛋白)利用多重皮下或腹腔内注射的方式免疫动物(例如兔、大鼠、小鼠、山羊、驴)产制。该抗原可任意选择地与载体缀合，诸如钥孔状帽贝血蓝素(KLH)或血清白蛋白。该抗原(不论有无载体蛋白质)系经无菌盐水稀释，且通常与佐剂(例如完全或不完全弗氏(Freund's)佐剂)组合以形成稳定乳液。在经过足够时间后，自该经免疫的动物的血液及/或腹水回收多克隆抗体。该多克隆抗体可根据该领域的标准方法自血清或腹水纯化，该等方法包括但不限于亲和性层析、离子交换层析、胶体电泳及透析。

[0218] 在一些实施方式中，该Wnt途径抑制剂系单克隆抗体。单克隆抗体可利用本领域技术人员已知的杂交瘤方法制备(见例如Kohler and Milstein,1975,Nature,256:495-497)。在一些实施方式中，使用杂交瘤方法系将小鼠、仓鼠或其他适当的宿主动物经上述方法免疫，以诱发产制将与该免疫抗原特异性结合的抗体的淋巴细胞。在一些实施方式中，淋巴细胞可于试管内免疫。在一些实施方式中，该免疫抗原可为人蛋白质或其一部分。在一些

实施方式中，该免疫抗原可为小鼠蛋白质或其一部分。

[0219] 在免疫后，淋巴细胞系经分离并利用例如聚乙二醇与适当的骨髓瘤细胞系融合，以形成接着可与未融合的淋巴细胞及骨髓瘤细胞分离的杂交瘤细胞。产制特异性拮抗选定抗原的单克隆抗体的杂交瘤可利用多种方法识别，该等方法包括但不限于免疫沉淀、免疫转渍及试管内结合试验(例如流式细胞分析、FACS、ELISA及放射性免疫测定)。该杂交瘤可利用标准方法于试管内增殖(J.W.Goding,1996,Monoclonal Antibodies:Principles and Practice,3rd Edition,Academic Press,San Diego,CA)，或于动物活体内(in vivo)以腹水肿瘤方式增殖。该单克隆抗体可根据该领域的标准方法自培养基或腹水液体纯化，该等方法包括但不限于亲和性层析、离子交换层析、胶体电泳及透析。

[0220] 在某些实施方式中，单克隆抗体可利用本领域技术人员已知的重组DNA技术制备。编码单克隆抗体的多核苷酸系自成熟B细胞或杂交瘤细胞分离，像是藉由使用寡核苷酸引物的RT-PCR以特异性扩增编码该抗体的重链及轻链的基因，该等多核苷酸的序列系利用公知技术测定。该经分离的编码重链及轻链的多核苷酸接着被选殖至适当表达载体，该载体在转染至原本不产制免疫球蛋白的宿主细胞诸如大肠杆菌(E.coli)、类人猿COS细胞、中国仓鼠卵巢(CHO)细胞或骨髓瘤细胞后产制单克隆抗体。在其他实施方式中，重组单克隆抗体或其片段可自噬菌体展示库分离。

[0221] 编码单克隆抗体的多核苷酸可进一步以多种不同方式使用重组DNA技术修饰，以产制可供选择的抗体。在一些实施方式中，例如小鼠单克隆抗体的轻链及重链的恒定结构域可被例如人抗体的该些区域取代以产制嵌合抗体，或以非免疫球蛋白多肽取代以产制融合抗体。在一些实施方式中，该等恒定区系经截短或移除以产制所欲的单克隆抗体的抗体片段。可变区的定点或高密度突变形成可被用于优化单克隆抗体的特异性、亲和性等。

[0222] 在一些实施方式中，该Wnt途径抑制剂系人化抗体。通常，人化抗体系其中CDR的氨基酸残基经源自具有所欲特异性、亲和性及/或结合能力的非人物种(例如小鼠、大鼠、兔、仓鼠等)的免疫球蛋白的CDR的氨基酸残基取代的人免疫球蛋白，该取代系利用本领域技术人员已知的方法进行。在一些实施方式中，人免疫球蛋白的Fv框架区氨基酸残基系由具有所欲特异性、亲和性及/或结合能力的非人物种的抗体的对应氨基酸残基取代。在一些实施方式中，该人化抗体可进一步藉由取代Fv框架区及/或该经取代的非人氨基酸残基内的额外氨基酸残基加以修饰，以精进优化抗体特异性、亲和性及/或能力。通常，该人化抗体将包含实质上所有的至少一个且通常两个可变结构域，该可变结构域包含所有或实质上所有的对应该非人免疫球蛋白的CDR，然而所有或实质上所有的框架区系具有人免疫球蛋白序列的框架区。在一些实施方式中，该人化抗体亦可包含至少部分的免疫球蛋白恒定区或恒定结构域(Fc)，通常为人免疫球蛋白的该部分。在某些实施方式中，该等人化抗体系用于治疗用途，因为当投予至人个体时它们可能减少抗原性及HAMA(人抗小鼠抗体)反应。用于制备人化抗体的方法系该领域所广为周知。

[0223] 在某些实施方式中，该Wnt途径抑制剂系人抗体。人抗体可利用该领域已知的多种技术直接制备。在一些实施方式中，可制备在试管内经免疫的永生化人B淋巴细胞或自经免疫而产制以标靶抗原为目标的抗体的个体分离。在一些实施方式中，该人抗体可选自噬菌体库，其中该噬菌体库表达人抗体。或者，噬菌体展示技术可被用来自未经免疫的捐赠者的免疫球蛋白可变区结构域基因贮库以试管内产制人抗体及抗体片段。用于产制及使用抗体

噬菌体库的技术系该领域所广为周知。该领域已知的亲和性成熟策略包括但不限于链改组(chain shuffling)(Marks et al.,1992,Bio/Technology,10:779-783)及定点突变形成可被用于产制高亲和性人抗体。

[0224] 在一些实施方式中,人抗体可于包含人免疫球蛋白基因座的转基因小鼠中制备。经免疫后,这些小鼠可产制整套人抗体而不产制内源性免疫球蛋白。此方法系于美国专利第5,545,807、5,545,806、5,569,825、5,625,126、5,633,425及5,661,016号中描述。

[0225] 本发明亦包含特异性识别至少一种人FZD蛋白或至少一种Wnt蛋白的双特异性抗体。双特异性抗体可特异性辨识及结合至少二种不同表位。该等不同的表位可位于相同分子内(例如二个不同表位位于人FZD5上)或位于不同分子上(例如一表位位于FZD5上,一不同表位位于第二蛋白上)。在一些实施方式中,该双特异性抗体系单克隆人或人化抗体。在一些实施方式中,该抗体可特异性识别及结合第一抗原标靶(例如FZD蛋白)及第二抗原标靶像是在淋巴细胞上的效应分子(例如CD2、CD3、CD28、CD80、或CD86)或Fc受体(例如CD64、CD32或CD16)以使细胞性防御机制集中于表达该第一抗原标靶的细胞。在一些实施方式中,该等抗体可被用于引导细胞毒性剂至表达特定标靶抗原的细胞。这些抗体具有抗原结合臂及与细胞毒性剂或放射性核种螯合剂诸如EOTUBE、DPTA、DOTA或TETA结合的臂。

[0226] 双特异性抗体可为完整抗体或抗体片段。本发明亦考虑超过两价的抗体。例如,可制备三特异性抗体(Tutt et al.,1991,J.Immunol.,147:60)。因此,在某些实施方式中,该等抗体系多特异性。用于制造双特异性及多特异性抗体的技术系本领域技术人员所知。

[0227] 在某些实施方式中,此处描述的抗体(或其他多肽)可为单特异性。例如,在某些实施方式中,抗体所包含的一或多个抗原结合部位的各者系能结合不同蛋白质上的同源性表位。在某些实施方式中,此处所述的单特异性抗体的抗原结合部位能结合例如FZD5及FZD7(即在FZD5及FZD7蛋白上皆能发现的相同表位)。

[0228] 在某些实施方式中,该Wnt途径抑制剂系包含抗原结合部位的抗体片段。抗体片段可具有与完整抗体不同的功能或能力,例如抗体片段可具有增加的肿瘤穿透。已知用于产制抗体片段的各种技术包括但不限于完整抗体的蛋白水解消化。在一些实施方式中,抗体片段包括由胃蛋白酶消化抗体分子所产制的F(ab')2片段。在一些实施方式中,抗体片段包括藉由减少F(ab')2片段的双硫键所产制的Fab片段。在其他实施方式中,抗体片段包括藉由以木瓜酶及还原剂处理抗体分子所产制的Fab片段。在某些实施方式中,抗体片段系经重组产制。在一些实施方式中,抗体片段包括Fv或单链Fv(scFv)片段。Fab、Fv及scFv抗体片段可在大肠杆菌或其他宿主细胞中表达及分泌,允许大量产制这些片段。在一些实施方式中,抗体片段系自如此处讨论的抗体噬菌体分子库分离。举例来说,可利用方法建构Fab表达库以允许快速有效地识别对FZD或Wnt蛋白或其衍生物、片段、类似物或同源物具有所欲特异性的单克隆Fab片段。在一些实施方式中,抗体片段系线性抗体片段。在某些实施方式中,抗体片段系单特异性或双特异性。在某些实施方式中,该Wnt途径抑制剂系scFv。各种技术可被用于产制对一或多种人FZD蛋白或一或多种人Wnt蛋白具有特异性的单链抗体。

[0229] 另外尤其以抗体片段来说所欲的是,修饰抗体以增加其血清半衰期。此可藉由例如使抗体片段中的适当区域发生突变以纳入救援受体结合表位至抗体片段中达成,或藉由将该表位纳入肽标签中然后使该肽标签与抗体片段的末端或中间融合(例如藉由DNA或肽合成)达成。在一些实施方式中,抗体系经修饰以减少其血清半衰期。

[0230] 异源缀合抗体亦属于本发明的范围内。异源缀合抗体系由二个共价连接的抗体组成。该等抗体被计划用于例如使免疫细胞以非所欲的细胞为标靶。亦考虑到该等异源缀合抗体可利用已知的合成蛋白质化学方法于试管内制备，包括该些涉及交联剂的方法。举例来说，免疫毒素可利用双硫交换反应或藉由形成硫醚键加以建构。为达此目的的适当试剂实例包括亚胺基硫醇盐及甲基-4-巯基丁亚氨酸酯。

[0231] 就本发明的目的而言，应了解的是经修饰的抗体可包含任何类型的提供该抗体与标靶(即人FZD蛋白或人Wnt蛋白)连接的可变区。在这方面，该可变区可包含或衍生自任何种类的可被诱导以启动体液性反应及产制拮抗该所欲的肿瘤相关抗原的免疫球蛋白的哺乳动物。因此，该经修饰的抗体的可变区可为例如人、小鼠、非人灵长动物(例如长尾猕猴(cynomolgus monkey)、猕猴等)或兔来源。在一些实施方式中，该经修饰的免疫球蛋白的可变区及恒定区皆为人来源。在其他实施方式中，兼容性抗体的可变区(通常源自非人来源)可经工程化或特别改质以促进该分子的结合特性或减少免疫原性。在这方面，可用于本发明的可变区可经人化或藉由纳入输入氨基酸序列以另行改变。

[0232] 在某些实施方式中，重链及轻链的可变结构域系藉由至少部分取代一或多个CDR及(若需要的话)部分取代框架区及序列修饰及/或改变加以改变。虽然CDR可能源自与框架区来源的抗体相同类型或甚至相同亚型的抗体，一般设想CDR将较佳地源自不同物种的抗体。要转移一可变结构域的抗原结合能力至另一可变结构域不一定需要将所有CDR取代成捐赠者可变区的所有CDR。相反地，可能只需要转移该些维持抗原结合部位的活性所需的残基即可。

[0233] 尽管对可变区进行改变，本领域技术人员将了解，本发明的经修饰的抗体将包含其中至少部分的一或多个恒定结构域已被删除或以其他方式改变的抗体(例如全长抗体或其免疫反应性片段)以提供所欲的生化特征，像是相较于包含原始或未经改变的恒定区的大约相同免疫原性的抗体具有增加的肿瘤定位及/或增加的血清半衰期。在一些实施方式中，该经修饰的抗体的恒定区将包含人恒定区。与本发明兼容的恒定区修饰包含添加、删除或取代一或多个结构域中的一或多个氨基酸。此处所揭示的经修饰的抗体可能包含对三个重链恒定结构域的一或多种(CH1、CH2或CH3)及/或对轻链恒定结构域(CL)的改变或修饰。在一些实施方式中，一或多个结构域系自该经修饰的抗体的恒定区部分或全部删除。在一些实施方式中，该经修饰的抗体将包含其中整个CH2结构域被移除的结构域删除建构体或变异体(ΔCH2建构体)。在一些实施方式中，该缺少的恒定区结构域系由短氨基酸间隔子(例如10个氨基酸残基)取代，以提供通常由该缺少恒定区授予的一些分子柔韧性。

[0234] 在一些实施方式中，该经修饰的抗体系经工程化以直接融合CH3结构域与该抗体的铰链区。在其他实施方式中，肽间隔子被插入铰链区与经修饰的CH2及/或CH3结构域之间。举例来说，其中CH2结构域被删除且剩余的CH3结构域(经修饰或未经修饰)系以5至20个氨基酸间隔子与铰链区连接的建构体可被表达。该间隔子可被添加以确保恒定区的调节组件维持自由及可接近，或该铰链区维持可弯折。然而，应注意氨基酸间隔子在一些情况中可能证实具有免疫原性，且诱发拮抗该建构体的非所欲免疫反应。因此，在某些实施方式中，任何添加至建构体的间隔子将为相对非免疫原性，以维持该经修饰的抗体的所欲生物性质。

[0235] 在一些实施方式中，该经修饰的抗体可能仅具有恒定结构域的部分删除或取代少

数或甚至单一个氨基酸。举例来说，在CH2结构域的选择区域中的单一氨基酸突变可能足以实质上减少Fc结合，因此增加癌细胞定位及/或肿瘤穿透。类似地，所欲的是单纯删除该一或多个恒定区结构域中控制特定效应功能(例如补体C1q结合)的部分。该恒定区的部分删除可增进该抗体的选择特性(血清半衰期)，同时保留其他与该主题恒定区结构域完整有关的所欲功能。另外，如上所述，该揭示抗体的恒定区可经由一或多个氨基酸的突变或取代修饰以增进该形成建构物的特性。在这方面可能扰乱由保守性结合位点所提供的活性(例如Fc结合)，然而实质上维持该经修饰的抗体的构造及免疫原性特性。在某些实施方式中，该经修饰的抗体包含添加一或多个氨基酸至恒定区以增进所欲特征诸如减少或增加效应功能或提供更多细胞毒素或碳水化合物连接位点。

[0236] 该领域已知的是恒定区媒介数种效应功能。举例来说，补体的C1成分与(结合至抗原的)IgG或IgM抗体的Fc区结合活化该补体系统。补体活化于细胞病原体的调理作用及溶解中至为重要。补体活化亦刺激发炎反应，且亦与自体免疫超敏性有关。此外，抗体的Fc区可与表达Fc受体(FcR)的细胞结合。有一些Fc受体对不同类型的抗体具有特异性，包括IgG(γ受体)、IgE(ε受体)、IgA(α受体)及IgM(μ受体)。抗体与细胞表面上的Fc受体结合引发多种重要且多变的生物反应，包括吞噬及破坏抗体包覆颗粒、清空免疫复合物、藉由杀手细胞溶解经抗体包覆的标靶细胞、释放发炎介质、胚胎转移及控制免疫球蛋白的产制。

[0237] 在某些实施方式中，该Wnt途径抑制剂系提供经改变的效应功能的抗体。这些经改变的效应功能可能影响该经投予的抗体的生物特性。举例来说，在一些实施方式中，删除或不活化(经由点突变或其他方法)恒定区结构域可能减少循环中经修饰的抗体(例如抗FZD抗体)与Fc受体结合，因此增加癌细胞定位及/或肿瘤穿透。在其他实施方式中，恒定区修饰增加或减少抗体的血清半衰期。在一些实施方式中，恒定区系经修饰以消除双硫键或寡糖基团。根据本发明对恒定区的修饰可轻易利用本领域技术人员广为周知的生化或分子工程技术进行。

[0238] 在某些实施方式中，Wnt途径抑制剂系不具有一或多种效应功能的抗体。例如在一些实施方式中，该抗体不具ADCC活性及/或CDC活性。在某些实施方式中，该抗体不与Fc受体及/或补体因子结合。在某些实施方式中，该抗体不具效应功能。

[0239] 本发明另包含实质上与此处前述的嵌合抗体、人化抗体、人抗体或彼等的抗体片段同源的变异体及相等物。这些可包含例如保守性取代突变，即以类似氨基酸取代一或多个氨基酸。例如，保守性取代系指以同类型的氨基酸取代另一者，像是例如以一酸性氨基酸取代另一酸性氨基酸、以一碱性氨基酸取代另一碱性氨基酸或以一中性氨基酸取代另一中性氨基酸。保守性氨基酸取代的目的系该领域广为周知并于此处描述。

[0240] 在某些实施方式中，此处所述的抗体系经分离。在某些实施方式中，此处所述的抗体系实质上纯的。

[0241] 在本发明的一些实施方式中，该Wnt途径抑制剂系多肽。该多肽可为包含与至少一种人FZD蛋白或至少一种Wnt蛋白结合的抗体或其片段的重组多肽、天然多肽或合成多肽。在本领域中咸信本发明的一些氨基酸序列可变化而不会对该蛋白的结构或功能造成显著影响。因此，本发明另包括多肽的变异体，该变异体显示实质活性或包括拮抗人FZD蛋白或Wnt蛋白的抗体的区域或彼等的片段。在一些实施方式中，FZD结合多肽或Wnt结合多肽的氨基酸序列变异体包括删除、插入、倒位、重复及/或其他类型的取代。

[0242] 该多肽、类似物及彼等的变异体可另经修饰以包含正常非该多肽的部分的额外化学基团。该等衍生化基团可增进该多肽的溶解性、生物半衰期及/或吸收。该等基团亦可减少或消除该多肽及变异体的任何非所欲的不良反应。有关化学基团的介绍可见Remington: The Science and Practice of Pharmacy,22st Edition,2012,Pharmaceutical Press, London。

[0243] 可用于本文所述的方法的经分离的多肽可藉由该领域已知的任何适当方法产制。该等方法从直接蛋白质合成方法至建构编码多肽序列的DNA序列及在适当宿主中表达该等序列皆可。在一些实施方式中,DNA序列系利用重组技术建构,其藉由分离或合成编码感兴趣的野生型蛋白质的DNA序列。可任意选择地,该序列可藉由定点突变形成突变以提供其功能性类似物。

[0244] 在一些实施方式中,编码感兴趣的多肽的DNA序列可藉由化学合成利用寡核苷酸合成器建构。寡核苷酸可根据该所欲多肽的氨基酸序列设计,并选择该些将产制感兴趣重组多肽的宿主细胞所偏好的密码子。标准方法可被用于合成编码经分离的感兴趣多肽的多核苷酸序列。举例来说,完全氨基酸序列可被用于建构反翻译基因。另外,可合成包含编码经分离的特定多肽的核苷酸序列的DNA寡聚体。例如,多个编码该所欲多肽的部分的小型寡核苷酸可被合成然后连接。个别寡核苷酸通常包含5'或3'悬端以用于互补组装。

[0245] 一经组装(藉由合成、定点突变形成或其他方法),该编码感兴趣的特定多肽的多核苷酸序列可被插入表达载体并可操作性连接适合该蛋白质于所欲宿主内表达的表达控制序列。适当组装可藉由核苷酸测序、限制酶定位及/或于适当宿主内表达生物活性多肽证实。如该领域所广为周知,为了在宿主内获得高表达量的经转染的基因,该基因必须与在选定的表达宿主内具功能性的转录及翻译表达控制序列可操作性连接。

[0246] 在某些实施方式中,重组表达载体被用于扩增及表达拮抗人FZD蛋白或Wnt蛋白的DNA编码结合剂(例如抗体或可溶性受体)或其片段。举例来说,重组表达载体可为可复制的DNA建构体,其具有与适当转录及/或翻译调节组件可操作性连接的编码FZD结合剂、Wnt结合剂、抗FZD抗体或其片段、抗Wnt抗体或其片段、或FZD-Fc可溶性受体的多肽链的合成性或cDNA衍生性DNA片段,该调节组件系源自哺乳动物、微生物、病毒或昆虫基因。转录单位通常包含下列的组合:(1)于基因表达中具有调节作用的基因组件,例如转录启动子或增强子,(2)经转录成mRNA然后翻译成蛋白质的结构或编码序列,及(3)适当的转录及翻译启动及终止序列。调节组件可包括操作子序列以控制转录。通常由复制起点授予的于宿主内复制的能力及有利转化物辨识的选择基因可被额外纳入。DNA区系"可操作性连接"当它们彼此之间系功能性相关。举例来说,信号肽的DNA(分泌前导序列)系可操作性连接多肽的DNA,若其被表达为参与该多肽分泌的前体;启动子系可操作性连接编码序列,若其控制该序列的转录;或核糖体结合位点系可操作性连接编码序列,若其位置系为了允许翻译。在一些实施方式中,适用于酵母菌表达系统的结构组件包括使宿主细胞得以胞外分泌经翻译的蛋白质的前导序列。在其他实施方式中,当重组蛋白质系于无前导或转运序列存在时表达,其可包括N端甲硫氨酸残基。此残基之后可任意选择地与该经表达的重组蛋白质切开以提供最终产物。

[0247] 表达控制序列及表达载体的选择取决于宿主选择。多样化的表达宿主/载体组合可被采用。可用于真核宿主的表达载体包括例如包含源自SV40、牛乳头状瘤病毒、腺病毒及

巨细胞病毒的表达控制序列的载体。可用于细菌宿主的表达载体包括已知的细菌质粒，像是源自大肠杆菌的质粒(包括pCR1、pBR322、pMB9及彼等的衍生物)，及广泛宿主范围质粒像是M13及其他丝状单股DNA噬菌体。

[0248] 用于表达FZD结合或Wnt结合剂(或用来作为抗原的蛋白)的适当宿主细胞包括原核生物、酵母菌细胞、昆虫细胞或在适当启动子控制下的高级真核细胞。原核生物包括革兰氏阴性或革兰氏阳性有机体，例如大肠杆菌(E.coli)或杆菌(Bacillus)。高级真核细胞包括如下所述的哺乳动物来源的株化细胞系。不含细胞的翻译系统亦可被采用。用于细菌性、真菌性、酵母菌性及哺乳动物细胞性宿主的适当选殖及表达载体系该领域所广为周知。有关蛋白质产制(包括抗体产制)方法的额外信息可见于例如美国专利公开号2008/0187954；美国专利第6,413,746及6,660,501号；及国际专利公开号WO 2004/009823。

[0249] 多种哺乳动物细胞培养系统被用于表达重组多肽。于哺乳动物细胞中表达重组蛋白可为较佳，因为这些蛋白通常经过正确折叠折叠、适当改质且具生物功能性。适当哺乳动物宿主细胞系的实例包括COS-7(猴肾来源)、L-929(小鼠纤维母细胞来源)、C127(小鼠乳房肿瘤来源)、3T3(小鼠纤维母细胞来源)、CHO(中国仓鼠卵巢来源)、HeLa(人子宫颈癌来源)、BHK(仓鼠肾纤维母细胞来源)、HEK-293(人胚胎肾来源)细胞系及彼等的变异株。哺乳动物表达载体可包含非转录组件(诸如复制起点、与所欲表达的基因相连的适当启动子及增强子，及其他5'或3'侧翼非转录序列)及5'或3'非翻译序列(诸如必要的核糖体结合位点、聚腺苷酸化位点、剪接供点及受点，及转录终止序列)。

[0250] 于昆虫细胞培养系统(例如杆状病毒)中表达重组蛋白质亦提供产制正确折叠及具生物功能的蛋白质的有效方法。用于在昆虫细胞中产制异源性蛋白质的杆状病毒系统系本领域技术人员所广为周知(见例如Luckow and Summers,1988,Bio/Technology,6:47)。

[0251] 因此，本发明提供包含此处所述的FZD结合剂或Wnt结合剂的细胞。在一些实施方式中，该等细胞产制此处所述的结合剂(例如抗体或可溶性受体)。在某些实施方式中，该等细胞产制抗体。在某些实施方式中，该等细胞产制抗体OMP-18R5。在一些实施方式中，该等细胞产制可溶性受体。在一些实施方式中，该等细胞产制FZD-Fc可溶性受体。在一些实施方式中，该等细胞产制FZD8-Fc可溶性受体。在一些实施方式中，该等细胞产制FZD8-Fc可溶性受体54F28。

[0252] 由经转化的宿主产制的蛋白质可根据任何适当方法纯化。标准方法包括层析(例如离子交换、亲和性及尺寸柱层析)、离心、差别溶解或藉由任何其他用于蛋白质纯化的标准技术。亲和性标签诸如六组氨酸、麦芽糖结合结构域、流感外套序列及麸胱甘肽-S-转移酶可被连接至该蛋白质以允许藉由通过适当亲和性管柱的轻易纯化。经分离的蛋白亦可经物理特征化，使用像是蛋白水解、质谱分析(MS)、核磁共振(NMR)、高效液相层析(HPLC)及x光结晶的技术。

[0253] 在一些实施方式中，源自分泌重组蛋白质至培养基的表达系统的上清液可利用商用蛋白质浓缩过滤器先行浓缩，例如使用阿密康(Amicon)或密里博(Millipore)Pellicon超过滤单位浓缩。在浓缩步骤之后，该浓缩液可被加至适当纯化基材。在一些实施方式中，可采用阴离子交换树脂，例如具有二乙基胺基乙基(DEAE)悬挂基团的基材或基质。该基材可为丙烯酰胺、洋菜糖、葡聚糖、纤维素或其他常用于蛋白质纯化的基材。在一些实施方式中，可采用阳离子交换步骤。适当的阳离子交换基材包括包含磺丙基或羧甲基的各种不可

溶基材。在一些实施方式中，可采用羟磷灰石基质，包括但不限于陶瓷羟磷灰石(CHT)。在某些实施方式中，一或多种应用疏水性反相HPLC基质(例如具有悬挂甲基或其他脂肪族基团的硅胶)的反相HPLC步骤可被采用以进一步纯化结合剂。上述的一些或所有纯化步骤的各种组合亦可被应用以提供均质性重组蛋白。

[0254] 在一些实施方式中，于细菌培养中生产的重组蛋白质可被分离，例如藉由自细胞团块初步萃取，接着进行一或多次浓缩、盐析、水性离子交换或大小排除层析步骤。HPLC可被使用于最终纯化步骤。用于表达重组蛋白的微生物细胞可藉由任何方便方法破碎，包括冷冻解冻循环、超声波震荡、机械破碎或使用细胞溶解剂。

[0255] 该领域已知的用于纯化抗体及其他蛋白质的方法亦包括例如该些于美国专利公开号2008/0312425、2008/0177048及2009/0187005所述者。

[0256] 在某些实施方式中，该Wnt结合剂或该FZD结合剂系非抗体的多肽。用于识别及产制以高亲和性与蛋白质标靶结合的非抗体多肽的各种方法系该领域所知。见例如Skerra,2007,Curr.Opin.Biotechnol.,18:295-304;Hosse et al.,2006,Protein Science,15:14-27;Gill et al.,2006,Curr.Opin.Biotechnol.,17:653-658;Nygren,2008,FEBS J.,275:2668-76;及Skerra,2008,FEBS J.,275:2677-83。在某些实施方式中，噬菌体展示技术可被用于生产及/或识别FZD结合或Wnt结合多肽。在某些实施方式中，该多肽包含选自蛋白A、蛋白G、脂质运载蛋白(lipocalin)、纤维粘连蛋白结构域、锚蛋白(ankyrin)共同重复结构域或硫氧还蛋白类型的蛋白质支架。

[0257] 在某些实施方式中，该结合剂可以数种缀合(即免疫缀合物或放射缀合物)或非缀合形式的任一者被使用。在某些实施方式中，抗体可以非缀合形式被使用以驾驭个体的天然防御机制，包括补体依赖性细胞毒性及抗体依赖性细胞毒性，以消灭该恶性或癌细胞。

[0258] 在一些实施方式中，该结合剂系与细胞毒性剂缀合。在一些实施方式中，该细胞毒性剂系化学治疗剂，包括但不限于甲胺喋呤(methotrexate)、甲烯土霉素(adriamicin)、多柔比星(doxorubicin)、霉法兰(melphalan)、丝裂霉素C(mitomycin C)、氯芥苯丁酸(chlorambucil)、正定霉素(daunorubicin)或其他插入剂。在一些实施方式中，该细胞毒性剂系细菌、真菌、植物或动物来源的酶活性毒素及彼等的片段，包括但不限于白喉毒素A链、白喉毒素的非结合活性片段、外毒素A链、蓖麻毒素A链、相思豆毒素(abrin)A链、莫迪素(modeccin)A链、α-次黄嘌呤(sarcin)、油桐(Aleurites fordii)蛋白、石竹素(dianthin)蛋白、美洲商陆(Phytolaca americana)蛋白(PAPI、PAPII及PAP-S)、苦瓜(momordica charantia)抑制剂、泻果素(curcin)、巴豆素(crotin)、肥皂草(saponaria officinalis)抑制剂、白树毒素(gelonin)、丝裂胶素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、伊诺霉素(enomycin)及新月毒素(trichothecene)。在一些实施方式中，该细胞毒性剂系放射性同位素以产制放射缀合物或经放射缀合的抗体。多种放射性核种可用于产制经放射缀合的抗体，包括但不限于^{90}Y、^{125}I、^{131}I、^{123}I、^{111}In、^{131}In、^{105}Rh、^{153}Sm、^{67}Cu、^{67}Ga、^{166}Ho、^{177}Lu、^{186}Re、^{188}Re及^{212}Bi。在一些实施方式中，本发明可产制抗体与一或多种小分子毒素的缀合物，该等毒素诸如卡利奇霉素(calicheamicin)、类美坦素(maytansinoids)、新月毒素(trichothene)、CC1065及具有毒素活性的该些毒素的衍生物。在某些实施方式中，抗体与细胞毒性剂的缀合物可利用各种双官能性蛋白偶合剂制备，例如N-琥珀酰亚胺基-3-(2-吡啶二硫代)丙酸酯(SPDP)、二亚胺环硫丁烷(IT)、亚氨酸酯的双官能基衍生物

(诸如己二亚胺二甲酯HCL)、活性酯的双官能基衍生物(诸如辛二酸二琥珀酰亚胺)、醛的双官能基衍生物(诸如戊二醛)、双迭氮化合物(诸如双(对-迭氮苯甲酰基)己二胺)、双重氮衍生物(诸如双-(对-重氮苯甲酰基)-乙二胺)、二异氰酸酯(诸如2,6-二异氰酸甲苯酯)及双活性氟化合物(诸如1,5-二氟-2,4-二硝苯)。

[0259] 在某些实施方式中,该Wnt途径抑制剂(例如抗体或可溶性受体)系至少一种Wnt蛋白(即1、2、3、4、5、6、7、8、9或10种Wnt蛋白)的拮抗剂。在某些实施方式中,该Wnt途径抑制剂抑制其所结合的Wnt蛋白的活性。在某些实施方式中,该Wnt途径抑制剂抑制至少约10%、至少约20%、至少约30%、至少约50%、至少约75%、至少约90%或约100%的其所结合的人Wnt蛋白的活性。

[0260] 在某些实施方式中,该Wnt途径抑制剂(例如抗体或可溶性受体)抑制至少一种人Wnt与适当受体的结合。在某些实施方式中,该Wnt途径抑制剂抑制至少一种人Wnt蛋白与一或多种人FZD蛋白的结合。在一些实施方式中,该至少一种Wnt蛋白系选自下列:Wnt1、Wnt2、Wnt2b/13、Wnt3、Wnt3a、Wnt4、Wnt5a、Wnt5b、Wnt6、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt9a、Wnt9b、Wnt10a、Wnt10b、Wnt11、及Wnt16。在一些实施方式中,该一或多种人FZD蛋白系选自下列:FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9及FZD10。在某些实施方式中,该Wnt途径抑制剂抑制一或多种Wnt蛋白与FZD1、FZD2、FZD4、FZD5、FZD7、及/或FZD8的结合。在某些实施方式中,该Wnt途径抑制剂抑制一或多种Wnt蛋白与FZD8的结合。在某些实施方式中,由该Wnt途径抑制剂对特定Wnt与FZD蛋白的结合的抑制系至少约10%、至少约25%、至少约50%、至少约75%、至少约90%或至少约95%。在某些实施方式中,抑制Wnt与FZD蛋白结合的剂亦抑制Wnt途径信号传导。在某些实施方式中,抑制人Wnt途径信号传导的Wnt途径抑制剂系抗体。在某些实施方式中,抑制人Wnt途径信号传导的Wnt途径抑制剂系FZD-Fc可溶性受体。在某些实施方式中,抑制人Wnt途径信号传导的Wnt途径抑制剂系FZD8-Fc可溶性受体。在某些实施方式中,抑制人Wnt途径信号传导的Wnt途径抑制剂系可溶性受体54F28。

[0261] 在某些实施方式中,本文中所述的该Wnt途径抑制剂(例如抗体或可溶性受体)为至少一种人Wnt蛋白的抑制剂且抑制Wnt活性。在某些实施方式中,该Wnt途径抑制剂抑制至少约10%、至少约20%、至少约30%、至少约50%、至少约75%、至少约90%或约100%的Wnt活性。在一些实施方式中,该Wnt途径抑制剂抑制一、二、三、四、五或更多种Wnt蛋白的活性。在一些实施方式中,该Wnt途径抑制剂抑制选自下列的至少一种人Wnt蛋白的活性:Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt4、Wnt5a、Wnt5b、Wnt6、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt9a、Wnt9b、Wnt10a、Wnt10b、Wnt11、及Wnt16。在一些实施方式中,该Wnt结合剂与至少一种选自Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a、及Wnt10b的Wnt蛋白结合。在某些实施方式中,该至少一种Wnt蛋白系选自Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt8a、Wnt8b、Wnt10a、及Wnt10b。在某些实施方式中,抑制人Wnt活性的Wnt途径抑制剂系抗体。在某些实施方式中,抑制人Wnt活性的Wnt途径抑制剂系FZD-Fc可溶性受体。在某些实施方式中,抑制人Wnt活性的Wnt途径抑制剂系FZD8-Fc可溶性受体。在某些实施方式中,抑制人Wnt活性的Wnt途径抑制剂系可溶性受体54F28。

[0262] 在某些实施方式中,本文所述的该Wnt途径抑制剂系至少一种人FZD蛋白的拮抗剂且抑制FZD活性。在某些实施方式中,该Wnt途径抑制剂抑制至少约10%、至少约20%、至少约30%、至少约50%、至少约75%、至少约90%或约100%的FZD活性。在一些实施方式中,该

Wnt途径抑制剂抑制一、二、三、四、五或更多种FZD蛋白的活性。在一些实施方式中，该Wnt途径抑制剂抑制选自下列的至少一种人FZD蛋白的活性：FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9及FZD10。在某些实施方式中，该Wnt途径抑制剂抑制FZD1、FZD2、FZD4、FZD5、FZD7、及/或FZD8的活性。在某些实施方式中，该Wnt途径抑制剂抑制FZD8的活性。在一些实施方式中，该Wnt途径抑制剂系抗FZD抗体。在某些实施方式中，该Wnt途径抑制剂系抗FZD抗体OMP-18R5。

[0263] 在某些实施方式中，本文所述的该Wnt途径抑制剂系至少一种人Wnt蛋白的拮抗剂且抑制Wnt信号传导。在某些实施方式中，该Wnt途径抑制剂抑制至少约10％、至少约20％、至少约30％、至少约50％、至少约75％、至少约90％或约100％的Wnt信号传导。在一些实施方式中，该Wnt途径抑制剂抑制一、二、三、四、五或更多种Wnt蛋白的信号传导。在一些实施方式中，该Wnt途径抑制剂抑制至少一种选自Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a、及Wnt10b的Wnt蛋白的信号传导。在某些实施方式中，抑制Wnt信号传导的Wnt途径抑制剂系抗体。在某些实施方式中，抑制Wnt信号传导的Wnt途径抑制剂系可溶性受体。在某些实施方式中，抑制Wnt信号传导的Wnt途径抑制剂系FZD-Fc可溶性受体。在某些实施方式中，抑制Wnt信号传导的Wnt途径抑制剂系FZD8-Fc可溶性受体。在某些实施方式中，抑制Wnt信号传导的Wnt途径抑制剂系可溶性受体54F28。

[0264] 在某些实施方式中，本文中所述的Wnt途径抑制剂系β-连环蛋白信号传导的拮抗剂。在某些实施方式中，该Wnt途径抑制剂抑制至少约10％、至少约20％、至少约30％、至少约50％、至少约75％、至少约90％或约100％的β-连环蛋白信号传导。在某些实施方式中，抑制β-连环蛋白信号传导的Wnt途径抑制剂系抗体。在某些实施方式中，抑制β-连环蛋白信号传导的Wnt途径抑制剂系抗FZD抗体。在某些实施方式中，抑制β-连环蛋白信号传导的Wnt途径抑制剂系抗体OMP-18R5。在某些实施方式中，抑制β-连环蛋白信号传导的Wnt途径抑制剂系可溶性受体。在某些实施方式中，抑制β-连环蛋白信号传导的Wnt途径抑制剂系FZD-Fc可溶性受体。在某些实施方式中，抑制β-连环蛋白信号传导的Wnt途径抑制剂系FZD8-Fc可溶性受体。

[0265] 在某些实施方式中，本文中所述的Wnt途径抑制剂抑制至少一种Wnt蛋白与受体的结合。在某些实施方式中，该Wnt途径抑制剂抑制至少一种人Wnt蛋白与一或多种其受体的结合。在一些实施方式中，该Wnt途径抑制剂抑制至少一种Wnt蛋白与至少一种FZD蛋白的结合。在一些实施方式中，该Wnt结合剂抑制至少一种Wnt蛋白与FZD1、FZD2、FZD3、FZD4、FDZ5、FDZ6、FDZ7、FDZ8、FDZ9、及/或FZD10的结合。在某些实施方式中，至少一种Wnt与至少一种FZD蛋白的结合系经抑制至少约10％、至少约25％、至少约50％、至少约75％、至少约90％或至少约95％。在某些实施方式中，抑制至少一种Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂另抑制Wnt途径信号传导及/或β-连环蛋白信号传导。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系抗体。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系抗FZD抗体。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系抗体OMP-18R5。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系可溶性受体。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系FZD-Fc可溶性受体。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系

FZD8-Fc可溶性受体。在某些实施方式中，抑制至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系FZD8-Fc可溶性受体54F28。

[0266] 在某些实施方式中，本文中所述的Wnt途径抑制剂阻断至少一种Wnt与受体的结合。在某些实施方式中，该Wnt途径抑制剂阻断至少一种人Wnt蛋白与一或多种其受体的结合。在一些实施方式中，该Wnt途径抑制剂抑制至少一种Wnt与至少一种FZD蛋白的结合。在一些实施方式中，该Wnt途径抑制剂阻断至少一种Wnt蛋白与FZD1、FZD2、FZD3、FZD4、FDZ5、FDZ6、FDZ7、FDZ8、FDZ9、及/或FZD10的结合。在某些实施方式中，至少一种Wnt与至少一种FZD蛋白的结合系经阻断至少约10％、至少约25％、至少约50％、至少约75％、至少约90％或至少约95％。在某些实施方式中，阻断至少一种Wnt蛋白与至少一种FZD蛋白结合的Wnt途径抑制剂另抑制Wnt途径信号传导及/或β-连环蛋白信号传导。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系抗体。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系抗FZD抗体。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系抗体OMP-18R5。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系可溶性受体。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系FZD-Fc可溶性受体。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系FZD8-Fc可溶性受体。在某些实施方式中，阻断至少一种人Wnt与至少一种FZD蛋白结合的Wnt途径抑制剂系可溶性受体54F28。

[0267] 在某些实施方式中，本文所述的Wnt途径抑制剂抑制Wnt途径信号传导。应了解的是，抑制Wnt途径信号传导的Wnt途径抑制剂在某些实施方式中可能抑制Wnt信号传导途径中藉由一或多种受体的信号传导，但不一定抑制藉由所有受体的信号传导。在某些选择性实施方式中，所有人受体的Wnt途径信号传导可能皆被抑制。在某些实施方式中，选自FZD1、FZD2、FZD3、FZD4、FDZ5、FDZ6、FDZ7、FDZ8、FDZ9、及FZD10的一或多种受体的Wnt途径信号传导系经抑制。在某些实施方式中，Wnt途径抑制剂对Wnt途径信号传导的抑制系指减少Wnt途径信号传导的量至少约10％、至少约25％、至少约50％、至少约75％、至少约90％或至少约95％。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系抗体。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系抗FZD抗体。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系抗体OMP-18R5。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系可溶性受体。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系FZD-Fc可溶性受体。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系FZD8-Fc可溶性受体。在一些实施方式中，抑制Wnt途径信号传导的Wnt途径抑制剂系可溶性受体54F28。

[0268] 在某些实施方式中，本文所述的Wnt途径抑制剂抑制β-连环蛋白的活化。应了解的是，抑制β-连环蛋白的活化的Wnt途径抑制剂在某些实施方式中可能抑制藉由一或多种受体的β-连环蛋白的活化，但不一定抑制藉由所有受体的β-连环蛋白的活化。在某些选择性实施方式中，藉由所有人受体的β-连环蛋白活化可能皆被抑制。在某些实施方式中，藉由选自FZD1、FZD2、FZD3、FZD4、FDZ5、FDZ6、FDZ7、FDZ8、FDZ9、及FZD10的一或多种受体的β-连环蛋白活化系经抑制。在某些实施方式中，该Wnt结合剂对β-连环蛋白活化的抑制系指减少β-连环蛋白活化量至少约10％、至少约25％、至少约50％、至少约75％、至少约90％或至少约

95％。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系抗体。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系抗FZD抗体。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系抗体OMP-18R5。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系可溶性受体。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系FZD-Fc可溶性受体。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系FZD8-Fc可溶性受体。在一些实施方式中，抑制β-连环蛋白的活化的Wnt途径抑制剂系可溶性受体54F28。

[0269] 用于测定Wnt途径抑制剂是否抑制β-连环蛋白信号传导的活体内及试管内测定系该领域所知。举例来说，可使用以细胞为基底的荧光素酶报告试验测量试管内的β-连环蛋白信号传导量，其利用含有多份TCF结合结构域与下游萤火虫荧光素酶报告基因的TCF/Luc报告载体(Gazit et al.,1999,Oncogene,18;5959-66;TOPflash,Millipore,Billerica MA)。在一或多种Wnt蛋白(例如由转染细胞表达或由Wnt条件培养基提供的Wnt)存在下，结合剂存在时的β-连环蛋白信号传导量系与无结合剂存在时的信号传导量比较。除了TCF/Luc报告子测定之外，结合剂(或候选剂)对β-连环蛋白信号传导的影响可于试管内或活体内藉由测量该剂对β-连环蛋白调节基因表达量的影响加以测定，如c-myc(He et al.,1998,Science,281:1509-12)、细胞周期素D1(Tetsu et al.,1999,Nature,398:422-6)及/或纤维粘连蛋白(Gradl et al.1999,Mol.Cell Biol.,19:5576-87)。在某些实施方式中，结合剂对β-连环蛋白信号传导的影响亦可能藉由测量该剂对Dishevelled-1、Dishevelled-2、Dishevelled-3、LRP5、LRP6及/或β-连环蛋白的磷酸化状态的影响检测。

[0270] 在某些实施方式中，Wnt途径抑制剂具有一或多种下列影响：抑制肿瘤细胞增生、抑制肿瘤生长、减少癌干细胞于肿瘤中的频率、减少肿瘤的肿瘤发生性、藉由减少癌干细胞于肿瘤中的频率以减少肿瘤的肿瘤发生性、刺激肿瘤细胞的细胞死亡、诱导肿瘤中的细胞分化、使致癌细胞分化成非致癌状态、诱导肿瘤细胞分化标志的表达、防止肿瘤细胞转移、或减少肿瘤细胞的存活。

[0271] 在某些实施方式中，Wnt途径抑制剂可抑制肿瘤生长。在某些实施方式中，Wnt途径抑制剂可抑制活体内(例如异种移植小鼠模型及/或于罹患癌的人)的肿瘤生长。在一些实施方式中，该肿瘤系选自结直肠肿瘤、结肠肿瘤、胰肿瘤、肺肿瘤、卵巢肿瘤、肝肿瘤、乳房肿瘤、肾肿瘤、前列腺肿瘤、胃肠道肿瘤、黑色素瘤、子宫颈肿瘤、膀胱肿瘤、神经胶母细胞瘤或头颈肿瘤的肿瘤。在某些实施方式中，该肿瘤系黑色素瘤。在某些实施方式中，该肿瘤系结直肠肿瘤。在某些实施方式中，该肿瘤系胰肿瘤。在某些实施方式中，该肿瘤系乳房肿瘤。在某些实施方式中，该肿瘤系Wnt依赖性肿瘤。

[0272] 在某些实施方式中，Wnt途径抑制剂可减少肿瘤的肿瘤发生性。在某些实施方式中，Wnt途径抑制剂可于动物模型诸如小鼠异种移植模型中减少包含癌干细胞的肿瘤的肿瘤发生性。在某些实施方式中，肿瘤中癌干细胞的数量或频率系减少至少约二倍、约三倍、约五倍、约十倍、约50倍、约100倍、或约1000倍。在某些实施方式中，癌干细胞的数量或频率减少系藉由使用动物模型的限制稀释试验测定。有关使用限制稀释试验以测定肿瘤中癌干细胞的数量或频率减少的其他实例及指南可见例如国际公开号WO 2008/042236、及美国专利公开号2008/0064049及2008/0178305。

[0273] 在某些实施方式中，本文描述的Wnt途径抑制剂于活体内具有至少1小时、至少约2

小时、至少约5小时、至少约10小时、至少约24小时、至少约2天、至少约3天、至少约1周、或至少约2周的活性。在某些实施方式中，该Wnt途径抑制剂系IgG(例如IgG1或IgG2)抗体，其于活体内具有至少1小时、至少约2小时、至少约5小时、至少约10小时、至少约24小时、至少约2天、至少约3天、至少约1周、或至少约2周的活性。在某些实施方式中，该Wnt途径抑制剂系融合蛋白，其于活体内具有至少1小时、至少约2小时、至少约5小时、至少约10小时、至少约24小时、至少约2天、至少约3天、至少约1周、或至少约2周的活性。

[0274] 在某些实施方式中，本文描述的Wnt途径抑制剂于小鼠、长尾猕猴(cynomolgus monkey)或人体内具有至少约5小时、至少约10小时、至少约24小时、至少约2天、至少约3天、至少约1周、或至少约2周的循环半衰期。在某些实施方式中，该Wnt途径抑制剂系于小鼠、长尾猕猴或人体内具有至少约5小时、至少约10小时、至少约24小时、至少约2天、至少约3天、至少约1周、或至少约2周的循环半衰期的IgG(例如IgG1或IgG2)抗体。在某些实施方式中，该Wnt途径抑制剂系融合蛋白，其于小鼠、长尾猕猴(cynomolgus monkey)或人体内具有至少约5小时、至少约10小时、至少约24小时、至少约2天、至少约3天、至少约1周、或至少约2周的循环半衰期。增加(或减少)剂诸如多肽及抗体的半衰期的方法系该领域所知。举例来说，增加IgG抗体循环半衰期的已知方法包括导入突变至Fc区，此增加pH 6.0时抗体对新生儿Fc受体(FcRn)的pH依赖性结合(见例如美国专利公开号2005/0276799、2007/0148164及2007/0122403)。增加缺乏Fc区的抗体片段的循环半衰期的已知方法包括像是PEG化的技术。

IV.试剂盒

[0275] 另外提供用于实施本发明的方法的试剂盒。所谓“试剂盒”系意图指任何包含至少一种用于特异性检测本发明的至少一种生物标记物的表达的试剂例如抗体、核酸探针等的制品(例如包装或容器)。该试剂盒可被促销、经销及/或贩卖为一种用于实施本发明的方法的单位。此外，该等试剂盒可包含描述该试剂盒及包括其使用的说明性材料的包装仿单。

[0276] 在一些实施方式中，试剂盒包含用于使用微阵列技术实施本发明的方法的试剂。在一些实施方式中，试剂盒包含用于使用qPCR测试实施本发明的方法的试剂。阳性及/或阴性对照可被包括于试剂盒中以验证根据本发明采用的试剂的活性及正确使用。对照可包括已知有受到关注的生物标记物存在或不存在的样本，或其他包含受到关注的生物标记物的样本。对照的设计及使用系为标准且为本领域技术人员的例行能力。

[0277] 在一些实施方式中，试剂盒包含选自SEQ ID NO:62至79的多核苷酸。在一些实施方式中，试剂盒包含：(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物、及包含SEQ ID NO:64的探针；(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物、及包含SEQ ID NO:67的探针；(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物、及包含SEQ ID NO:70的探针；(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物、及包含SEQ ID NO:73的探针；(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物、及包含SEQ ID NO:76的探针；以及(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物、及包含SEQ ID NO:79的探针。

[0278] 应进一步理解的是，在本发明的方法中的任何或所有步骤可由人员实施或以自动化方式进行。因此，样本制备、生物标记物表达的检测等的步骤可经自动化。

[0279] 本发明的实施方式可藉由参考下列非限制性实施例进一步定义，以下实施例描述

本发明的某些抗体的详细制备及使用本发明的抗体的方法。本领域技术人员将显而易见的是，许多在材料及方法上的调整可加以实施而不背离本发明的范围。

实施例1

鉴别对OMP-18R5与紫杉醇(taxol)的组合治疗有反应的肿瘤

[0280] 乳房肿瘤异种移植模型OMP-B34、OMP-B39、OMP-B44、OMP-B59、OMP-B60、UM-T01、UM-T03及UM-PE13系自最小继代、衍生自病患的肿瘤样品于OncoMed Pharmaceuticals或密西根大学建立。六至八周龄的NOD/SCID小鼠系经皮下注射2-4×10^4个OMP-B34、OMP-B39、OMP-B44、OMP-B59、OMP-B60、UM-T01、UM-T03或UM-PE13肿瘤细胞。让肿瘤生长直到肿瘤达到平均体积100至150mm^3。肿瘤小鼠系经随机分组成四组(每组n＝10)并经对照抗体1B711(15mg/kg)、抗FZD抗体OMP-18R5(15mg/kg)、紫杉醇(10mg/kg)或OMP-18R5(15mg/kg)与紫杉醇(10mg/kg)的组合治疗。抗体及/或紫杉醇的治疗系经每周投予。监测肿瘤生长，于所示时间点以电子卡尺测量肿瘤体积。数据以平均值±SEM表示。

[0281] 为了决定肿瘤是否对抗FZD抗体OMP-18R5有反应，单剂肿瘤体积数据系与对照比较，而OMP-18R5与紫杉醇的组合治疗系与紫杉醇单剂比较。在本试验中，“有反应者”肿瘤系定义为相较于紫杉醇作为单剂时的肿瘤生长抑制，OMP-18R5与紫杉醇的组合显示显著较高肿瘤生长抑制的肿瘤。

[0282] 各异种移植模型的结果系显示于图1A至H。T检定系于各时间点进行。多重比较使用双向重复测量变异数分析，接着进行邦弗朗尼校正。T检定及双向重复测量变异数分析系利用GraphPad Prism5(GraphPad Software Inc.)进行。肿瘤OMP-B59、OMP-B60、UM-T03及UM-PE13系经显示为有反应者，然而肿瘤OMP-B34、OMP-B39、OMP-B44及UM-T01系经显示为无反应者。结果示于表1。

表1

肿瘤	肿瘤亚型	分类
OMP-B34	TNBC	无反应者
OMP-B39	TNBC	无反应者
OMP-B44	TNBC	无反应者
OMP-B59	TNBC	有反应者
OMP-B60	TNBC	有反应者
UM-T01	TNBC	无反应者
UM-T03	ER+PR+HER2+	有反应者
UM-PE13	TNBC	有反应者

实施例2

鉴别预测性生物标记物

[0283] 微阵列分析系于对OMP-18R5与紫杉醇的组合治疗无反应(“无反应者”)的未经处理的乳房肿瘤OMP-B34、OMP-B39、OMP-B44，及UM-T01以及对OMP-18R5与紫杉醇的组合治疗有反应(“有反应者”)的未经处理的肿瘤OMP-B59、OMP-B60、UM-T03及UM-PE13上进行。依照制造商说明使用RNeasy纤维组织迷你试剂盒(Qiagen,Valencia CA)及DNA酶处理，自各肿瘤分离RNA。样本系储存于-80℃。RNA系于Agilent 2100生物分析仪上检视，藉由存在完整28S及18S核糖体波峰可证实完整性。所有RNA样本具有260/280比>1.8。自各肿瘤分离的总

RNA系利用Ovation RNA扩增系统V2(NuGEN,San Carlos,CA)加以扩增。经扩增的反义单股cDNA系利用FL-Ovation cDNA生物素模块V2(NuGEN)加以分段及生物素基化。在与阵列杂交前,cDNA及经分段的cDNA的质量系藉由分光亮度计及生物分析器评估。使该经处理的RNA与Affymetrix HG-U133 plus 2.0微阵列(Affymetrix,Santa Clara,CA)杂交,如厂商技术手册所述。在杂交后,该微阵列系经清洗、扫描及分析。微阵列数据系经处理成探针组级的数据,其系使用GeneChip-RMA(Wu et al.,2004,J.Amer.Stat.Assn.,99:909-917)。可能与鼠标记交叉杂交的探针组系经移除。要将数据摘要成基因等级并且确保选择具有最强信号的探针组,在所有定位于一个基因的探针组使用最大表达。移除具有低表达(以log2尺度而言<5)或几乎零变异(<0.01)的基因。基因系经标准化至N(0,1),此系藉由将平均值减去log2尺度表达并除以各基因的标准偏差。

[0284] 分析系利用来自数种信号传导途径的基因进行,该等信号传导途径包括典型、平面细胞极性、Wnt/Ca+2、Wnt信号传导负调节、细胞命运、组织极性、细胞生长及增生、细胞移动、细胞周期及细胞恒定(见表2)。

表2

基因代号	蛋白名称
AES	氨基端分裂增强子
APC	大肠腺瘤息肉蛋白
AXIN1	Axin-1
BCL9	B 细胞 CLL／淋巴瘤 9 蛋白
BTRC	F-box/含 WD 重复蛋白 1A
CCND1	G1/S 特定周期蛋白 D1
CCND2	G1/S 特定周期蛋白 D2
CCND3	G1/S 特定周期蛋白 D3
CSNK1A1	酪蛋白激酶 I 异构体 α
CSNK1D	酪蛋白激酶 I 异构体 δ
CSNK1G1	酪蛋白激酶 I 异构体 γ-1
CSNK2A1	酪蛋白激酶 II 次单位 α
CTBP1	C 端结合蛋白 1
CTBP2	C 端结合蛋白 2
CTNNB1	连环蛋白 β-1
CTNNBIP1	β-连环蛋白交互反应蛋白 1
CXXC4	CXXC 型锌指蛋白 4
DAAM1	蓬乱蛋白相关形态发生活化子 1

DIXDC1	Dixin
DKK1	Dickkopf相关蛋白 1
DVL1	区段极性蓬乱蛋白同源物 DVL-1
DVL2	区段极性蓬乱蛋白同源物 DVL-2
EP300	组蛋白乙酰基转移酶 p300
FBXW11	F-box/含 WD 重复蛋白 11
FBXW2	F-box/含 WD 重复蛋白 2
FBXW4	F-box/含 WD 重复蛋白 4
FGF4	纤维母细胞生长因子 4
FOSL1	Fos 相关抗原 1
FOXN1	叉头盒蛋白 N1
FRAT1	原致癌基因 FRAT1
FRZB	分泌型卷曲相关蛋白 3
FSHB	促滤泡素次单位 β
FZD1	卷曲蛋白 1
FZD2	卷曲蛋白 2
FZD3	卷曲蛋白 3
FZD4	卷曲蛋白 4
FZD5	卷曲蛋白 5
FZD6	卷曲蛋白 6
FZD7	卷曲蛋白 7
FZD8	卷曲蛋白 8
GSK3A	肝糖合成酶激酶 3α
GSK3B	肝糖合成酶激酶 4α
JUN	转录因子 AP-1
KREMEN1	Kremen 蛋白 1
LEF1	淋巴样增强子结合因子 1
LRP5	低密度脂蛋白受体相关蛋白 5

LRP6	低密度脂蛋白受体相关蛋白 6
MYC	Myc 原致癌基因蛋白
NKD1	蛋白裸角皮同源物
NLK	丝氨酸 / 苏氨酸蛋白激酶 NLK
PITX2	脑下垂体同源匣 2
PORCN	蛋白半胱氨酸 N 棕榈酰基转移酶 porcupine
PPP2CA	丝氨酸 / 苏氨酸蛋白磷酸酶 2A 酶催化次单位 α 异构体
PPP2R1A	丝氨酸 / 苏氨酸蛋白磷酸酶 2A 65kDa 调节次单位 A α 异构体
PYGO1	Pygopus 同源物 1
RHOU	Rho 相关 GTP 结合蛋白 RhoU
SENP2	Sentrin 特定蛋白酶 2
SFRP1	分泌型卷曲相关蛋白 1
SFRP4	分泌型卷曲相关蛋白 4
SLC9A3R1	Na(+)/H(+)交换调节辅因子 NHE-RF1
SOX17	转录因子 SOX-17
T	Brachyury 蛋白
TCF7	转录因子 7
TCF7L1	转录因子 7 样 1
TLE1	转导蛋白样增强子蛋白 1
TLE2	转导蛋白样增强子蛋白 2
WIF1	Wnt 抑制因子 1
WISP1	WNT1 诱导性信号传导途径蛋白 1
WNT1	原致癌基因 Wnt-1
WNT2	蛋白 Wnt-2
WNT2B	蛋白 Wnt-2B
WNT3	蛋白 Wnt-3
WNT3A	蛋白 Wnt-3a
WNT4	蛋白 Wnt-4

WNT5A	蛋白 Wnt-5a
WNT5B	蛋白 Wnt-5b
WNT6	蛋白 Wnt-6
WNT7A	蛋白 Wnt-7a
WNT7B	蛋白 Wnt-7b
WNT8A	蛋白 Wnt-8a
WNT9A	蛋白 Wnt-9a
WNT10A	蛋白 Wnt-10a
WNT11	蛋白 Wnt-11
WNT16	蛋白 Wnt-16

[0285] 支持向量机递归特征删除(SVM-RFE)方法(Guyon et al,2002,Machine Learning,46:389-422)系用于鉴别可区别有反应与无反应肿瘤的基因,且支持向量机(SVM)方法(Cortes and Vapnik,1995,Machine Learning,20:273-297)系用于分类。留一交叉验证(LOOCV)方法被用于选择基因的数量且也用于测量模型的阳性预测值(PPV)、阴性预测值(NPV)、敏感性及特异性。使用8个乳房肿瘤的包含FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的生物标记物标签达到最佳表达,其中PPV＝NPV＝敏感性＝特异性＝100％(见表2)。如图3所示,主组分分析(PCA)显示6基因生物标记物标签导致近乎完美的分开8个乳房肿瘤。此外,在6基因生物标记物标签与实施例1所述的活体内实验的肿瘤体积比(RTV)之间观察到强烈相关性(相关性＝0.95,p值＝0.0003;交叉验证相关性＝0.89,p值＝0.00027;图4)。

[0286] 判定值系根据训练数据由SVM模型决定。以6基因生物标记物标签而言,判定值可由6基因标准化表达的加权总合计算:0.4560427*FBXW2+0.3378467*CCND2-0.4809354*RHOU+0.409029*CTBP2+0.3291529*WIF1+02926374*DKK1+0.04662682。阳性判定值显示肿瘤经预测为有反应者,而阴性判定值显示肿瘤经预测为无反应者。此外,分类机率可藉由适配逻辑回归至判定值上获得。具有高于0.5的机率的肿瘤将被预测为有反应者,而具有低于0.5的机率的肿瘤将被预测为无反应者。

实施例3

活体内验证预测性生物标记物

[0287] 六个额外的乳癌肿瘤系选自OncoMed肿瘤库,并如实施例1所述进行微阵列分析。该六个乳癌肿瘤系OMP-B29、OMP-B71、OMP-B84、OMP-B90、UM-T02及UM-T06。如本文所述,使用6基因生物标记物标签的分类机率分析以预测这些肿瘤各者对抗FZD抗体OMP-18R5与紫杉醇的组合治疗的反应(见图5)。同时,在如实施例1所述的活体内异种移植模型中评估该六个肿瘤(见图6A至F)。如实施例1a所述,在活体内模型中的“有反应者”系显示相较于紫杉醇作为单剂时的肿瘤生长抑制,OMP-18R5与紫杉醇的组合显示显著较高肿瘤生长抑制的肿瘤。根据分类机率的预测系与活体内异种移植模型的结果比较。结果示于表3。

表3

肿瘤	肿瘤亚型	分类机率	判定值	预测	体内反应
OMP-B29	ER+PR+HER2-	0.3344	-0.5928	无反应者	无反应者
OMP-B71	ER+PR+HER2-	0.9897	1.6789	有反应者	有反应者
OMP-B84	ER+PR+HER2-	0.4324	-0.4002	无反应者	无反应者
OMP-B90	TNBC	0.8152	0.492	有反应者	有反应者
UM-T02	TNBC	0.4387	-0.3972	无反应者	无反应者
UM-T06	ER+PR+HER2-	0.1385	-1.0778	无反应者	无反应者

[0288] 如表3所示，六个乳癌肿瘤各者的反应系由6基因生物标记物标签使用判定值及分类机率正确预测。

实施例4

6基因生物标记物标签的盛行率预估

[0289] 生物标记物标签的盛行率可被定义为根据生物标记物标签经预测为有反应者的族群比例。6基因生物标记物标签在HER2阴性(HER2-)及三阴性乳癌(TNBC)族群中的盛行率系藉由将6基因生物标记物标签应用于三个公众可用的乳癌微阵列数据组预估。Cremoux2001数据组系汇总自有226名病患的Affymetrix U133plus2微阵列，包括145名HER2-及81名HER2+病患，其中51名TNBC病患被包括于HER2-组中。Wang2011数据组系汇总自有115名病患的Affymetrix U133plus2微阵列，包括79名HER2-及36名HER2+病患，其中28名TNBC病患被包括于HER2-组中。Prat2010数据组系汇总自有333名病患的Agilent人1A微阵列，包括215名HER2-及118名HER2+病患，其中57名TNBC病患被包括于HER2-组中。公众数据的前处理包括下载数据、抽取定位至六个基因的探针组及瓦解(collapsing)探针组至六个基因。基因等级的表达数据系藉由减去平均值并除以公众数据中各基因的标准偏差经进一步标准化。利用训练数据建立的SVM模型被用于分类公众数据。获得分类机率且经预测为有反应者(机率>0.5)的比例系根据6基因生物标记物标签计算。

[0290] 如图7所示，在3个数据组中的6基因生物标记物标签的预测盛行率非常类似(约60％)。此预测建议大量乳癌病患族群将对抗FZD抗体OMP-18R5与紫杉醇的组合治疗有反应。

实施例5

6基因生物标记物标签的qPCR测试

[0291] qPCR测试系经发展以测定肿瘤样本中FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量。引物及探针系利用公众可得的mRNA序列设计。产制引物及探针并在利用人新鲜冷冻(FF)及经福尔马林固定的石蜡包埋(FFPE)人组织样本进行的理想化及验证测试中使用该等引物及探针。特定引物及探针系列于表4(所有序列皆为5'至3'方向)。四个参考基因被用于标准化，包括TOP1(拓扑异构酶1)、GUSB(β-葡萄糖苷酸酶)、SDHA(琥珀酸去氢酶)及PUM1(pumilio同源物1)。

表4

基因	引物/探针	序列	SEQ ID NO
CCND2	正向引物	GCTGTCTCTGATCCGCAAGC	SEQ ID NO:62
	反向引物	GACGGTGGGTACATGGCAAAC	SEQ ID NO:63
	探针	CCTTCATTGCTCTGTGTGCCACCGAC	SEQ ID NO:64
CTBP2	正向引物	ATCCGTGGGGAGACGCTG	SEQ ID NO:65
	反向引物	CTCGAACTGCAACCGCCTG	SEQ ID NO:66
	探针	CCCGTGCGACCAAAGCCAATGAGG	SEQ ID NO:67
DKK1	正向引物	GACCATTGACAACTACCAGCCGTA	SEQ ID NO:68
	反向引物	TGGGACTAGCGCAGTACTCATC	SEQ ID NO:69
	探针	TGCCGCACTCCTCGTCCTCTG	SEQ ID NO:70
FBXW2	正向引物	GCCAGTTATGATATTCTCAGGGTCA	SEQ ID NO:71
	反向引物	AGCAGGGCAAAGATATCTCCAAA	SEQ ID NO:72

	探针	AGACTCCTGAGATAGCAAACTTGGCCT	SEQ ID NO:73
RHOU1	正向引物	CCCACCGAGTACATCCCTACTG	SEQ ID NO:74
	反向引物	CAGTGTCACAGAGTTGGAGTCTCA	SEQ ID NO:75
	探针	CGCCCATCCACAGACACCACCG	SEQ ID NO:76
WIF1	正向引物	GTTCCAAAGGTTACCAGGGAGAC	SEQ ID NO:77
	反向引物	GTTGGGTTCATGGCAGGTTCC	SEQ ID NO:78
	探针	CCAGGCTCGCAGACAGGCTTTGAAC	SEQ ID NO:79

[0292] qPCR系于自18个异种移植乳房肿瘤获得的总RNA上进行。肿瘤样品系经收集并立即快速冷冻并储存于-80℃直到分离RNA。总RNA系根据制造商程序利用RNeasy纤维迷你试剂盒(Qiagen,Valencia CA,PN#74704)萃取,其间使用TissueLyzer均质及DNase I处理。在Bioanalyzer 2100(Agilent,Santa Clara,CA)上检视RNA,确认RNA完整(RIN值>6.0)。所有RNA具有A260/A280比>1.8。

[0293] qPCR系以二步骤方式进行。首先,cDNA系自总RNA合成,使用如Applied Biosystems User Bulletin 2中所述的随机六聚体。TaqMan通用PCR预混试剂(Applied Biosystems,Foster City,CA.Cat#4304437及4326708)系根据制造商程序用于后续qPCR反应。基因表达的量系利用循环临界(Ct)方法自三重(triplicate)反应测定。循环临界通常被认为是信号超过检测临界所需的循环次数。Ct值与样本中的标靶核酸的量成反比。六个基因的Ct系利用四个参考基因的Ct值加以标准化。18个异种移植样本的6基因标签的经标准化Ct系显示于表5。

表5

	FBXW2	CCND2	RHOU	CTBP2	WIF1	DKK1
OMP-B84	0.8425	12.5125	4.6775	1.0775	16.4025	5.2575
OMP-B71	0.98375	14.52375	6.46875	0.08875	4.56875	1.14375
OMP-B59	0.83875	2.67375	6.43875	-0.6012	4.90375	10.7888
OMP-B86	2.4725	11.5125	2.5425	1.3275	-0.8825	1.1125
OMP-B39	1.03	12.54	1.44	2.225	2.045	6.365
OMP-B90 p1	1.175	6.955	6.87	1.535	17.535	10.87
OMP-B94	1.67375	1.52875	5.95375	1.56875	9.34875	4.37875
OMP-B40	1.03	16.455	6.775	0.73	16.455	14.985
OMP-B29	1.445	13.63	6.425	0.695	13.63	4.185
OMP-B60	1.6725	14.7775	6.9825	0.4775	-0.8025	8.6775
OMP-B90 p2	0.75875	14.18375	5.78875	0.13875	15.54375	10.7388
UM-T06	1.19875	11.51875	4.27875	1.34375	8.87375	6.26375
OMP-B44	1.61	11.765	4.755	0.505	7.225	9.61
UM-T02	2.255	13.215	4.195	1.075	16.125	4.225
UM-T 3	1.67625	12.58625	5.83625	0.20125	16.21625	4.17125
OMP-B34	0.08625	0.58625	6.21125	0.53125	9.02125	0.06125
UM-PE13	0.925	15.185	4.055	-0.7	15.185	6.62
UM-T01	2.20375	15.11375	6.44375	-0.1062	15.11375	15.1138

[0294] 判定值可藉由6基因自qPCR测试产生的数据的经标准化表达的加权总合计算。这些判定值与自微阵列数据分析产生的判定值不同，然而二个模型的预测能力非常类似。

[0295] 应了解此处所描述的实施例及实施方式仅供说明示范的目的，各种对于彼等的修饰或改变将由本领域技术人员建议且将被纳入本申请案的精神与范围内。

[0296] 本文所引述的所有公开文献、专利及专利申请案皆以参照方式完整纳入此处，犹如个别公开文献、专利或专利申请案系特别且个别明示以参照方式纳入。

[0297] 本说明书中揭示的序列如下：

OMP-18R5重链CDR1(SEQ ID NO:1)

GFTFSHYTLS

OMP-18R5重链CDR2(SEQ ID NO:2)

VISGDGSYTYYADSVKG

OMP-18R5重链CDR3(SEQ ID NO:3)

NFIKYVFAN

OMP-18R5轻链CDR1(SEQ ID NO:4)

SGDNIGSFYVH

OMP-18R5轻链CDR2(SEQ ID NO:5)

DKSNRPSG

OMP-18R5轻链CDR3(SEQ ID NO:6)

QSYANTLSL

OMP-18R5重链可变区氨基酸序列(SEQ ID NO:7)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRFTISSD NSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSS

OMP-18R5轻链可变区氨基酸序列(SEQ ID NO:8)

DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLT ISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG

含画底线的预测信号序列的OMP-18R5重链氨基酸序列(SEQ ID NO:9)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGD GSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSASTKGPSVFPLAP CSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK

含画底线的预测信号序列的OMP-18R5轻链氨基酸序列(SEQ ID NO:10)

MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRP SGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFS HYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFAN WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK

不含预测信号序列的OMP-18R5轻链氨基酸序列(SEQ ID NO:12)

DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLT ISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

不含预测信号序列的人FZD1Fri结构域氨基酸序列(SEQ ID NO:13)

QQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKV QCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELCVGQNTSDKG T

不含预测信号序列的人FZD2Fri结构域氨基酸序列(SEQ ID NO:14)

QFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMY APVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQICVGQNHSEDG

不含预测信号序列的人FZD3Fri结构域氨基酸序列(SEQ ID NO:15)

HSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRV TLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPYPRLVDL

不含预测信号序列的人FZD4Fri结构域氨基酸序列(SEQ ID NO:16)

FGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEK INIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEV

不含预测信号序列的人FZD5Fri结构域氨基酸序列(SEQ ID NO:17)

ASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATT

不含预测信号序列的人FZD6 Fri结构域氨基酸序列(SEQ ID NO:18)

HSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNTETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFDPHTEFLG

不含预测信号序列的人FZD7 Fri结构域氨基酸序列(SEQ ID NO:19)

QPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEICVGQNTSDGSG

不含预测信号序列的人FZD8 Fri结构域氨基酸序列(SEQ ID NO:20)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTT

不含预测信号序列的人FZD9 Fri结构域氨基酸序列(SEQ ID NO:21)

LEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALCMEAPENA

不含预测信号序列的人FZD10 Fri结构域氨基酸序列(SEQ ID NO:22)

ISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLCMEAPNNG

人FZD1氨基酸116至227(SEQ ID NO:23)

CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELC

人FZD2氨基酸39至150(SEQ ID NO:24)

CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQIC

人FZD3氨基酸28至133(SEQ ID NO:25)

CEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDC

人FZD4氨基酸48至161(SEQ ID NO:26)

CDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMC

人FZD5氨基酸33至147(SEQ ID NO:27)

CQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLC

人FZD6氨基酸24至129(SEQ ID NO:28)

CEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYC

人FZD7氨基酸49至160(SEQ ID NO:28)

CQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEIC

人FZD8氨基酸35至148(SEQ ID NO:30)

CQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPC
RSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLC

人FZD9氨基酸39至152(SEQ ID NO:31)

CQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPAC
RPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALC

人FZD10氨基酸34至147(SEQ ID NO:32)

CQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPAC
RVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLC

不含预测信号序列的人FZD8Fri结构域氨基酸序列(变异体)(SEQ IDNO:33)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY
KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDL

人IgG_1 Fc区(SEQ ID NO:34)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

人IgG_1 Fc区(变异体)(SEQ ID NO:35)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N-
VFSCSVMHEALHNHYTQKSLSLSPGK

人IgG_1 Fc区(SEQ ID NO:36)

KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

人IgG_1 Fc区(SEQ ID NO:37)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

人IgG_2 Fc区(SEQ ID NO:38)

CVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc变异体54F03氨基酸序列(无预测信号序列)(SEQ ID NO:39)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY
KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGRADKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW

LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc变异体54F16、54F17、54F18、54F23、54F25、54F27、54F29、54F31及54F34氨基酸序列(无预测信号序列)(SEQ ID NO:40)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY
KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc变异体54F19、54F20、54F24、54F26、54F28、54F30、54F32、54F34及54F35氨基酸序列(无预测信号序列)(SEQ ID NO:41)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY
KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVKNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

含信号序列的FZD8-Fc变异体54F03氨基酸序列(SEQ ID NO:42)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFW
PLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMD
YNRTDLTTGRADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGK

含信号序列的FZD8-Fc变异体54F16氨基酸序列(SEQ ID NO:43)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFW
PLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMD
YNRTDLTTKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGK

含信号序列的FZD8-Fc变异体54F26(SEQ ID NO:44)

MEWGYLLEVTSLLAALFLLQRSPIVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFW
PLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMD
YNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

含信号序列的FZD8-Fc变异体54F28(SEQ ID NO:45)

MEWGYLLEVTSLLAALLLLQRSPFVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFW

PLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMD
YNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

人Wnt1 C端多半胱氨酸区(aa 288-370)(SEQ ID NO:46)

DLVYFEKSPNFCTYSGRLGTAGTAGRACNSSSPALDGCELLCCGRGHRTRTQRVTERCNCTFHWCCHVSCRNC
THTRVLHECL

人Wnt2 C端多半胱氨酸区(aa 267-360)(SEQ ID NO:47)

DLVYFENSPDYCIRDREAGSLGTAGRVCNLTSRGMDSCEVMCCGRGYDTSHVTRMTKCGCKFHWCCAVRCQDC
LEALDVHTCKAPKNADWTTAT

人Wnt2b C端多半胱氨酸区(aa 298-391)(SEQ ID NO:48)

DLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSKGTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAVRCKEC
RNTVDVHTCKAPKKAEWLDQT

人Wnt3C端多半胱氨酸区(aa 273-355)(SEQ ID NO:49)

DLVYYENSPNFCEPNPETGSFGTRDRTCNVTSHGIDGCDLLCCGRGHNTRTEKRKEKCHCIFHWCCYVSCQEC
IRIYDVHTCK

人Wnt3a C端多半胱氨酸区(aa 270-352)(SEQ ID NO:50)

DLVYYEASPNFCEPNPETGSFGTRDRTCNVSSHGIDGCDLLCCGRGHNARAERRREKCRCVFHWCCYVSCQEC
TRVYDVHTCK

人Wnt7a C端多半胱氨酸区(aa 267-359)(SEQ ID NO:51)

DLVYIEKSPNYCEEDPVTGSVGTQGRACNKTAPQASGCDLMCCGRGYNTHQYARVWQCNCKFHWCCYVKCNTC
SERTEMYTCK

人Wnt7b C端多半胱氨酸区(aa 267-349)(SEQ ID NO:52)

DLVYIEKSPNYCEEDAATGSVGTQGRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNCKFHWCCFVKCNTC
SERTEVFTCK

人Wnt8a C端多半胱氨酸区(aa 248-355)(SEQ ID NO:53)

ELIFLEESPDYCTCNSSLGIYGTEGRECLQNSHNTSRWERRSCGRLCTECGLQVEERKTEVISSCNCKFQWCC
TVKCDQCRHVVSKYYCARSPGSAQSLGRVWFGVYI

人Wnt8b C端多半胱氨酸区(aa 245-351)(SEQ ID NO:54)

ELVHLEDSPDYCLENKTLGLLGTEGRECLRRGRALGRWELRSCRRLCGDCGLAVEERRAETVSSCNCKFHWCC
AVRCEQCRRRVTKYFCSRAERPRGGAAHKPGRKP

人Wnt10a C端多半胱氨酸区(aa 335-417)(SEQ ID NO:55)

DLVYFEKSPDFCEREPRLDSAGTVGRLCNKSSAGSDGCGSMCCGRGHNILRQTRSERCHCRFHWCCFVVCEEC
RITEWVSVCK

人Wnt10b C端多半胱氨酸区(aa 307-389)(SEQ ID NO:56)

ELVYFEKSPDFCERDPTMGSPGTRGRACNKTSRLLDGCGSLCCGRGHNVLRQTRVERCHCRFHWCCYVLCDEC
KVTEWVNVCK

接头(SEQ ID NO:57)

ESGGGGVT

接头(SEQ ID NO:58)

LESGGGGVT

接头(SEQ ID NO:59)

GRAQVT

接头(SEQ ID NO:60)

WRAQVT

接头(SEQ ID NO:61)

ARGRAQVT

CCND2正向引物(SEQ ID NO:62)

GCTGTCTCTGATCCGCAAGC

CCND2反向引物(SEQ ID NO:63)

GACGGTGGGTACATGGCAAAC

CCND2探针(SEQ ID NO:64)

CCTTCATTGCTCTGTGTGCCACCGAC

CTBP2正向引物(SEQ ID NO:65)

ATCCGTGGGGAGACGCTG

CTBP2反向引物(SEQ ID NO:66)

CTCGAACTGCAACCGCCTG

CTBP2探针(SEQ ID NO:67)

CCCGTGCGACCAAAGCCAATGAGG

DKK1正向引物(SEQ ID NO:68)

GACCATTGACAACTACCAGCCGTA

DKK1反向引物(SEQ ID NO:69)

TGGGACTAGCGCAGTACTCATC

DKK1探针(SEQ ID NO:70)

TGCCGCACTCCTCGTCCTCTG

FBXW2正向引物(SEQ ID NO:71)

GCCAGTTATGATATTCTCAGGGTCA

FBXW2反向引物(SEQ ID NO:72)

AGCAGGGCAAAGATATCTCCAAA

FBXW2探针(SEQ ID NO:73)

AGACTCCTGAGATAGCAAACTTGGCCT

RHOU1正向引物(SEQ ID NO:74)

CCCACCGAGTACATCCCTACTG

RHOU1反向引物(SEQ ID NO:75)

CAGTGTCACAGAGTTGGAGTCTCA

RHOU1探针(SEQ ID NO:76)

CGCCCATCCACAGACACCACCG

WIF1正向引物(SEQ ID NO:77)

GTTCCAAAGGTTACCAGGGAGAC

WIF1反向引物(SEQ ID NO:78)

GTTGGGTTCATGGCAGGTTCC

WIF1探针(SEQ ID NO:79)

CCAGGCTCGCAGACAGGCTTTGAAC

序列表

<110> 昂科梅德制药有限公司

<120> 与Wnt途径抑制剂有关的预测性生物标记物的鉴别

<130> 2293.116PC02

<140> To Be Assigned
<141> Herewith

<150> 61/975,339
<151> 2014-04-04

<150> 61/910,663
<151> 2013-12-02

<160> 79

<170> PatentIn version 3.5

<210> 1
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 18R5 重链 CDR1

<400> 1

Gly Phe Thr Phe Ser His Tyr Thr Leu Ser
1 5 10

[0001]

<210> 2
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> 重链 CDR2

<400> 2

Val Ile Ser Gly Asp Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 3
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 重链 CDR3

<400> 3

Asn Phe Ile Lys Tyr Val Phe Ala Asn
1 5

<210> 4
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> 轻链 CDR1

<400> 4

Ser Gly Asp Asn Ile Gly Ser Phe Tyr Val His
1 5 10

<210> 5
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 轻链 CDR2

<400> 5

Asp Lys Ser Asn Arg Pro Ser Gly
1 5

<210> 6
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 轻链 CDR3

<400> 6

Gln Ser Tyr Ala Asn Thr Leu Ser Leu
1 5

<210> 7
<211> 118
<212> PRT
<213> 人工序列

[0002] <220>
<223> 重链可变区氨基酸序列

<400> 7

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
20 25 30

Thr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Val Ile Ser Gly Asp Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Phe Ile Lys Tyr Val Phe Ala Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 8

<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 轻链可变区氨基酸序列

<400> 8

Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Ser Cys Ser Gly Asp Asn Ile Gly Ser Phe Tyr Val
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Asp Lys Ser Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Ala Asn Thr Leu Ser Leu
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105

[0003]

<210> 9
<211> 463
<212> PRT
<213> 人工序列

<220>
<223> 重链氨基酸序列

<220>
<221> MISC_FEATURE
<222> (1)..(19)
<223> 预测信号序列

<400> 9

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser His Tyr Thr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Ser Tyr Thr Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn
85 90 95

```
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
                100                 105                 110

Tyr Tyr Cys Ala Arg Asn Phe Ile Lys Tyr Val Phe Ala Asn Trp Gly
            115                 120                 125

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        130                 135                 140

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
225                 230                 235                 240
```

[0004]

```
Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400
```

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
405 410 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
420 425 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
435 440 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 10
<211> 232
<212> PRT
<213> 人工序列

<220>
<223> 轻链氨基酸序列

<220>
<221> MISC_FEATURE
<222> (1)..(19)
<223> 预测信号序列

<400> 10

Met Ala Trp Ala Leu Leu Leu Leu Thr Leu Leu Thr Gln Gly Thr Gly
1 5 10 15

[0005]

Ser Trp Ala Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala
20 25 30

Pro Gly Gln Thr Ala Arg Ile Ser Cys Ser Gly Asp Asn Ile Gly Ser
35 40 45

Phe Tyr Val His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu
50 55 60

Val Ile Tyr Asp Lys Ser Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe
65 70 75 80

Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr
85 90 95

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Ala Asn Thr
100 105 110

Leu Ser Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
115 120 125

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
130 135 140

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
145 150 155 160

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
165 170 175

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
180 185 190

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
195 200 205

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
210 215 220

Thr Val Ala Pro Thr Glu Cys Ser
225 230

<210> 11
<211> 444
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的重链氨基酸序列

<400> 11

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
20 25 30

Thr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

[0006]

Ser Val Ile Ser Gly Asp Gly Ser Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Phe Ile Lys Tyr Val Phe Ala Asn Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser

195 200 205

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
210 215 220

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
290 295 300

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
340 345 350

[0007]

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
385 390 395 400

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
405 410 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
420 425 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440

<210> 12
<211> 213
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的轻链氨基酸序列

<400> 12

Asp Ile Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Ser Cys Ser Gly Asp Asn Ile Gly Ser Phe Tyr Val
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Asp Lys Ser Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Ala Asn Thr Leu Ser Leu
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys Ala
100 105 110

Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala
115 120 125

Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly Ala
130 135 140

Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly Val
145 150 155 160

[0008] Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser
165 170 175

Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser Tyr
180 185 190

Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala
195 200 205

Pro Thr Glu Cys Ser
210

<210> 13
<211> 151
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的Fri 结构域氨基酸序列

<400> 13

Gln Gln Pro Pro Pro Pro Pro Gln Gln Gln Gln Ser Gly Gln Gln Tyr
1 5 10 15

Asn Gly Glu Arg Gly Ile Ser Val Pro Asp His Gly Tyr Cys Gln Pro
20 25 30

Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln Thr Ile Met
35 40 45

Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly Leu Glu Val

50 55 60

His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Ala Glu Leu Lys
65 70 75 80

Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val Leu Glu Gln
85 90 95

Ala Leu Pro Pro Cys Arg Ser Leu Cys Glu Arg Ala Arg Gln Gly Cys
100 105 110

Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Asp Thr Leu Lys
115 120 125

Cys Glu Lys Phe Pro Val His Gly Ala Gly Glu Leu Cys Val Gly Gln
130 135 140

Asn Thr Ser Asp Lys Gly Thr
145 150

<210> 14
<211> 136
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的Fri 结构域氨基酸序列

<400> 14

[0009]

Gln Phe His Gly Glu Lys Gly Ile Ser Ile Pro Asp His Gly Phe Cys
1 5 10 15

Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln Thr
20 25 30

Ile Met Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly Leu
35 40 45

Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Pro Glu
50 55 60

Leu Arg Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val Leu
65 70 75 80

Glu Gln Ala Ile Pro Pro Cys Arg Ser Ile Cys Glu Arg Ala Arg Gln
85 90 95

Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Glu Arg
100 105 110

Leu Arg Cys Glu His Phe Pro Arg His Gly Ala Glu Gln Ile Cys Val
115 120 125

Gly Gln Asn His Ser Glu Asp Gly
130 135

<210> 15
<211> 121
<212> PRT

<213> 人工序列

<220>
<223> 不含预测信号序列的Fri 结构域氨基酸序列

<400> 15

His Ser Leu Phe Ser Cys Glu Pro Ile Thr Leu Arg Met Cys Gln Asp
1 5 10 15

Leu Pro Tyr Asn Thr Thr Phe Met Pro Asn Leu Leu Asn His Tyr Asp
20 25 30

Gln Gln Thr Ala Ala Leu Ala Met Glu Pro Phe His Pro Met Val Asn
35 40 45

Leu Asp Cys Ser Arg Asp Phe Arg Pro Phe Leu Cys Ala Leu Tyr Ala
50 55 60

Pro Ile Cys Met Glu Tyr Gly Arg Val Thr Leu Pro Cys Arg Arg Leu
65 70 75 80

Cys Gln Arg Ala Tyr Ser Glu Cys Ser Lys Leu Met Glu Met Phe Gly
85 90 95

Val Pro Trp Pro Glu Asp Met Glu Cys Ser Arg Phe Pro Asp Cys Asp
100 105 110

Glu Pro Tyr Pro Arg Leu Val Asp Leu
115 120

[0010]

<210> 16
<211> 131
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的Fri 结构域氨基酸序列

<400> 16

Phe Gly Asp Glu Glu Glu Arg Arg Cys Asp Pro Ile Arg Ile Ser Met
1 5 10 15

Cys Gln Asn Leu Gly Tyr Asn Val Thr Lys Met Pro Asn Leu Val Gly
20 25 30

His Glu Leu Gln Thr Asp Ala Glu Leu Gln Leu Thr Thr Phe Thr Pro
35 40 45

Leu Ile Gln Tyr Gly Cys Ser Ser Gln Leu Gln Phe Phe Leu Cys Ser
50 55 60

Val Tyr Val Pro Met Cys Thr Glu Lys Ile Asn Ile Pro Ile Gly Pro
65 70 75 80

Cys Gly Gly Met Cys Leu Ser Val Lys Arg Arg Cys Glu Pro Val Leu
85 90 95

Lys Glu Phe Gly Phe Ala Trp Pro Glu Ser Leu Asn Cys Ser Lys Phe
100 105 110

Pro Pro Gln Asn Asp His Asn His Met Cys Met Glu Gly Pro Gly Asp
115 120 125

Glu Glu Val
130

<210> 17
<211> 131
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的人FZD5 Fri结构域氨基酸序列

<400> 17

Ala Ser Lys Ala Pro Val Cys Gln Glu Ile Thr Val Pro Met Cys Arg
1 5 10 15

Gly Ile Gly Tyr Asn Leu Thr His Met Pro Asn Gln Phe Asn His Asp
20 25 30

Thr Gln Asp Glu Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val
35 40 45

Glu Ile Gln Cys Ser Pro Asp Leu Arg Phe Phe Leu Cys Ser Met Tyr
50 55 60

Thr Pro Ile Cys Leu Pro Asp Tyr His Lys Pro Leu Pro Pro Cys Arg
65 70 75 80

[0011]

Ser Val Cys Glu Arg Ala Lys Ala Gly Cys Ser Pro Leu Met Arg Gln
85 90 95

Tyr Gly Phe Ala Trp Pro Glu Arg Met Ser Cys Asp Arg Leu Pro Val
100 105 110

Leu Gly Arg Asp Ala Glu Val Leu Cys Met Asp Tyr Asn Arg Ser Glu
115 120 125

Ala Thr Thr
130

<210> 18
<211> 127
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的人FZD6 Fri 结构域氨基酸序列

<400> 18

His Ser Leu Phe Thr Cys Glu Pro Ile Thr Val Pro Arg Cys Met Lys
1 5 10 15

Met Ala Tyr Asn Met Thr Phe Phe Pro Asn Leu Met Gly His Tyr Asp
20 25 30

Gln Ser Ile Ala Ala Val Glu Met Glu His Phe Leu Pro Leu Ala Asn
35 40 45

Leu Glu Cys Ser Pro Asn Ile Glu Thr Phe Leu Cys Lys Ala Phe Val
50 55 60

Pro Thr Cys Ile Glu Gln Ile His Val Val Pro Pro Cys Arg Lys Leu
65 70 75 80

Cys Glu Lys Val Tyr Ser Asp Cys Lys Lys Leu Ile Asp Thr Phe Gly
85 90 95

Ile Arg Trp Pro Glu Glu Leu Glu Cys Asp Arg Leu Gln Tyr Cys Asp
100 105 110

Glu Thr Val Pro Val Thr Phe Asp Pro His Thr Glu Phe Leu Gly
115 120 125

<210> 19
<211> 138
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的人FZD7 Fri 结构域氨基酸序列

<400> 19

Gln Pro Tyr His Gly Glu Lys Gly Ile Ser Val Pro Asp His Gly Phe
1 5 10 15

Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
20 25 30

[0012]

Thr Ile Leu Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly
35 40 45

Leu Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Pro
50 55 60

Glu Leu Arg Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val
65 70 75 80

Leu Asp Gln Ala Ile Pro Pro Cys Arg Ser Leu Cys Glu Arg Ala Arg
85 90 95

Gln Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Glu
100 105 110

Arg Leu Arg Cys Glu Asn Phe Pro Val His Gly Ala Gly Glu Ile Cys
115 120 125

Val Gly Gln Asn Thr Ser Asp Gly Ser Gly
130 135

<210> 20
<211> 131
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的人FZD8 Fri 结构域氨基酸序列

<400> 20

Ala Ser Ala Lys Glu Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys
1 5 10 15

Lys Gly Ile Gly Tyr Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His
20 25 30

Asp Thr Gln Asp Glu Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu
35 40 45

Val Glu Ile Gln Cys Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met
50 55 60

Tyr Thr Pro Ile Cys Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys
65 70 75 80

Arg Ser Val Cys Glu Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg
85 90 95

Gln Tyr Gly Phe Ala Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro
100 105 110

Glu Gln Gly Asn Pro Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp
115 120 125

Leu Thr Thr
130

[0013]

<210> 21
<211> 137
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的人FZD9 Fri 结构域氨基酸序列

<400> 21

Leu Glu Ile Gly Arg Phe Asp Pro Glu Arg Gly Arg Gly Ala Ala Pro
1 5 10 15

Cys Gln Ala Val Glu Ile Pro Met Cys Arg Gly Ile Gly Tyr Asn Leu
20 25 30

Thr Arg Met Pro Asn Leu Leu Gly His Thr Ser Gln Gly Glu Ala Ala
35 40 45

Ala Glu Leu Ala Glu Phe Ala Pro Leu Val Gln Tyr Gly Cys His Ser
50 55 60

His Leu Arg Phe Phe Leu Cys Ser Leu Tyr Ala Pro Met Cys Thr Asp
65 70 75 80

Gln Val Ser Thr Pro Ile Pro Ala Cys Arg Pro Met Cys Glu Gln Ala
85 90 95

Arg Leu Arg Cys Ala Pro Ile Met Glu Gln Phe Asn Phe Gly Trp Pro
100 105 110

Asp Ser Leu Asp Cys Ala Arg Leu Pro Thr Arg Asn Asp Pro His Ala

115 120 125

Leu Cys Met Glu Ala Pro Glu Asn Ala
130 135

<210> 22
<211> 134
<212> PRT
<213> 人工序列

<220>
<223> 不含预测信号序列的人 FZD10 Fri 结构域氨基酸序列

<400> 22

Ile Ser Ser Met Asp Met Glu Arg Pro Gly Asp Gly Lys Cys Gln Pro
1 5 10 15

Ile Glu Ile Pro Met Cys Lys Asp Ile Gly Tyr Asn Met Thr Arg Met
20 25 30

Pro Asn Leu Met Gly His Glu Asn Gln Arg Glu Ala Ala Ile Gln Leu
35 40 45

His Glu Phe Ala Pro Leu Val Glu Tyr Gly Cys His Gly His Leu Arg
50 55 60

Phe Phe Leu Cys Ser Leu Tyr Ala Pro Met Cys Thr Glu Gln Val Ser
65 70 75 80

[0014] Thr Pro Ile Pro Ala Cys Arg Val Met Cys Glu Gln Ala Arg Leu Lys
85 90 95

Cys Ser Pro Ile Met Glu Gln Phe Asn Phe Lys Trp Pro Asp Ser Leu
100 105 110

Asp Cys Arg Lys Leu Pro Asn Lys Asn Asp Pro Asn Tyr Leu Cys Met
115 120 125

Glu Ala Pro Asn Asn Gly
130

<210> 23
<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 人FZD1 氨基酸116-227

<400> 23

Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
1 5 10 15

Thr Ile Met Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly
20 25 30

Leu Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Ala
35 40 45

Glu Leu Lys Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val

50 55 60

Leu Glu Gln Ala Leu Pro Pro Cys Arg Ser Leu Cys Glu Arg Ala Arg
65 70 75 80

Gln Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Asp
85 90 95

Thr Leu Lys Cys Glu Lys Phe Pro Val His Gly Ala Gly Glu Leu Cys
100 105 110

<210> 24
<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD2 氨基酸39-150

<400> 24

Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
1 5 10 15

Thr Ile Met Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly
20 25 30

Leu Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Pro
35 40 45

[0015] Glu Leu Arg Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val
50 55 60

Leu Glu Gln Ala Ile Pro Pro Cys Arg Ser Ile Cys Glu Arg Ala Arg
65 70 75 80

Gln Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Glu
85 90 95

Arg Leu Arg Cys Glu His Phe Pro Arg His Gly Ala Glu Gln Ile Cys
100 105 110

<210> 25
<211> 106
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD3 氨基酸28-133

<400> 25

Cys Glu Pro Ile Thr Leu Arg Met Cys Gln Asp Leu Pro Tyr Asn Thr
1 5 10 15

Thr Phe Met Pro Asn Leu Leu Asn His Tyr Asp Gln Gln Thr Ala Ala
20 25 30

Leu Ala Met Glu Pro Phe His Pro Met Val Asn Leu Asp Cys Ser Arg
35 40 45

Asp Phe Arg Pro Phe Leu Cys Ala Leu Tyr Ala Pro Ile Cys Met Glu

50 55 60

Tyr Gly Arg Val Thr Leu Pro Cys Arg Arg Leu Cys Gln Arg Ala Tyr
65 70 75 80

Ser Glu Cys Ser Lys Leu Met Glu Met Phe Gly Val Pro Trp Pro Glu
85 90 95

Asp Met Glu Cys Ser Arg Phe Pro Asp Cys
100 105

<210> 26
<211> 114
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD4 氨基酸48-161

<400> 26

Cys Asp Pro Ile Arg Ile Ser Met Cys Gln Asn Leu Gly Tyr Asn Val
1 5 10 15

Thr Lys Met Pro Asn Leu Val Gly His Glu Leu Gln Thr Asp Ala Glu
20 25 30

Leu Gln Leu Thr Thr Phe Thr Pro Leu Ile Gln Tyr Gly Cys Ser Ser
35 40 45

[0016] Gln Leu Gln Phe Phe Leu Cys Ser Val Tyr Val Pro Met Cys Thr Glu
50 55 60

Lys Ile Asn Ile Pro Ile Gly Pro Cys Gly Gly Met Cys Leu Ser Val
65 70 75 80

Lys Arg Arg Cys Glu Pro Val Leu Lys Glu Phe Gly Phe Ala Trp Pro
85 90 95

Glu Ser Leu Asn Cys Ser Lys Phe Pro Pro Gln Asn Asp His Asn His
100 105 110

Met Cys

<210> 27
<211> 115
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD5 氨基酸33-147

<400> 27

Cys Gln Glu Ile Thr Val Pro Met Cys Arg Gly Ile Gly Tyr Asn Leu
1 5 10 15

Thr His Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu Ala Gly
20 25 30

Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys Ser Pro

35 40 45

Asp Leu Arg Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys Leu Pro
50 55 60

Asp Tyr His Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu Arg Ala
65 70 75 80

Lys Ala Gly Cys Ser Pro Leu Met Arg Gln Tyr Gly Phe Ala Trp Pro
85 90 95

Glu Arg Met Ser Cys Asp Arg Leu Pro Val Leu Gly Arg Asp Ala Glu
100 105 110

Val Leu Cys
115

<210> 28
<211> 106
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD6 氨基酸24-129

<400> 28

Cys Glu Pro Ile Thr Val Pro Arg Cys Met Lys Met Ala Tyr Asn Met
1 5 10 15

[0017] Thr Phe Phe Pro Asn Leu Met Gly His Tyr Asp Gln Ser Ile Ala Ala
20 25 30

Val Glu Met Glu His Phe Leu Pro Leu Ala Asn Leu Glu Cys Ser Pro
35 40 45

Asn Ile Glu Thr Phe Leu Cys Lys Ala Phe Val Pro Thr Cys Ile Glu
50 55 60

Gln Ile His Val Val Pro Pro Cys Arg Lys Leu Cys Glu Lys Val Tyr
65 70 75 80

Ser Asp Cys Lys Lys Leu Ile Asp Thr Phe Gly Ile Arg Trp Pro Glu
85 90 95

Glu Leu Glu Cys Asp Arg Leu Gln Tyr Cys
100 105

<210> 29
<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD7 氨基酸49-160

<400> 29

Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
1 5 10 15

Thr Ile Leu Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly

20 25 30

Leu Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Pro
35 40 45

Glu Leu Arg Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val
50 55 60

Leu Asp Gln Ala Ile Pro Pro Cys Arg Ser Leu Cys Glu Arg Ala Arg
65 70 75 80

Gln Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Glu
85 90 95

Arg Leu Arg Cys Glu Asn Phe Pro Val His Gly Ala Gly Glu Ile Cys
100 105 110

<210> 30
<211> 114
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD8 氨基酸35 148

<400> 30

Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr Asn Tyr
1 5 10 15

[0018]

Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu Ala Gly
20 25 30

Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys Ser Pro
35 40 45

Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys Leu Glu
50 55 60

Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu Arg Ala
65 70 75 80

Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala Trp Pro
85 90 95

Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro Asp Thr
100 105 110

Leu Cys

<210> 31
<211> 114
<212> PRT
<213> 人工序列

<220>
<223> 人 FZD9 氨基酸39-152

<400> 31

Cys Gln Ala Val Glu Ile Pro Met Cys Arg Gly Ile Gly Tyr Asn Leu

1 5 10 15

Thr Arg Met Pro Asn Leu Leu Gly His Thr Ser Gln Gly Glu Ala Ala
20 25 30

Ala Glu Leu Ala Glu Phe Ala Pro Leu Val Gln Tyr Gly Cys His Ser
35 40 45

His Leu Arg Phe Phe Leu Cys Ser Leu Tyr Ala Pro Met Cys Thr Asp
50 55 60

Gln Val Ser Thr Pro Ile Pro Ala Cys Arg Pro Met Cys Glu Gln Ala
65 70 75 80

Arg Leu Arg Cys Ala Pro Ile Met Glu Gln Phe Asn Phe Gly Trp Pro
85 90 95

Asp Ser Leu Asp Cys Ala Arg Leu Pro Thr Arg Asn Asp Pro His Ala
100 105 110

Leu Cys

<210> 32
<211> 114
<212> PRT
<213> 人工序列

[0019]

<220>
<223> 人 FZD10 氨基酸34-147

<400> 32

Cys Gln Pro Ile Glu Ile Pro Met Cys Lys Asp Ile Gly Tyr Asn Met
1 5 10 15

Thr Arg Met Pro Asn Leu Met Gly His Glu Asn Gln Arg Glu Ala Ala
20 25 30

Ile Gln Leu His Glu Phe Ala Pro Leu Val Glu Tyr Gly Cys His Gly
35 40 45

His Leu Arg Phe Phe Leu Cys Ser Leu Tyr Ala Pro Met Cys Thr Glu
50 55 60

Gln Val Ser Thr Pro Ile Pro Ala Cys Arg Val Met Cys Glu Gln Ala
65 70 75 80

Arg Leu Lys Cys Ser Pro Ile Met Glu Gln Phe Asn Phe Lys Trp Pro
85 90 95

Asp Ser Leu Asp Cys Arg Lys Leu Pro Asn Lys Asn Asp Pro Asn Tyr
100 105 110

Leu Cys

<210> 33
<211> 129
<212> PRT

<213> 人工序列

<220>
<223> 不含预测信号序列的人 FZD8 Fri 结构域氨基酸序列（变异体）

<400> 33

Ala Ser Ala Lys Glu Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys
1 5 10 15

Lys Gly Ile Gly Tyr Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His
20 25 30

Asp Thr Gln Asp Glu Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu
35 40 45

Val Glu Ile Gln Cys Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met
50 55 60

Tyr Thr Pro Ile Cys Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys
65 70 75 80

Arg Ser Val Cys Glu Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg
85 90 95

Gln Tyr Gly Phe Ala Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro
100 105 110

Glu Gln Gly Asn Pro Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp
115 120 125

[0020]

Leu

<210> 34
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 人 IgG1 Fc 区

<400> 34

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220

Pro Gly Lys
225

[0021]

<210> 35
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 人 IgG1 Fc 区（变异体）

<400> 35

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val

115 120 125

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
130 135 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220

Pro Gly Lys
225

<210> 36
<211> 230
<212> PRT
<213> 人工序列

<220>
[0022] <223> 人 IgG1 Fc 区

<400> 36

Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
1 5 10 15

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
20 25 30

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
35 40 45

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
50 55 60

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
65 70 75 80

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
85 90 95

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
100 105 110

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
115 120 125

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
130 135 140

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
145 150 155 160

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
165 170 175

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
180 185 190

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
195 200 205

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
210 215 220

Ser Leu Ser Pro Gly Lys
225 230

<210> 37
<211> 232
<212> PRT
<213> 人工序列

<220>
<223> 人 IgG1 Fc 区

<400> 37

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

[0023]

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr

165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 38
<211> 224
<212> PRT
<213> 人工序列

<220>
<223> 人 IgG2 Fc 区

<400> 38

Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
1 5 10 15

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
20 25 30

[0024] Thr Pro Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp Pro
35 40 45

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50 55 60

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val
65 70 75 80

Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
85 90 95

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr
100 105 110

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
115 120 125

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
130 135 140

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
145 150 155 160

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp
165 170 175

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
180 185 190

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
195 200 205

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215 220

<210> 39
<211> 361
<212> PRT
<213> 人工序列

<220>
<223> FZD8-Fc 变异体 54F03氨基酸序列（无预测信号序列）

<400> 39

Ala Ser Ala Lys Glu Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys
1 5 10 15

Lys Gly Ile Gly Tyr Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His
20 25 30

Asp Thr Gln Asp Glu Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu
35 40 45

Val Glu Ile Gln Cys Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met
50 55 60

Tyr Thr Pro Ile Cys Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys
65 70 75 80

[0025]

Arg Ser Val Cys Glu Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg
85 90 95

Gln Tyr Gly Phe Ala Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro
100 105 110

Glu Gln Gly Asn Pro Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp
115 120 125

Leu Thr Thr Gly Arg Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro
130 135 140

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
145 150 155 160

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
165 170 175

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
180 185 190

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
195 200 205

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
210 215 220

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys

225 230 235 240

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
245 250 255

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
260 265 270

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
275 280 285

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
290 295 300

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
305 310 315 320

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
325 330 335

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
340 345 350

Lys Ser Leu Ser Leu Ser Pro Gly Lys
355 360

[0026]

<210> 40
<211> 361
<212> PRT
<213> 人工序列

<220>
<223> FZD8-Fc 变异体 54F16, 54F17, 54F18, 54F23, 54F25, 54F27, 54F29, 54F31, 和 54F34氨基酸序列 (无预测信号序列)

<400> 40

Ala Ser Ala Lys Glu Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys
1 5 10 15

Lys Gly Ile Gly Tyr Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His
20 25 30

Asp Thr Gln Asp Glu Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu
35 40 45

Val Glu Ile Gln Cys Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met
50 55 60

Tyr Thr Pro Ile Cys Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys
65 70 75 80

Arg Ser Val Cys Glu Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg
85 90 95

Gln Tyr Gly Phe Ala Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro
100 105 110

Glu Gln Gly Asn Pro Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp
115 120 125

Leu Thr Thr Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
130 135 140

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
145 150 155 160

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
165 170 175

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
180 185 190

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
195 200 205

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
210 215 220

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
225 230 235 240

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
245 250 255

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
260 265 270

[0027] Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
275 280 285

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
290 295 300

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
305 310 315 320

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
325 330 335

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
340 345 350

Lys Ser Leu Ser Leu Ser Pro Gly Lys
355 360

<210> 41
<211> 363
<212> PRT
<213> 人工序列

<220>
<223> FZD8-Fc 变异体 54F19, 54F20, 54F24, 54F26, 54F28, 54F30, 54F32, 54F34 和 54F35氨基酸序列（无预测信号序列）

<400> 41

Ala Ser Ala Lys Glu Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys
1 5 10 15

```
Lys Gly Ile Gly Tyr Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His
            20                  25                  30

Asp Thr Gln Asp Glu Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu
        35                  40                  45

Val Glu Ile Gln Cys Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met
    50                  55                  60

Tyr Thr Pro Ile Cys Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys
65                  70                  75                  80

Arg Ser Val Cys Glu Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg
                85                  90                  95

Gln Tyr Gly Phe Ala Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro
            100                 105                 110

Glu Gln Gly Asn Pro Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp
        115                 120                 125

Leu Thr Thr Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
    130                 135                 140

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
145                 150                 155                 160
```

[0028]

```
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                165                 170                 175

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            180                 185                 190

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        195                 200                 205

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    210                 215                 220

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
225                 230                 235                 240

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                245                 250                 255

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
            260                 265                 270

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        275                 280                 285

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    290                 295                 300

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
305                 310                 315                 320
```

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
325 330 335

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
340 345 350

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
355 360

<210> 42
<211> 388
<212> PRT
<213> 人工序列

<220>
<223> 含信号序列的FZD8-Fc 变异体 54F03氨基酸序列

<400> 42

Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
1 5 10 15

Ala Leu Leu Gln Arg Ser Ser Gly Ala Ala Ala Ala Ser Ala Lys Glu
20 25 30

Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr
35 40 45

Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu
50 55 60

[0029]

Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys
65 70 75 80

Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys
85 90 95

Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu
100 105 110

Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala
115 120 125

Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro
130 135 140

Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp Leu Thr Thr Gly Arg
145 150 155 160

Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
165 170 175

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
180 185 190

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
195 200 205

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu

210 215 220

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
225 230 235 240

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
245 250 255

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
260 265 270

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
275 280 285

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
290 295 300

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
305 310 315 320

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
325 330 335

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
340 345 350

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
355 360 365

[0030]

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
370 375 380

Ser Pro Gly Lys
385

<210> 43
<211> 388
<212> PRT
<213> 人工序列

<220>
<223> 含信号序列的FZD8-Fc 变异体 54F16氨基酸序列

<400> 43

Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
1 5 10 15

Ala Leu Leu Gln Arg Ser Ser Gly Ala Ala Ala Ala Ser Ala Lys Glu
20 25 30

Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr
35 40 45

Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu
50 55 60

Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys
65 70 75 80

Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys
85 90 95

Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu
100 105 110

Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala
115 120 125

Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro
130 135 140

Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp Leu Thr Thr Lys Ser
145 150 155 160

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
165 170 175

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
180 185 190

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
195 200 205

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
210 215 220

[0031] Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
225 230 235 240

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
245 250 255

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
260 265 270

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
275 280 285

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
290 295 300

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
305 310 315 320

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
325 330 335

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
340 345 350

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
355 360 365

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
370 375 380

Ser Pro Gly Lys
385

<210> 44
<211> 390
<212> PRT
<213> 人工序列

<220>
<223> 含信号序列的FZD8-Fc 变异体 54F26

<400> 44

Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
1 5 10 15

Phe Leu Leu Gln Arg Ser Pro Ile Val His Ala Ala Ser Ala Lys Glu
20 25 30

Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr
35 40 45

Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu
50 55 60

Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys
65 70 75 80

Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys
85 90 95

[0032]

Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu
100 105 110

Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala
115 120 125

Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro
130 135 140

Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp Leu Thr Thr Glu Pro
145 150 155 160

Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
165 170 175

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
180 185 190

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
195 200 205

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
210 215 220

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
225 230 235 240

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp

245 250 255

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
260 265 270

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
275 280 285

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
290 295 300

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
305 310 315 320

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
325 330 335

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
340 345 350

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
355 360 365

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
370 375 380

Ser Leu Ser Pro Gly Lys
385 390

[0033]

<210> 45
<211> 390
<212> PRT
<213> 人工序列

<220>
<223> 含信号序列的FZD8-Fc 变异体 54F28

<400> 45

Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
1 5 10 15

Leu Leu Leu Gln Arg Ser Pro Phe Val His Ala Ala Ser Ala Lys Glu
20 25 30

Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr
35 40 45

Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu
50 55 60

Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys
65 70 75 80

Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys
85 90 95

Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu
100 105 110

Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala
115 120 125

Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro
130 135 140

Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp Leu Thr Thr Glu Pro
145 150 155 160

Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
165 170 175

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
180 185 190

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
195 200 205

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
210 215 220

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
225 230 235 240

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
245 250 255

[0034] Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
260 265 270

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
275 280 285

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
290 295 300

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
305 310 315 320

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
325 330 335

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
340 345 350

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
355 360 365

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
370 375 380

Ser Leu Ser Pro Gly Lys
385 390

<210> 46
<211> 83
<212> PRT

<213> 人工序列

<220>
<223> 人 Wnt1 C-端多半胱氨酸区 (aa 288-370)

<400> 46

Asp Leu Val Tyr Phe Glu Lys Ser Pro Asn Phe Cys Thr Tyr Ser Gly
1 5 10 15

Arg Leu Gly Thr Ala Gly Thr Ala Gly Arg Ala Cys Asn Ser Ser Ser
20 25 30

Pro Ala Leu Asp Gly Cys Glu Leu Leu Cys Cys Gly Arg Gly His Arg
35 40 45

Thr Arg Thr Gln Arg Val Thr Glu Arg Cys Asn Cys Thr Phe His Trp
50 55 60

Cys Cys His Val Ser Cys Arg Asn Cys Thr His Thr Arg Val Leu His
65 70 75 80

Glu Cys Leu

<210> 47
<211> 94
<212> PRT
<213> 人工序列

<220>
[0035] <223> 人 Wnt2 C-端多半胱氨酸区(aa 267-360)

<400> 47

Asp Leu Val Tyr Phe Glu Asn Ser Pro Asp Tyr Cys Ile Arg Asp Arg
1 5 10 15

Glu Ala Gly Ser Leu Gly Thr Ala Gly Arg Val Cys Asn Leu Thr Ser
20 25 30

Arg Gly Met Asp Ser Cys Glu Val Met Cys Cys Gly Arg Gly Tyr Asp
35 40 45

Thr Ser His Val Thr Arg Met Thr Lys Cys Gly Cys Lys Phe His Trp
50 55 60

Cys Cys Ala Val Arg Cys Gln Asp Cys Leu Glu Ala Leu Asp Val His
65 70 75 80

Thr Cys Lys Ala Pro Lys Asn Ala Asp Trp Thr Thr Ala Thr
85 90

<210> 48
<211> 94
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt2b C-端多半胱氨酸区 (aa 298-391)

<400> 48

Asp Leu Val Tyr Phe Asp Asn Ser Pro Asp Tyr Cys Val Leu Asp Lys

1 5 10 15

Ala Ala Gly Ser Leu Gly Thr Ala Gly Arg Val Cys Ser Lys Thr Ser
20 25 30

Lys Gly Thr Asp Gly Cys Glu Ile Met Cys Cys Gly Arg Gly Tyr Asp
35 40 45

Thr Thr Arg Val Thr Arg Val Thr Gln Cys Glu Cys Lys Phe His Trp
50 55 60

Cys Cys Ala Val Arg Cys Lys Glu Cys Arg Asn Thr Val Asp Val His
65 70 75 80

Thr Cys Lys Ala Pro Lys Lys Ala Glu Trp Leu Asp Gln Thr
85 90

<210> 49
<211> 83
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt3 C-端多半胱氨酸区 (aa 273-355)

<400> 49

Asp Leu Val Tyr Tyr Glu Asn Ser Pro Asn Phe Cys Glu Pro Asn Pro
1 5 10 15

[0036]

Glu Thr Gly Ser Phe Gly Thr Arg Asp Arg Thr Cys Asn Val Thr Ser
20 25 30

His Gly Ile Asp Gly Cys Asp Leu Leu Cys Cys Gly Arg Gly His Asn
35 40 45

Thr Arg Thr Glu Lys Arg Lys Glu Lys Cys His Cys Ile Phe His Trp
50 55 60

Cys Cys Tyr Val Ser Cys Gln Glu Cys Ile Arg Ile Tyr Asp Val His
65 70 75 80

Thr Cys Lys

<210> 50
<211> 83
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt3a C-端多半胱氨酸区 (aa 270-352)

<400> 50

Asp Leu Val Tyr Tyr Glu Ala Ser Pro Asn Phe Cys Glu Pro Asn Pro
1 5 10 15

Glu Thr Gly Ser Phe Gly Thr Arg Asp Arg Thr Cys Asn Val Ser Ser
20 25 30

His Gly Ile Asp Gly Cys Asp Leu Leu Cys Cys Gly Arg Gly His Asn

35 40 45

Ala Arg Ala Glu Arg Arg Arg Glu Lys Cys Arg Cys Val Phe His Trp
50 55 60

Cys Cys Tyr Val Ser Cys Gln Glu Cys Thr Arg Val Tyr Asp Val His
65 70 75 80

Thr Cys Lys

<210> 51
<211> 83
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt7a C-端多半胱氨酸区 (aa 267-359)

<400> 51

Asp Leu Val Tyr Ile Glu Lys Ser Pro Asn Tyr Cys Glu Glu Asp Pro
1 5 10 15

Val Thr Gly Ser Val Gly Thr Gln Gly Arg Ala Cys Asn Lys Thr Ala
20 25 30

Pro Gln Ala Ser Gly Cys Asp Leu Met Cys Cys Gly Arg Gly Tyr Asn
35 40 45

[0037] Thr His Gln Tyr Ala Arg Val Trp Gln Cys Asn Cys Lys Phe His Trp
50 55 60

Cys Cys Tyr Val Lys Cys Asn Thr Cys Ser Glu Arg Thr Glu Met Tyr
65 70 75 80

Thr Cys Lys

<210> 52
<211> 83
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt7b C-端多半胱氨酸区 (aa 267-349)

<400> 52

Asp Leu Val Tyr Ile Glu Lys Ser Pro Asn Tyr Cys Glu Glu Asp Ala
1 5 10 15

Ala Thr Gly Ser Val Gly Thr Gln Gly Arg Leu Cys Asn Arg Thr Ser
20 25 30

Pro Gly Ala Asp Gly Cys Asp Thr Met Cys Cys Gly Arg Gly Tyr Asn
35 40 45

Thr His Gln Tyr Thr Lys Val Trp Gln Cys Asn Cys Lys Phe His Trp
50 55 60

Cys Cys Phe Val Lys Cys Asn Thr Cys Ser Glu Arg Thr Glu Val Phe

65 70 75 80

Thr Cys Lys

<210> 53
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt8a C-端多半胱氨酸区 (aa 248-355)

<400> 53

Glu Leu Ile Phe Leu Glu Glu Ser Pro Asp Tyr Cys Thr Cys Asn Ser
1 5 10 15

Ser Leu Gly Ile Tyr Gly Thr Glu Gly Arg Glu Cys Leu Gln Asn Ser
20 25 30

His Asn Thr Ser Arg Trp Glu Arg Arg Ser Cys Gly Arg Leu Cys Thr
35 40 45

Glu Cys Gly Leu Gln Val Glu Glu Arg Lys Thr Glu Val Ile Ser Ser
50 55 60

Cys Asn Cys Lys Phe Gln Trp Cys Cys Thr Val Lys Cys Asp Gln Cys
65 70 75 80

[0038] Arg His Val Val Ser Lys Tyr Tyr Cys Ala Arg Ser Pro Gly Ser Ala
85 90 95

Gln Ser Leu Gly Arg Val Trp Phe Gly Val Tyr Ile
100 105

<210> 54
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt8b C-端多半胱氨酸区 (aa 245-351)

<400> 54

Glu Leu Val His Leu Glu Asp Ser Pro Asp Tyr Cys Leu Glu Asn Lys
1 5 10 15

Thr Leu Gly Leu Leu Gly Thr Glu Gly Arg Glu Cys Leu Arg Arg Gly
20 25 30

Arg Ala Leu Gly Arg Trp Glu Leu Arg Ser Cys Arg Arg Leu Cys Gly
35 40 45

Asp Cys Gly Leu Ala Val Glu Glu Arg Arg Ala Glu Thr Val Ser Ser
50 55 60

Cys Asn Cys Lys Phe His Trp Cys Cys Ala Val Arg Cys Glu Gln Cys
65 70 75 80

Arg Arg Arg Val Thr Lys Tyr Phe Cys Ser Arg Ala Glu Arg Pro Arg

85 90 95

Gly Gly Ala Ala His Lys Pro Gly Arg Lys Pro
100 105

<210> 55
<211> 83
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt10a C-端多半胱氨酸区 (aa 335-417)

<400> 55

Asp Leu Val Tyr Phe Glu Lys Ser Pro Asp Phe Cys Glu Arg Glu Pro
1 5 10 15

Arg Leu Asp Ser Ala Gly Thr Val Gly Arg Leu Cys Asn Lys Ser Ser
20 25 30

Ala Gly Ser Asp Gly Cys Gly Ser Met Cys Cys Gly Arg Gly His Asn
35 40 45

Ile Leu Arg Gln Thr Arg Ser Glu Arg Cys His Cys Arg Phe His Trp
50 55 60

Cys Cys Phe Val Val Cys Glu Glu Cys Arg Ile Thr Glu Trp Val Ser
65 70 75 80

[0039] Val Cys Lys

<210> 56
<211> 83
<212> PRT
<213> 人工序列

<220>
<223> 人 Wnt10b C-端多半胱氨酸区 (aa 307-389)

<400> 56

Glu Leu Val Tyr Phe Glu Lys Ser Pro Asp Phe Cys Glu Arg Asp Pro
1 5 10 15

Thr Met Gly Ser Pro Gly Thr Arg Gly Arg Ala Cys Asn Lys Thr Ser
20 25 30

Arg Leu Leu Asp Gly Cys Gly Ser Leu Cys Cys Gly Arg Gly His Asn
35 40 45

Val Leu Arg Gln Thr Arg Val Glu Arg Cys His Cys Arg Phe His Trp
50 55 60

Cys Cys Tyr Val Leu Cys Asp Glu Cys Lys Val Thr Glu Trp Val Asn
65 70 75 80

Val Cys Lys

<210> 57

<210> 8
<212> PRT
<213> 人工序列

<220>
<223> 接头

<400> 57

Glu Ser Gly Gly Gly Gly Val Thr
1 5

<210> 58
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 接头

<400> 58

Leu Glu Ser Gly Gly Gly Gly Val Thr
1 5

<210> 59
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 接头

<400> 59

[0040] Gly Arg Ala Gln Val Thr
1 5

<210> 60
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 接头

<400> 60

Trp Arg Ala Gln Val Thr
1 5

<210> 61
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 接头

<400> 61

Ala Arg Gly Arg Ala Gln Val Thr
1 5

<210> 62
<211> 20
<212> DNA
<213> 人工序列

<220>
<223> CCND2 正向引物

<400> 62
gctgtctctg atccgcaagc 20

<210> 63
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> CCND2 反向引物

<400> 63
gacggtgggt acatggcaaa c 21

<210> 64
<211> 26
<212> DNA
<213> 人工序列

<220>
<223> CCND2 探针

<400> 64
ccttcattgc tctgtgtgcc accgac 26

<210> 65
<211> 19
<212> DNA
<213> 人工序列

<220>
<223> CTBP2 反向引物

[0041] <400> 65
ctcgaactgc aaccgcctg 19

<210> 66
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> CTBP2 探针

<400> 66
cccgtgcgac caaagccaat gagg 24

<210> 67
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> DKK1 正向引物

<400> 67
gaccattgac aactaccagc cgta 24

<210> 68
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> DKK1 反向引物

<400> 68
tgggactagc gcagtactca tc 22

<210> 69
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> DKK1 探针

<400> 69
tgccgcactc ctcgtcctct g 21

<210> 70
<211> 25
<212> DNA
<213> 人工序列

<220>
<223> FBXW2 正向引物

<400> 70
gccagttatg atattctcag ggtca 25

<210> 71
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> FBXW2 反向引物

<400> 71
agcagggcaa agatatctcc aaa 23

[0042]

<210> 72
<211> 27
<212> DNA
<213> 人工序列

<220>
<223> FBXW2 探针

<400> 72
agactcctga gatagcaaac ttggcct 27

<210> 73
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> RHOU1 正向引物

<400> 73
cccaccgagt acatccctac tg 22

<210> 74
<211> 24
<212> DNA
<213> 人工序列

<220>
<223> RHOU1 反向引物

<400> 74
cagtgtcaca gagttggagt ctca 24

<210> 75
<211> 22
<212> DNA

<213> 人工序列

<220>
<223> RHOU1 探针

<400> 75
cgcccatcca cagacaccac cg 22

<210> 76
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> WIF1 正向引物

<400> 76
gttccaaagg ttaccaggga gac 23

<210> 77
<211> 21
<212> DNA
<213> 人工序列

<220>
[0043] <223> WIF1 反向引物

<400> 77
gttgggttca tggcaggttc c 21

<210> 78
<211> 25
<212> DNA
<213> 人工序列

<220>
<223> WIF1 探针

<400> 78
ccaggctcgc agacaggctt tgaac 25

<210> 79
<211> 18
<212> DNA
<213> 人工序列

<220>
<223> CTBP2 正向引物

<400> 79
atccgtgggg agacgctg 18

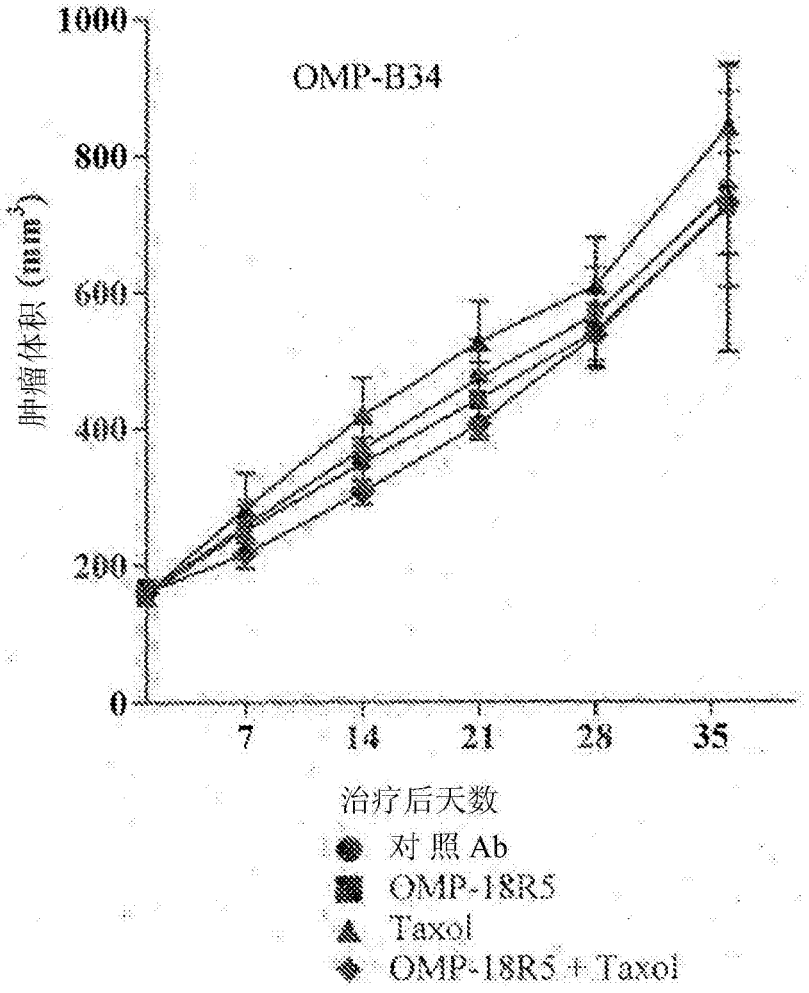

OMP-B34
肿瘤体积 (mm³)
1000
800
600
400
200
0
7
14
21
28
35
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1A

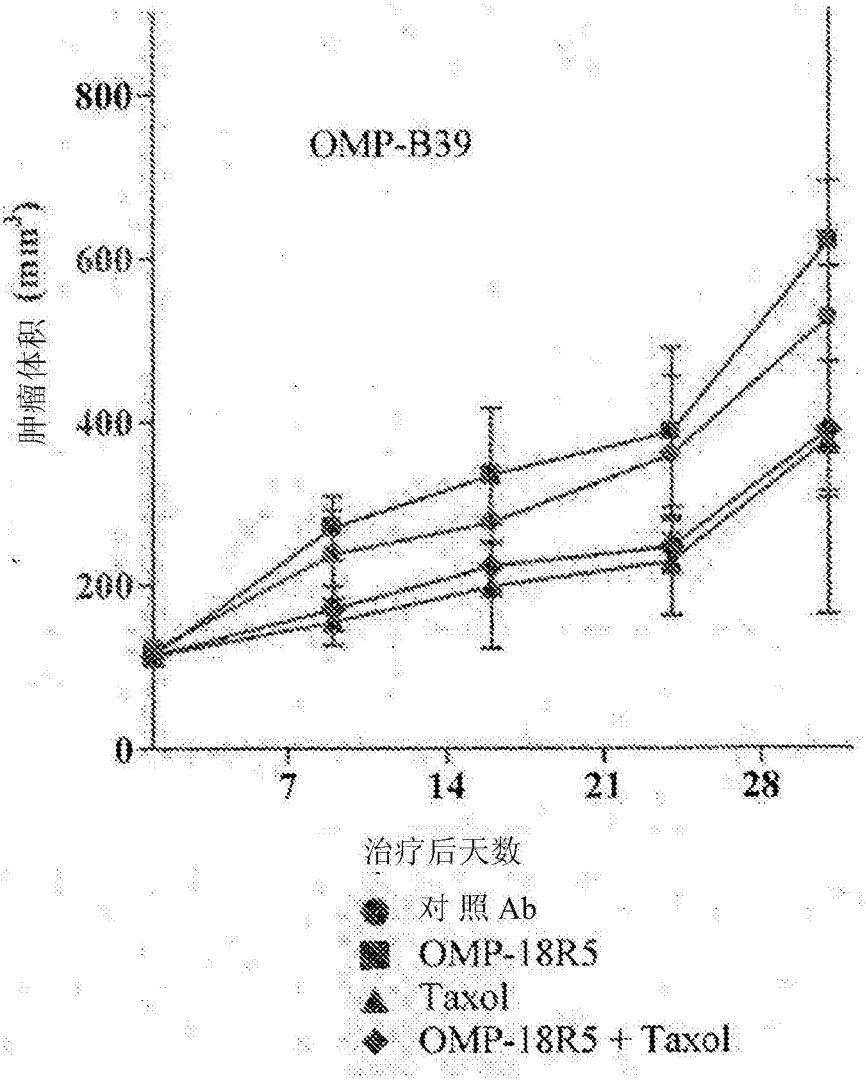

OMP-B39
肿瘤体积 (mm³)
800
600
400
200
0
7
14
21
28
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1B

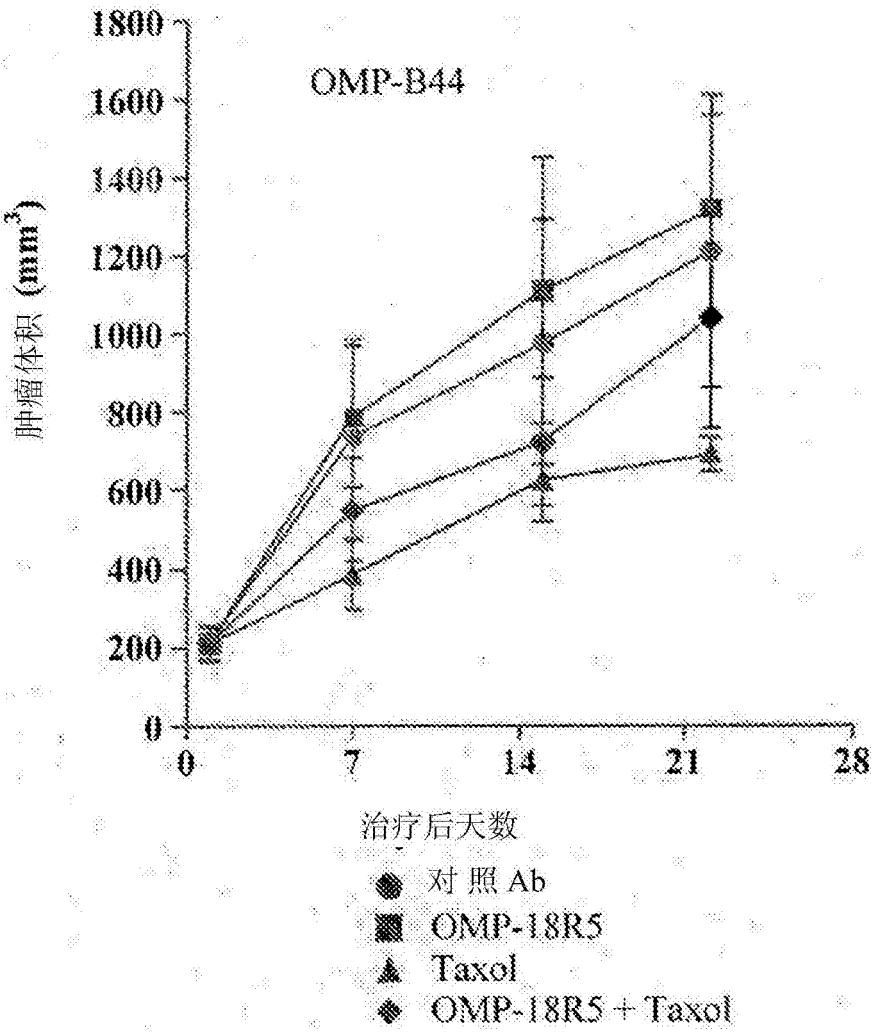

OMP-B44
肿瘤体积 (mm^3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1C

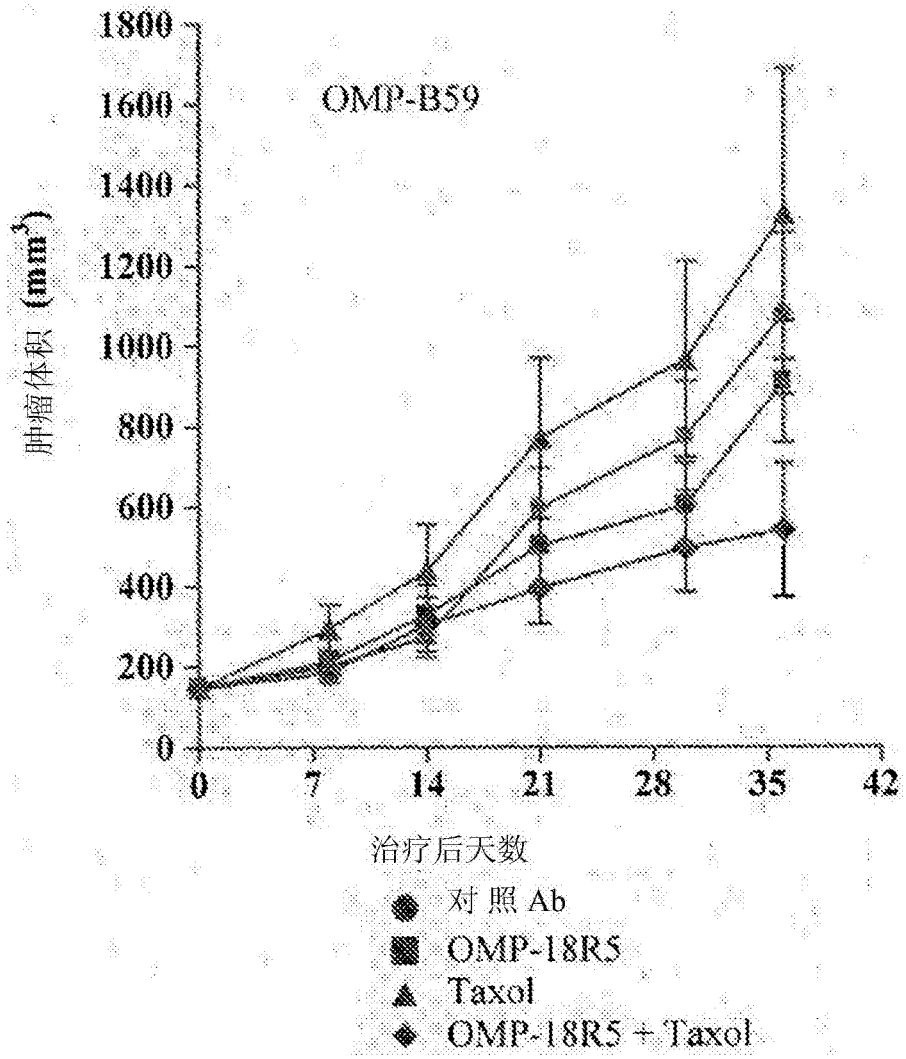

OMP-B59
肿瘤体积 (mm^3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
35
42
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1D

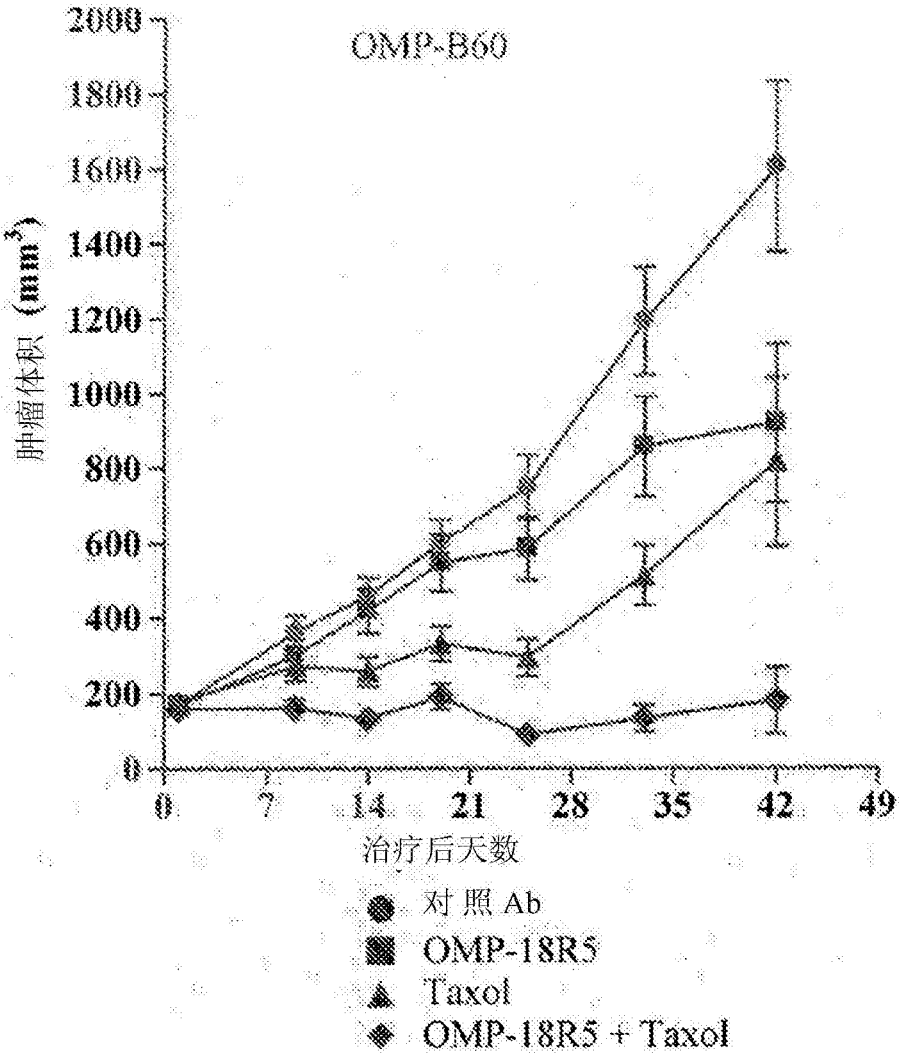

OMP-B60
肿瘤体积 (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
35
42
49
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1E

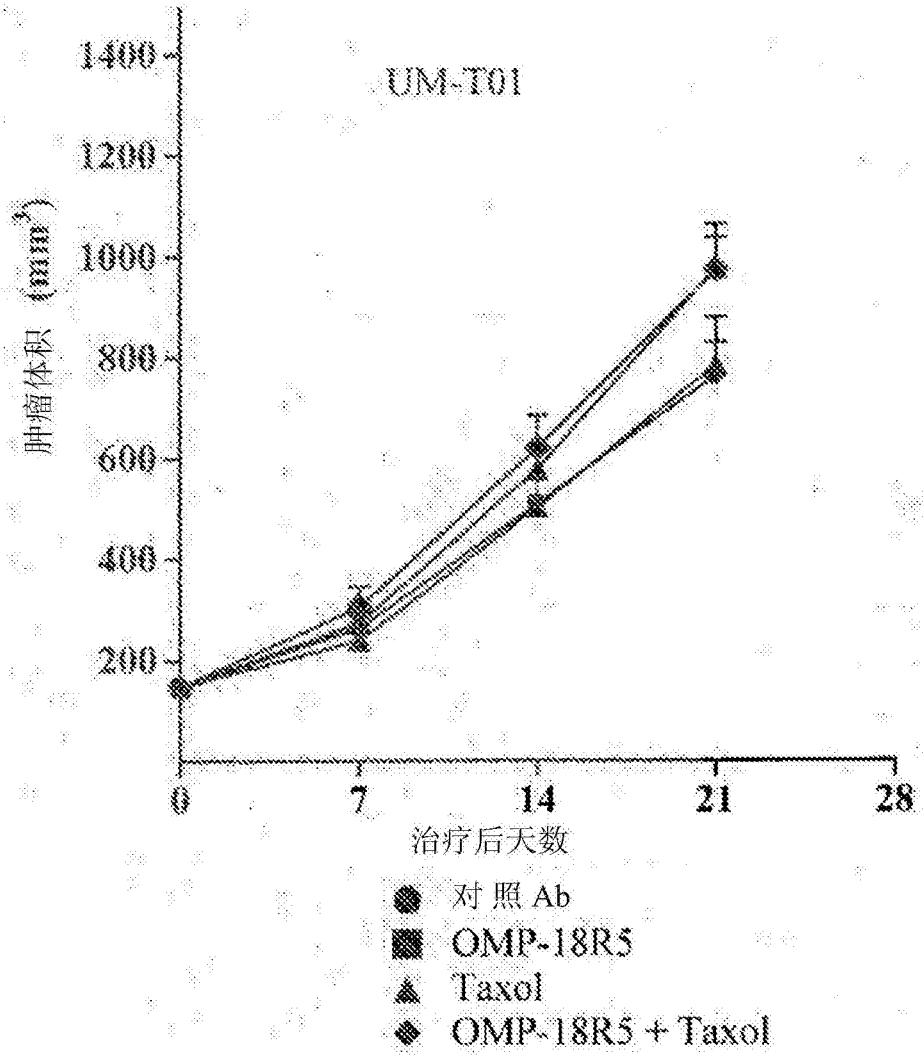

UM-T01
肿瘤体积 (mm^3)
1400
1200
1000
800
600
400
200
0
7
14
21
28
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1F

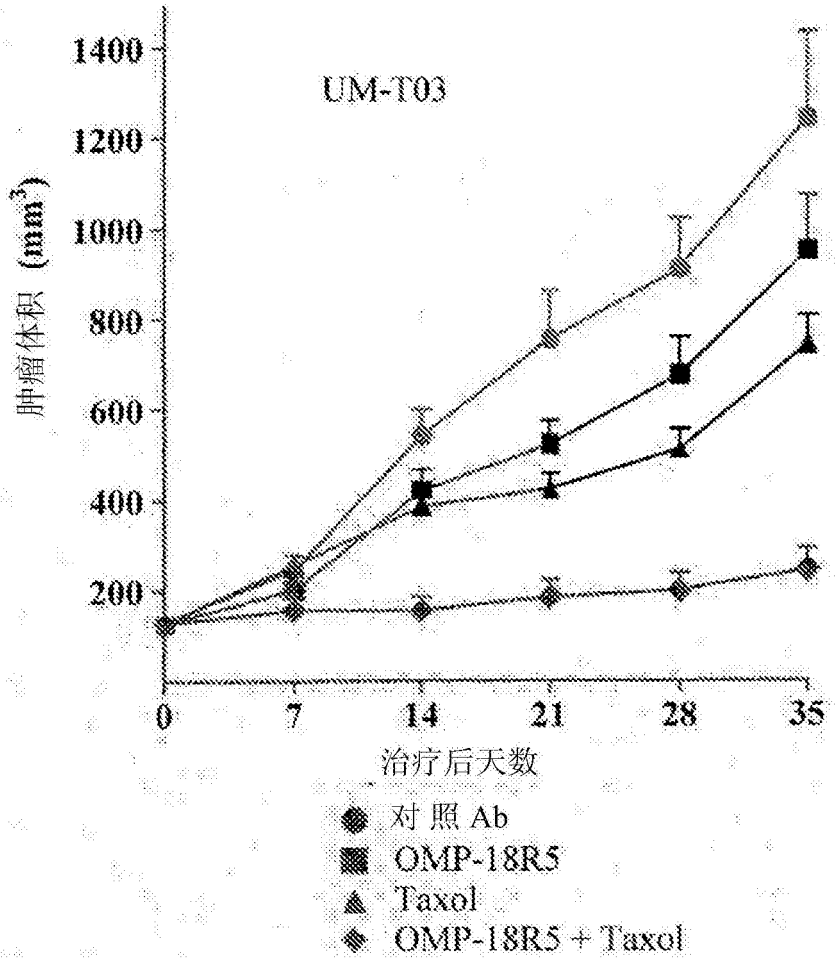

UM-T03
肿瘤体积 (mm3)
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1G

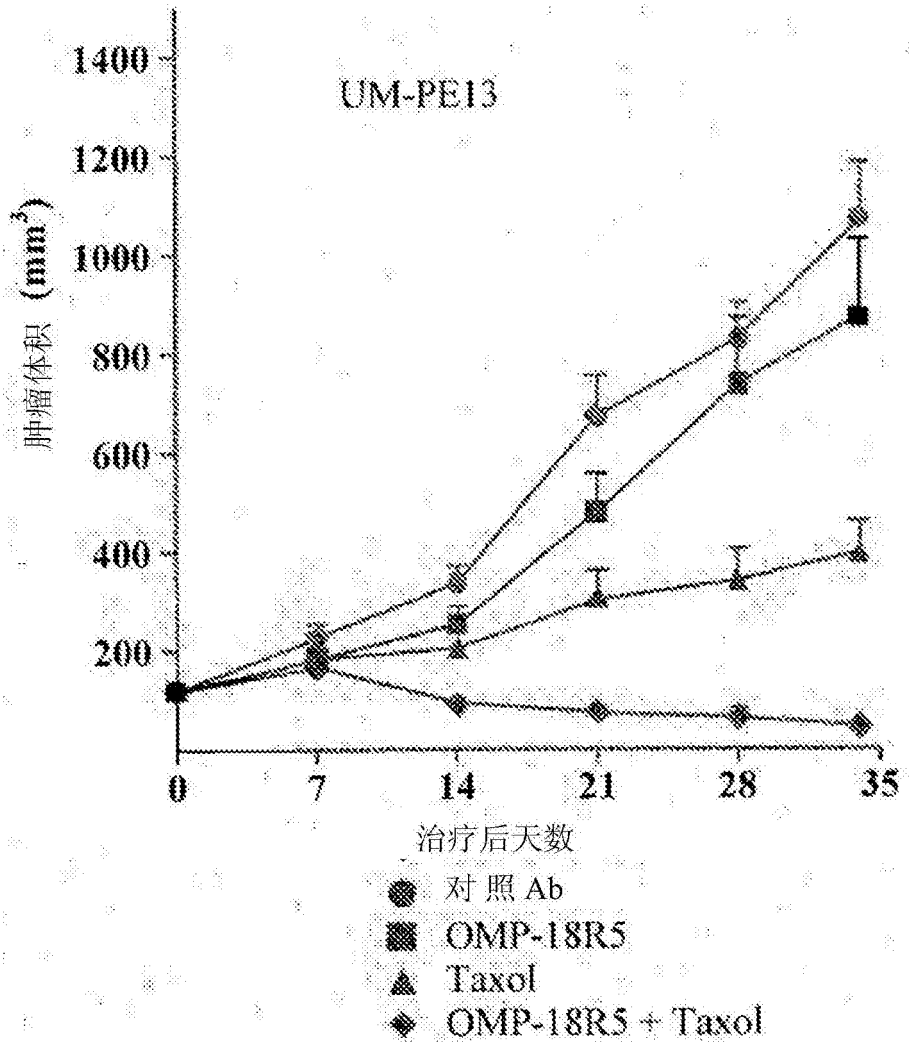

UM-PE13
肿瘤体积 (mm³)
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图1H

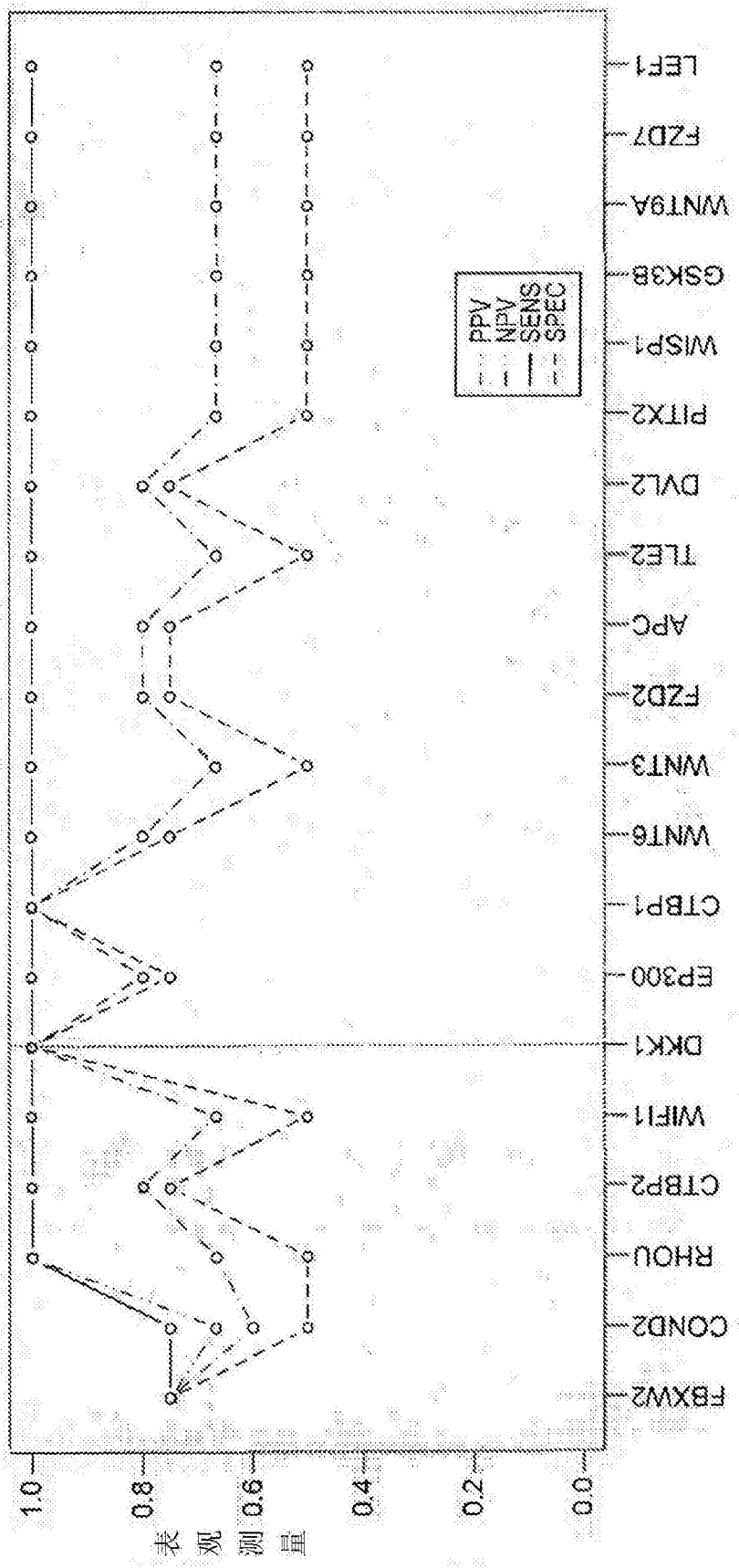

PPV
NPV
SENS
SPEC
LEF1
FZD7
WNT9A
GSK3B
WISP1
PITX2
DVL2
TLE2
APC
FZD2
WNT3
WNT6
CTBP1
EP300
DKK1
WIF11
CTBP2
RHOU
COND2
FBXW2
1.0
0.8
0.6
0.4
0.2
0.0
表 现 测 量

图2

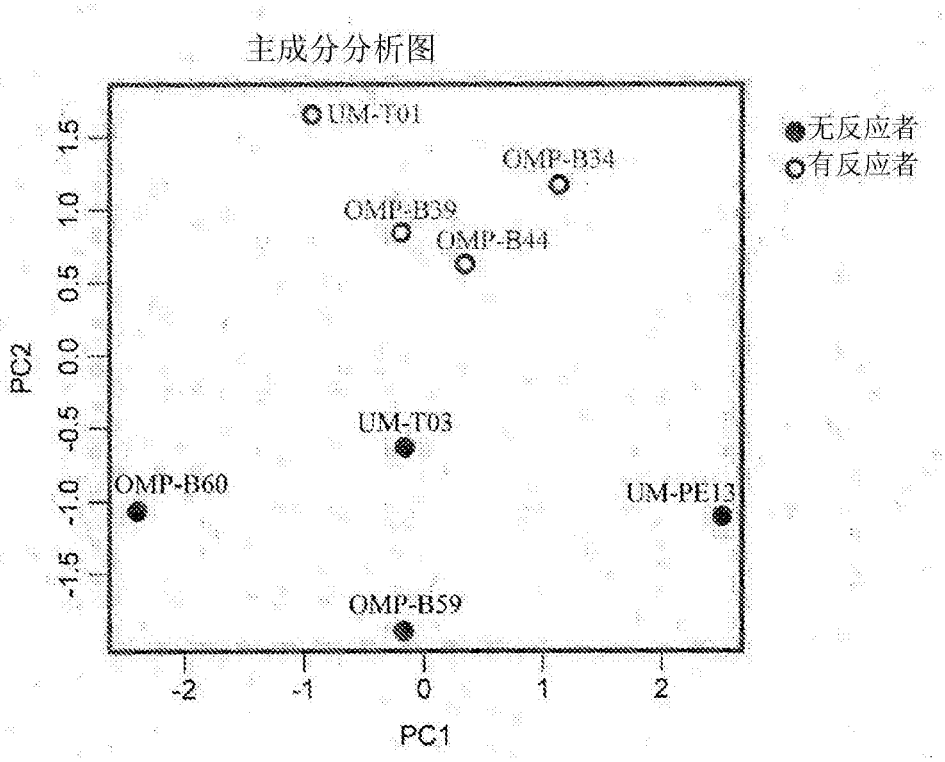

主成分分析图
UM-T01
OMP-B34
OMP-B39
OMP-B44
UM-T03
OMP-B60
UM-PE13
OMP-B59
无反应者
有反应者
PC2
PC1
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2
-1
0
1
2

图3

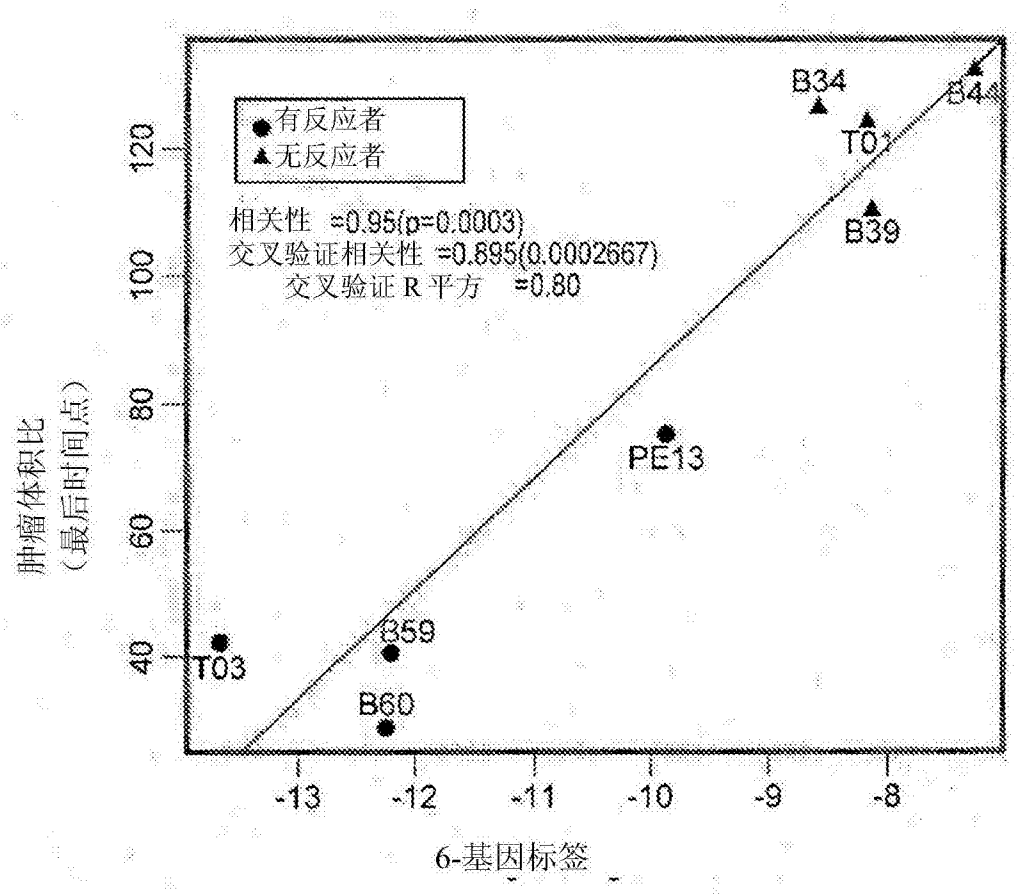

有反应者
无反应者
相关性 =0.95(p=0.0003)
交叉验证相关性 =0.895(0.0002667)
交叉验证 R 平方 =0.80
肿瘤体积比
（最后时间点）
120
100
80
60
40
B34
B4
T01
B39
PE13
B59
T03
B60
-13
-12
-11
-10
-9
-8
6-基因标签

图4

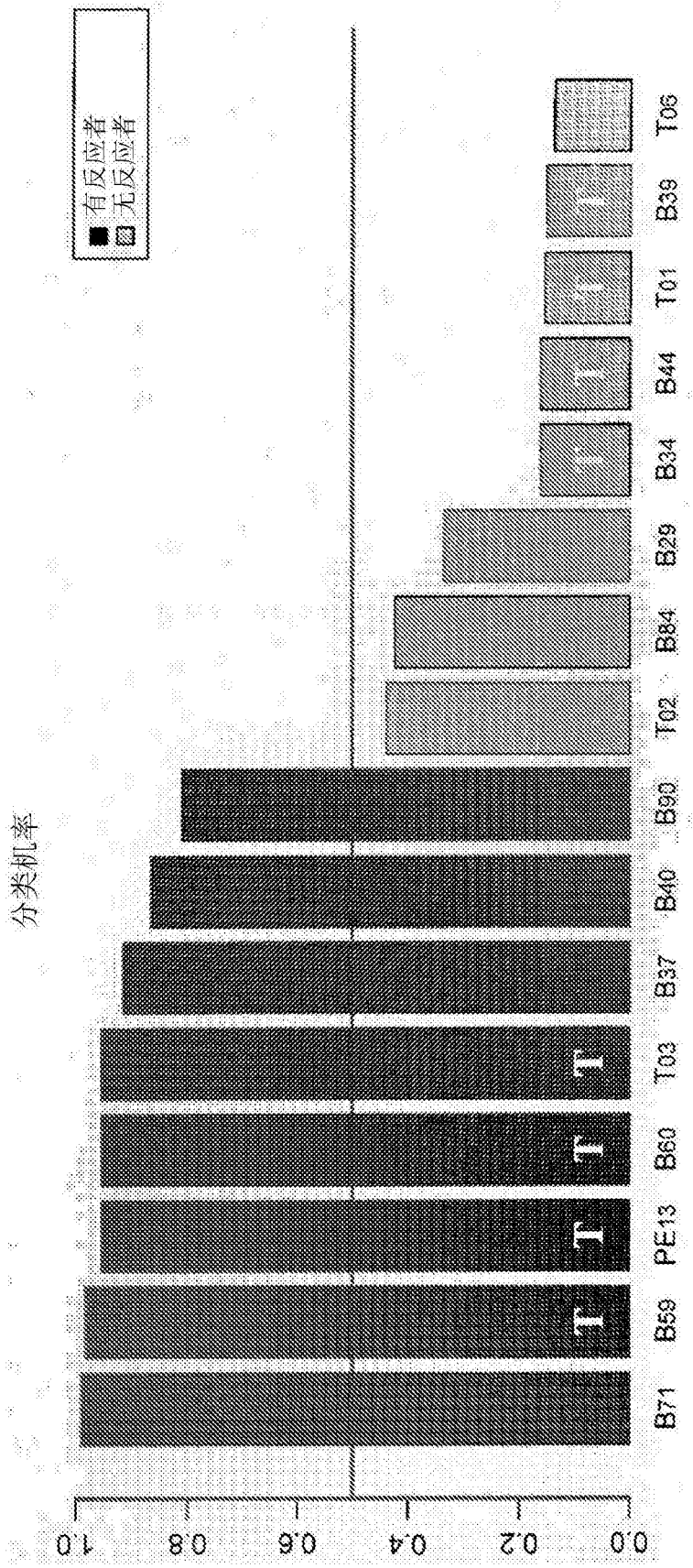

有反应者
无反应者
分类机率
0.0
0.2
0.4
0.6
0.8
1.0
B71
B59
PE13
B60
T03
B37
B40
B90
T02
B84
B29
B34
B44
T01
B39
T06

图5

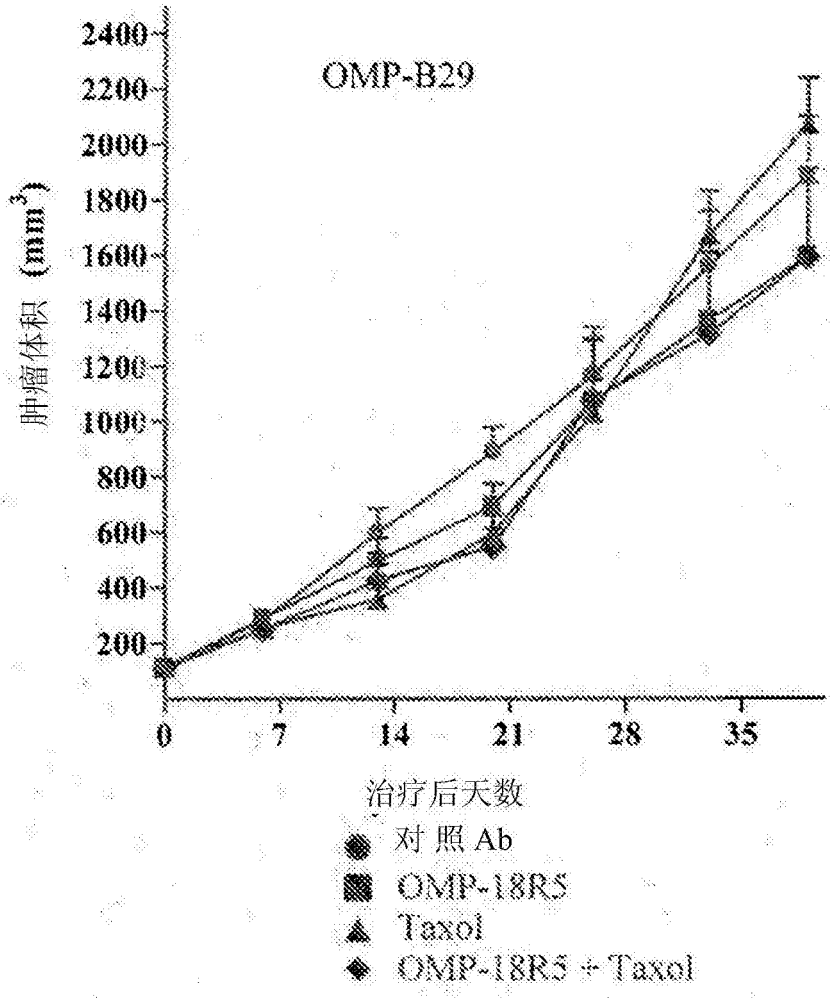

OMP-B29
肿瘤体积 (mm³)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图6A

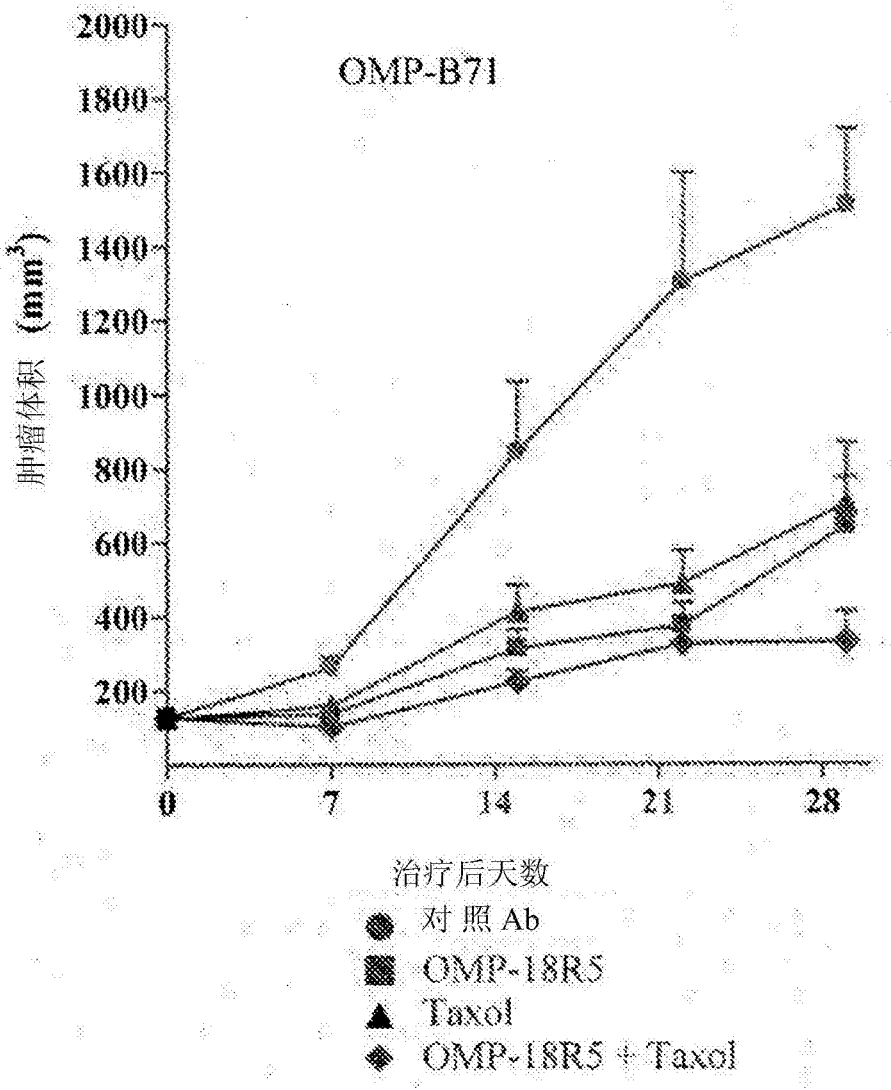

OMP-B71
肿瘤体积 (mm³)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7
14
21
28
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图6B

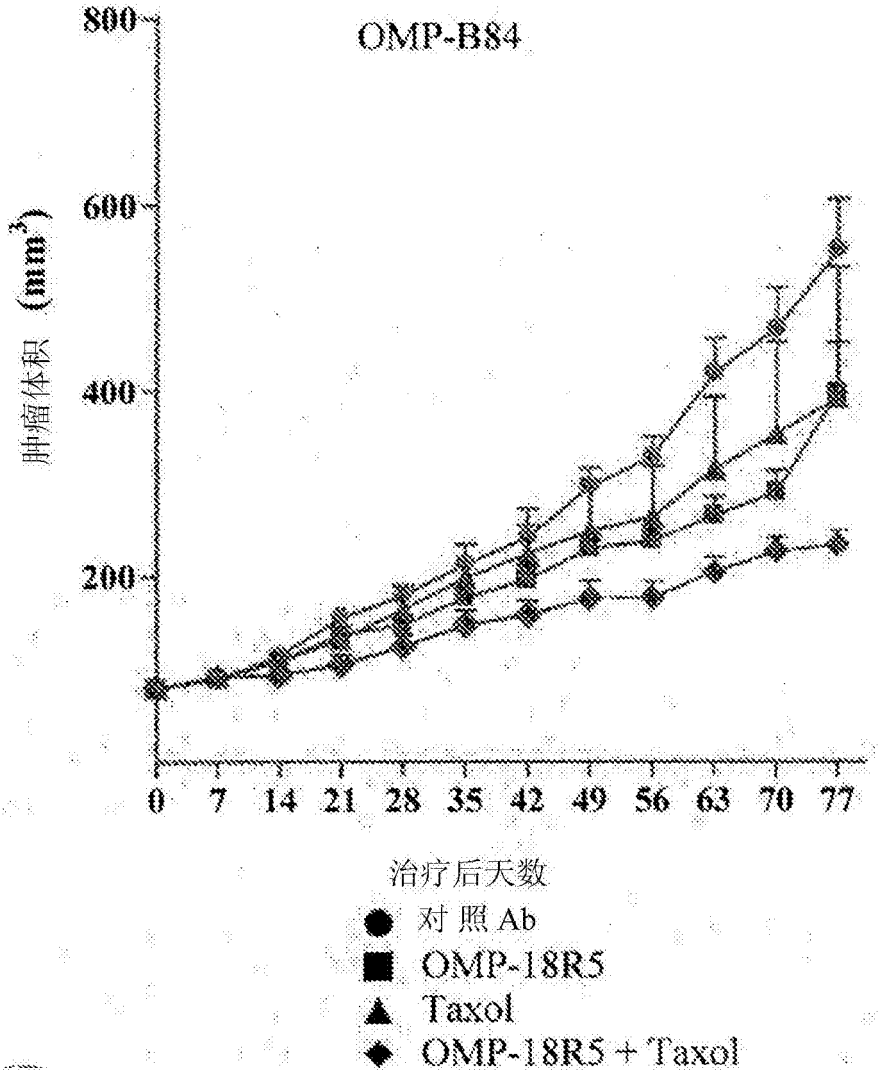

OMP-B84
800
600
400
200
肿瘤体积 (mm³)
0 7 14 21 28 35 42 49 56 63 70 77
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图6C

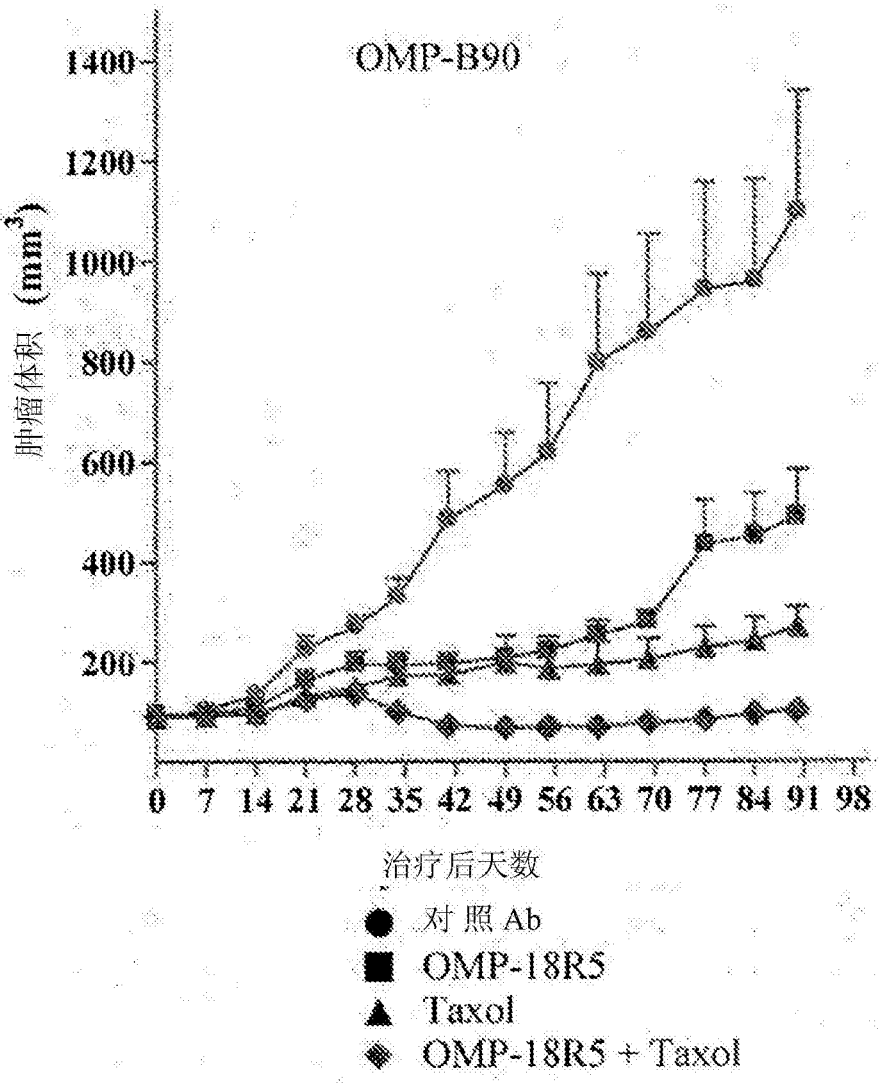

OMP-B90
肿瘤体积 (mm3)
1400
1200
1000
800
600
400
200
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图6D

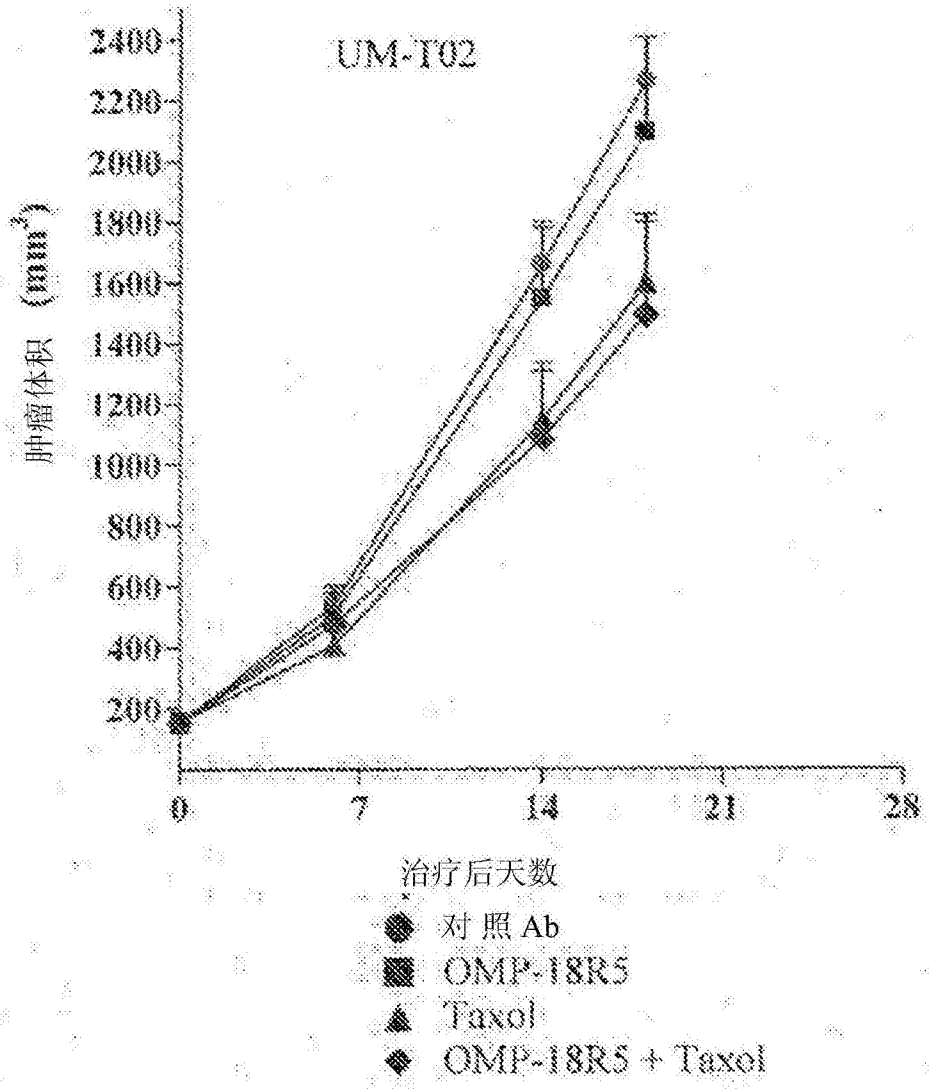

UM-T02
肿瘤体积 (mm³)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7
14
21
28
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图6E

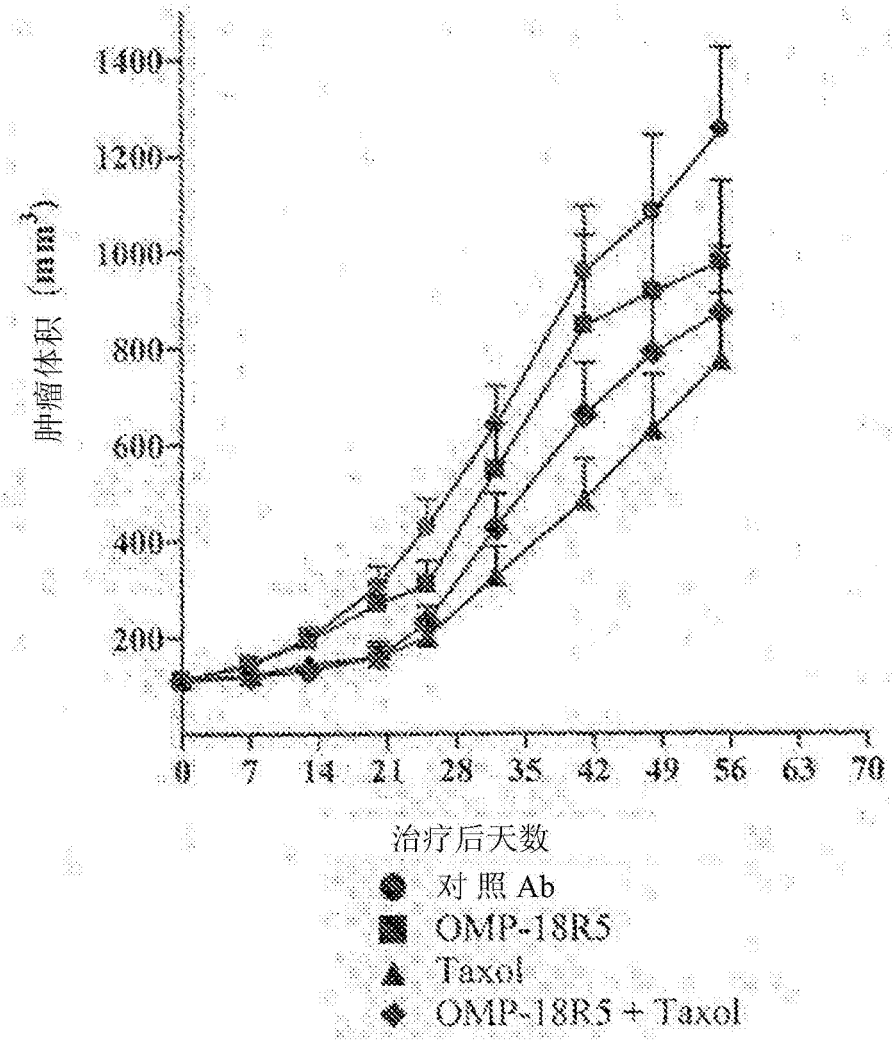

肿瘤体积 (mm³)
1400
1200
1000
800
600
400
200
0
7
14
21
28
35
42
49
56
63
70
治疗后天数
对照 Ab
OMP-18R5
Taxol
OMP-18R5 + Taxol

图6F

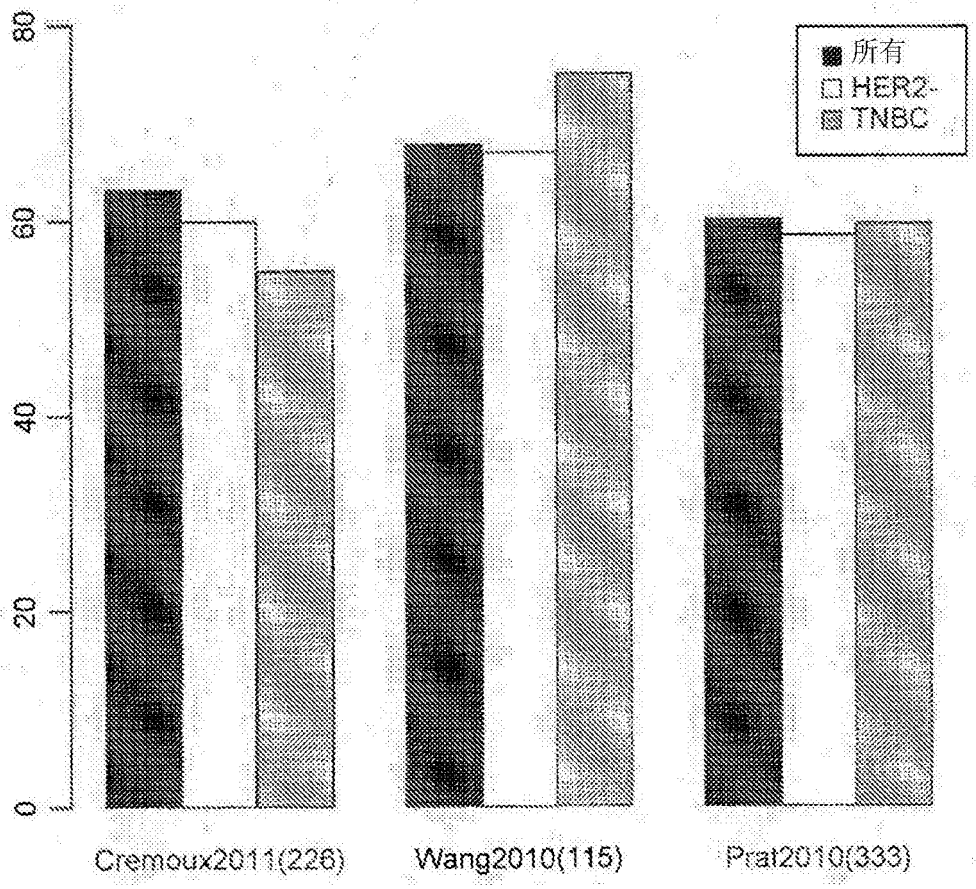

80
60
40
20
0
所有
HER2-
TNBC
Cremoux2011(226)
Wang2010(115)
Prat2010(333)

图7

1.一种治疗病患的乳癌的方法，其包含：

(a)鉴别该病患是否可能对Wnt途径抑制剂的治疗有反应，其中该Wnt途径抑制剂是特异性结合人FZD1、FZD2、FZD5、FZD7和FZD8的抗体，其包括包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3；

其中该鉴别包含：

(i)获得该病患乳癌的样本；

(ii)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及

(iii)根据所述生物标记物的表达量，鉴别可能对治疗有反应的病患；以及

(b)对该可能对治疗有反应的病患投予有效量的Wnt途径抑制剂。

2.一种鉴别可能对Wnt途径抑制剂的治疗有反应或无反应的人乳房肿瘤的方法，其中该Wnt途径抑制剂是特异性结合人FZD1、FZD2、FZD5、FZD7和FZD8的抗体，其包括包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3；

其中该方法包含：

(a)获得该乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及

(c)根据所述生物标记物的表达量，鉴别该肿瘤为可能对治疗有反应或无反应。。

3.一种分类人乳房肿瘤为可能对Wnt途径抑制剂的治疗有反应或无反应的方法，其中该Wnt途径抑制剂是特异性结合人FZD1、FZD2、FZD5、FZD7和FZD8的抗体，其包括包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3；

其中该方法包含：

(a)获得该乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及

(c)根据所述生物标记物的表达，分类该肿瘤为可能对治疗有反应或无反应。

4.一种测定人乳房肿瘤对Wnt途径抑制剂的治疗的反应性的方法，其中该Wnt途径抑制剂是特异性结合人FZD1、FZD2、FZD5、FZD7和FZD8的抗体，其包括包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3；

其中该方法包含：

(a)获得该乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及

(c)根据所述生物标记物的表达，判定该肿瘤对治疗的反应性。

5.一种鉴别对Wnt途径抑制剂的治疗可能有反应的乳癌病患的方法，其中该Wnt途径抑制剂是特异性结合人FZD1、FZD2、FZD5、FZD7和FZD8的抗体，其包括包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3；

其中该方法包含：

(a)获得该病患乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及

(c)根据所述生物标记物的表达量，鉴别可能对治疗有反应的病患。

6.一种选择乳癌病患以接受Wnt途径抑制剂的治疗的方法，其中该Wnt途径抑制剂是特异性结合人FZD1、FZD2、FZD5、FZD7和FZD8的抗体，其包括包含GFTFSHYTLS(SEQ ID NO:1)的重链CDR1，包含VISGDGSYTYYADSVKG(SEQ ID NO:2)的重链CDR2，包含NFIKYVFAN(SEQ ID NO:3)的重链CDR3，包含SGDNIGSFYVH(SEQ ID NO:4)的轻链CDR1，包含DKSNRPSG(SEQ ID NO:5)的轻链CDR2，及包含QSYANTLSL(SEQ ID NO:6)的轻链CDR3；

其中该方法包含：

(a)获得该病患乳房肿瘤的样本；

(b)测量该样本中的生物标记物标签的各生物标记物的表达量，其中该生物标记物标签包含生物标记物FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1；以及

(c)根据所述生物标记物的表达量，选择该将接受治疗的病患。

7.如权利要求1至6中任一项所述的方法，其中各生物标记物的表达是通过PCR为基础的测试、qPCR测试、微阵列或RNA测序来测量。

8.如权利要求1至6中任一项所述的方法，其中FBXW2、CCND2、RHOU、CTBP2、WIF1、DKK1的表达量是通过使用选自SEQ ID NO:62-79的多核苷酸来测量。

9.如权利要求8所述的方法，其中FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的表达量是利用下列测量：

(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物及包含SEQ ID NO:64的探针；

(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物及包含SEQ ID NO:67的探针；

(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物及包含SEQ ID NO:70的探针；

(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物及包含SEQ ID NO:73的探针；

(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物及包含SEQ ID NO:76的探针；以及

(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物及包含SEQ ID NO:79的探针。

10.如权利要求1至9中任一项所述的方法，其中该Wnt途径抑制剂是包括包含SEQ ID

NO:7的重链可变区和包含SEQ ID NO:8的轻链可变区的抗体。

11.如权利要求第1至9中任一项所述的方法，其中该Wnt途径抑制剂是包含重链可变区及轻链可变区的抗体，该抗体是由保藏于美国菌种保存中心编号为PTA-9541的质粒编码。

12.如权利要求1至11中任一项所述的方法，其中该抗体是单克隆抗体、重组抗体、嵌合抗体、双特异性抗体、人化抗体、人抗体或包含抗原结合部位的抗体片段。

13.如权利要求1至9中任一项的方法，其中该Wnt途径抑制剂是抗体OMP-18R5。

14.如权利要求1-13中任一项所述的方法，其中该乳房肿瘤或乳癌是HER2阴性乳房肿瘤。

15.如权利要求1-13中任一项所述的方法，其中该乳房肿瘤或乳癌是三阴性乳癌(TNBC)肿瘤。

16.如权利要求1至15中任一项所述的方法，其中该Wnt途径抑制剂的治疗是与一或多种额外治疗剂组合。

17.如权利要求16所述的方法，其中该额外治疗剂是化学治疗剂。

18.如权利要求16所述的方法，其中该额外治疗剂是太平洋紫杉醇(paclitaxel)。

19.如权利要求16所述的方法，其中该额外治疗剂是经nab结合的太平洋紫杉醇(ABRAXANE)。

20.如权利要求1至19中任一项所述的方法，其中该样本是组织样本或肿瘤活体样本。

21.如权利要求1至20中任一项所述的方法，其中该样本是经福尔马林固定的石蜡包埋(FFPE)样本。

22.一种用于检测乳房肿瘤或乳癌样本中的FBXW2、CCND2、RHOU、CTBP2、WIF1及DKK1的试剂盒，其中该试剂盒包含选自SEQ ID NO:62至79的多核苷酸。

23.如权利要求22的试剂盒，其包含：

(a)SEQ ID NO:62的正向引物、SEQ ID NO:63的反向引物及包含SEQ ID NO:64的探针；

(b)SEQ ID NO:65的正向引物、SEQ ID NO:66的反向引物及包含SEQ ID NO:67的探针；

(c)SEQ ID NO:68的正向引物、SEQ ID NO:69的反向引物及包含SEQ ID NO:70的探针；

(d)SEQ ID NO:71的正向引物、SEQ ID NO:72的反向引物及包含SEQ ID NO:73的探针；

(e)SEQ ID NO:74的正向引物、SEQ ID NO:75的反向引物及包含SEQ ID NO:76的探针；以及

(f)SEQ ID NO:77的正向引物、SEQ ID NO:78的反向引物及包含SEQ ID NO:79的探针。