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(54) Title: METHODS AND MONITORING OF TREATMENT WITH A WNT PATHWAY INHIBITOR

(57) Abstract: Methods for treating diseases such as cancer comprising administering a Wnt pathway inhibitor, either alone or in combination with other anti-cancer agents, and monitoring for skeletal-related side effects and/or toxicity.

METHODS AND MONITORING OF TREATMENT WITH A WNT PATHWAY INHIBITOR

CROSS-REFERENCE TO RELATED APPLICATONS

[0001] This application claims priority benefit of U.S. Provisional Application No. 61/760,523, filed February 4, 2013, which is hereby incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0002] The present invention relates to the field of treating diseases with a Wnt pathway inhibitor. More particularly, the invention provides methods for treating cancer comprising administering a Wnt pathway inhibitor, either alone or in combination with other anti-cancer agents, and monitoring for side effects and/or toxicity.

BACKGROUND OF THE INVENTION

[0003] Cancer is one of the leading causes of death in the developed world, with over one million people diagnosed with cancer and 500,000 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 (Siegel et al., 2011, *CA: A Cancer J. Clin.* 61:212-236).

[0004] Signaling pathways normally connect extracellular signals to the nucleus leading to expression of genes that directly or indirectly control cell growth, differentiation, survival, and death. 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 has been identified as a potential target for cancer therapy. 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. The activation of the Wnt pathway may maintain tumor cells in an undifferentiated state and/or lead to uncontrolled proliferation. Thus carcinogenesis can 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 secreted lipid-modified glycoproteins of which 19 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 seven transmembrane domain proteins of the G-protein coupled receptor (GPCR) superfamily and contain a large extracellular N-terminal ligand binding domain with 10 conserved cysteines, known as a cysteine-rich domain (CRD) or 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), and FZD receptors have been 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). Additional evidence for the role of Wnt signaling in breast cancer has since accumulated. For instance, transgenic over-expression of β-catenin in the mammary glands 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. Approximately 5-10% of all colorectal cancers are hereditary with one of the main forms being familial adenomatous polyposis (FAP), 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] Similar to breast cancer and colon cancer, melanoma often has constitutive activation of the Wnt pathway, as indicated by the nuclear accumulation of β-catenin. Activation of the Wnt/β-catenin pathway in some melanoma tumors and cell lines is due to modifications in pathway components, such as APC, ICAT, LEF1 and β-catenin (see e.g., Larue et al., 2006, *Frontiers Biosci.*, 11:733-742). However, there are conflicting reports in the literature as to the exact role of Wnt/β-catenin signaling in melanoma. For example, one study found that elevated levels of nuclear β-catenin correlated with improved survival from melanoma, and that activated Wnt/β-catenin signaling was associated with decreased cell proliferation (Chien et al., 2009, *PNAS*, 106:1193-1198).

[0010] Chemotherapy is a well-established therapeutic approach for numerous cancers, but its efficacy can be limited by side effects and/or toxicity. In addition, targeted therapies such as the anti-ErbB2 receptor (HER2) antibody trastuzumab (HERCEPTIN), tyrosine kinase inhibitors imatinib (GLEEVEC), dasatinib (SPRYCEL), nilotibib (TASIGNA), sunitinib (SUTENT), sorafenib (NEXAVAR), the anti-VEGF antibody bevacizumab (AVASTIN), and anti-angiogenesis drugs sunitinib (SUTENT) and sorafenib (NEXAVAR), are known to cause, or are likely to cause, side effects and/or toxicity in subjects who take them. Thus, new methods to identify drug-induced side effects, monitor those side effects, and/or mitigate those side effects so that effective cancer therapy can continue are still needed.

BRIEF SUMMARY OF THE INVENTION

[0011] The present invention provides improved methods for treating diseases comprising administering to a subject a therapeutically effective amount of a Wnt pathway inhibitor. For example, in one aspect the invention provides methods of screening for, detecting, identifying, monitoring, reducing, preventing, attenuating, and/or mitigating a skeletal-related side effect and/or toxicity related to treatment with a Wnt pathway inhibitor. In some embodiments, the methods comprise determining the level of a bone turnover marker in a sample from a patient who has received, is receiving, will receive, or is being considered for initial or further treatment with a Wnt pathway inhibitor, including but not limited to an anti-Frizzled (FZD) antibody or a soluble FZD receptor.

[0012] In another aspect, the invention provides methods of identifying a subject as eligible for treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject, determining the level of a biomarker in the sample, and identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the biomarker is below a predetermined level. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the method of identifying a subject as eligible for treatment

with a Wnt pathway inhibitor, comprises: obtaining a biological sample from the subject, determining the level of a bone resorption biomarker in the sample, and identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level. In some embodiments, the bone resorption biomarker is collagen type 1 cross-linked C-telopeptide (β-CTX).

[0013] In one aspect, the invention provides methods of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of skeletal-related side effects and/or toxicity, comprising: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein an increase in the level of the biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the method of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of skeletal-related side effects and/or toxicity, comprises: obtaining a biological sample from the subject receiving treatment, determining the level of a bone resorption biomarker in the sample, and comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein an increase in the level of the bone resorption biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the bone resorption biomarker is β-CTX.

[0014] In another aspect, the invention provides methods of detecting the development of skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein an increase in the level of the biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the method of detecting the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprises: obtaining a biological sample from the subject receiving treatment, determining the level of a bone resorption biomarker in the sample, and comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein an increase in the level of the bone resorption biomarker indicates development of a skeletal-related side effect and/or toxicity. In some embodiments, the bone resorption biomarker is β-CTX.

[0015] In another aspect, the invention provides methods for identifying skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker,

wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then a skeletal-related side effect and/or toxicity is indicated. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the method for identifying skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprises: obtaining a biological sample from the subject receiving treatment, determining the level of a bone resorption biomarker in the sample, and comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then a skeletal-related side effect and/or toxicity is indicated. In some embodiments, the bone resorption biomarker is β-CTX.

[0016] In another aspect, the invention provides methods for monitoring skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then a skeletal-related side effect and/or toxicity is indicated. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the method for monitoring skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprises: obtaining a biological sample from the subject receiving treatment, determining the level of a bone resorption biomarker in the sample, and comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then a skeletal-related side effect and/or toxicity is indicated. In some embodiments, the bone resorption biomarker is β-CTX.

[0017] In some aspects and/or embodiments of the methods described herein, wherein if the bone resorption biomarker level (e.g., β-CTX) in a sample increases 2-fold or greater as compared to a predetermined level, the subject is administered a therapeutically effective amount of an anti-resorptive medication. In some embodiments, the bone resorption biomarker is β-CTX and the predetermined level is less than about 1000pg/ml. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0018] In another aspect, the invention provides methods of reducing skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject receiving treatment, determining the level of a bone resorptive biomarker in the sample, comparing the level of the bone resorptive biomarker in the sample to a predetermined level of the bone resorptive biomarker, and administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of the bone resorptive biomarker in the

sample is higher than the predetermined level of the bone resorptive biomarker. In some embodiments, the increase in the resorptive biomarker is about 1.5-fold or greater, about 2-fold or greater, about 2.5-fold or greater, or about 3-fold or greater than the predetermined level of the bone resorptive biomarker. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0019] In another aspect, the invention provides methods of preventing or attenuating the development of skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor, determining the level of a bone resorptive biomarker in the sample, comparing the level of the bone resorptive biomarker in the sample to a predetermined level of the bone resorptive biomarker, administering to the subject a therapeutically effective amount of an anti-resorptive medication, and administering to the subject the Wnt pathway inhibitor. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0020] In another aspect, the invention provides methods of ameliorating skeletal-related side effects and/or toxicity in a subject administered a Wnt pathway inhibitor, comprising: determining the level of a bone resorptive biomarker in a sample, and administering to the subject a therapeutically effective amount of an anti-resorptive medication. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0021] In another aspect, the invention provides methods of screening a subject for the risk of skeletal-related side effects and/or toxicity from treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor, determining the level of a bone resorption biomarker in the sample, and comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level then the subject is at risk for skeletal-related side effects and/or toxicity. In some embodiments, if the subject is at risk for skeletal-related side effects and/or toxicity, the subject is administered a therapeutically effective amount of a therapeutic agent directed to the skeletal-related side effect and/or toxicity prior to treatment with the Wnt pathway inhibitor. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the therapeutic agent directed to skeletal-related side effects is a bisphosphonate.

[0022] In another aspect, the invention provides methods of treating cancer in a subject, comprising: administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor, and determining the level of a bone resorption biomarker in a sample from the subject. In some embodiments, the method of treating cancer further comprises comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker. In some embodiments, the method

of treating cancer further comprises comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity. In some embodiments, the method of treating cancer further comprises comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker is higher than the predetermined level of the bone resorption biomarker then the subject is administered a therapeutically effective amount of an anti-resorptive medication. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0023] In another aspect, the invention provides methods of inhibiting tumor growth in a subject, comprising: administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor, and determining the level of a bone resorption biomarker in a sample from the subject. In some embodiments, the method of inhibiting tumor growth further comprises comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker. In some embodiments, the method of inhibiting tumor growth further comprises comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity. In some embodiments, the method of inhibiting tumor growth further comprises comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein if the level of the bone resorption biomarker is higher than the predetermined level of the bone resorption biomarker then the subject is administered a therapeutically effective amount of an anti-resorptive medication. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0024] In some aspects and/or embodiments of the methods described herein, the biological sample is blood, serum, or plasma. In some embodiments, the biological sample is a "fasting sample". As used herein, a "fasting sample" refers to a sample taken from an individual who has not eaten food and has not drank any liquids for at least 9 – 12 hours. In some embodiments, the predetermined level is about 1500pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 1200pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 1000pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 800pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 600pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 400pg/ml or less in a blood, serum, or plasma

sample. In some embodiments, the predetermined level of a biomarker (e.g., a bone turnover marker) is the amount of the biomarker in a sample obtained at an earlier date. In some embodiments, the predetermined level of a biomarker (e.g., a bone turnover marker) is the amount of the biomarker in a sample obtained prior to treatment. In some embodiments, the predetermined level of a biomarker (e.g., a bone turnover marker) is the amount of the biomarker in a sample obtained at an initial screening. In some embodiments, the predetermined level of a biomarker (e.g., a bone turnover marker) is a normal reference level. In some embodiments, the predetermined level of a biomarker is a baseline level. In some embodiments, the baseline level is the amount of the biomarker determined at an initial screening (e.g., prior to treatment). In some embodiments the bone resorption biomarker is β-CTX. In some embodiments, the predetermined level for β-CTX is about 1000pg/ml or less in blood, serum, or plasma.

[0025] In some aspects and/or embodiments of the methods described herein, a biological sample is obtained approximately every week, every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, or every 6 weeks.

[0026] In certain embodiments of each of the aforementioned aspects, as well as other aspects and embodiments described elsewhere herein, the Wnt pathway inhibitor is an antibody that specifically binds at least one human Wnt protein. Non-limiting examples of anti-Wnt antibodies have been described in, for example, U.S. Patent Publication No. 2012/0027778 and International Publication WO 2011/088127. In some embodiments, the Wnt pathway inhibitor is an antibody that specifically binds at least one human FZD protein. Non-limiting examples of anti-FZD antibodies have been described in, for example, U.S. Patent No. 7,982,013. In some embodiments, the Wnt pathway inhibitor is a soluble FZD receptor. Non-limiting examples of soluble FZD receptors have been described in, for example, U.S. Patent Nos. 7,723,477 and 8,324,361 and U.S. Patent Publication No. 2011/0305695.

[0027] In some embodiments, 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/or (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).

[0028] In certain embodiments of each of the aforementioned aspects, as well as other aspects and embodiments described elsewhere herein, the Wnt pathway inhibitor is an antibody comprising (a) a heavy chain variable region having at least about 90%, at least about 95%, or 100% sequence identity to SEQ ID NO:7; and/or (b) a light chain variable region having at least about 90%, at least about 95%, or 100% sequence identity to SEQ ID NO:8. In some embodiments, the Wnt pathway inhibitor is antibody OMP-18R5.

[0029] In certain embodiments of each of the aforementioned aspects, as well as other aspects and embodiments described elsewhere herein, the Wnt pathway inhibitor is a recombinant antibody. In some embodiments, the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, or a human antibody. In some embodiments, the antibody is an antibody fragment comprising an antigen-binding site. In certain embodiments, the antibody or antibody fragment is monovalent, monospecific, or bivalent. In some embodiments, the antibody is a bispecific antibody or a multispecific antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG2 antibody. In certain embodiments, the antibody is isolated. In other embodiments, the antibody is substantially pure.

[0030] In some embodiments, the Wnt pathway inhibitor is an antibody that binds at least one human FZD with a dissociation constant (K_D) of about 10nM to about 0.1nM.

[0031] In certain embodiments, the Wnt pathway inhibitor comprises the same heavy and light chain amino acid sequences as an antibody encoded by a plasmid deposited with ATCC having deposit no. PTA-9541. In certain embodiments, the Wnt pathway inhibitor comprises the same heavy chain variable region and light chain variable region amino acid sequences as an antibody encoded by a plasmid deposited with ATCC having deposit no. PTA-9541. In certain embodiments, the Wnt pathway inhibitor is encoded by the plasmid having ATCC deposit no. PTA-9541 which was deposited with American Type Culture Collection (ATCC), at 10801 University Boulevard, Manassas, VA, 20110, under the conditions of the Budapest Treaty on September 29, 2008. In certain embodiments, the Wnt pathway inhibitor competes for specific binding to a human FZD with an antibody encoded by the plasmid deposited with ATCC having deposit no. PTA-9541.

[0032] In any of the aspects and/or embodiments of the methods described herein, the subject has cancer. In some embodiments, the cancer is selected from the group consisting of: lung cancer, pancreatic cancer, breast cancer, colon cancer, colorectal cancer, melanoma, gastrointestinal cancer, gastric cancer, renal cancer, ovarian cancer, liver cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, glioma, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, hepatocellular carcinoma (HCC), neuroendocrine cancer, thyroid cancer, adenocarcinoma, and head and neck cancer. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is pancreatic cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is ovarian cancer. In some embodiments, the cancer is liver cancer. In some embodiments, the cancer is HCC.

[0033] In any of the aspects and/or embodiments of the methods described herein, the subject is treated with the Wnt pathway inhibitor in combination with one or more additional anti-cancer agents. In some embodiments, the one or more additional anti-cancer agents are chemotherapeutic agents. In some embodiments, the additional anti-cancer agent is paclitaxel or albumin-bound paclitaxel. In some

embodiments, the additional anti-cancer agent is gemcitabine. In some embodiments, the additional anti-cancer agents are gemcitabine and albumin-bound paclitaxel. In some embodiments, the additional anti-cancer agent is docetaxel. In some embodiments, the additional anti-cancer agent is carboplatin. In some embodiments, the additional anti-cancer agents are carboplatin and paclitaxel or albumin-bound paclitaxel. In some embodiments, the additional anti-cancer agent is sorafenib.

[0034] 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 DESCRIPTION OF THE FIGURES

[0035] Figure 1. Inhibition of breast tumor growth *in vivo* with intermittent dosing of a Wnt pathway inhibitor. Mice were treated with paclitaxel (-●-), 5mg/kg OMP-18R5 in combination with paclitaxel (-■-), 10mg/kg OMP-18R5 in combination with paclitaxel (-▲-), 25mg/kg OMP-18R5 in combination with paclitaxel (-▼-), or 45mg/kg OMP-18R5 in combination with paclitaxel (-◆-). Data is shown as tumor volume (mm^3) over days post-treatment. OMP-18R5 was administered intraperitoneally once every three weeks (indicated by arrows) and paclitaxel was administered at 10mg/kg once a week.

[0036] Figure 2. Inhibition of breast tumor growth *in vivo* with intermittent dosing of a Wnt pathway inhibitor. Mice were treated with paclitaxel (-■-), 25mg/kg OMP-18R5 in combination with paclitaxel once every 4 weeks (-▼-), 25mg/kg OMP-18R5 in combination with paclitaxel once every 2 weeks (-▲-), or 25mg/kg OMP-18R5 in combination with paclitaxel once a week (-●-). Data is shown as tumor volume (mm^3) over days post-treatment. OMP-18R5 was administered intraperitoneally and paclitaxel was administered at 15mg/kg once a week.

[0037] Figure 3. Effect of OMP-18R5 on bone formation in mice.

[0038] Figure 4. Effect of zoledronic acid on bone formation in mice treated with OMP-18R5.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The present invention relates to treating diseases with a Wnt pathway inhibitor. More particularly, the invention provides methods for treating cancer comprising administering a Wnt pathway inhibitor, either alone or in combination with other anti-cancer agents, and monitoring for skeletal-related side effects and/or toxicity, including those related to the Wnt pathway inhibitor.

[0040] The anti-FZD antibody OMP-18R5 was administered to subjects in a Phase 1a single agent dose escalation trial. The data from this early trial, as well as results from animal studies suggested that

administration of a Wnt pathway inhibitor such as an anti-FZD antibody or a FZD8-Fc soluble receptor may result in skeletal-related side effects and/or toxicity in certain patients. Furthermore, the Phase 1a study showed that increased β-CTX levels may be an early indicator that a patient being treated with a Wnt pathway inhibitor is at risk of developing skeletal-related side effects and/or toxicities, allowing for intervention with appropriate medications.

[0041] These results made it desirable to develop risk mitigation and monitoring strategies for skeletal-related side effects and/or toxicities as described herein for subjects receiving treatment with a Wnt pathway inhibitor (e.g., an anti-FZD antibody or a soluble FZD receptor) as a single agent or in combination with additional anti-cancer agents.

I. Definitions

[0042] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0043] The terms "antagonist" and "antagonistic" as used herein refer to any molecule that partially or fully blocks, inhibits, reduces, or neutralizes a biological activity of a target and/or signaling pathway (e.g., the Wnt pathway). The term "antagonist" is used herein to include any molecule that partially or fully blocks, inhibits, reduces, or neutralizes the activity of a protein (e.g., a FZD protein or a Wnt protein). Suitable antagonist molecules specifically include, but are not limited to, antagonist antibodies, antibody fragments, soluble receptors, or small molecules.

[0044] The terms "modulation" and "modulate" as used herein refer to a change or an alteration in a biological activity. Modulation includes, but is not limited to, stimulating or inhibiting an activity. Modulation may be an increase or a decrease in activity (e.g., a decrease in Wnt pathway signaling), a change in binding characteristics, or any other change in the biological, functional, or immunological properties associated with the activity of a protein, pathway, or other biological point of interest.

[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 recognition 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 recognition site (e.g., 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 an 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 regions of the heavy and light chains each consist 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, with the CDRs from the other chain, contribute to the formation of the antigen-binding sites 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 recognition site (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 forms of non-human (e.g., murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulins in which residues of the CDRs are replaced by residues from the CDRs of a non-human species (e.g., mouse, rat, rabbit, or hamster) that have the desired specificity, affinity, and/or binding capability (Jones et al., 1986, *Nature*, 321:522-525; Riechmann et al., 1988, *Nature*, 332:323-327; Verhoeyen et al., 1988, *Science*, 239:1534-1536). In some instances, the Fv framework region residues of a human

immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, and/or binding capability. The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or binding capability. In general, the humanized antibody will comprise substantially all of at least one, and typically two or three, variable domains 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 consensus sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin.

[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. This definition of a human antibody specifically excludes a humanized antibody comprising non-human CDRs.

[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 region of both light and heavy chains corresponds to the variable region of antibodies 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 in antibodies derived from another species (usually human).

[0052] The phrase "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, Marks et al., 1992, *Bio/Technology* 10:779-783, describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., 1994, *PNAS*, 91:3809-3813; Schier et al., 1995, *Gene*, 169:147-155; Yelton et al., 1995, *J. Immunol.* 155:1994-2004; Jackson et al., 1995, *J. Immunol.*, 154:3310-9; and Hawkins et al., 1992, *J. Mol. Biol.*, 226:889-896. Site-directed mutagenesis may also be used to obtain affinity-matured antibodies.

[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. It is understood that, in certain embodiments, an antibody or binding moiety 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, an antibody may, in certain embodiments, specifically bind more than one target. In certain embodiments, multiple targets may be bound by the same antigen-binding site on the 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 some embodiments, an antibody may be multispecific and comprise at least two antigen-binding sites with differing specificities. By way of non-limiting example, a bispecific antibody may comprise one antigen-binding site that recognizes an epitope on one protein and further comprise a second, different antigen-binding site that recognizes a different epitope on a second protein. Generally, but not necessarily, reference to binding means specific binding.

[0055] As used herein the term "soluble receptor" refers to an N-terminal extracellular fragment (or a portion thereof) of a receptor protein preceding the first transmembrane domain of the receptor that can be secreted from a cell in soluble form.

[0056] 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 the Fri domain are also included in this term.

[0057] 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).

[0058] 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.

[0059] 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.

[0060] 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(s), i.e., the one or more RSPO protein(s) 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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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.

[0066] 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 the 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 invade neighboring body structures.

[0067] 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 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.

[0068] 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.

[0069] 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).

[0070] 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).

[0071] The term "subject" 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 "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0072] 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.

[0073] 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 binding agent (e.g., an antibody) of the present disclosure, and which does not destroy the activity of the binding agent. The excipient, carrier, or adjuvant should be non-toxic when administered with a binding agent in doses sufficient to deliver a therapeutic effect.

[0074] 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.

[0075] 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 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 or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer cells into soft tissue and bone; inhibition of or an absence of tumor or cancer cell metastasis; inhibition 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 effects.

[0076] As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[0077] 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.

[0078] As used herein, reference to "about" or "approximately" a value or parameter includes (and describes) embodiments that are directed to that value or parameter. For example, description referring to "about X" includes description of "X".

[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. Wnt pathway inhibitors

[0080] The present invention provides Wnt pathway inhibitors for use in methods of inhibiting tumor growth and/or for use in methods of treating cancer.

[0081] In certain embodiments, the Wnt pathway inhibitors are agents that bind one or more human Frizzled proteins (FZD). These agents are referred to herein as "FZD-binding agents". 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.

[0082] 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.

[0083] 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".

[0084] In certain embodiments, the FZD-binding agent (e.g., antibody) specifically binds the extracellular domain (ECD) within 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).

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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).

[0090] For example, the specific binding of an antibody to a human FZD protein may be determined using ELISA. An ELISA assay comprises preparing antigen, coating wells of a 96 well microtiter plate with antigen, adding to the well the FZD-binding agent (e.g., an antibody) conjugated to a detectable compound such as an enzymatic substrate (e.g. horseradish peroxidase or alkaline phosphatase), incubating for a period of time and detecting the presence of the FZD-binding agent bound to the antigen. In some embodiments, the FZD-binding antibody or agent is not conjugated to a detectable compound, but instead a second conjugated antibody that recognizes the FZD-binding antibody or agent (e.g., an anti-Fc antibody) is added to the well. In some embodiments, instead of coating the well with the antigen, the FZD-binding antibody or agent can be coated to the well and a second antibody conjugated to a detectable compound can be added following the addition of the antigen to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase and/or optimize the signal detected as well as other variations of ELISAs that may be used.

[0091] In another example, the specific binding of an antibody to a human FZD protein may be determined using FACS. A FACS screening assay may comprise generating a cDNA construct that expresses an antigen as a fusion protein, transfecting the construct into cells, expressing the antigen on the surface of the cells, mixing the FZD-binding antibody or other FZD-binding agent with the transfected cells, and incubating for a period of time. The cells bound by a FZD-binding antibody or other FZD-binding agent may be identified by using a secondary antibody conjugated to a detectable compound (e.g., PE-conjugated anti-Fc antibody) and a flow cytometer. One of skill in the art would be knowledgeable as to the parameters that can be modified to optimize the signal detected as well as other variations of FACS that may enhance screening (e.g., screening for blocking antibodies).

[0092] The binding affinity of an antibody or other binding-agent to an antigen (e.g., a FZD protein) and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^{3}H or ^{125}I), or fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of unlabeled antigen followed by the detection of the antibody bound to the labeled antigen. The affinity of the antibody for an antigen (e.g., a FZD protein) and the binding off-rates can be determined from the data by Scatchard plot analysis. In some embodiments, Biacore kinetic analysis is used to determine the binding on and off rates of antibodies or agents that bind an antigen (e.g., a FZD protein). Biacore kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized antigen (e.g., a FZD protein) on their surface.

[0093] 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).

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[0100] 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.

[0101] 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.

[0102] 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).

[0103] In certain embodiments, a FZD-binding agent comprises, consists essentially of, or consists of, the antibody OMP-18R5.

[0104] 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 (e.g., antibody) competes for specific binding to one or more human FZD proteins with an antibody that comprises a heavy chain variable region and a light chain variable region encoded by the plasmid deposited with ATCC having deposit no. PTA-9541. 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.

[0105] 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.

[0106] In certain embodiments, the Wnt pathway inhibitors are agents that bind one or more human Wnt proteins. These agents are referred to herein as "Wnt-binding agents". 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.

[0107] 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.

[0108] 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.

[0109] 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 to which the agent or antibody binds that is 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).

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] For example, the specific binding of an antibody to a human Wnt protein may be determined using ELISA. An ELISA assay comprises preparing antigen, coating wells of a 96 well microtiter plate with antigen, adding to the well the Wnt-binding agent (e.g., an antibody) conjugated to a detectable compound such as an enzymatic substrate (e.g. horseradish peroxidase or alkaline phosphatase), incubating for a period of time and detecting the presence of the Wnt-binding agent bound to the antigen. In some embodiments, the Wnt-binding antibody or agent is not conjugated to a detectable compound, but instead a second conjugated antibody that recognizes the Wnt-binding antibody or agent (e.g., an anti-Fc antibody) is added to the well. In some embodiments, instead of coating the well with the antigen, the Wnt-binding antibody or agent can be coated to the well and a second antibody conjugated to a detectable compound can be added following the addition of the antigen to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase and/or optimize the signal detected as well as other variations of ELISAs that may be used.

[0115] In another example, the specific binding of an antibody to a human Wnt protein may be determined using FACS. A FACS screening assay may comprise generating a cDNA construct that expresses an antigen as a fusion protein, transfecting the construct into cells, expressing the antigen on the surface of the cells, mixing the Wnt-binding antibody with the transfected cells, and incubating for a period of time. The cells bound by the Wnt-binding antibody may be identified by using a secondary

antibody conjugated to a detectable compound (e.g., PE-conjugated anti-Fc antibody) and a flow cytometer. One of skill in the art would be knowledgeable as to the parameters that can be modified to optimize the signal detected as well as other variations of FACS that may enhance screening (e.g., screening for blocking antibodies).

[0116] The binding affinity of a Wnt-binding agent to an antigen (e.g., a Wnt protein) and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays such as those described above for FZD-binding agents.

[0117] 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 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. 2011/0305695.

[0118] 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.

[0119] 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.

[0120] 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).

[0121] 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.

[0122] 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.

[0123] 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).

[0124] 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.

[0125] 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.

[0126] 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).

[0127] 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).

[0128] 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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.

[0137] In some embodiments, the Wnt-binding agent is OMP-54F28 (also referred to as 54F28). In some embodiments, the Wnt-binding agent is not OMP-54F28.

[0138] 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.

[0139] 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.

[0140] 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.

[0141] 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.

[0142] 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.

[0143] 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.

[0144] 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.

[0145] 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.

[0146] 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.

[0147] 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.

[0148] 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.

[0149] 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.

[0150] 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".

[0151] 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.

[0152] 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.

[0153] 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.

[0154] 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.

[0155] 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 (see e.g., McCafferty et al., 1990, *Nature*, 348:552-554; Clackson et al., 1991, *Nature*, 352:624-628; and Marks et al., 1991, *J. Mol. Biol.*, 222:581-597).

[0156] 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.

[0157] In some embodiments, the Wnt pathway inhibitor is a humanized antibody. Typically, humanized antibodies are human immunoglobulins in which residues from the CDRs are replaced by residues from a CDR 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, the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from 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 residues either in the Fv framework region and/or within the replaced non-human 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 consensus 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.

[0158] 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 (see, e.g., Cole et al., 1985, *Monoclonal*

Antibodies and Cancer Therapy, Alan R. Liss, p. 77; Boemer et al., 1991, *J. Immunol.*, 147:86-95; and U.S. Patent Nos. 5,750,373; 5,567,610; and 5,229,275). In some embodiments, the human antibody can be selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, *Nature Biotechnology*, 14:309-314; Sheets et al., 1998, *PNAS*, 95:6157-6162; Hoogenboom and Winter, 1991, *J. Mol. Biol.*, 227:381; Marks et al., 1991, *J. Mol. Biol.*, 222:581). 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 described in U.S. Patent Nos. 5,969,108; 6,172,197; 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915; 6,593,081; 6,300,064; 6,653,068; 6,706,484; and 7,264,963; and Rothe et al., 2008, *J. Mol. Bio.*, 376:1182-1200. 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.

[0159] 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.

[0160] 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.

[0161] Techniques for making bispecific antibodies are known by those skilled in the art, see for example, Millstein et al., 1983, *Nature*, 305:537-539; Brennan et al., 1985, *Science*, 229:81; Suresh et al., 1986, *Methods in Enzymol.*, 121:120; Traunecker et al., 1991, *EMBO J.*, 10:3655-3659; Shalaby et al., 1992, *J. Exp. Med.*, 175:217-225; Kostelny et al., 1992, *J. Immunol.*, 148:1547-1553; Gruber et al., 1994, *J. Immunol.*, 152:5368; U.S. Patent No. 5,731,168; and U.S. Patent Publication No. 2011/0123532. 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.

[0162] 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).

[0163] 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 (Huse et al., 1989, *Science*, 246:1275-1281) 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.

[0164] 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.

[0165] 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 (U.S. Patent No. 4,676,980). 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.

[0166] 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.

[0167] 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.

[0168] 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.

[0169] 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.

[0170] 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.

[0171] 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.

[0172] 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.

[0173] 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.

[0174] 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.

[0175] Thus, the present invention provides methods for producing an antibody. In some embodiments, the method for producing an antibody comprises using hybridoma techniques. In some embodiments, a method for producing an antibody that binds a human FZD protein is provided. In some embodiments, a method for producing an antibody that binds a human Wnt protein is provided. In some embodiments, the method of generating an antibody comprises screening a human phage library. In some

embodiments, the antibody is identified using a membrane-bound heterodimeric molecule comprising a single antigen-binding site. In some non-limiting embodiments, the antibody is identified using methods and polypeptides described in U.S. Patent Publication No. 2011/0287979.

[0176] The present invention further provides methods of identifying an antibody that binds at least one FZD protein. In some embodiments, the antibody is identified by screening by FACS for binding to a FZD protein or a portion thereof. In some embodiments, the antibody is identified by screening using ELISA for binding to a FZD protein. In some embodiments, the antibody is identified by screening by FACS for blocking of binding of a FZD protein to a human Wnt protein. In some embodiments, the antibody is identified by screening for inhibition or blocking of Wnt pathway signaling.

[0177] The present invention further provides methods of identifying an antibody that binds at least one Wnt protein. In some embodiments, the antibody is identified by screening by FACS for binding to a Wnt protein or a portion thereof. In some embodiments, the antibody is identified by screening using ELISA for binding to a Wnt protein. In some embodiments, the antibody is identified by screening by FACS for blocking of binding of a Wnt protein to a human FZD protein. In some embodiments, the antibody is identified by screening for inhibition or blocking of Wnt pathway signaling.

[0178] In some embodiments, a method of generating an antibody to at least one human FZD protein comprises screening an antibody-expressing library for antibodies that bind a human FZD protein. In some embodiments, the antibody-expressing library is a phage library. In some embodiments, the antibody-expressing library is a mammalian cell library. In some embodiments, the screening comprises panning. In some embodiments, antibodies identified in a first screening, are screened again using a different FZD protein thereby identifying an antibody that binds the first FZD protein and a second FZD protein. In some embodiments, the antibody identified in the screening binds the first FZD protein and at least one other FZD protein. In certain embodiments, the at least one other FZD protein is selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In certain embodiments, the antibody identified in the screening binds FZD1, FZD2, FZD5, FZD7, and FZD8. In some embodiments, the antibody identified in the screening is a FZD antagonist. In some embodiments, the antibody identified by the methods described herein inhibits the Wnt pathway. In some embodiments, the antibody identified in the screening inhibits β-catenin signaling.

[0179] In some embodiments, a method of generating an antibody to at least one human Wnt protein comprises screening an antibody-expressing library for antibodies that bind a human Wnt protein. In some embodiments, the antibody-expressing library is a phage library. In some embodiments, the antibody-expressing library is a mammalian cell library. In some embodiments, the screening comprises panning. In some embodiments, antibodies identified in a first screening, are screened again using a different Wnt protein thereby identifying an antibody that binds a first Wnt protein and a second Wnt protein. In some embodiments, the antibody identified in the screening binds a first Wnt protein and at

least one other Wnt protein. In certain embodiments, the at least one other FZD protein is selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b. In some embodiments, the antibody identified in the screening is a Wnt antagonist. In some embodiments, the antibody identified by the methods described herein inhibits the Wnt pathway. In some embodiments, the antibody identified in the screening inhibits β-catenin signaling.

[0180] In certain embodiments, the antibodies described herein are isolated. In certain embodiments, the antibodies described herein are substantially pure.

[0181] 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.

[0182] 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.

[0183] The isolated polypeptides 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.

[0184] 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.

[0185] 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.

[0186] 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.

[0187] 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.

[0188] 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 described by Pouwels et al. (1985, *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, NY). 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.

[0189] 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.

[0190] 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).

[0191] 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 OMP-54F28.

[0192] 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.

[0193] 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.

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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).

[0199] 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.

[0200] 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 OMP-54F28.

[0201] 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 OMP-54F28.

[0202] 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.

[0203] 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 OMP-54F28.

[0204] 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.

[0205] 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 OMP-54F28.

[0206] 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 OMP-54F28.

[0207] 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 OMP-54F28.

[0208] 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 OMP-54F28.

[0209] *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.

[0210] 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.

[0211] 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, hepatocellular tumor, thyroid tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, neuroendocrine 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 lung tumor. In some embodiments, the tumor is an ovarian tumor. In some embodiments, the tumor is a liver tumor. In certain embodiments, the tumor is a neuroendocrine tumor. In certain embodiments, the tumor is a Wnt-dependent tumor.

[0212] 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.

[0213] 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.

[0214] 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.

III. Methods of use and pharmaceutical compositions

[0215] The present invention provides methods of treating diseases such as cancer with a Wnt pathway inhibitor, while screening for, monitoring, reducing, preventing, attenuating, and/or mitigating side effects and/or toxicities, including, but not limited to skeletal-related side effects and/or toxicities associated with the Wnt pathway inhibitor. Side effects and/or toxicities associated with cancer treatment may include, but are not limited to, fatigue, vomiting, nausea, diarrhea, pain, hair loss, neutropenia, anemia, thrombocytopenia, cardiovascular-related complications, skeletal-related complications, and any combination thereof. As used herein, "skeletal-related complications" (e.g., skeletal-related side effects and/or toxicities) include but are not limited to, osteopenia, osteoporosis, bone fractures (including silent fractures), and combinations thereof. Thus, in some aspects and/or embodiments of the methods described herein, the screening for, monitoring, reducing, preventing, attenuating, and/or mitigating skeletal-related side effects and/or toxicities is screening for, monitoring, reducing, preventing, attenuating, and/or mitigating bone density loss and/or fracture risk. Often bone density loss is asymptomatic and/or early signs of skeletal-related side effects are not evident with, for example, bone density scanning.

[0216] Bone metabolism is a continuous dual process of bone formation and bone destruction. Bone destruction is referred to as bone resorption and is carried out by osteoclasts, while bone formation is carried out by osteoblasts. In adults, the dual processes of bone formation and bone destruction are in balance, maintaining a constant, homeostatically controlled amount of bone. Bone metabolism may be assessed and/or monitored by measurement of biomarkers (e.g., enzymes, proteins, and/or degradation products) released during bone formation and bone resorption. These biomarkers are often referred to as "bone turnover markers", and include bone formation markers and bone resorption markers. Bone formation biomarkers include serum total alkaline phosphatase, serum bone-specific alkaline phosphatase, serum osteocalcin, serum procollagen type 1 amino-terminal propeptide (P1NP) and serum procollagen type 1 carboxy-terminal propeptide (P1CP). Bone resorption biomarkers include, urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypryidinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), and tartrate-resistant acid phosphatase 5b.

[0217] Approximately 90% of the organic matrix of bone is type 1 collagen, a helical protein that is cross-linked at the N- and C-terminal ends of the molecule. During bone resorption, osteoclasts secrete a

mixture of acid and neutral proteases that degrade the collagen fibrils into molecular fragments including C-telopeptide (CTX). As bone ages, the alpha form of aspartic acid present in CTX converts to the beta form (β-CTX). β-CTX is released into the bloodstream during bone resorption and serves as a specific marker for the degradation of mature type 1 collagen.

[0218] Bone turnover markers have been used to monitor anti-resorptive therapies (e.g., hormone replacement therapies and bisphosphonate therapies) in post-menopausal women, as well as in individuals diagnosed with osteopenia. In addition, bone turnover markers may be used to assess drug-induced osteoporosis resulting from therapy with hormonal and non-hormonal drugs. These drugs may include, but are not limited to, glucocorticoids, thyroid hormone, aromatase inhibitors, ovarian suppressing agents, androgen deprivation therapy, thiazolidinediones, selective serotonin reuptake inhibitors, anticonvulsants, heparins, oral anticoagulants, loop diuretics, calcineurin inhibitors, anti-retroviral therapy, and proton pump inhibitors. Bone turnover markers have not previously been used to assess the effect of Wnt pathway inhibitors. Accordingly, in some embodiments, the present invention provides methods for using bone turnover markers to monitor skeletal-related side effects and/or toxicities in subjects being treated with a Wnt pathway inhibitor. In some embodiments, the methods use a bone formation biomarker to monitor and/or detect decreased levels of bone formation. In some embodiments, the methods use a bone resorption biomarker to monitor and/or detect increased levels of bone resorption. In some embodiments, monitoring the level of a bone formation biomarker gives an early indication of decreased levels of bone formation and/or increased risk of bone fracture, osteopenia, and/or osteoporosis. In some embodiments, monitoring the level of a bone resorption biomarker gives an early indication of increased levels of bone resorption and/or increased risk of bone fracture, osteopenia, and/or osteoporosis. In some embodiments, the methods detect skeletal-related side effects and/or toxicities prior to any evidence of skeletal dysfunction as evaluated by bone density scans.

[0219] In certain embodiments, the skeletal-related side effects and/or toxicities that are detected, identified, monitored, reduced, prevented, attenuated, and/or screened for are skeletal-related side effects and/or toxicities caused by, associated with, and/or related to administration of a Wnt pathway inhibitor or treatment with a Wnt pathway inhibitor. In certain embodiments, the skeletal-related side effects and/or toxicities are related to the Wnt pathway inhibitor. In certain embodiments, the skeletal-related side effects and/or toxicities are related to the activity of the Wnt pathway inhibitor. In certain embodiments, the skeletal-related side effects and/or toxicities are related to a Wnt pathway inhibitor that is an anti-FZD antibody. In certain embodiments, the skeletal-related side effects and/or toxicities are related to a Wnt pathway inhibitor that is anti-FZD antibody OMP-18R5. In certain embodiments, the skeletal-related side effects and/or toxicities are related to the Wnt pathway inhibitor that is a FZD soluble receptor. In certain embodiments, the skeletal-related side effects and/or toxicities are related to the Wnt pathway inhibitor

that is a FZD8-Fc soluble receptor. In certain embodiments, the skeletal-related side effects and/or toxicities are related to the Wnt pathway inhibitor that is FZD8-Fc soluble receptor OMP-54F28.

[0220] The invention provides methods for selecting a subject for treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample, and selecting the subject for treatment with the Wnt pathway inhibitor if the level of the biomarker is below a predetermined level. In some embodiments, the methods for selecting a subject for treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject, determining the level of a biomarker in the sample, and selecting the subject for treatment with the Wnt pathway inhibitor if the level of the biomarker is below a predetermined level. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the bone turnover marker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is β-CTX.

[0221] In some embodiments, the method of selecting a subject for treatment with a Wnt pathway inhibitor comprises: obtaining a biological sample from the subject, determining the level of a bone turnover marker in the sample, and selecting the subject for treatment with the Wnt pathway inhibitor if the level of the bone turnover marker is below a predetermined level. In some embodiments, the biological sample is urine, blood, serum, or plasma. In some embodiments, the bone turnover marker is a bone resorptive biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorptive biomarker is CTX or β-CTX. Thus, in some embodiments, the methods of selecting a subject for treatment with a Wnt pathway inhibitor, comprising: obtaining a biological sample from the subject, determining the level of β-CTX in the sample, and selecting the subject for treatment with the Wnt pathway inhibitor if the level of β-CTX is below a predetermined level.

[0222] The invention provides methods of identifying a subject as eligible for treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample, and identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the biomarker is below a predetermined level. In some embodiments, the methods of identifying a subject as eligible for treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject, determining the level of a biomarker in the sample, and identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the biomarker is below a predetermined level. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein

(BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the methods of identifying a subject as eligible for treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject, determining the level of β-CTX in the sample, and identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of β-CTX is below a predetermined level.

[0223] The invention also provides methods of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of skeletal-related side effects and/or toxicity, comprising: determining the level of a biomarker in a sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein an increase in the level of the biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the methods of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of skeletal-related side effects and/or toxicity comprise: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein an increase in the level of the biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, a method of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of skeletal-related side effects and/or toxicity, comprises: obtaining a biological sample from the subject receiving treatment, determining the level of β-CTX in the sample, and comparing the level of β-CTX in the sample to a predetermined level of β-CTX, wherein an increase in the level of β-CTX indicates development of skeletal-related side effects and/or toxicity.

[0224] The invention also provides methods of detecting the development of skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample, and comparing the level of a biomarker in the sample to a predetermined level of the biomarker, wherein an increase in the level of the biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the methods of detecting the development of skeletal-related side effects and/or toxicity in a subject receiving treatment

with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of a biomarker in the sample to a predetermined level of the biomarker, wherein an increase in the level of the biomarker indicates development of skeletal-related side effects and/or toxicity. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, the methods of detecting the development of skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject receiving treatment, determining the level of β-CTX in the sample, and comparing the level of β-CTX in the sample to a predetermined level of β-CTX, wherein an increase in the level of β-CTX indicates development of skeletal-related side effects and/or toxicity.

[0225] The invention also provides methods for identifying skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then a skeletal-related side effect and/or toxicity is indicated. In some embodiments, the methods for identifying skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then a skeletal-related side effect and/or toxicity is indicated. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments,

the bone resorption biomarker is β-CTX. In some embodiments, a method for identifying a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprises: obtaining a biological sample from the subject receiving treatment, determining the level of β-CTX in the sample, and comparing the level of β-CTX in the sample to a predetermined level of β-CTX, wherein if the level of β-CTX in the sample is higher than the predetermined level of β-CTX then a skeletal-related side effect and/or toxicity is indicated.

[0226] The invention also provides methods for monitoring skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then skeletal-related side effects and/or toxicity is indicated. In some embodiments, the methods for monitoring skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then skeletal-related side effects and/or toxicity is indicated. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, a method for monitoring cardiotoxicity in a subject receiving treatment with a Wnt pathway inhibitor comprises: obtaining a biological sample from the subject receiving treatment, determining the level of β-CTX in the sample, and comparing the level of β-CTX in the sample to a predetermined level of β-CTX, wherein if the level of β-CTX in the sample is higher than the predetermined level of β-CTX then a skeletal-related side effect and/or toxicity is indicated.

[0227] The invention also provides methods of reducing skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample from the subject, comparing the level of the biomarker in the sample to a predetermined level of the biomarker, and administering to the subject a therapeutically effective amount of an anti-resorptive medication such as a bisphosphonate if the level of the biomarker in the sample is higher than the predetermined level of the biomarker. In some embodiments, the methods of reducing

skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject receiving treatment, determining the level of a biomarker in the sample, comparing the level of the biomarker in the sample to a predetermined level of the biomarker, and administering to the subject a therapeutically effective amount of an anti-resorptive medication such as a bisphosphonate if the level of the biomarker in the sample is higher than the predetermined level of the biomarker. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, a method for reducing skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprises: obtaining a biological sample from the subject receiving treatment, determining the level of β-CTX in the sample, and comparing the level of β-CTX in the sample to a predetermined level of β-CTX, and administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of β-CTX in the sample is higher than the predetermined level of β-CTX. In some embodiments, the anti-resorptive medication is a bisphosphonate.

[0228] The invention also provides methods of preventing or attenuating the development of skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample from the subject, comparing the level of the biomarker in the sample to a predetermined level of the biomarker; administering to the subject a therapeutically effective amount of an anti-resorptive medication, and administering to the subject the Wnt pathway inhibitor. In some embodiments, the methods of preventing or attenuating the development of skeletal-related side effects and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor, determining the level of a biomarker in the sample, comparing the level of the biomarker in the sample to a predetermined level of the biomarker; administering to the subject a therapeutically effective amount of an anti-resorptive medication, and administering to the subject the Wnt pathway inhibitor. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption

biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, a method of preventing or attenuating the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor comprises: obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor, determining the level of β-CTX in the sample, comparing the level of β-CTX in the sample to a predetermined level of β-CTX; administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of β-CTX in the sample is higher than the predetermined level of β-CTX; and administering to the subject the Wnt pathway inhibitor.

[0229] In some embodiments of the methods described herein, the predetermined level is about 1500pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 1200pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 1000pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 800pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 600pg/ml or less in a blood, serum, or plasma sample. In some embodiments, the predetermined level is about 400pg/ml or less in a blood, serum, or plasma sample. In the context of predetermined levels of β-CTX, the term "about" means the referenced amount plus or minus 10% of that referenced amount.

[0230] In some embodiments, the predetermined level of a biomarker (e.g., a bone resorption biomarker or β-CTX) is the amount of the biomarker in a sample obtained at an earlier date. In some embodiments, the predetermined level of a biomarker (e.g., a bone resorption biomarker or β-CTX) is the amount of the biomarker in a sample obtained at an initial screening. In some embodiments, the predetermined level of a biomarker (e.g., a bone resorption biomarker or β-CTX) is the amount of the biomarker in a sample obtained prior to treatment. In some embodiments, the predetermined level of a biomarker (e.g., a bone resorption biomarker or β-CTX) is the amount of the biomarker in a sample obtained at an initial screening. In some embodiments, the predetermined level of a biomarker (e.g., a bone resorption biomarker or β-CTX) is a normal reference level. In some embodiments, the predetermined level of a biomarker (e.g., a bone resorption biomarker or β-CTX) is a baseline level. In some embodiments, the baseline level is the amount of the biomarker determined at an initial screening. In some embodiments, the baseline level is the amount of the biomarker determined prior to treatment.

[0231] In some embodiments, if the β-CTX level in the sample is increased 2-fold or greater (i.e., a doubling or greater) as compared to a predetermined level, the subject is administered a therapeutically effective amount of an anti-resorptive medication. In some embodiments, if the β-CTX level in the

sample is increased 2-fold or greater (i.e., a doubling or greater) as compared to a baseline level, the subject is administered a therapeutically effective amount of an anti-resorptive medication.

[0232] In any of the methods described herein, a biological sample is obtained approximately every week, every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, or every 6 weeks.

[0233] In some embodiments of any of the methods described herein, the subjects are evaluated using a DEXA (dual energy X-ray absorptiometry) bone density scan. This technique is the most commonly used test for measuring bone mineral density (BMD). The DEXA output includes a T-score, which compares the subject's bone density to a 30-35 year old person, and a Z-score, which compares the subject's bone density to the average bone density of someone their age and gender. The T-score is used to determine if an individual has osteopenia or osteoporosis according to a standard scale. A T-score greater than -1 is considered normal bone density; a T-score between -1 and -2.5, is considered osteopenia; a T-score less than -2.5 is considered osteoporosis; and a T-score less than -2.5 and 1+ osteoporotic fractures is considered severe (established) osteoporosis. In some embodiments, a skeletal-related side effect and/or toxicity is indicated if the T-score declines to less than -2.5 in the total femur or vertebrae L1-L4. In some embodiments, a skeletal-related side effect and/or toxicity is indicated if the T-score declines to less than -2.0 in the total femur or vertebrae L1-L4. In some embodiments, a skeletal-related side effect and/or toxicity is indicated if the T-score declines to less than -1.5 in the total femur or vertebrae L1-L4. In some embodiments, a skeletal-related side effect and/or toxicity is indicated if the T-score declines to less than -1.0 in the total femur or vertebrae L1-L4.

[0234] The invention also provides methods of ameliorating skeletal-related side effects and/or toxicity in a subject administered a Wnt pathway inhibitor, comprising: administering to the subject a therapeutically effective amount of an anti-resorptive medication.

[0235] The invention also provides methods of screening a subject for the risk of skeletal-related side effects and/or toxicity from treatment with a Wnt pathway inhibitor, comprising: determining the level of a biomarker in a sample from the subject, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then the subject is at risk for skeletal-related side effects and/or toxicity. In some embodiments, the methods of screening a subject for the risk of skeletal-related side effects and/or toxicity from treatment with a Wnt pathway inhibitor comprise: obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor, determining the level of a biomarker in the sample, and comparing the level of the biomarker in the sample to a predetermined level of the biomarker, wherein if the level of the biomarker in the sample is higher than the predetermined level of the biomarker then the subject is at risk for skeletal-related side effects and/or toxicity. In some embodiments, the skeletal-related side effect and/or toxicity is an increased risk of bone fracture. In some embodiments, the skeletal-related side effect and/or toxicity is osteopenia or osteoporosis. In some

embodiments, the biomarker is a bone turnover marker. In some embodiments, the biomarker is a bone resorption biomarker. In some embodiments, the bone resorption biomarker is urinary hydroxyproline, urinary total pyridinoline (PYD), urinary free deoxypyridinoline (DPD), urinary collagen type 1 cross-linked N-telopeptide (NTX), urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), bone sialoprotein (BSP), or tartrate-resistant acid phosphatase 5b. In some embodiments, the bone resorption biomarker is CTX. In some embodiments, the bone resorption biomarker is β-CTX. In some embodiments, a method of screening a subject for the risk of a skeletal-related side effect and/or toxicity from treatment with a Wnt pathway inhibitor comprises: obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor, determining the level of β-CTX in the sample, and comparing the level of β-CTX in the sample to a predetermined level of β-CTX, wherein if the level of β-CTX in the sample is higher than the predetermined level of β-CTX then the subject is at risk for a skeletal-related side effect and/or toxicity. In some embodiments, the predetermined level of β-CTX is a value determined at an initial screening. In some embodiments, the predetermined level of β-CTX is from about 400 to 1200pg/ml. In some embodiments, if the subject is at risk for a skeletal-related side effect and/or toxicity, the subject is administered a therapeutically effective amount of an anti-resorptive medication prior to treatment with the Wnt pathway inhibitor.

[0236] In some embodiments of the methods described herein, the anti-resorptive medication is a bisphosphonate. It is believed that bisphosphonates prevent loss of bone mass by "inducing" osteoclasts to undergo apoptosis and thereby inhibiting the digestion of bone. In some embodiments, the bisphosphonate is selected from the group consisting of: etidronate, clodronate, tiludronate, pamidronate, neridronate, olpadronate, alendronate (FOSAMAX), ibandronate (BONIVA), risedronate (ACTONEL), and zoledronic acid (RECLAST). In some embodiments, the bisphosphonate is zoledronic acid. In some embodiments, the anti-resorptive medication is anti-RANKL antibody denosumab (PROLIA).

[0237] In any of the methods described herein, the Wnt pathway inhibitor is an anti-FZD antibody. In any of the methods described herein, the Wnt pathway inhibitor is an anti-Wnt antibody. In any of the methods described herein, the Wnt pathway inhibitor is a FZD soluble receptor.

[0238] In certain embodiments of any of the methods described herein, 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).

[0239] In certain embodiments of any of the methods described herein, 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.

[0240] In certain embodiments, the Wnt pathway inhibitor comprises the same heavy chain variable region and the same light chain variable region sequences as OMP-18R5. In some embodiments, the Wnt pathway inhibitor is antibody OMP-18R5. OMP-18R5 is an IgG2 human monoclonal antibody that binds human FZD1, FZD2, FZD5, FZD7, and FZD8 receptors and has been previously described in U.S. Patent No. 7,982,013.

[0241] In certain embodiments, the Wnt pathway inhibitor comprises the same heavy and light chain amino acid sequences as an antibody encoded by a plasmid deposited with ATCC having deposit no. PTA-9541. In certain embodiments, the Wnt pathway inhibitor is encoded by the plasmid having ATCC deposit no. PTA-9541 which was deposited with American Type Culture Collection (ATCC), at 10801 University Boulevard, Manassas, VA, 20110, under the conditions of the Budapest Treaty on September 29, 2008. In certain embodiments, the Wnt pathway inhibitor competes for specific binding to a human FZD with an antibody encoded by the plasmid deposited with ATCC having deposit no. PTA-9541.

[0242] In certain embodiments of any of the methods described herein, the Wnt pathway inhibitor is a FZD soluble receptor. In some embodiments, the Wnt pathway inhibitor is a FZD8 soluble receptor comprising SEQ ID NO:20, SEQ ID NO:30, or SEQ ID NO:33. In some embodiments, the Wnt pathway inhibitor is a FZD8 soluble receptor comprising SEQ ID NO:20. In some embodiments, the Wnt pathway inhibitor is a FZD8 soluble receptor comprising SEQ ID NO:30. In some embodiments, the Wnt pathway inhibitor is a FZD8 soluble receptor comprising SEQ ID NO:33.

[0243] In certain embodiments of any of the methods described herein, the Wnt pathway inhibitor is a FZD-Fc soluble receptor. In some embodiments, the Wnt pathway inhibitor is a FZD8-Fc soluble receptor. In some embodiments, the Wnt pathway inhibitor is a FZD8-Fc soluble receptor comprising SEQ ID NO:39, SEQ ID NO:40, or SEQ ID NO:41. In some embodiments, the Wnt pathway inhibitor is a FZD8-Fc soluble receptor comprising SEQ ID NO:39. In some embodiments, the Wnt pathway inhibitor is a FZD8-Fc soluble receptor comprising SEQ ID NO:40. In some embodiments, the Wnt pathway inhibitor is a FZD8-Fc soluble receptor comprising SEQ ID NO:41. In some embodiments, the Wnt pathway inhibitor is OMP-54F28. In some embodiments, the Wnt pathway inhibitor is not OMP-54F28.

[0244] In some embodiments, the subject has cancer. In some embodiments, the cancer is selected from the group consisting of: lung cancer, breast cancer, colon cancer, colorectal cancer, melanoma, pancreatic cancer, gastrointestinal cancer, renal cancer, ovarian cancer, liver cancer, hepatocellular carcinoma (HCC), endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroendocrine cancer, neuroblastoma, glioma, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, and head and neck cancer. As used herein, "lung cancer" refers to all lung cancers including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). In certain embodiments, the cancer is a hematological cancer, such as a lymphoma or leukemia. In some embodiments, the cancer is

breast cancer. In certain embodiments, the cancer is NSCLC. In certain embodiments, the cancer is ovarian cancer. In certain embodiments, the cancer is pancreatic cancer. In some embodiments, the cancer is liver cancer. In certain embodiments, the cancer is not a neuroendocrine cancer.

[0245] Thus, the invention also provides methods of treating cancer. In some embodiments, the methods comprise a method of treating cancer in a subject in need thereof, comprising: (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; and (b) determining the level of a bone resorption biomarker in a sample from the subject. In some embodiments, a method of treating cancer comprises (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; (b) determining the level of a bone resorption biomarker in a sample from the subject; and (c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker. In some embodiments, a method of treating cancer comprises (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; (b) determining the level of a bone resorption biomarker in a sample from the subject; and (c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity. In some embodiments, a method of treating cancer comprises (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; (b) determining the level of a bone resorption biomarker in a sample from the subject; and (c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is administered a therapeutically effective amount of an anti-resorptive medication.

[0246] The invention also provides methods of inhibiting tumor growth. In some embodiments, the methods comprise a method of inhibiting tumor growth in a subject in need thereof, comprising: (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; and (b) determining the level of a bone resorption biomarker in a sample from the subject. In some embodiments, a method of inhibiting tumor growth comprises (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; (b) determining the level of a bone resorption biomarker in a sample from the subject; and (c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker. In some embodiments, a method of inhibiting tumor growth comprises (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; (b) determining the level of a bone resorption biomarker in a sample from the subject; and (c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-

related side effect and/or toxicity. In some embodiments, a method of inhibiting tumor growth comprises (a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; (b) determining the level of a bone resorption biomarker in a sample from the subject; and (c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is administered a therapeutically effective amount of an anti-resorptive medication.

[0247] In some embodiments, the biological sample is a body fluid. In some embodiments, the biological sample is blood, plasma, serum, or urine. In some embodiments, the biological sample is a venous whole blood specimen. In some embodiments, the biological sample is a venous whole blood specimen using EDTA or heparin as an anticoagulant. In some embodiments, the biological sample is a plasma specimen. In some embodiments, the biological sample is a plasma specimen using EDTA or heparin as an anticoagulant. Samples of body fluids may be obtained by any method known in the art. In some embodiments, the biological sample is a frozen tissue sample or is fresh tissue sample.

[0248] Assays for measuring or determining the level of a bone resorption biomarker (e.g., β-CTX) in a sample are known to those of skilled in the art. For example, in some embodiments an immunoassay that quantitatively measures β-CTX levels in whole blood or plasma specimens is used. In some embodiments, the sample contains EDTA as an anticoagulant. In some embodiments, the sample contains heparin as an anticoagulant. In some embodiments, the immunoassay comprises two highly specific monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR of β-CTX, wherein the aspartic acid residue is β-isomerized. In order to obtain a specific signal in the immunoassay, two chains of EKAHD-β-GGR must be cross-linked. In some embodiments, a sample and appropriate controls are placed into streptavidin-coated microtiter wells, followed by a solution containing biotinylated monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR of β-CTX. After incubation and washing, a chromogenic substrate solution is added to microtiter wells. After incubation, the reaction is stopped. Absorbance of the microtiter wells is read and the β-CTX concentration is determined.

[0249] In some embodiments, the Wnt pathway inhibitor is administered as an initial dose of about 0.5mg/kg. For example, antibody OMP-18R5 is diluted with 5% dextrose in water (USP) to a total volume of 250mL. The OMP-18R5 is delivered through a 0.22-micron filter over 30 minutes as an intravenous infusion. In some embodiments, subsequent doses are administered in a similar manner.

[0250] In another aspect of the invention, the methods described herein may further comprise administering one or more additional therapeutic agents. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the Wnt pathway inhibitor. Pharmaceutical compositions comprising a Wnt pathway inhibitor and an additional therapeutic

agent(s) are also provided. In some embodiments, the one or more additional therapeutic agents comprise 1, 2, 3, or more additional therapeutic agents.

[0251] Combination therapy with at least two therapeutic agents often uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action may result in additive or synergetic effects. Combination therapy may allow for a lower dose of each agent than is used in monotherapy, thereby reducing side effects and/or toxicities. Combination therapy may increase the therapeutic index of one or both of the therapeutic agents. Combination therapy may decrease the likelihood that resistant cancer cells will develop. In some embodiments, combination therapy comprises a therapeutic agent that primarily affects (e.g., inhibits or kills) non-tumorigenic cells and a therapeutic agent that primarily affects (e.g., inhibits or kills) tumorigenic CSCs. Thus, in some embodiments, the Wnt pathway inhibitor is administered in combination with at least one additional therapeutic agent. In some embodiments, an anti-FZD antibody is administered in combination with at least one additional therapeutic agent. In some embodiments, the anti-FZD antibody OMP-18R5 is administered in combination with at least one additional therapeutic agent. In some embodiments, a FZD soluble receptor is administered in combination with at least one additional therapeutic agent. In some embodiments, the FZD8-Fc soluble receptor is administered in combination with at least one additional therapeutic agent.

[0252] Therapeutic agents that may be administered in combination with the Wnt pathway inhibitor 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 antibody or soluble receptor) 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.

[0253] Chemotherapeutic agents useful in the instant invention include, but are not limited to, alkylating agents such as thiotepa and cyclophosphamide (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 cisplatin. In certain embodiments, the additional therapeutic agent is carboplatin. In certain embodiments, the additional therapeutic agent is paclitaxel. In certain embodiments, where the chemotherapeutic agent administered in combination with a Wnt pathway inhibitor is carboplatin, the cancer or tumor being treated is lung cancer or a lung tumor.

[0254] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapeutic 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. In certain embodiments, the additional therapeutic agent is irinotecan.

[0255] 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. In certain embodiments, the additional therapeutic agent is gemcitabine. In some embodiments, the additional therapeutic agent is pemetrexed. In certain embodiments, where the chemotherapeutic agent administered in combination with a Wnt pathway inhibitor is gemcitabine, the cancer or tumor being treated is pancreatic cancer or a pancreatic tumor. In certain embodiments, where the chemotherapeutic agent administered in combination with a Wnt pathway inhibitor is pemetrexed, the cancer or tumor being treated is lung cancer or a lung tumor. In some embodiments, the Wnt pathway inhibitor is administered in combination with pemetrexed and carboplatin. In some embodiments, an anti-FZD antibody or a FZD soluble receptor is administered in combination with gemcitabine to treat pancreatic cancer. In some embodiments, the anti-FZD antibody OMP-18R5 or the FZD8-Fc soluble receptor OMP-54F28 is administered in combination with gemcitabine to treat pancreatic cancer. In some embodiments, an anti-FZD antibody or a FZD soluble receptor is administered in combination with gemcitabine and albumin-bound paclitaxel to treat pancreatic cancer. In some embodiments, the anti-FZD antibody OMP-18R5 or the FZD8-Fc soluble receptor OMP-54F28 is administered in combination with gemcitabine and albumin-bound paclitaxel to treat pancreatic cancer. In some embodiments, an anti-FZD antibody or a FZD soluble receptor is administered in combination with carboplatin and paclitaxel or albumin-bound paclitaxel to treat ovarian cancer. In some embodiments, the anti-FZD antibody OMP-18R5 or the FZD8-Fc soluble receptor OMP-54F28 is administered in combination with carboplatin and paclitaxel or albumin-bound paclitaxel to treat ovarian cancer.

[0256] 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 (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 some embodiments, an anti-FZD antibody or a FZD soluble receptor is administered in combination with paclitaxel or albumin-bound paclitaxel to treat breast cancer. In some embodiments, the anti-FZD antibody OMP-18R5 or the FZD8-Fc soluble receptor OMP-54F28 is administered in combination with paclitaxel or albumin-bound paclitaxel to treat breast cancer. 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 lung cancer. In some embodiments, an anti-FZD antibody or a FZD soluble receptor is administered in combination with docetaxel to treat lung cancer. In some embodiments, the anti-FZD antibody OMP-18R5 or the FZD8-Fc soluble receptor OMP-54F28 is administered in combination with docetaxel to treat lung cancer.

[0257] 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 (e.g. an antibody) of the present invention with a small molecule that acts as an inhibitor against additional tumor-associated proteins including, but not limited to, EGFR, ErbB2, HER2, and/or VEGF. In certain embodiments, the additional therapeutic agent is a small molecule that inhibits protein kinases. In certain embodiments, the additional therapeutic agent is a small molecule that inhibits tyrosine protein kinases. In some embodiments, an anti-FZD antibody or a FZD soluble receptor is administered in combination with a protein kinase inhibitor (e.g., sorafenib) to treat liver cancer, (e.g., HCC). In some embodiments, the anti-FZD antibody OMP-18R5 or the FZD8-Fc soluble receptor OMP-54F28 is administered in combination with a protein kinase inhibitor (e.g., sorafenib) to treat liver cancer, (e.g., HCC). 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 a small molecule inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is a small molecule inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is a small molecule inhibitor of the BMP pathway. In some embodiments, the additional therapeutic agent is a small molecule that inhibits β-catenin signaling.

[0258] In some embodiments, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of a Wnt pathway inhibitor (e.g. an antibody) of the present invention with other antibodies against additional tumor-associated proteins including, but not limited to, antibodies that bind EGFR, ErbB2, HER2, and/or VEGF.

In certain embodiments, the additional therapeutic agent is an antibody that is an anti-cancer stem cell marker antibody. In some embodiments, the additional therapeutic agent is an antibody that binds a component of the Notch pathway. In some embodiments, the additional therapeutic agent is an antibody that binds a component of the Wnt pathway. In certain embodiments, the additional therapeutic agent is an antibody that inhibits a cancer stem cell pathway. In some embodiments, the additional therapeutic agent is an antibody inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is an antibody inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is an antibody inhibitor of the BMP pathway. In some embodiments, the additional therapeutic agent is an antibody that inhibits β-catenin signaling. In certain embodiments, the additional therapeutic agent is an antibody that is an angiogenesis inhibitor or modulator (e.g., an anti-VEGF or VEGF receptor antibody). In certain embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), trastuzumab (HERCEPTIN), panitumumab (VECTIBIX), or cetuximab (ERBITUX). 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.

[0259] Furthermore, treatment with a Wnt pathway inhibitor described herein can include combination treatment with other biologic molecules, such as one or more cytokines (e.g., lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of tumors, cancer cells, or any other therapy deemed necessary by a treating physician.

[0260] It will be appreciated that the combination of a Wnt pathway inhibitor and an additional therapeutic agent may be administered in any order or concurrently. In some embodiments, the Wnt pathway inhibitor is administered to subjects that have previously undergone treatment with a second therapeutic agent. In certain other embodiments, the Wnt pathway inhibitor and a second therapeutic agent is administered substantially simultaneously or concurrently. For example, a subject may be given a Wnt pathway inhibitor (e.g., an antibody) while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In certain embodiments, a Wnt pathway inhibitor is administered within 1 year of the treatment with a second therapeutic agent. In certain alternative embodiments, a Wnt pathway inhibitor is administered within 10, 8, 6, 4, or 2 months of any treatment with a second therapeutic agent. In certain other embodiments, a Wnt pathway inhibitor is administered within 4, 3, 2, or 1 weeks of any treatment with a second therapeutic agent. In some embodiments, a Wnt pathway inhibitor is administered within 5, 4, 3, 2, or 1 days of any treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[0261] As is known to those of skill in the art, administration of any therapeutic agent may lead to side effects and/or toxicities. In some cases, the side effects and/or toxicities are so severe as to preclude

administration of the particular agent at a therapeutically effective dose. In some cases, drug therapy must be discontinued, and other agents may be tried. However, many agents in the same therapeutic class often display similar side effects and/or toxicities, meaning that the subject either has to stop therapy, or if possible, suffer from the unpleasant side effects associated with the therapeutic agent.

[0262] Side effects from therapeutic agents may include, but are not limited to, hives, skin rashes, itching, nausea, vomiting, decreased appetite, diarrhea, chills, fever, fatigue, muscle aches and pain, headaches, low blood pressure, high blood pressure, hypokalemia, low blood counts, bleeding, and cardiac problems.

[0263] Thus, in some embodiments, the methods described herein include using an intermittent dosing regimen, which may reduce side effects and/or toxicities associated with administration of a Wnt pathway inhibitor. As used herein, "intermittent dosing" refers to a dosing regimen using a dosing interval of more than once a week, e.g., dosing once every 2 weeks, once every 3 weeks, once every 4 weeks, etc. In some embodiments, a method for treating a subject comprises administering to the subject an effective dose of a Wnt pathway inhibitor (e.g., an anti-FZD antibody or a FZD soluble receptor) according to an intermittent dosing regimen. In some embodiments, the method comprises administering to the subject an effective dose of a Wnt pathway inhibitor (e.g., an anti-FZD antibody or a FZD soluble receptor) according to an intermittent dosing regimen, and increasing the therapeutic index of the Wnt pathway inhibitor. In some embodiments, the intermittent dosing regimen comprises administering an initial dose of a Wnt pathway inhibitor to the subject, and administering subsequent doses of the Wnt pathway inhibitor about once every 2 weeks. In some embodiments, the intermittent dosing regimen comprises administering an initial dose of a Wnt pathway inhibitor to the subject, and administering subsequent doses of the Wnt pathway inhibitor about once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises administering an initial dose of a Wnt pathway inhibitor to the subject, and administering subsequent doses of the Wnt pathway inhibitor about once every 4 weeks.

[0264] In some embodiments, the subsequent doses in an intermittent dosing regimen are about the same amount or less than the initial dose. In other embodiments, the subsequent doses are a greater amount than the initial dose. As is known by those of skill in the art, doses used will vary depending on the clinical goals to be achieved. In some embodiments, the initial dose is about 0.25mg/kg to about 20mg/kg. In some embodiments, the initial dose is about 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20mg/kg. In certain embodiments, the initial dose is about 0.5mg/kg. In certain embodiments, the initial dose is about 1mg/kg. In certain embodiments, the initial dose is about 2.5mg/kg. In certain embodiments, the initial dose is about 5mg/kg. In certain embodiments, the initial dose is about 7.5mg/kg. In certain embodiments, the initial dose is about 10mg/kg. In certain embodiments, the initial dose is about 12.5mg/kg. In certain embodiments, the initial dose is about 15mg/kg. In certain embodiments, the initial dose is about 20mg/kg. In some embodiments, the

subsequent doses are about 0.25mg/kg to about 20mg/kg. In certain embodiments, the subsequent doses are about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20mg/kg. In certain embodiments, the subsequent doses are about 0.5mg/kg. In certain embodiments, the subsequent doses are about 1mg/kg. In certain embodiments, the subsequent doses are about 2.5mg/kg. In certain embodiments, the subsequent doses are about 5mg/kg. In some embodiments, the subsequent doses are about 7.5mg/kg. In some embodiments, the subsequent doses are about 10mg/kg. In some embodiments, the subsequent doses are about 12.5mg/kg. In some embodiments, the subsequent doses are about 15mg/kg. In some embodiments, the subsequent doses are about 20mg/kg.

[0265] In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 2.5mg/kg and (b) administering subsequent doses of about 2.5mg/kg once every 2 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 5mg/kg and (b) administering subsequent doses of about 5mg/kg once every 2 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 2.5mg/kg and (b) administering subsequent doses of about 2.5mg/kg once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 5mg/kg and (b) administering subsequent doses of about 5mg/kg once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 10mg/kg and (b) administering subsequent doses of about 10mg/kg once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 15mg/kg and (b) administering subsequent doses of about 15mg/kg once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 20mg/kg and (b) administering subsequent doses of about 20mg/kg once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 2.5mg/kg and (b) administering subsequent doses of about 2.5mg/kg once every 4 weeks. In some embodiments, the intermittent dosing regimen comprises: (a) administering to the subject an initial dose of a Wnt pathway inhibitor of about 5mg/kg and (b) administering subsequent doses of about 5mg/kg once every 4 weeks. In certain embodiments, the initial dose and the maintenance doses are different, for example, the initial dose is about 5mg/kg and the subsequent doses are about 2.5mg/kg. In certain embodiments, an intermittent dosing regimen may comprise a loading dose, for example, the initial dose is about 20mg/kg and the subsequent doses are about 2.5mg/kg or about 5mg/kg administered once every 2 weeks, once every 3 weeks, or once every 4 weeks.

[0266] In some embodiments of the methods described herein, a method of treating cancer comprises administering a therapeutically effective amount of OMP-18R5 to a subject in need thereof at a dosage of (a) at least about 0.5mg/kg about every one to two weeks or (b) at least about 1.0mg/kg about every three weeks. In some embodiments, a method of treating cancer comprises administering a therapeutically effective amount of OMP-18R5 to a subject in need thereof at a dosage of about 0.5mg/kg to about 1.0mg/kg about every one to two weeks. In some embodiments, a method of treating cancer comprises administering a therapeutically effective amount of OMP-18R5 to a subject in need thereof at a dosage of about 1.0mg/kg to about 10.0 mg/kg about every three weeks. In some embodiments, a method of treating cancer comprises administering a therapeutically effective amount of OMP-18R5 to a subject in need thereof at a dosage of about 10mg/kg to about 20.0 mg/kg about every three weeks.

[0267] In certain embodiments, the method for treating cancer in a human patient comprises administering to the patient a dose of a Wnt pathway inhibitor once every 3 weeks, and repeating this administration for a total of 3, 4, 5, 6, 7, 8, or more cycles. In certain embodiments, the method for treating cancer in a human patient comprises administering to the patient a dose of a Wnt pathway inhibitor of about 10mg/kg once every 3 weeks, and repeating this administration for a total of 3, 4, 5, 6, 7, 8, or more cycles. In certain embodiments, the method for treating cancer in a human patient comprises administering to the patient a dose of a Wnt pathway inhibitor of about 15mg/kg once every 3 weeks, and repeating this administration for a total of 3, 4, 5, 6, 7, 8, or more cycles. In certain embodiments, the method for treating cancer in a human patient comprises administering to the patient a dose of a Wnt pathway inhibitor of about 20mg/kg once every 3 weeks, and repeating this administration for a total of 3, 4, 5, 6, 7, 8, or more cycles. In some embodiments, the administration is repeated for 4 cycles. In some embodiments, the administration is repeated for 5 cycles. In some embodiments, the administration is repeated for 6 cycles. In some embodiments, the administration is repeated for 7 cycles. In some embodiments, the administration is repeated for 8 cycles.

[0268] Another aspect of the present invention is directed to methods for reducing toxicity of a Wnt pathway inhibitor in a human subject comprises administering to the subject the Wnt pathway inhibitor using an intermittent dosing regimen. Another aspect of the present invention is directed to methods for reducing side effects of a Wnt pathway inhibitor in a human subject comprises administering to the subject the Wnt pathway inhibitor using an intermittent dosing regimen. Another aspect of the present invention is directed to methods for increasing the therapeutic index of a Wnt pathway inhibitor in a human subject comprises administering to the subject the Wnt pathway inhibitor using an intermittent dosing regimen.

[0269] The choice of delivery method for the initial and subsequent doses is made according to the ability of the subject to tolerate introduction of the Wnt pathway inhibitor into the body. Thus, in any of the aspects and/or embodiments described herein, the administration of the Wnt pathway inhibitor may be

by intravenous injection or intravenously. In some embodiments, the administration is by intravenous infusion. In any of the aspects and/or embodiments described herein, the administration of the Wnt pathway inhibitor may be by a non-intravenous route.

[0270] In certain embodiments, the treatment involves the administration of a Wnt pathway inhibitor (e.g. an antibody) of the present invention in combination with radiation therapy. Treatment with a Wnt pathway inhibitor can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Dosing schedules for such radiation therapy can be determined by the skilled medical practitioner.

[0271] Embodiments of the present disclosure can be further defined by reference to the following non-limiting examples, which describe the use of a Wnt pathway inhibitor for treatment of cancer. 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

Intermittent dosing with anti-FZD antibody OMP-18R5 in a breast xenograft model and effect on tumor growth

[0272] UM-PE13 breast tumor cells (20,000 cells) were injected subcutaneously into 6-8 week old NOD/SCID mice. The animals were randomized into groups (n = 10 per group) and treated with anti-FZD antibody OMP-18R5 in combination with paclitaxel (Taxol) and paclitaxel alone. Paclitaxel was administered at 10mg/kg weekly and OMP-18R5 was administered at doses of 5, 10, 25, or 45mg/kg once every 3 weeks. The agents were administered intraperitoneally. Tumor volumes were measured on the indicated days with electronic calipers.

[0273] As shown in Figure 1, OMP-18R5 in combination with paclitaxel administered every 3 weeks was efficacious in reducing PE-13 tumor growth at doses as low as 5mg/kg or 10mg/kg. This tumor growth inhibition was greater than the growth inhibition seem with paclitaxel alone when administered weekly. Higher doses of OMP-18R5, 25mg/kg and 45mg/kg, in combination with paclitaxel inhibited tumor growth to an even greater extent and tumor regression was observed at later time points. These results demonstrate that the efficacy of anti-FZD antibody treatment in combination with a chemotherapeutic agent such as paclitaxel is maintained with intermittent dosing regimens.

Example 2

Effect of intermittent dosing with anti-FZD antibody OMP-18R5 on bone formation

[0274] UM-PE13 breast tumor cells (20,000 cells) were injected subcutaneously into 6-8 week old NOD/SCID mice. The animals were randomized into groups (n = 10 per group) and treated with anti-FZD antibody OMP-18R5 in combination with paclitaxel (Taxol) or paclitaxel alone. Paclitaxel was administered at 15mg/kg once a week and OMP-18R5 was administered at 25mg/kg once every 4 weeks, once every 2 weeks or once a week. The agents were administered intraperitoneally. Tumor volumes were measured on the indicated days with electronic calipers.

[0275] As shown in Figure 2, OMP-18R5 in combination with paclitaxel administered at 25mg/kg was efficacious in reducing PE-13 tumor growth with dosing once a week, once every 2 weeks, and once every 4 weeks. Tumor growth inhibition with OMP-18R5 in combination with paclitaxel was greater than the growth inhibition seen with paclitaxel alone.

[0276] At the ending of dosing on day 77, trabecular bone formation was assessed in the OMP-18R5 treated mice as compared to mice treated with control (paclitaxel alone).

[0277] Tissue sections were prepared from the tibia of control and OMP-18R5-treated mice and stained with hemotoxylin and eosin (H&E). The light pink staining regions highlighted by the white arrows correspond to trabecular bone.

[0278] As observed in Figure 3, there was a reduction in bone loss with treatment of OMP-18R5 at 25mg/kg once every 2 weeks as compared to treatment of 25mg/kg once every week. Importantly, treatment of OMP-18R5 at 25mg/kg every 4 weeks appeared to have no perceptible effect on bone formation.

Example 3

Effect of zolendronic acid in reducing the effect of OMP-18R5 on bone formation

[0279] NOD/SCID mice were randomized into groups (n = 5 per group) and treated with anti-FZD antibody OMP-18R5 or OMP-18R5 in combination with zolendronic acid. Mice were treated with 20mg/kg OMP-18R5 on days 1 and 15 only, or 20mg/kg OMP-18R5 on days 1 and 15 in combination with a single IV dose of 100ug/kg zoledronic acid on day 1. At the end of dosing on day 29, femurs and tibias from mice treated with OMP-18R5 alone were compared to femurs and tibias from mice treated with the combination of OMP-18R5 and zoledronic acid and to mice treated with a control antibody.

[0280] Tissues sections of femur and tibia were prepared as described in Example 2.

[0281] As shown in Figure 4, a single IV administration of zoledronic acid to mice treated with OMP-18R5 resulted in subchondral bone formation comparable to mice treated with a control antibody. Additional studies have demonstrated that co-administration of zolendronic acid does not affect the anti-

tumor efficacy of OMP-18R5. These data support the hypothesis that bisphosphonate administration may be protective against the catabolic effects of Wnt inhibition, providing a path to preserve bone integrity and allow the benefits of targeting the Wnt pathway.

Example 4

Phase 1a Study of OMP-18R5 in patients with solid tumors

[0282] The study is an open-label Phase 1a dose-escalation study of OMP-18R5 in patients with a solid tumor for which there is no remaining standard curative therapy and no therapy with a demonstrated survival benefit. The primary objectives of the study are to determine the safety and the maximum tolerated dose of OMP-18R5. The secondary objectives are to determine the rate of immunogenicity, the preliminary efficacy, and the pharmacokinetics of OMP-18R5.

[0283] The patients in the initial portion of the trial were treated with a dosing regimen of OMP-18R5 of 0.5mg/kg every week (n = 3) and 1.0mg/kg every week (n = 5). One patient who received 0.5mg/kg once a week developed fractures of their anterior ribs and lumbar spine after receiving study drug for approximately 100 days. As a result, in the current phase of the trial (study is ongoing and patients are still being enrolled) less frequent dosing is being utilized. Specifically, the dose levels are 0.5mg/kg once every two weeks (n = 3), and 1mg/kg (n = 4), 2.5 mg/kg (n = 3), 5mg/kg (n = 3), 10 mg/kg (n = 3), 15mg/kg (n = 5), and 20mg/kg (n = 5) once every 3 weeks (as of January 10, 2014). Cohorts of 3 subjects are treated and evaluated for dose-limiting toxicities (DLTs) through Day 28. If 0 of 3 subjects have a DLT, escalation to the next dose cohort occurs. If 1 of 3 subjects experiences a DLT, 3 additional subjects are treated. If 2 or more subjects experience a DLT, no further subjects are dosed at that level and 3 additional subjects are added to the preceding dose cohort unless 6 subjects have already been treated at that dose level. Tumor assessments are performed on Day 56 and then every 56 days thereafter. Patients with stable disease or a response at Day 56 will be allowed to continue to receive OMP-18R5 until disease progression.

[0284] After a patient experienced a skeletal-related (bone fracture) event, samples from the first 8 patients were used to measure four bone turnover markers - bone specific alkaline phosphatase, procollagen type 1 N-terminal propeptide (P1NP), osteocalcin, and collagen type 1 cross-linked C-telopeptide (β-CTX). While no change during therapy was noted for bone specific alkaline phosphatase, P1NP, and osteocalcin, an increase in β-CTX was noted in all 7 subjects who had at least one follow-up value (Table 1, increased β-CTX values are underlined).

Table 1

Patient	Tumor Type	Dose (mg/kg)	Day	β-CTX
1	Colorectal	0.5 QW	Day 0	570
2	Colorectal	0.5 QW	Day 0 Day 28 Treatment Terminated	196 308 217
3	Neuroendocrine (carcinoid)	0.5 QW	Day 0 Day 28 Day 56 Treatment Terminated	219 825 896 708
4	Leiomyosarcoma	1 QW	Day 0 Treatment Terminated	298 401
5	Breast	1 QW	Day 0 Day 28 Treatment Terminated	229 681 370
6	Colorectal	1 QW	Day 0 Day 28	162 598
7	Colon	1 QW	Day 0 Day 28 Treatment Terminated	144 301
8	Pancreatic	1 QW	Day 0 Day 28	406 551

[0285] Thus, β-CTX appeared to be an early and sensitive biomarker of the effect of OMP-18R5 on bone.

[0286] Based on the initial Phase 1a study results, the study protocol was amended to include monitoring for skeletal-related side effects and/or toxicities with DEXA bone density scans, bone scans, and measurements of bone turnover biomarkers bone specific alkaline phosphatase, P1NP, osteocalcin, and β-CTX. The amended protocol also included a strategy for treatment of skeletal-related side effects and/or toxicities. Any patient who had at least a doubling of their β-CTX level from their screening value or a T-score decline to less than -2.5 in the total femur or L1-L4 DEXA scan measurement would be administered an anti-resorptive medication, specifically the bisphosphonate zoledronic acid. The zoledronic acid will be administered intravenously at a dose of 5 mg at the time of the doubling of the β-CTX value or decline in T-score.

[0287] Table 2 shows the results (as of January 10, 2014) from the 26 patients who were subsequently enrolled and treated with less frequent dosing (i.e., intermittent dosing) of OMP-18R5 (β-

CTX values at least twice as high as baseline are underlined and an asterisk indicates when zoledronic acid was administered).

Table 2

Patient	Tumor Type	Dose (mg/kg)	Day	β-CTX	T-score
9	Melanoma	0.5 Q2W	Day 0	203	-0.7
			Day 28	195	
			Day 56	287	-0.9
10	Neuroendocrine (pancreas)	0.5 Q2W	Day 0	306	-1.4
			Day 28	286	
			Day 56	304	-1.0
			Day 84*	664	
			Day 112	270	-0.9
			Day 140	288	
			Day 168	413	-1.0
			Day 196	372	
			Day 224	377	-0.9
			Day 252	363	
			Day 280	424	-1.0
			Day 308	505	
			Day 336	499	-1.2
			Day 364	420	
			Day 392	430	-1.3
			Day 420	402	
			Day 448	461	
				374	-1.5
11	Colorectal	0.5 Q2W	Day 0	308	0.9
			Day 42	358	
			Treatment Terminated	327	0.9
12	Neuroendocrine (carcinoid)	1 Q3W	Day 0	689	-1.4
			Day 28	846	
			Day 56	707	-1.3
			Day 84	350	
			Day 112	759	-1.4
			Day 140	526	
			Day 168	967	-1.8
			Day 196	688	
			Day 224	1216	-1.7
			Day 252	1174	

			Day 280 Day 308 Day 336 Day 364 Day 392* Day 420 Day 448 Day 476 Day 504 Day 532 Day 560	1223 1045 890 1380 1332 218 274 246 214 510 341	-1.7 -1.9 -2.0 -2.3 -2.1
13	Bladder	1 Q3W	Day 0 Treatment Terminated	618 876	-0.9 -1.2
14	Colon	1 Q3W	Day 0 Day 28 Treatment Terminated	471 760 688	+2.4 +2.2
15	Colon	1 Q3W	Day 0 Day 28 Day 56 Treatment Terminated	340 469 586 156	-0.7 -0.8
16	Breast	2.5 Q3W	Day 0 Day 28* Treatment Terminated	386 805 345	-0.7 -0.8
17	Thymic	2.5 Q3W	Day 0 Day 28	232 309	-1
18	Desmoid	2.5 Q3W	Day 0 Day 28 Treatment Terminated	607 555 824	-0.9 -1.0
19	Esophagus	5 Q3W	Day 0 Day 28 Treatment Terminated*	648 811 1336	+0.1 -0.1
20	Medullary thyroid	5 Q3W	Day 0 Day 28 Day 56	561 665 1111	-1.0 -1.0
21	Colorectal	5 Q3W	Day 0	629	-0.1
22	Cervical	10 Q3W	Day 0 Day 28	367 697	-1.2
23	Chondrosarcoma	10 Q3W	Day 0 Day28* Treatment Terminated	568 1449 199	-1.3 -1.6

24	Appendix	10 Q3W	Day 0 Day 28* Day 56	114 652 172	-1.3
25	Neuroendocrine	15 Q3W	Day 0 Day 28	927 ND	-0.7
26	Small cell carcinoma, anal	15 Q3W	Day 0 Day 28 Day 56*	596 1171 1278	-1.3 -1.5
27	Breast	15 Q3W	Day 0 Day 28	492 ND	-1.9
28	Colorectal	15 Q3W	Day 0 Day 28 Day 56* Day 84	385 667 898 259	+0.6 +0.4
29	Colorectal	15 Q3W	Day 0 Day 28 Treatment Terminated	964 905 907	-1.6
30	Adenoid cystic adenocarcinoma	20 Q3W	Day 0 Day 28 Day 56	290 306 375	+1.5
31	Colorectal	20 Q3W	Day 0 Day 28 Day 56	434 ND 848	
32	Adenoid cystic adenocarcinoma	20 Q3W	Day 0 Day 28	550 148	-2.3
33	HCC	20 Q3W	Day 0 Day 28	473 979	+0.8
34	Small bowel adenocarcinoma	20 Q3W	Day 0	596	

[0288] At the January 2013 time point, only two of the first ten additional patients (patients 9-18) had a doubling of their β-CTX (patient 10 from a value of 306 at baseline to a value of 664 at Day 84; and patient 16 from a value of 386 at baseline to a value of 805 at Day 28). At the January 10, 2014 time point, nine of the 26 additional patients (patients 9-34) had a doubling of their β-CTX. These data suggest that less frequent dosing of OMP-18R5 at the dose levels studied results in fewer rises in β-CTX and less bone toxicity. According to the amended protocol, patient 10 was administered an intravenous dose of 5mg of zoledronic acid. Following the administration of zoledronic acid, the β-CTX value returned to approximately baseline, a value of 270 at day 112, and remained at approximately that level in subsequent measurements. Patient 16 also received zoledronic acid for doubling of their β-CTX level, and their β-

CTX levels also returned to baseline after treatment. Patient 12 received zoledronic acid for doubling of their β-CTX level and subsequently their β-CTX level was reduced to approximately a third of their baseline level. Patients 16, 19, 23, 24, 26, and 28 were all treated with zoledronic acid with subsequent reductions in their β-CTX levels. These data suggest that zoledronic acid blocks and/or inhibits the bone resorptive properties of OMP-18R5, and can be used to mitigate this skeletal-related side effect.

[0289] At the January 2013 time point, none of the patients enrolled in the study had a significant change in their bone mineral density (BMD) as assessed by DEXA scans (T-scores) while on treatment with OMP-18R5 (Table 3). At the January 10, 2014 time point, none of the patients enrolled in the study had a significant change in their BMD as assessed by DEXA scans (T-scores) while on treatment with OMP-18R5 (Table 2).

Table 3

Patient	DEXA timepoint	Location	T-Score
1	Screening	AP spine L1-L4	-1.6
	Termination	AP spine L1-L4	-1.9
	Screening	AP spine L3	-2.0
	Termination	AP spine L3-L4	-2.1
	Screening	Dual femur neck left	-1.8
	Termination	Dual femur neck right	-1.7
	Screening	Dual femur total mean	-1.7
	Termination	Dual femur total mean	-2.2
3	Screening	AP spine L1-L2	-0.1
	Screening	AP spine L1-L4	+0.2
	Termination	AP spine L1-L4	+0.7
	Termination	AP spine L3-L4	+0.5
	Screening	Dual femur neck left	-0.1
	Termination	Dual femur neck right	+0.2
	Screening	Dual femur total mean	+1.0
	Termination	Dual femur total mean	+0.7
5	Screening	Femur	-1.2
	Termination	Femur	-1.0
	Screening	Lumbar spine	-0.6
	Termination	Lumbar spine	-0.5
7	Screening	Femur	+1.2
	Termination	Femur	+0.7
	Screening	Lumbar spine	+0.9
	Termination	Lumbar spine	+0.9
9	Screening	Lumbar spine	-0.7

	Termination	Lumbar spine	-0.9
	Screening	Hip	+0.2
	Termination	Hip	+0.2
10	Screening	AP spine L1-L2	-0.9
	Screening	AP spine L1-L4	-0.4
	Screening	Dual femur neck left	-1.4
	Screening	Dual femur total mean	-0.9
	Day 56	Lumbar spine	-0.3
	Day 56	Hip	-0.8
11	Screening	Femur	+1.0
	Termination	Hip	+0.9
	Screening	Lumbar spine	+0.9
	Termination	Lumbar spine	+1.1
13	Screening	Lumbar spine	+0.1
	Termination	Lumbar spine	+0.3
	Screening	Hip	-0.9
	Termination	Hip	-1.2
14	Screening	Lumbar spine	+3.6
	Termination	Lumbar spine	+3.9
	Screening	Hip	+2.4
	Termination	Hip	+2.2
16	Screening	Lumbar spine	+0.7
	Termination	Lumbar spine	+0.8

[0290] These data suggest that osteopenic patients can be treated with OMP-18R5 without a significant risk of developing a further decline in their bone mineral density. Furthermore, it confirms that β-CTX appears to be an early and sensitive biomarker of skeletal-related side effects and/or toxicities resulting from treatment with a Wnt pathway inhibitor. Finally, the study has shown that the skeletal-related side effects tied to treatment with OMP-18R5 appear to be manageable and reversible.

Example 5

Phase 1a Study of OMP-54F28 in patients with solid tumors

[0291] The study is an open-label Phase 1a dose-escalation study of OMP-54F28 in patients with a solid tumor for which there is no remaining standard curative therapy. The primary objectives of the study are to determine the safety and the maximum tolerated dose of OMP-54F28. The secondary objectives are to determine the rate of immunogenicity, the preliminary efficacy, and the pharmacokinetics of OMP-54F28.

[0292] The patients in the initial portion of the trial were treated with a dosing regimen of OMP-54F28 of 0.5mg/kg (n = 3), 1.0mg/kg (n = 3), 2.5mg/kg (n = 3), 5mg/kg (n = 5), 10mg/kg (n = 3), 15mg/kg (n = 3) and 20mg/kg (n = 5) once every 3 weeks. This study is ongoing and patients are still being enrolled. Cohorts of 3 subjects are treated and evaluated for dose-limiting toxicities (DLTs) through Day 28. If 0 of 3 subjects have a DLT, escalation to the next dose cohort occurs. If 1 of 3 subjects experiences a DLT, 3 additional subjects are treated. If 2 or more subjects experience a DLT, no further subjects are dosed at that level and 3 additional subjects are added to the preceding dose cohort unless 6 subjects have already been treated at that dose level. Tumor assessments are performed on Day 56 and then every 56 days thereafter. Patients with stable disease or a response at Day 56 will be allowed to continue to receive OMP-54F28 until disease progression.

[0293] Based on information gathered from the Phase 1 OMP-18R5 study, any patient who has at least a doubling of their β-CTX level from their screening value or a T-score decline to less than -2.5 in their total femur or L1-L4 DEXA scan measurement will be administered zoledronic acid. The zoledronic acid will be administered intravenously at a dose of 5 mg at the time of the doubling of the β-CTX value or decline in T-score.

[0294] Table 4 shows the results (as of January 2013) from the first 6 patients who were enrolled and treated with OMP-54F28 once every 3 weeks (β-CTX values at least twice as high as baseline are underlined).

Table 4

Patient	Tumor Type	Dose (mg/kg)	Day	β-CTX
1	Ovarian	0.5 Q3W	Day 0 Day 28 Day 56 Treatment Terminated	215 144 119 104
2	Colorectal	0.5 Q3W	Day 0 Day 28 Treatment Terminated	538 604 <u>1122</u>
3	Pancreatic	0.5 Q3W	Day 0 Day 28 Day 56 Day 84	497 360 414 614
4	Adenocystic	1 Q3W	Day 0 Day 28	346 289
5	Renal cell	1 Q3W	Day 0 Day 28	657 346

6	Neuroendocrine Cervical	1 Q3W	Day 0 Day 28	262 238

[0295] Table 5 shows the results (as of January 9, 2014) from the first 25 patients who were enrolled and treated with OMP-54F28 once every 3 weeks (β-CTX values at least twice as high as baseline are underlined and an asterisk indicates when zoledronic acid was administered).

Table 5

Patient	Tumor Type	Dose (mg/kg)	Day	β-CTX	T-score
1	Ovarian	0.5 Q3W	Day 0 Day 28 Day 56 Treatment Terminated	215 144 119 104	+0.1 +0.5
2	Colorectal	0.5 Q3W	Day 0 Day 28 Treatment Terminated	538 604 <u>1122</u>	+0.5
3	Pancreatic	0.5 Q3W	Day 0 Day 28 Day 56 Day 84 Day 112 Day 140 Day 168* Day 196	497 360 414 614 605 605 <u>1009</u> 251	-0.1 +2.4 +2.1 +1.7
4	Adenocystic	1 Q3W	Day 0 Day 28 Day 56	346 289 277	+1.2 +0.6
5	Renal cell	1 Q3W	Day 0 Day 28 Day 56 Day 84 Day 112 Day 140	657 346 344 359 359 300	+0.5 +0.5 +0.5
6	Large Cell Neuroendocrine Cervical	1 Q3W	Day 0 Day 28 Day 56	262 238 245	+0.1 +0.1
7	Colorectal	2.5 Q3W	Day 0 Day 28	890 1450	-1.2

			Treatment Terminated	972	-1.4
8	NSCLC	2.5 Q3W	Day 0 Day 28 Treatment Terminated	396 378 497	-0.1 -0.4
9	Cholangio-carcinoma	2.5 Q3W	Day 0 Day 28	725 485	-0.5 -0.5
10	Urothelial carcinoma	5 Q3W	Day 0 Day 28 Day 56	634 570 484	+3.0 +2.2
11	Colorectal	5 Q3W	Day 0 Day 28 Treatment Terminated	563 852 744	-1.3 -0.8
12	Cervical	5 Q3W	Day 0 Day 28 Day 56	605 479 558	-0.9 -0.5
13	Renal cell	5 Q3W	Day 0 Day 28 Day 56	250 387 378	0.0 +0.6
14	Desmoid	5 Q3W	Day 0 Day 28 Day 56 Day 84 Day 112 Day 140 Day 168 Day 196 Day 224	354 167 362 242 233 245 114 296 276	-1.3 -1.0 -1.3 -1.4
15	Leiomyo-sarcoma	10 Q3W	Day 0 Day 28	386 300	-1.8
16	Desmoid	10 Q3W	Day 0 Day 28 Day 56* Day 84 Day 112 Day 140 Day 175 Day 196	355 435 <u>806</u> 180 182 192 319 222	-0.6 -0.9 -0.8
17	HCC	10 Q3W	Day 0 Day 28 Day 56	207 148 114	+1.2 +1.1

18	Colorectal	15 Q3W	Day 0 Day 28 Treatment Terminated	796 864 529	-0.5 -0.5
19	Pancreatic	15 Q3W	Day 0 Day 28 Treatment Terminated	770 824 610	-1.2
20	Osteo-carcinoma	15 Q3W	Day 0 Day 28 Treatment Terminated	518 512 476	-0.8 -0.3
21	Testicular	20 Q3W	Day 0 Day 28 Day 56 Day 84*	191 239 309 482	-0.1 -0.2
22	NSCLC	20 Q3W	Day 0 Day 28 Day 56 Day 84	648 851 698 402	-0.2 -0.2
23	Thyroid	20 Q3W	Day 0 Day 28* Day 56	262 731 251	+0.1
24	Basal cell	20 Q3W	Day 0 Day 28	660 670	-0.6
25	Pancreatic	20 Q3W	Day 0 Day 28	511 882	

[0296] At the January 2013 time point, Patient 2 had a doubling of their β-CTX from a value of 538 at baseline to a value of 1122 at Day 42. This patient's disease progressed and treatment with OMP-54F28 was stopped. At the January 9, 2014 time point, five of the 25 patients had a doubling of their β-CTX. Patients 3, 16, 21 and 28 were treated with zoledronic acid and subsequently their β-CTX levels were reduced to baseline levels or levels lower than baseline. Similar to results seen with OMP-18R5 treatment, these initial data suggest that treatment with OMP-54F28 at dose levels of 0.5mg/kg, 1.0mg/kg, 2.5mg/kg, 5mg/kg, 10mg/kg, 15mg/kg, and 20mg/kg once every 3 weeks results in few rises in β-CTX and less bone toxicity. These early results from treatment with OMP-54F28 are further evidence that the skeletal-related side effects tied to treatment with Wnt pathway inhibitors appear to be manageable with reasonable mitigation strategies.

[0297] 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.

[0298] 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.

[0299] 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

WHAT IS CLAIMED IS:

1. A method of selecting a subject for treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) selecting the subject for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level.

2. A method of identifying a subject as eligible for treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level.

3. A method of selecting a subject for treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) selecting the subject for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level.

4. A method of identifying a subject as eligible for treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level.

5. The method of any one of claims 1-4, wherein the biological sample is blood, serum, or plasma.

6. The method of any one of claims 1-5, wherein the bone resorption biomarker is β-CTX.

7. The method of claim 6, wherein the predetermined level of β-CTX in blood, serum, or plasma is a level of β-CTX determined at an earlier timepoint or at an initial screening.

8. The method of claim 6, wherein the predetermined level of β-CTX in blood, serum, or plasma is about 1000pg/ml or less.

9. The method of any one of claims 1-8, which further comprises administering the Wnt pathway inhibitor to the subject if the level of the bone resorption biomarker is below the predetermined level.

10. A method of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of a skeletal-related side effect and/or toxicity, comprising:

(a) obtaining a biological sample from the subject receiving treatment;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein an increase in the level of the bone resorption biomarker indicates development of a skeletal-related side effect and/or toxicity.

11. A method of detecting the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject receiving treatment;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein an increase in the level of the bone resorption biomarker indicates development of a skeletal-related side effect and/or toxicity.

12. A method for identifying a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject receiving treatment;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level then a skeletal-related side effect and/or toxicity is indicated.

13. A method for monitoring a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject receiving treatment;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein if the level of the bone resorption biomarker in the biological sample from the subject is higher than the predetermined level then a skeletal-related side effect and/or toxicity is indicated.

14. A method of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of a skeletal-related side effect and/or toxicity, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein an increase in the level of the bone resorption biomarker indicates development of a skeletal-related side effect and/or toxicity.

15. A method of detecting the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein an increase in the level of the bone resorption biomarker indicates development of a skeletal-related side effect and/or toxicity.

16. A method for identifying a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level, then a skeletal-related side effect and/or toxicity is indicated.

17. A method for monitoring a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level, then a skeletal-related side effect and/or toxicity is indicated.

18. The method of any one of claims 10-17, wherein the sample is blood, serum, or plasma.

19. The method of any one of claims 10-18, wherein the skeletal-related side effect and/or toxicity is an increased risk of bone fracture, osteopenia, or osteoporosis.

20. The method of any one of claims 10-19, wherein the predetermined level of the bone resorption biomarker is the amount of bone resorption biomarker in a sample obtained from the subject at an earlier date.

21. The method of any one of claims 10-20, wherein the predetermined level of the bone resorption biomarker is the amount of bone resorption biomarker in a sample obtained from the subject prior to treatment.

22. The method of any one of claims 10-21, wherein the predetermined level of the bone resorption biomarker is a baseline level.

23. The method of any one of claims 10-22, wherein if the bone resorption biomarker level is above a predetermined level for any one sample, the subject is administered a therapeutically effective amount of an anti-resorptive medication.

24. The method of any one of claims 10-22, wherein if the bone resorption biomarker level is 2-fold or more above a predetermined level, the subject is administered a therapeutically effective amount of an anti-resorptive medication.

25. The method of claim 10-24, wherein the bone resorption biomarker is β-CTX.

26. A method for reducing a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject receiving treatment;

(b) determining the level of a bone resorption biomarker in the sample;

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; and

(d) administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker.

27. A method of preventing or attenuating the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor;

(b) determining the level of a bone resorption biomarker in the sample;

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

(d) administering to the subject a therapeutically effective amount of an anti-resorptive medication; and

(e) administering to the subject the Wnt pathway inhibitor.

28. A method of screening a subject for the risk of a skeletal-related side effect and/or toxicity from treatment with a Wnt pathway inhibitor, comprising:

(a) obtaining a biological sample from the subject prior to treatment with the Wnt pathway inhibitor;

(b) determining the level of a bone resorption biomarker in the sample; and

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity.

29. A method for reducing a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject;

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; and

(c) administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker.

30. A method of preventing or attenuating the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject prior to treatment with the Wnt pathway inhibitor;

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

(c) administering to the subject a therapeutically effective amount of an anti-resorptive medication; and

(d) administering to the subject the Wnt pathway inhibitor.

31. A method of screening a subject for the risk of a skeletal-related side effect and/or toxicity from treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity.

32. A method of treating cancer in a subject in need thereof, comprising:

(a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; and

(b) determining the level of a bone resorption biomarker in a sample from the subject.

33. A method of claim 32, further comprising:

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity.

34. A method of claim 32, further comprising:

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is administered a therapeutically effective amount of an anti-resorptive medication.

35. The method of any one of claims 26-34, wherein the biological sample is blood, serum, or plasma.

36. The method of any one of claims 26-35, wherein the bone resorption biomarker is β-CTX.

37. The method of claim 36, wherein if the β-CTX level is 2-fold or greater as compared to a predetermined level, then the subject is administered a therapeutically effective amount of an anti-resorptive medication.

38. The method of claim 28 or claim 31, wherein if the subject is at risk for a skeletal-related side effect and/or toxicity, the subject is administered a therapeutically effective amount of an anti-resorptive medication prior to treatment with the Wnt pathway inhibitor.

39. A method for reducing a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising administering to the subject a therapeutically effective amount of an anti-resorptive medication.

40. A method of preventing or attenuating the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising administering to the subject a therapeutically effective amount of an anti-resorptive medication.

41. The method of any one of claims 26-40, wherein the skeletal-related side effect and/or toxicity is an increased risk of bone fracture, osteopenia, or osteoporosis.

42. The method of any one of claims 1-41, wherein the Wnt pathway inhibitor is an antibody that specifically binds at least one Frizzled (FZD) protein or portion thereof.

43. The method of claim 42, 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.

44. The method of any one of claims 1-41, 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).

45. The method of any one of claims 1-41, 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.

46. The method according to any one of claims 1-45, wherein the Wnt pathway inhibitor is antibody OMP-18R5.

47. The method of claim 46, wherein OMP-18R5 is administered intravenously to the subject in need thereof at a dosage of (a) at least about 0.5 mg/kg about every one to two weeks or (b) at least about 1.0 mg/kg about every three weeks.

48. The method of claim 46, wherein OMP-18R5 is administered at a dosage of about 0.5 mg/kg to about 1.0 mg/kg about every one to two weeks.

49. The method of claim 46, wherein OMP-18R5 is administered at a dosage of about 1.0 mg/kg to about 10.0 mg/kg about every three weeks.

50. The method according to any one of claims 1-41, wherein the Wnt pathway inhibitor is a Wnt-binding agent.

51. The method of claim 50, wherein the Wnt-binding agent is an antibody.

52. The method according to any one of claims 1-41, 50, or 51, wherein the Wnt pathway inhibitor is an antibody that specifically binds at least one Wnt protein.

53. The method of claim 52, wherein the antibody specifically binds at least one Wnt protein selected from the group consisting of: Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b.

54. The method according to any one of claims 42-45 or 51-53, wherein the antibody is a monoclonal antibody, a recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, or a antibody fragment comprising an antigen-binding site.

55. The method according to any one of claims 42-54, wherein the antibody is a monospecific antibody or a bispecific antibody.

56. The method according to any one of claims 42-45 or 51-55, wherein the antibody is an IgG1 antibody or an IgG2 antibody.

57. The method according to any one of claims 1-41, wherein the Wnt pathway inhibitor is a soluble receptor.

58. The method of claim 57, wherein the Wnt-binding agent is a soluble receptor.

59. The method of claim 57 or claim 58, wherein the soluble receptor comprises a Fri domain of a human FZD protein.

60. The method of claim 59, wherein the Fri domain of the human FZD protein consists 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.

61. The method of claim 59, wherein the Fri domain of the human FZD protein consists essentially of the Fri domain of FZD8.

62. The method of claim 59, wherein the Fri domain of the human FZD protein 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.

63. The method of claim 62, wherein the Fri domain of the human FZD protein consists essentially of SEQ ID NO:20 or SEQ ID NO:30.

64. The method according to any one of claims 59-63, wherein the Fri domain of the human FZD protein is directly linked to a non-FZD polypeptide.

65. The method according to any one of claims 59-63, wherein the Fri domain of the human FZD protein is connected to a non-FZD polypeptide by a linker.

66. The method of claim 64 or claim 65, wherein the non-FZD polypeptide comprises a human Fc region.

67. The method according to any one of claims 64-66, wherein 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.

68. The method of claim 57, wherein the Wnt-binding agent comprises:

(a) a first polypeptide consisting essentially 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, or SEQ ID NO:33; and

(b) a second polypeptide consisting essentially of 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.

69. The method of claim 57, wherein the Wnt-binding agent comprises:

(a) a first polypeptide consisting essentially 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, or SEQ ID NO:33; and

(b) a second polypeptide consisting essentially of 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.

70. The method of claim 68 or claim 69, wherein the first polypeptide consists essentially of SEQ ID NO:20.

71. The method of claim 68 or claim 69, wherein the first polypeptide consists essentially of SEQ ID NO:20, and wherein the second polypeptide consists essentially of SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:35.

72. The method of claim 68 or claim 69, wherein the first polypeptide consists essentially of SEQ ID NO:30.

73. The method of claim 68 or claim 69, wherein the first polypeptide consists essentially of SEQ ID NO:58, and wherein the second polypeptide consists essentially of SEQ ID NO:36, SEQ ID NO:37, or SEQ ID NO:35.

74. The method of claim 57, wherein the Wnt-binding agent comprises SEQ ID NO:39, SEQ ID NO:40, or SEQ ID NO:41.

75. The method of claim 57, wherein the Wnt-binding agent comprises SEQ ID NO:41.

76. The method of claim 57, wherein the Wnt-binding agent is FZD8-Fc soluble receptor OMP-54F28.

77. The method according to any one of claims 27-29, 30, or 35-76, wherein the anti-resorptive medication is a bisphosphonate or denosumab.

78. The method of claim 77, wherein the bisphosphonate is selected from the group consisting of: etidronate, clodronate, tiludronate, pamidronate, neridronate, olpadronate, alendronate, ibandronate, risedronate, and zoledronic acid.

79. The method of claim 77 or claim 78, wherein the bisphosphonate is zoledronic acid.

80. The method of any one of claims 1-79, wherein the subject has cancer.

81. The method of claim 80, wherein the cancer is selected from the group consisting of: lung cancer, breast cancer, colon cancer, colorectal cancer, melanoma, pancreatic cancer, gastrointestinal cancer, renal cancer, ovarian cancer, neuroendocrine cancer, liver cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, glioma, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, and head and neck cancer.

82. The method of any one of claims 1-81, wherein the subject is treated with the Wnt pathway inhibitor in combination with one or more additional anti-cancer agents.

83. The method of any one of claims 1-81, wherein the skeletal-related side effect and/or toxicity is related to the Wnt pathway inhibitor.

84. The method of any one of claims 1-81, wherein the skeletal-related side effect and/or toxicity is an increased risk of bone fracture, osteopenia, or osteoporosis.

85. A method of monitoring a subject receiving treatment with a Wnt pathway inhibitor for the development of a skeletal-related side effect and/or toxicity, wherein the Wnt pathway inhibitor is an anti-FZD antibody or a FZD soluble receptor, the method comprising:

(a) determining the level of a bone resorption biomarker in a sample obtained from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein the predetermined level is a level measured prior to treatment with the Wnt pathway inhibitor;

wherein an increase in the level of the bone resorption biomarker indicates development of a skeletal-related side effect and/or toxicity.

86. A method for reducing a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, wherein the Wnt pathway inhibitor is an anti-FZD antibody or a FZD soluble receptor, the method comprising:

(a) determining the level of a bone resorption biomarker in a sample obtained from the subject;

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker, wherein the predetermined level is a level measured prior to treatment with the Wnt pathway inhibitor; and

(c) administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker.

AMENDED CLAIMS
received by the International Bureau on 13 June 2014 (13.06.2014)

What is claimed is:

1. A method of selecting a subject for treatment with a Wnt pathway inhibitor, comprising:
   (a) determining the level of a bone resorption biomarker in a sample from the subject; and
   (b) selecting the subject for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level;
   wherein the Wnt pathway inhibitor is
      (i) an antibody that specifically binds at least one frizzled (FZD) protein or
      (ii) a soluble receptor comprising a Fri domain of human FZD8.

2. A method of identifying a subject as eligible for treatment with a Wnt pathway inhibitor, comprising:
   (a) determining the level of a bone resorption biomarker in a sample from the subject; and
   (b) identifying the subject as eligible for treatment with the Wnt pathway inhibitor if the level of the bone resorption biomarker is below a predetermined level;
   wherein the Wnt pathway inhibitor is
      (i) an antibody that specifically binds at least one frizzled (FZD) protein or
      (ii) a soluble receptor comprising a Fri domain of human FZD8.

3. The method of claim 1 or claim 2, which comprises administering the Wnt pathway inhibitor to the subject if the level of the bone resorption biomarker is below the predetermined level.

4. A method for identifying a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:
   (a) determining the level of a bone resorption biomarker in a sample from the subject; and
   (b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;
   wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level, then a skeletal-related side effect and/or toxicity is indicated.

5. A method for monitoring a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:
   (a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level, then a skeletal-related side effect and/or toxicity is indicated.

6. The method of any one of claims 1 to 5, wherein the bone resorption biomarker is β-CTX.

7. The method of claim 6, wherein the predetermined level of β-CTX is:
(a) a level of β-CTX determined at an earlier timepoint;
(b) a level of β-CTX determined at an initial screening;
(c) a level of β-CTX determined prior to treatment;
(d) a baseline level; or
(e) about 1000pg/ml or less.

8. The method of any one of claims 4 to 7, wherein if the bone resorption biomarker level is:
(a) above a predetermined level for any one sample or
(b) 2-fold or more above a predetermined level;
the subject is administered a therapeutically effective amount of an anti-resorptive medication.

9. A method for reducing a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:
(a) determining the level of a bone resorption biomarker in a sample from the subject;
(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; and
(c) administering to the subject a therapeutically effective amount of an anti-resorptive medication if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker.

10. A method of preventing or attenuating the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising:
(a) determining the level of a bone resorption biomarker in a sample from the subject prior to treatment with the Wnt pathway inhibitor;
(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker;

(c) administering to the subject a therapeutically effective amount of an anti-resorptive medication; and

(d) administering to the subject the Wnt pathway inhibitor.

11. A method of screening a subject for the risk of a skeletal-related side effect and/or toxicity from treatment with a Wnt pathway inhibitor, comprising:

(a) determining the level of a bone resorption biomarker in a sample from the subject; and

(b) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity.

12. A method of treating cancer in a subject in need thereof, comprising:

(a) administering to the subject a therapeutically effective amount of a Wnt pathway inhibitor; and

(b) determining the level of a bone resorption biomarker in a sample from the subject.

13. The method of claim 12, further comprising:

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is at risk for a skeletal-related side effect and/or toxicity; or

(c) comparing the level of the bone resorption biomarker in the sample to a predetermined level of the bone resorption biomarker; wherein if the level of the bone resorption biomarker in the sample is higher than the predetermined level of the bone resorption biomarker then the subject is administered a therapeutically effective amount of an anti-resorptive medication.

14. The method of any one of claims 1 to 13, wherein the biological sample is blood, serum, or plasma.

15. The method of any one of claims 1 to 14, wherein the bone resorption biomarker is β-CTX.

16. The method of claim 15, wherein if the β-CTX level is 2-fold or greater as compared to a predetermined level, then the subject is administered a therapeutically effective amount of an anti-resorptive medication.

17. The method of claim 11 or claim 13, wherein if the subject is at risk for a skeletal-related side effect and/or toxicity, the subject is administered a therapeutically effective amount of an anti-resorptive medication prior to treatment with the Wnt pathway inhibitor.

18. A method for reducing a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising administering to the subject a therapeutically effective amount of an anti-resorptive medication.

19. A method of preventing or attenuating the development of a skeletal-related side effect and/or toxicity in a subject receiving treatment with a Wnt pathway inhibitor, comprising administering to the subject a therapeutically effective amount of an anti-resorptive medication.

20. The method of any one of claims 4 to 11 or 13 to 19, wherein the skeletal-related side effect and/or toxicity is an increased risk of bone fracture, osteopenia, or osteoporosis.

21. The method of any one of claims 1 to 20, 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).

22. The method of any one of claims 1 to 20, 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.

23. The method of claim 21 or claim 22, wherein the antibody is a monoclonal antibody, a recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, a bispecific

antibody, an IgG1 antibody, an IgG2 antibody, or a antibody fragment comprising an antigen-binding site.

24. The method of any one of claims 1 to 23, wherein the Wnt pathway inhibitor is antibody OMP-18R5.

25. The method of any one of claims 1 to 20, wherein the Wnt pathway inhibitor is a soluble receptor comprising a Fri domain of a human FZD8 protein.

26. The method of claim 25, wherein the Fri domain of the human FZD protein comprises SEQ ID NO:20.

27. The method of claim 25 or claim 26, wherein the soluble receptor comprises a human Fc region.

28. The method of any one of claims 1 to 20 or 25 to 27, wherein the Wnt pathway inhibitor comprises SEQ ID NO:41.

29. The method of any one of claims 1 to 20 or 25 to 28, wherein the Wnt pathway inhibitor is FZD8-Fc soluble receptor OMP-54F28.

30. The method according to any one of claims 8 to 10 or 13 to 29, wherein the anti-resorptive medication is a bisphosphonate or denosumab.

31. The method of claim 30, wherein the bisphosphonate is selected from the group consisting of: zoledronic acid, etidronate, clodronate, tiludronate, pamidronate, neridronate, olpadronate, alendronate, ibandronate, and risedronate.

32. The method of any one of claims 1 to 31, wherein the subject has cancer.

33. The method of claim 32, wherein the cancer is selected from the group consisting of: lung cancer, breast cancer, colon cancer, colorectal cancer, melanoma, pancreatic cancer, gastrointestinal cancer, renal cancer, ovarian cancer, neuroendocrine cancer, liver cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, glioma, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, and head and neck cancer.

34. The method of any one of claims 1 to 33, wherein the subject is treated with the Wnt pathway inhibitor in combination with one or more additional anti-cancer agents.

35. The method of any one of claims 4 to 11 or 13 to 34, wherein the skeletal-related side effect and/or toxicity is related to the Wnt pathway inhibitor.

Figure 1

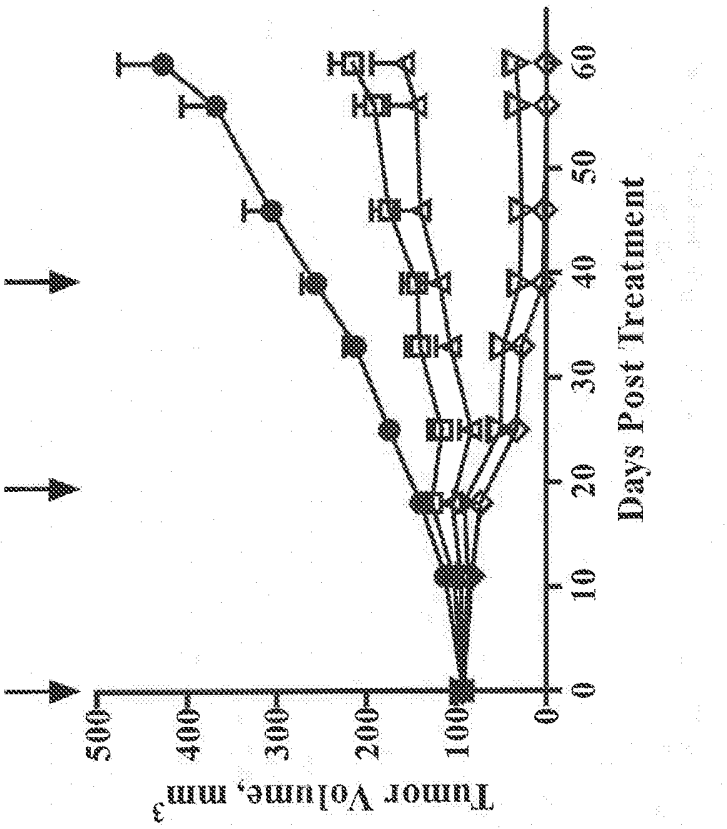
Paclitaxel
5 mg/kg 18R5+Paclitaxel
10 mg/kg 18R5+Paclitaxel
25 mg/kg 18R5+Paclitaxel
45 mg/kg 18R5+Paclitaxel
18R5 dose frequency: Q3W
Taxol dose frequency: Q1W
Tumor Volume, mm3
500
400
300
200
100
0
0
10
20
30
40
50
60
Days Post Treatment

Figure 2

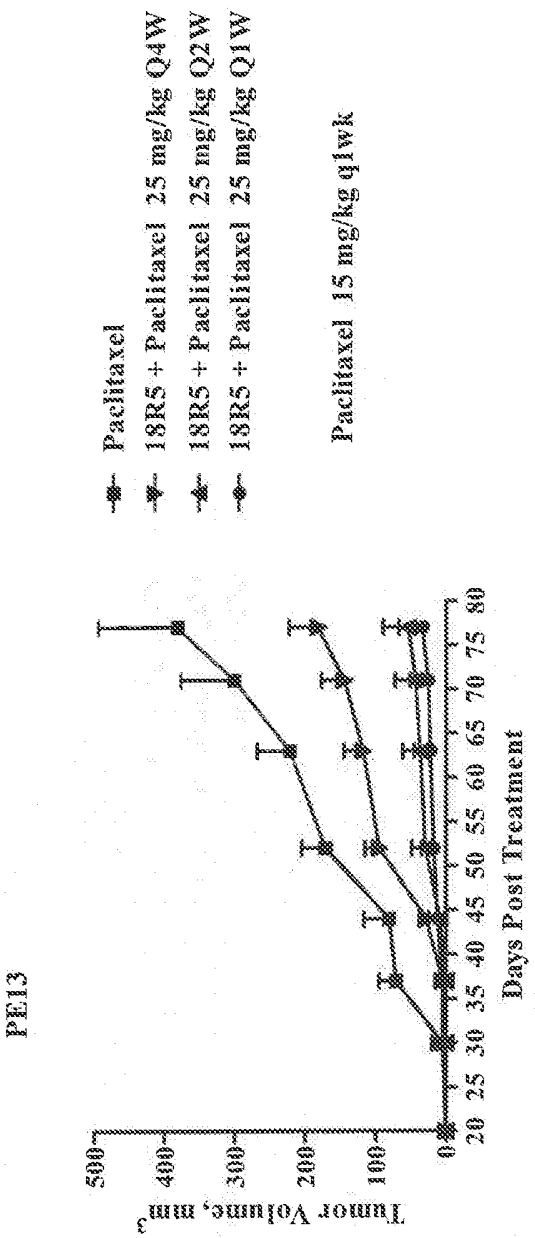
PE13
Paclitaxel
18R5 + Paclitaxel 25 mg/kg Q4W
18R5 + Paclitaxel 25 mg/kg Q2W
18R5 + Paclitaxel 25 mg/kg Q1W
Paclitaxel 15 mg/kg q1wk
Tumor Volume, mm3
500
400
300
200
100
0
20 25 30 35 40 45 50 55 60 65 70 75 80
Days Post Treatment

Figure 3

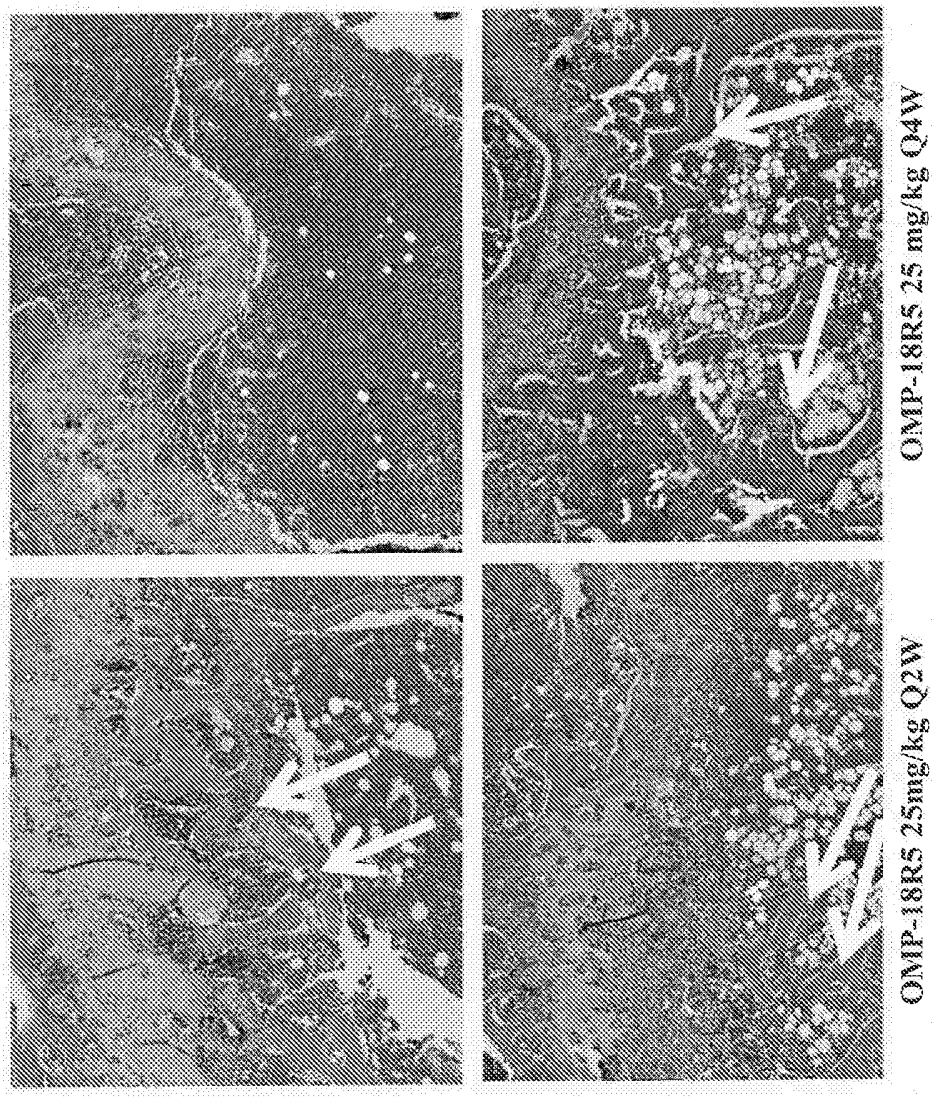
Control
OMP-18R5 25mg/kg Q1W
OMP-18R5 25mg/kg Q2W
OMP-18R5 25 mg/kg Q4W

Figure 4

Control
OMP-18R5
OMP-18R5 + zoledronic acid
Zoledronic acid (100 mg/kg)
OMP-18R5 (20 mg/kg)
Two weeks
Two weeks
Analyze effects on bone
OMP-18R5 (20 mg/kg)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/014443

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 19/08 (2014.01)
USPC - 514/16.7
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) - A01K 2267/03; A61K 39/395; A61P 19/08; C07K 14/705:; G01N 33/50 (2014.01)
USPC - 424/130.1, 133.1; 435/325, 326; 514/16.7, 16.9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12N 2501/415; C12Q 2600/136, 2600/156, 2600/158; G01N 33/5041 (2014.02)

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

PatBase, Google Patents, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2012/058393 A2 (RICHARDS et al) 03 May 2012 (03.05.2012) entire document	1-5, 26, 27, 30, 32, 34, 35 ---------- 10-18, 28, 29, 31, 33, 38, 85, 86
X	US 2009/0023905 A1 (ASKEW et al) 22 January 2009 (22.01.2009) entire document	39, 40
Y	US 2009/0263400 A1 (URDEA et al) 22 October 2009 (22.10.2009) entire document	10-18, 28, 29, 31, 33, 38, 85
Y	US 2012/0027778 A1 (GURNEY) 02 February 2012 (02.02.2012) entire document	85, 86
A	US 2005/0130199 A1 (CARSON et al) 16 June 2005 (16.06.2005) entire document	1- 5, 10-18, 26-35, 38-40, 85, 86
A	GAUDIO et al. 'Increased Sclerostin Serum Levels Associated with Bone Formation and Resorption Markers in Patients with Immobilization-Induced Bone Loss.' J Clin Endocrinol Metab. 95(5): 2248-53. 19 March 2010. entire document	1- 5, 10-18, 26-35, 38-40, 85, and 86
P, X	WHEATER et al. 'The clinical utility of bone marker measurements in osteoporosis.' J Transl Med. 11(201): 1-14. 29 August 2013. entire document	1- 5, 10-18, 26-35, 38-40, 85, and 86

☐ 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
27 March 2014	15 APR 2014
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: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/014443

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.: 6-9, 19-25, 36, 37, 41-84
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

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权利要求书7页 说明书74页
序列表39页
PCT/RO/134表1页 附图4页
按照条约第19条修改的权利要求书3页

(54)发明名称

使用Wnt途径抑制剂进行治疗的方法及对该治疗的监测

(57)摘要

本发明涉及治疗疾病例如癌的方法，其包含单独投予Wnt途径抑制剂或与其他抗癌剂组合投予，及监测骨骼相关不良反应及/或毒性。

CN 105073195 A

1. 一种选择受试者以使用 Wnt 途径抑制剂进行治疗的方法，其包含：

(a) 自该受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 若该骨吸收生物标志的量系低于预设量，则选择该受试者以使用该 Wnt 途径抑制剂进行治疗。

2. 一种识别受试者为适合使用 Wnt 途径抑制剂进行治疗的方法，其包含：

(a) 自该受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 若该骨吸收生物标志的量系低于预设量，则识别该受试者为适合使用该 Wnt 途径抑制剂进行治疗。

3. 一种选择受试者以使用 Wnt 途径抑制剂进行治疗的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 若该骨吸收生物标志的量系低于预设量，则选择该受试者以使用该 Wnt 途径抑制剂进行治疗。

4. 一种识别受试者为适合使用 Wnt 途径抑制剂进行治疗的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 若该骨吸收生物标志的量系低于预设量，则识别该受试者为适合使用该 Wnt 途径抑制剂进行治疗。

5. 如权利要求第 1 至 4 项中任一项的方法，其中该生物样本系血液、血清或血浆。

6. 如权利要求第 1 至 5 项中任一项的方法，其中该骨吸收生物标志系 β-CTX。

7. 如权利要求第 6 项的方法，其中该 β-CTX 于血液、血清或血浆中的预设量系于稍早时间点或在初次筛选时测定的 β-CTX 的量。

8. 如权利要求第 6 项的方法，其中该 β-CTX 于血液、血清或血浆中的预设量系约 1000pg/ml 或更低。

9. 如权利要求第 1 至 8 项中任一项的方法，若该骨吸收生物标志的量系低于该预设量，所述方法进一步包含对该受试者投予该 Wnt 途径抑制剂。

10. 一种监测接受 Wnt 途径抑制剂治疗的受试者是否发展骨骼相关不良反应及 / 或毒性的方法，其包含：

(a) 自该接受治疗的受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。

11. 一种对接受 Wnt 途径抑制剂治疗的受试者检测骨骼相关不良反应及 / 或毒性发展的方法，其包含：

(a) 自该接受治疗的受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。

12. 一种对接受 Wnt 途径抑制剂治疗的受试者识别骨骼相关不良反应及 / 或毒性的方

法，其包含：

(a) 自该接受治疗的受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中若该样本中骨吸收生物标志的量系高于该预设量，则显示骨骼相关不良反应及/或毒性。

13. 一种对接受 Wnt 途径抑制剂治疗的受试者监测骨骼相关不良反应及/或毒性的方法，其包含：

(a) 自该接受治疗的受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中若来自该受试者的生物样本中骨吸收生物标志的量系高于该预设量，则显示骨骼相关不良反应及/或毒性。

14. 一种监测接受 Wnt 途径抑制剂治疗的受试者是否发展骨骼相关不良反应及/或毒性的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及/或毒性。

15. 一种对接受 Wnt 途径抑制剂治疗的受试者检测骨骼相关不良反应及/或毒性发展的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及/或毒性。

16. 一种对接受 Wnt 途径抑制剂治疗的受试者识别骨骼相关不良反应及/或毒性的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中若该样本中骨吸收生物标志的量系高于该预设量，则显示骨骼相关不良反应及/或毒性。

17. 一种对接受 Wnt 途径抑制剂治疗的受试者监测骨骼相关不良反应及/或毒性的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

其中若该样本中骨吸收生物标志的量系高于该预设量，则显示骨骼相关不良反应及/或毒性。

18. 如权利要求第 10 至 17 项中任一项的方法，其中该样本系血液、血清或血浆。

19. 如权利要求第 10 至 18 项中任一项的方法，其中该骨骼相关不良反应及/或毒性系增加骨折、骨量减少或骨质疏松症的风险。

20. 如权利要求第 10 至 19 项中任一项的方法，其中该骨吸收生物标志的预设量系在较

早日期自该受试者获得的样本中骨吸收生物标志的量。

21. 如权利要求第 10 至 20 项中任一项的方法，其中该骨吸收生物标志的预设量系在治疗前自该受试者获得的样本中骨吸收生物标志的量。

22. 如权利要求第 10 至 21 项中任一项的方法，其中该骨吸收生物标志的预设量系基准量。

23. 如权利要求第 10 至 22 项中任一项的方法，其中若任一样本的该骨吸收生物标志量系高于预设量，则对该受试者投予治疗有效量的抗吸收药物。

24. 如权利要求第 10 至 22 项中任一项的方法，其中若该骨吸收生物标志量系为预设量的 2 倍或更多，则对该受试者投予治疗有效量的抗吸收药物。

25. 如权利要求第 10 至 24 项的方法，其中该骨吸收生物标志系 β-CTX。

26. 一种使接受 Wnt 途径抑制剂治疗的受试者减少骨骼相关不良反应及 / 或毒性的方法，其包含：

(a) 自该接受治疗的受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；

(c) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；及

(d) 若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。

27. 一种使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法，其包含：

(a) 在使用 Wnt 途径抑制剂治疗之前，自该受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；

(c) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

(d) 对该受试者投予治疗有效量的抗吸收药物；及

(e) 对该受试者投予该 Wnt 途径抑制剂。

28. 一种筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法，其包含：

(a) 在使用 Wnt 途径抑制剂治疗之前，自该受试者获得生物样本；

(b) 测定该样本中骨吸收生物标志的量；及

(c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量；其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。

29. 一种使接受 Wnt 途径抑制剂治疗的受试者减少骨骼相关不良反应及 / 或毒性的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；及

(c) 若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。

30. 一种使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法，其包含：

(a) 在使用 Wnt 途径抑制剂进行治疗之前，测定来自该受试者的样本中骨吸收生物标志的量；

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量；

(c) 对该受试者投予治疗有效量的抗吸收药物；及

(d) 对该受试者投予该 Wnt 途径抑制剂。

31. 一种筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法，其包含：

(a) 测定来自该受试者的样本中骨吸收生物标志的量；及

(b) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量；其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。

32. 一种对有需要治疗的受试者治疗癌症的方法，其包含：

(a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂；及

(b) 测定来自该受试者的样本中骨吸收生物标志的量。

33. 如权利要求第 32 项的方法，其另包含：

(c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量；其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。

34. 如权利要求第 32 项的方法，其另包含：

(c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量；其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。

35. 如权利要求第 26 至 34 项中任一项的方法，其中该生物样本系血液、血清或血浆。

36. 如权利要求第 26 至 35 项中任一项的方法，其中该骨吸收生物标志系 β-CTX。

37. 如权利要求第 36 项的方法，其中若该 β-CTX 的量系为预设量的 2 倍或更多，则对该受试者投予治疗有效量的抗吸收药物。

38. 如权利要求第 28 或 31 项的方法，其中若该受试者具有骨骼相关不良反应及 / 或毒性的风险，则在使用该 Wnt 途径抑制剂进行治疗之前，对该受试者投予治疗有效量的抗吸收药物。

39. 一种使接受 Wnt 途径抑制剂治疗的受试者减少骨骼相关不良反应及 / 或毒性的方法的方法，其包含对该受试者投予治疗有效量的抗吸收药物。

40. 一种使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法，其包含对该受试者投予治疗有效量的抗吸收药物。

41. 如权利要求第 26 至 40 项中任一项的方法，其中该骨骼相关不良反应及 / 或毒性系增加骨折、骨量减少或骨质疏松症的风险。

42. 如权利要求第 1 至 41 项中任一项的方法，其中该 Wnt 途径抑制剂系与至少一种卷曲 (FZD) 蛋白或它的部分特异性结合的抗体。

43. 如权利要求第 42 项的方法，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。

44. 如权利要求第 1 至 41 项中任一项的方法，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)，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)。

45. 如权利要求第 1 至 41 项中任一项的方法，其中该 Wnt 途径抑制剂系抗体，其包含：

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。

46. 如权利要求第 1 至 45 项中任一项的方法，其中该 Wnt 途径抑制剂系抗体 OMP-18R5。

47. 如权利要求第 46 项的方法，其中 OMP-18R5 系经静脉投予至需要该剂的受试者，剂量为 (a) 约每一至二周至少约 0.5mg/kg，或 (b) 约每三周至少约 1.0mg/kg。

48. 如权利要求第 46 项的方法，其中 OMP-18R5 系以约每一至二周约 0.5mg/kg 至约 1.0mg/kg 的剂量投予。

49. 如权利要求第 46 项的方法，其中 OMP-18R5 系以约每三周约 1.0mg/kg 至约 10.0mg/kg 的剂量投予。

50. 如权利要求第 1 至 41 项中任一项的方法，其中该 Wnt 途径抑制剂系 Wnt 结合剂。

51. 如权利要求第 50 项的方法，其中该 Wnt 结合剂系抗体。

52. 如权利要求第 1 至 41、50 或 51 项中任一项的方法，其中该 Wnt 途径抑制剂系与至少一种 Wnt 蛋白特异性结合的抗体。

53. 如权利要求第 52 项的方法，其中该抗体与选自下列的至少一种 Wnt 蛋白特异性结合：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a 及 Wnt10b。

54. 如权利要求第 42 至 45 或 51 至 53 项中任一项的方法，其中该抗体系单克隆抗体、重组抗体、嵌合抗体、人源化抗体、人抗体或包含抗原结合部位的抗体片段。

55. 如权利要求第 42 至 54 项中任一项的方法，其中该抗体系单特异性抗体或双特异性抗体。

56. 如权利要求第 42 至 45 或 51 至 55 项中任一项的方法，其中该抗体系 IgG1 抗体或 IgG2 抗体。

57. 如权利要求第 1 至 41 项中任一项的方法，其中该 Wnt 途径抑制剂系可溶性受体。

58. 如权利要求第 57 项的方法，其中该 Wnt 结合剂系可溶性受体。

59. 如权利要求第 57 或 58 项的方法，其中该可溶性受体包含人 FZD 蛋白的 Fri 结构域。

60. 如权利要求第 59 项的方法，其中该人 FZD 蛋白的 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。

61. 如权利要求第 59 项的方法，其中该人 FZD 蛋白的 Fri 结构域实质上由 FZD8 的 Fri 结构域组成。

62. 如权利要求第 59 项的方法，其中该人 FZD 蛋白的 Fri 结构域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。

63. 如权利要求第 62 项的方法，其中该人 FZD 蛋白的 Fri 结构域实质上由 SEQ ID NO:20 或 SEQ ID NO:30 组成。

64. 如权利要求第 59 至 63 项中任一项的方法，其中该人 FZD 蛋白的 Fri 结构域系与非 FZD 多肽直接连接。

65. 如权利要求第 59 至 63 项中任一项的方法，其中该人 FZD 蛋白的 Fri 结构域系藉由连接子与非 FZD 多肽连接。

66. 如权利要求第 64 或 65 项的方法，其中该非 FZD 多肽包含人 Fc 区。

67. 如权利要求第 64 至 66 项中任一项的方法，其中该非 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。

68. 如权利要求第 57 项的方法，其中该 Wnt 结合剂包含：

(a) 第一多肽，其实质上由 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 组成；及

(b) 第二多肽，其实质上由 SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37 或 SEQ ID NO:38 组成；

其中该第一多肽系与该第二多肽直接连接。

69. 如权利要求第 57 项的方法，其中该 Wnt 结合剂包含：

(a) 第一多肽，其实质上由 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 组成；及

(b) 第二多肽，其实质上由 SEQ ID NO:34、SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37 或 SEQ ID NO:38 组成；

其中该第一多肽系藉由连接子与该第二多肽直接连接。

70. 如权利要求第 68 或 69 项的方法，其中该第一多肽实质上由 SEQ ID NO:20 组成。

71. 如权利要求第 68 或 69 的方法，其中该第一多肽实质上由 SEQ ID NO:20 组成，且其中该第二多肽实质上由 SEQ ID NO:36、SEQ ID NO:37 或 SEQ ID NO:35 组成。

72. 如权利要求第 68 或 69 项的方法，其中该第一多肽实质上由 SEQ ID NO:30 组成。

73. 如权利要求第 68 或 69 的方法，其中该第一多肽实质上由 SEQ ID NO:58 组成，且其中该第二多肽实质上由 SEQ ID NO:36、SEQ ID NO:37 或 SEQ ID NO:35 组成。

74. 如权利要求第 57 项的方法，其中该 Wnt 结合剂包含 SEQ ID NO:39、SEQ ID NO:40 或 SEQ ID NO:41。

75. 如权利要求第 57 项的方法，其中该 Wnt 结合剂包含 SEQ ID NO:41。

76. 如权利要求第 57 项的方法，其中该 Wnt 结合剂系 FZD8-Fc 可溶性受体 OMP-54F28。

77. 如权利要求第 27 至 29、30 或 35 至 76 项中任一项的方法，其中该抗吸收药物系双

膦酸盐或迪诺单抗。

78. 如权利要求第 77 项的方法，其中该双膦酸盐系选自：羟乙膦酸盐、氯屈膦酸盐、替鲁膦酸盐、帕米膦酸盐、奈立膦酸盐、奥帕膦酸盐、阿仑膦酸盐、伊班膦酸盐、利塞膦酸盐及唑来膦酸。

79. 如权利要求第 77 或 78 项的方法，其中该双膦酸盐系唑来膦酸。

80. 如权利要求第 1 至 79 项中任一项的方法，其中该受试者罹患癌症。

81. 如权利要求第 80 项的方法，其中该癌症系选自：肺癌、乳癌、结肠癌、结直肠癌、黑色素瘤、胰癌、胃肠癌、肾癌、卵巢癌、神经内分泌癌、肝癌、子宫内膜癌、肾癌、前列腺癌、甲状腺癌、神经胚细胞瘤、神经胶瘤、多形性神经胶质母细胞瘤、子宫颈癌、胃癌、膀胱癌、肝肿瘤及头颈癌。

82. 如权利要求第 1 至 81 项中任一项的方法，其中该受试者系经 Wnt 途径抑制剂与一或多种额外抗癌剂的组合治疗。

83. 如权利要求第 1 至 81 项中任一项的方法，其中该骨骼相关不良反应及 / 或毒性系与该 Wnt 途径抑制剂有关。

84. 如权利要求第 1 至 81 项中任一项的方法，其中该骨骼相关不良反应及 / 或毒性系增加骨折、骨量减少或骨质疏松症的风险。

85. 一种监测接受 Wnt 途径抑制剂治疗的受试者发展骨骼相关不良反应及 / 或毒性的方法，其中该 Wnt 途径抑制剂系抗 FZD 抗体或 FZD 可溶性受体，该方法包含：

(a) 测定自该受试者获得的样本中骨吸收生物标志的量；及

(b) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中该预设量系于使用该 Wnt 途径抑制剂进行治疗之前测量的量；

其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。

86. 一种减少接受 Wnt 途径抑制剂治疗的受试者的骨骼相关不良反应及 / 或毒性的方法，其中该 Wnt 途径抑制剂系抗 FZD 抗体或 FZD 可溶性受体，该方法包含：

(a) 测定自该受试者获得的样本中骨吸收生物标志的量；

(b) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中该预设量系于使用该 Wnt 途径抑制剂进行治疗之前测量的量；及

(c) 若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。

使用 Wnt 途径抑制剂进行治疗的方法及对该治疗的监测

相关申请案的交互引用

[0001] 本申请案主张美国临时专利申请案 61/760,523(2013 年 2 月 4 日提出) 的优先权权益,其以引用方式整体纳入本文。

技术领域

[0002] 本发明关于使用 Wnt 途径抑制剂治疗疾病的领域。更特别地,本发明提供治疗癌的方法,该方法包含单独投予 Wnt 途径抑制剂或与其他抗癌剂组合投予,及监测不良反应及 / 或毒性的方法。

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超过一百万人被诊断出癌症而且有 500,000 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。癌症有超过 200 种不同的种类,其中四种 :乳癌、肺癌、结直肠癌及前列腺癌,占所有新病例的几乎一半 (Siegel et al.,2011,CA:ACancer J.Clin.61:212-236)。

[0004] 信号传导途径通常连接细胞外信号至细胞核,导致表达直接或间接控制细胞生长、分化、存活、及死亡的基因。在许多种类的癌中,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 信号传导途径已被认为是有效的癌治疗标靶。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。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殖。因此癌的发生可藉由超越控制正常发育及组织修复的恒定机制进行 (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 基因编码分泌型脂肪修饰糖蛋白,其中 19 种已在哺乳动物中识别。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。该等 FZD 受体为 G 蛋白偶合受体 (GPCR) 超家族的七个跨膜结构域蛋白,且包含具有 10 个保守性半胱氨酸的大型胞外 N 端配体结合结构域,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),FZD 受体已被分成活化典型 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)。其他 Wnt 信号传导在乳癌角色的证据自此开始累积。举例来说，基因转殖 β-连环蛋白于乳腺中的过度表达导致增殖及腺癌 (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 途径的活化也和结直肠癌有关。大约 5 至 10％的所有结直肠癌为遗传性，其中一种主要形式为家族性腺瘤息肉症 (FAP)，这是一种体染色体显性疾病，其中大约 80％的患者包含大肠腺瘤息肉 (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殖发育及肿瘤生长 (Oshima et al., 1997, Cancer Res., 57:1644-9；Harada et al., 1999, EMBO J., 18:5931-42)。

[0009] 黑色素瘤和乳癌及结肠癌类似，通常具有 Wnt 途径的组成性活化，如细胞核累积 β-连环蛋白所示。一些黑色素瘤及细胞系中的 Wnt/β-连环蛋白途径活化是因为途径成分的修饰所致，如 APC、ICAT、LEF1 及 β-连环蛋白（见例如 Larue et al., 2006, Frontiers Biosci., 11:733-742)。然而，文献中关于 Wnt/β-连环蛋白信号传导于黑色素瘤的确切角色有所冲突。举例来说，一项研究发现细胞核 β-连环蛋白浓度上升和黑色素瘤存活改善有关，而经活化的 Wnt/β-连环蛋白信号传导却与细胞增殖减少有关 (Chien et al., 2009, PNAS, 106:1193-1198)。

[0010] 化学治疗是发展成熟的治疗许多癌症的手段，但其疗效可受限于不良反应及／或毒性。此外，标靶疗法例如抗 ErbB2 受体 (HER2) 抗体曲妥珠单抗 (trastuzumab) (HERCEPTIN)、酪氨酸激酶抑制剂伊马替尼 (imatinib) (GLEEVEC)、达沙替尼 (dasatinib) (SPRYCEL)、尼罗替尼 (nilotibib) (TASIGNA)、舒尼替尼 (sunitinib) (SUTENT)、索拉非尼 (sorafenib) (NEXAVAR)、抗 VEGF 抗体贝伐珠单抗 (bevacizumab) (AVASTIN)、及抗血管新生药物舒尼替尼 (sunitinib) (SUTENT) 及索拉非尼 (sorafenib) (NEXAVAR)，已知会造成或可能造成用药受试者的不良反应及／或毒性。因此，能识别药物引发的不良反应、监测该等不良反应、及／或减轻该等不良反应，以使有效的癌症疗法得以继续的新颖方法仍有需要。

发明内容

[0011] 本发明提供治疗疾病的改良方法，该方法包含对受试者投予治疗有效量的 Wnt 途径抑制剂。例如，在一方面中，本发明提供筛选、检测、识别、监测、减少、预防、减弱、及／或减轻与 Wnt 途径抑制剂治疗有关的骨骼相关不良反应及／或毒性的方法。在一些实施方式

中，该等方法包含测定来自病患的样本中骨转换标志的量，该病患已接受、正在接受、将要接受、或考虑接受Wnt途径抑制剂的初次或进一步治疗，该Wnt途径抑制剂包括但不限于抗卷曲(FZD)抗体或可溶性FZD受体。

[0012] 在另一方面中，本发明提供识别受试者为适合使用Wnt途径抑制剂进行治疗的方法，其包含：自该受试者获得生物样本、测定该样本中生物标志的量、及若该生物标志的量系低于预设量，则识别该受试者为适合使用该Wnt途径抑制剂进行治疗。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该识别受试者为适合使用Wnt途径抑制剂进行治疗的方法包含：自该受试者获得生物样本、测定该样本中骨吸收生物标志的量、及若该骨吸收生物标志的量系低于预设量，则识别该受试者为适合使用该Wnt途径抑制剂进行治疗。在一些实施方式中，该骨吸收生物标志系第1型胶原蛋白交联的C-端肽(β-CTX)。

[0013] 在一方面中，本发明提供监测接受Wnt途径抑制剂治疗的受试者是否发展骨骼相关不良反应及/或毒性的方法，其包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中该生物标志的量增加显示发展骨骼相关不良反应及/或毒性。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该监测接受Wnt途径抑制剂治疗的受试者是否发展骨骼相关不良反应及/或毒性的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中骨吸收生物标志的量、及比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及/或毒性。在一些实施方式中，该骨吸收生物标志系β-CTX。

[0014] 在另一方面中，本发明提供对接受Wnt途径抑制剂治疗的受试者检测骨骼相关不良反应及/或毒性发展的方法，其包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中该生物标志的量增加显示发展骨骼相关不良反应及/或毒性。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该对接受Wnt途径抑制剂治疗的受试者检测骨骼相关不良反应及/或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中骨吸收生物标志的量、及比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中该骨吸收生物标志的量增加显示发展骨骼相关不良反应及/或毒性。在一些实施方式中，该骨吸收生物标志系β-CTX。

[0015] 在另一方面中，本发明提供对接受Wnt途径抑制剂治疗的受试者识别骨骼相关不良反应及/或毒性发展的方法，其包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则显示骨骼相关不良反应及/或毒性。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该对接受Wnt途径抑制剂治疗的受试者识别骨骼相关不良反应及/或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中骨吸收生物标志的量、及比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则显示骨骼相关不良反应及/或毒性。在一些实施方式中，该骨吸收生物标志系β-CTX。

[0016] 在另一态样中，本发明提供对接受 Wnt 途径抑制剂治疗的受试者监测骨骼相关不良反应及 / 或毒性发展的方法，其包含 ：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则显示骨骼相关不良反应及 / 或毒性。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该对接受 Wnt 途径抑制剂治疗的受试者监测骨骼相关不良反应及 / 或毒性发展的方法包含 ：自该接受治疗的受试者获得生物样本、测定该样本中骨吸收生物标志的量、及比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则显示骨骼相关不良反应及 / 或毒性。在一些实施方式中，该骨吸收生物标志系 β -CTX。

[0017] 在本文中所描述的方法的一些方面及 / 或实施方式中，其中若样本中该骨吸收生物标志的量 (例如 β -CTX) 相较于预设量增加 2 倍或更高，则对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，该骨吸收生物标志系 β -CTX 且该预设量系小于约 1000pg/ml。在一些实施方式中，该抗吸收药物剂系双膦酸盐。

[0018] 在另一方面中，本发明提供使接受 Wnt 途径抑制剂治疗的受试者减少骨骼相关不良反应及 / 或毒性的方法，其包含 ：自该接受治疗的受试者获得生物样本、测定该样本中骨吸收生物标志的量、比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量、及若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，该吸收生物标志增加为该骨吸收生物标志的预设量的约 1.5 倍或更高、约 2 倍或更高、约 2.5 倍或更高、或约 3 倍或更高。在一些实施方式中，该骨吸收生物标志系 β -CTX。在一些实施方式中，该抗吸收药物剂系双膦酸盐。

[0019] 在另一方面中，本发明提供使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法，其包含 ：在使用 Wnt 途径抑制剂治疗之前，自该接受治疗的受试者获得生物样本、测定该样本中骨吸收生物标志的量、比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量、对该受试者投予治疗有效量的抗吸收药物、及对该受试者投予该 Wnt 途径抑制剂。在一些实施方式中，该骨吸收生物标志系 β -CTX。在一些实施方式中，该抗吸收药物剂系双膦酸盐。

[0020] 在另一方面中，本发明提供对投予 Wnt 途径抑制剂的受试者改善骨骼相关不良反应及 / 或毒性的方法，其包含 ：测定样本中骨吸收生物标志的量、及对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，该骨吸收生物标志系 β -CTX。在一些实施方式中，该抗吸收药物剂系双膦酸盐。

[0021] 在另一方面中，本发明提供筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法，其包含 ：在使用 Wnt 途径抑制剂治疗之前，自该受试者获得生物样本、测定该样本中骨吸收生物标志的量、及比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该样本中骨吸收生物标志的量系高于该预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中，若该受试者具有骨骼相关不良反应及 / 或毒性的风险，则在使用该 Wnt 途径抑制剂治疗之前，对该受试者投予治疗有效量的针对该骨骼相关不良反应及 / 或毒性的治疗剂。在一些实施方式中，该骨吸收生物标志系 β -CTX。在一些实施方式中，该针对骨骼相关不良反应的治疗剂系双膦酸盐。

[0022] 在另一方面中，本发明提供对受试者治疗癌症的方法，其包含：对该受试者投予治疗有效量的 Wnt 途径抑制剂、及测定来自该受试者的样本中骨吸收生物标志的量。在一些实施方式中，该治疗癌症的方法另包含比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量。在一些实施方式中，该治疗癌症的方法另包含比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中，该治疗癌症的方法另包含比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，该抗吸收药物剂系双膦酸盐。

[0023] 在另一方面中，本发明提供抑制受试者的肿瘤生长的方法，其包含：对该受试者投予治疗有效量的 Wnt 途径抑制剂、及测定来自该受试者的样本中骨吸收生物标志的量。在一些实施方式中，该抑制肿瘤生长的方法另包含比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量。在一些实施方式中，该抑制肿瘤生长的方法另包含比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中，该抑制肿瘤生长的方法另包含比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量，其中若该骨吸收生物标志的量系高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，该抗吸收药物剂系双膦酸盐。

[0024] 在本文所述的方法的一些方面及 / 或实施方式中，该生物样本系血液、血清或血浆。在一些实施方式中，该生物样本系“空腹样本”。如本文所使用，“空腹样本”系指自尚未进食且尚未饮用任何饮料至少 9 至 12 个小时的受试者所采集的样本。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 1500pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 1200pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 1000pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 800pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 600pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 400pg/ml 或更低。在一些实施方式中，该生物标志（例如骨转换标志）的预设量系该生物标志于稍早日期所获得的样本中的量。在一些实施方式中，该生物标志（例如骨转换标志）的预设量系该生物标志于治疗前所获得的样本中的量。在一些实施方式中，该生物标志（例如骨转换标志）的预设量系该生物标志于最初筛选时所获得的样本中的量。在一些实施方式中，该生物标志（例如骨转换标志）的预设量系正常参考量。在一些实施方式中，该生物标志的预设量系基准量。在一些实施方式中，该基准量系在最初筛选时（例如治疗前）所测定的该生物标志的量。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，该 β-CTX 的预设量系在血液、血清、或血浆中约 1000pg/ml 或更低。

[0025] 在本文所述的方法的一些方面及 / 或实施方式中，生物样本系于约每周、每 2 周、每 3 周、每 4 周、每 5 周、或每 6 周获得。

[0026] 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实施方式中，

该 Wnt 途径抑制剂系与至少一种人 Wnt 蛋白特异性结合的抗体。抗 Wnt 抗体的非限制性实例已于例如美国专利公开号 2012/0027778 及国际专利公开号 WO 2011/088127 中描述。在一些实施方式中，该 Wnt 途径抑制剂系与至少一种人 FZD 蛋白特异性结合的抗体。抗 FZD 抗体的非限制性实例已于例如美国专利第 7,982,013 号中描述。在一些实施方式中，该 Wnt 途径抑制剂系可溶性 FZD 受体。可溶性 FZD 受体的非限制性实例已于例如美国专利第 7,723,477 及 8,324,361 号及美国专利公开号 2011/0305695 中描述。

[0027] 在一些实施方式中，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 / 或 (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。

[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实施方式中，该 Wnt 途径抑制剂系包含下列的抗体：(a) 与 SEQ ID NO:7 具有至少约 90％、至少约 95％、或 100％序列一致性的重链可变区；及 / 或 (b) 与 SEQ ID NO:8 具有至少约 90％、至少约 95％、或 100％序列一致性的轻链可变区。在一些实施方式中，该 Wnt 途径抑制剂系抗体 OMP-18R5。

[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，该 Wnt 途径抑制剂系重组抗体。在一些实施方式中，该抗体系单克隆抗体、嵌合抗体、人源化抗体、或人抗体。在一些实施方式中，该抗体系包含抗原结合部位的抗体片段。在某些实施方式中，该抗体或抗体片段系单价、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 IgG1 抗体。在一些实施方式中，该抗体系 IgG2 抗体。在某些实施方式中，该抗体系经分离。在其他实施方式中，该抗体系实质上纯的。

[0030] 在一些实施方式中，该 Wnt 途径抑制剂系以约 10nM 至约 0.1nM 的解离常数 (K_D) 与至少一种人 FZD 结合的抗体。

[0031] 在某些实施方式中，该 Wnt 途径抑制剂包含与保藏于美国菌种保存中心 (ATCC) 的编号 PTA-9541 的质粒所编码的抗体相同的重链及轻链氨基酸序列。在某些实施方式中，该 Wnt 途径抑制剂包含与保藏于美国菌种保存中心 (ATCC) 的编号 PTA-9541 的质粒所编码的抗体相同的重链可变区及轻链可变区氨基酸序列。在某些实施方式中，该 Wnt 途径抑制剂系由具有 ATCC 保藏编号 PTA-9541 的质粒所编码，该质粒依据布达佩斯条约的规定于 2008 年 9 月 29 日保藏于美国菌种保存中心 (ATCC)(地址：10801University Boulevard,Manassas,VA,20110)。在某些实施方式中，该 Wnt 途径抑制剂与保藏于美国菌种保存中心 (ATCC) 的编号 PTA-9541 的质粒所编码的抗体，竞争与人 FZD 的特异性结合。

[0032] 在本文所述的方法的任何方面及 / 或实施方式中，该受试者罹患癌症。在一些实施方式中，该癌症系选自：肺癌、胰癌、乳癌、结肠癌、结直肠癌、黑色素瘤、胰癌、胃肠癌、肾癌 (renal cancer)、卵巢癌、肝癌、子宫内膜癌、肾癌 (kidney cancer)、前列腺癌、甲状腺癌、神经胚细胞瘤、神经胶瘤、多形性神经胶质母细胞瘤、子宫颈癌、胃癌、膀胱癌、肝肿瘤、肝细胞癌 (HCC)、神经内分泌癌、甲状腺癌、腺癌、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，该癌系胰癌。在一些实施方式中，该癌系肺癌。在一些实施方式中，

该癌系非小细胞肺癌 (NSCLC)。在一些实施方式中,该癌系卵巢癌。在一些实施方式中,该癌系肝癌。在一些实施方式中,该癌系 HCC。

[0033] 在本文所述的方法的任何方面及 / 或实施方式中,该受试者系经 Wnt 途径抑制剂与一或多种额外抗癌剂的组合治疗。在一些实施方式中,该一或多种额外抗癌剂系化学治疗剂。在一些实施方式中,该额外抗癌剂系太平洋紫杉醇 (paclitaxel) 或与白蛋白结合的太平洋紫杉醇。在一些实施方式中,该额外抗癌剂系吉西他滨 (gemcitabine)。在一些实施方式中,该额外抗癌剂系吉西他滨及与白蛋白结合的太平洋紫杉醇。在一些实施方式中,该额外抗癌剂系多西紫杉醇 (docetaxel)。在一些实施方式中,该额外抗癌剂系卡铂 (carboplatin)。在一些实施方式中,该额外抗癌剂系卡铂及太平洋紫杉醇或与白蛋白结合的太平洋紫杉醇。在一些实施方式中,该额外抗癌剂系索拉非尼 (sorafenib)。

[0034] 本发明的方面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之一或多者。

附图简要说明

[0035] 图 1 :间歇性投予 Wnt 途径抑制剂对活体内乳房肿瘤生长的抑制。使用下列治疗小鼠 :太平洋紫杉醇 (- ● -)、5mg/kg OMP-18R5 与太平洋紫杉醇的组合 (- ■ -)、10mg/kg OMP-18R5 与太平洋紫杉醇的组合 (- ▲ -)、25mg/kgOMP-18R5 与太平洋紫杉醇的组合 (- ▼ -)、或 45mg/kg OMP-18R5 与太平洋紫杉醇的组合 (- ◆ -)。数据以治疗后天数的肿瘤体积 (mm3) 显示。OMP-18R5 系经腹膜内投予,每三周一次 (如箭头所示),太平洋紫杉醇系以 10 mg/kg 的剂量每周投予一次。

[0036] 图 2 :间歇性投予 Wnt 途径抑制剂对活体内乳房肿瘤生长的抑制。使用下列治疗小鼠 :太平洋紫杉醇 (- ■ -)、25mg/kg OMP-18R5 与太平洋紫杉醇 (每 4 周一次) 的组合 (- ▼ -)、25mg/kg OMP-18R5 与太平洋紫杉醇 (每 2 周一次) 的组合 (- ▲ -)、或 25mg/kg OMP-18R5 与太平洋紫杉醇 (每周一次) 的组合 (- ● -)。数据以治疗后天数的肿瘤体积 (mm3) 显示。OMP-18R5 系经腹膜内投予,太平洋紫杉醇系以 15 mg/kg 的剂量每周投予一次。

[0037] 图 3 :OMP-18R5 对小鼠骨形成的影响。

[0038] 图 4 :唑来膦酸 (zoledronic acid) 对 OMP-18R5 治疗小鼠的骨形成的影响。

具体实施方式

本发明的详细说明

[0039] 本发明关于使用 Wnt 途径抑制剂治疗疾病。更特别地,本发明提供治疗癌的方法,该方法包含单独投予 Wnt 途径抑制剂或与其他抗癌剂组合投予,及监测骨骼相关不良反应及 / 或毒性 (包括与 Wnt 途径抑制剂有关者) 的方法。

[0040] 抗 FZD 抗体 OMP-18R5 系于第 1a 期单剂药物增量临床试验中投予给受试者。此早期试验的数据以及动物试验的结果显示,投予 Wnt 途径抑制剂如抗 FZD 抗体或 FZD8-Fc 可溶性受体,可能在某些病患导致骨骼相关不良反应及 / 或毒性。另外,该第 1a 期试验显示 β-CTX 的量增加,可能是接受 Wnt 途径抑制剂治疗的病患,可能发生骨骼相关不良反应及 / 或毒性的早期指标,因此允许适当药物的介入治疗。

[0041] 这些结果使吾等想针对如本文所述的骨骼相关不良反应及/或毒性发展风险降低及监测策略，以用于接受 Wnt 途径抑制剂（例如抗 FZD 抗体或可溶性 FZD 受体）作为单一剂治疗或与额外抗癌剂组合治疗的受试者。

I. 定义

[0042] 为了促进对本发明的了解，以下定义一些用语及用词。

[0043] 本文所使用的用语“拮抗剂”及“拮抗性”，系指部分或完全阻断、抑制、减少、或中和标靶及/或信号传导途径（例如 Wnt 途径）的生物活性的任何分子。本文所使用的用语“拮抗剂”包括部分或完全阻断、抑制、减少、或中和蛋白（例如 FZD 蛋白或 Wnt 蛋白）的活性的任何分子。适当的拮抗剂分子特别包括但不限于拮抗剂抗体、抗体片段、可溶性受体、或小分子。

[0044] 本文所使用的用语“调节”系指生物活性的改变或变化。调节包括但不限于刺激或抑制活性。调节可为增加或减少活性（例如减少 Wnt 途径信号传导）、改变结合特性，或任何其他与蛋白、途径、或其他生物关注点的活性有关的生物性、功能性、或免疫性性质的改变。

[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识别部位，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、人源化抗体、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识别部位（例如抗原结合部位）的经修饰的免疫球蛋白分子，只要该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 或其亚型（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亚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。本文所使用的“抗体片段”包含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）。重链及轻链的可变区各由四个框架区 (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，并与来自其他链中的 CDR 一起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] 本文所使用的用语“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物。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识别部位（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”系指由多种技术包括但不限于杂交瘤产制、噬菌体选择、重组表达及基因转殖动物制备的该等抗体。

[0049] 本文所使用的用语“人源化抗体”系指非人（例如小鼠）抗体的形式，该形式系包含最少非人序列的特定免疫球蛋白链、嵌合性免疫球蛋白或其片段。通常，人源化抗体系其中 CDR 的残基经非人物种（例如小鼠、大鼠、兔或仓鼠）的 CDR 残基置换的人免疫球蛋白，其具有所欲的特异性、亲和性及 / 或结合能力 (Jones et al., 1986, Nature, 321:522-525 ; Riechmann et al., 1988, Nature, 332:323-327 ; Verhoeyen et al., 1988, Science, 239:1534-1536)。在一些情况中，人免疫球蛋白的 Fv 框架区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残基置换。该人源化抗体可进一步藉由替换 Fv 框架区及 / 或该经置换的非人残基内的额外残基加以修饰，以精进优化抗体特异性、亲和性及 / 或结合能力。通常，该人源化抗体将包含实质上所有的至少一个且通常两或三个可变结构域，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共同序列的框架区。该人源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。

[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。此人抗体的定义特别排除包含非人 CDR 的人源化抗体。

[0051] 本文所使用的用语“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，然而该恒定区对应源自另一物种（通常是人）的抗体中的序列。

[0052] 本文所使用的用词「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，该等改变导致该抗体对抗原的亲和性增加，相较于不具有该等改变的母体抗体。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。举例来说，Marks et al., 1992, Bio/Technology 10:779-783 描述藉由 VH 及 VL 结构域替换产生的亲和性成熟。CDR 及 / 或框架残基的随机突变形成系由 Barbas et al., 1994, PNAS, 91:3809-3813 ; Schier et al., 1995, Gene, 169:147-155 ; Yelton et al., 1995, J. Immunol. 155:1994-2004 ; Jackson et al., 1995, J. Immunol., 154:3310-9 ; 及 Hawkins et al., 1992, J. Mol. Biol., 226:889-896 所述。定点突变形成亦可被用来获得亲和性成熟抗体。

[0053] 用语“表位”及“抗原决定簇”在本文可交换使用，系指可被特定抗体识别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迭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，然而藉由三级折迭形成的表位（又称为构型表位）通常在蛋白质变性时丧失。表

位通常包括至少 3 个及更常地至少 5 或 8 至 10 个呈独特空间构型的氨基酸。

[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。由于不同物种之间的同源性蛋白质具有序列一致性，因此特异性结合可包括识别超过一个物种的蛋白质的抗体（例如人 FZD 及小鼠 FZD）。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识别超过一种蛋白的抗体（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基团可能或可能不与第二标靶特异性结合。因此，“特异性结合”不一定表示（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，抗体与一种以上的标靶特异性结合。在某些实施方式中，多重标靶可能由抗体上的相同的抗原结合部位结合。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。在一些实施方式中，抗体可能为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，双特异性抗体可包含识别一蛋白上的表位的一抗原结合部位，另包含识别第二蛋白上的不同表位的第二、不同抗原结合部位。一般来说（但不必然），所谓的结合系指特异性结合。

[0055] 本文使用的用语“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 N 端胞外片段（或它的部分），其可以可溶形式自细胞分泌。

[0056] 本文使用的用语“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 Fri 结构域的 FZD 可溶性受体亦包括于此用语中。

[0057] 用语“多肽”和“肽”以及“蛋白”在本文可交换使用，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，以及包含该领域已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）。

[0058] 用语“多核苷酸”及“核酸”在本文可交换使用，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 / 或其类似物，或任何可藉由 DNA 或 RNA 聚合酶被纳入聚合物中的底物。

[0059] 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置换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 软件包及其变化性产品。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。

[0060] “保守性氨基酸置换”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替代的置换。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，以苯丙氨酸替代酪氨酸系保守性置换。较佳地，本发明的多肽及抗体的序列中的保守性置换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（即该一或多种 RSPO 蛋白）的结合。识别不消除抗原结合性的核苷酸及氨基酸保守性置换的方法系该领域所广为周知。

[0061] 本文所使用的用语“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，及包封于脂质体中的 DNA 或 RNA 表达载体。

[0062] 经“分离”的多肽、抗体、多核苷酸、载体、细胞或组合物系呈现未见于天然中的形式的多肽、抗体、多核苷酸、载体、细胞或组合物。经分离的多肽、抗体、多核苷酸、载体、细胞或组合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、细胞或组合物系实质上纯的。

[0063] 本文所使用的用语“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。

[0064] 本文所使用的用语“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。

[0065] 本文所使用的用语“肿瘤”及“瘤 (neoplasm)”系指任何由过度细胞生长或增殖所导致的组织团块，不论是良性（非癌性）或包括癌前性病灶的恶性（癌性）。

[0066] 本文所使用的用语“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以入侵邻近身体结构的细胞。

[0067] 用语“癌干细胞”、“CSC”、“肿瘤干细胞”及“肿瘤起始细胞”在本文可交换使用，系指源自癌或肿瘤且具有下列特性的细胞：(1) 具有广泛增殖能力，(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的增殖或发育能力，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。

[0068] 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）。本文使用的用语“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。

[0069] 本文所使用的用语“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）及增殖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。

[0070] 本文所使用的用语“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。

[0071] 用语“受试者”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、啮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，关于人受试者的用语“受试者”及“病患”在本文可交换使用。

[0072] 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。

[0073] 用语“医药上可接受的赋形剂、载剂或佐剂”或“可接受的医药载剂”系指可与本发明的至少一种结合剂（例如抗体）一起投予至受试者的赋形剂、载剂或佐剂，且该赋形剂、载剂或佐剂不破坏该结合剂的活性。该赋形剂、载剂或佐剂当与足以达到治疗效应的剂量的结合剂一起投予时应不具毒性。

[0074] 用语“有效量”或“治疗有效量”或“治疗效应”系指有效“治疗”受试者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。

[0075] 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，受试者系经本发明的方法成功“治疗”，若该病患显示下列一或多项：癌细胞的数量减少或完全消失；肿瘤大小减少；抑制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

制或缺乏肿瘤或癌细胞转移；抑制或缺乏癌生长；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；或一些效应的组合。

[0076] 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。

[0077] 应了解的是，只要本文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，只要本文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。

[0078] 如本文所使用，提及“约”或“大约”某数值或参数时，其包括（且描述）与该数值或参数相关的实施方式。举例来说，“约 X”的叙述包括“X”的叙述。

[0079] 当使用于本文诸如“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. Wnt 途径抑制剂

[0080] 本发明提供用于抑制肿瘤生长的方法及 / 或用于治疗癌症的方法中的 Wnt 途径抑制剂。

[0081] 在某些实施方式中，该 Wnt 途径抑制剂系与一或多种人卷曲蛋白 (FZD) 结合的剂。该等剂在本文中被称为“FZD 结合剂”。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。

[0082] 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。

[0083] 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”。

[0084] 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 蛋白内的胞外域 (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，且提供于本文为 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)。

[0085] 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。

[0086] 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。

[0087] 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}。

[0088] 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，该抗体系人源化抗体。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。

[0089] 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、Western 印迹分析、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）。

[0090] 举例来说，抗体与人 FZD 蛋白的特异性结合可利用 ELISA 测定。ELISA 测定包含准备抗原，以抗原包覆 96 孔微量板的孔槽，添加与可检测的化合物诸如酶底物（例如辣根过氧化酶或碱性磷酸酶）缀合的 FZD 结合剂（例如抗体）至孔槽，培养一段时间后，检测与该抗原结合的 FZD 结合剂的存在。在一些实施方式中，该 FZD 结合抗体或剂不与可检测的化合物缀合，而是将识别该 FZD 结合抗体或剂或抗体的第二缀合抗体（例如抗 Fc 抗体）加入孔槽中。在一些实施方式中，不以抗原包覆孔槽，反而是以 FZD 结合抗体或剂包覆孔槽，并于添加抗原至该经包覆的孔槽后加入与可检测的化合物缀合的第二抗体。该领域技术人员将知道可经修改以增加及 / 或优化检测信号的参数以及可使用的 ELISA 的其他变量。

[0091] 在另一实例中，抗体与人 FZD 蛋白的特异性结合可利用 FACS 测定。FACS 筛选测定可包含产制 cDNA 建构物以表达抗原为融合蛋白，将该建构物转染至细胞中，使该抗原表达在细胞表面，使该 FZD 结合抗体或其他 FZD 结合剂与该经转染的细胞混合，并培养一段时间。被 FZD 结合抗体或其他 FZD 结合剂结合的细胞，可利用与可检测的化合物缀合的二级抗体（例如 PE 缀合抗 Fc 抗体）及流式细胞分析识别。该领域技术人员将知道可经修改以优化经检测的信号的参数以及其他可增进筛选（例如筛选阻断抗体）的 FACS 变数。

[0092] 抗体或其他结合剂与抗原（例如 FZD 蛋白）的结合亲和性，及抗体 - 抗原交互反应的解离速率，可藉由竞争性结合测定决定。竞争性结合测定的一实例系放射性免疫测定，其包含在渐增量的未经标记的抗原存在下，培养经标记的抗原（例如 ^{3}H 或 ^{125}I）或其片段或变异体与感兴趣的抗体，之后检测与该经标记的抗原结合的抗体。该抗体对抗原（例如 FZD 蛋白）的亲和性及结合解离速率可由斯卡查德 (Scatchard) 图分析的数据决定。在一些实施方式中，Biacore 动力学分析系用于测定与抗原（例如 FZD 蛋白）结合的抗体或剂的结合及解离速率。Biacore 动力学分析包含分析抗体与芯片表面上经固定的抗原（例如 FZD 蛋白）的结合及解离。

[0093] 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。

[0094] 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，或其包含 1、2、3、或 4 个氨基酸置换的变异体；(b) 包含 VISGDGSYTYYADSVKG(SEQ ID NO:2) 的重链 CDR2，或其包含 1、2、3、或 4 个氨基酸置换的变异体；(c) 包含 NFIKYVFAN(SEQ ID NO:3) 的重链 CDR3，或其包含 1、2、3、或 4 个氨基酸置换的变异体；(d) 包含 SGDNIGSFYVH(SEQ ID NO:4) 的轻链 CDR1，或其包含 1、2、3、或 4 个氨基酸置换的变异体；(e) 包含 DKSNRPSG(SEQ ID NO:5) 的轻链 CDR2，或其包含 1、2、3、或 4 个氨基酸置换的变异体；及 (f) 包含 QSYANTLSL(SEQ ID NO:6) 的轻链 CDR3，或其包含 1、2、3、或 4 个氨基酸置换的变异体。在某些实施方式中，该氨基酸置换系保守性置换。

[0095] 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。

[0096] 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。

[0097] 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。

[0098] 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。

[0099] 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）氨基酸置换的 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。

[0100] 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。

[0101] 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。

[0102] 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。

[0103] 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。

[0104] 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（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：由保藏于 ATCC 的编号 PTA-9541 的质粒所编码的重链可变区及轻链可变区。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 结合剂或抗体于活体外（in vitro）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。

[0105] 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

的表位重迭。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 蛋白上的表位重迭。

[0106] 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。该等剂在本文被称为“Wnt 结合剂”。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、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a、及 Wnt10b。

[0107] 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。

[0108] 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一、二、三、四、或五种选自 Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a、及 Wnt10b 的人 Wnt 蛋白特异性结合。Wnt 结合剂的非限制性实例可见于国际公开号 WO 2011/088127。

[0109] 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 结合剂与位于该剂或抗体所结合的一或多种 Wnt 蛋白内的结构域结合，该一或多种 Wnt 蛋白系选自下列：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)。

[0110] 在某些实施方式中，该 Wnt 结合剂以约 1 μM 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。

[0111] 在某些实施方式中，该 Wnt 结合剂以约 1 μM 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。

[0112] 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，该抗体系人源化抗体。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。

[0113] 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。

[0114] 举例来说，抗体与人 Wnt 蛋白的特异性结合可利用 ELISA 测定。ELISA 测定包含准备抗原，以抗原包覆 96 孔微量板的孔槽，添加与可检测的化合物诸如酶底物（例如辣根过氧化酶或碱性磷酸酶）缀合的 Wnt 结合剂（例如抗体）至孔槽，培养一段时间后，检测与该抗原结合的 Wnt 结合剂的存在。在一些实施方式中，该 Wnt 结合抗体或剂不与可检测的化合物缀合，而是将识别该 Wnt 结合抗体或剂或抗体的第二缀合抗体（例如抗 Fc 抗体）加入孔槽中。在一些实施方式中，不以抗原包覆孔槽，反而是以 Wnt 结合抗体或剂包覆孔槽，并于添加抗原至该经包覆的孔槽后加入与可检测的化合物缀合的第二抗体。该领域技术人员将知道可经修改以增加及 / 或优化检测信号的参数以及可使用的 ELISA 的其他变量。

[0115] 在另一实例中，抗体与人 Wnt 蛋白的特异性结合可利用 FACS 测定。FACS 筛选测定可包含产制 cDNA 建构物以表达抗原为融合蛋白质，将该建构物转染至细胞中，使该抗原表达在细胞表面，使该 Wnt 结合抗体与该经转染的细胞混合，并培养一段时间。被 Wnt 结合抗体结合的细胞可利用与可检测的化合物缀合的二级抗体（例如与 PE 缀合的抗 Fc 抗体）及流式细胞分析识别。该领域技术人员将了解可经修改以优化经检测的信号的参数以及其他可增进筛选（例如筛选阻断抗体）的 FACS 变数。

[0116] Wnt 结合剂与抗原（例如 Wnt 蛋白）的结合亲和性，及抗体 - 抗原交互反应的解离速率，可藉由竞争性结合测定决定，诸如上述用于 FZD 结合剂者。

[0117] 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 FZD Fri 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 2011/0305695 中。

[0118] 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。该领域的技艺人士对于对应各种 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。

[0119] 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。

[0120] 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。

[0121] 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。

[0122] 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。

[0123] 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) 保守性置换且能与 Wnt 蛋白结合。

[0124] 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连接子区。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 IgG1Fc 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置换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。

[0125] 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。本文所使用的“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（即不含中介肽连接子）。在一些实施方式中，该第一多肽系与该 Fc 区经由连接子相连。

[0126] 本文使用的用语“连接子”系指插入第一多肽（例如 FZD 成分）与第二多肽（例如 Fc 区）之间的连接子。在一些实施方式中，该连接子系肽连接子。连接子不应不良影响该多肽的表达、分泌或生物活性。连接子应不具抗原性且不应诱发免疫反应。适当的连接子系该领域技术人员所知，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连接子的氨基酸包括苏氨酸及丙氨酸残基。连接子的长度范围广泛，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。连接子可能包括但不限于 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)。本文所使用的连接子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。

[0127] 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 区的连接子。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，该连接子包含 ESGGGGVT(SEQ ID NO:57) 或 LESGGGGVT(SEQ ID NO:58)。

[0128] 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。

[0129] 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连接子与该第二多肽连接。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。

[0130] 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。

[0131] 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连接子与该第二多肽连接。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。

[0132] 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）氨基酸置换及 / 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置换及 / 或删除，藉此导致具有一种 N 端的实质上相同的多肽。

[0133] 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。

[0134] 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）保守性置换。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。

[0135] 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）保守性置换。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。

[0136] 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）保守性置换。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。

[0137] 在一些实施方式中，该 Wnt 结合剂系 OMP-54F28（又称为 54F28）。在一些实施方式中，该 Wnt 结合剂不是 OMP-54F28。

[0138] 在某些实施方式中，Wnt 结合剂系多肽，其包含选自 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 的氨基酸序列。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。

[0139] 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。

[0140] 在某些实施方式中，Wnt 结合剂包含免疫球蛋白的 Fc 区。该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、删除或置换一或多个结构域中的一或多个氨基酸。本文所揭示的

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者(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)置换,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。

[0141] 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元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。

[0142] 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置换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置换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),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。

[0143] 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。

[0144] 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。

[0145] 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。

[0146] 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，包含连接子的融合蛋白已被发现比不包含连接子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 细胞表位系经识别及 / 或预测，可对这些区域进行修饰（例如氨基酸置换）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。

[0147] 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）或多肽的组合物。在一些实施方式中，该组合物包含多肽，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，该组合物包含多肽，其中 100％的该多肽具有 ASA 的 N 端序列。在一些实施方式中，该组合物包含多肽，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，该组合物包含多肽，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，该组合物包含多肽，其中至少 95％的该多肽具有 ASA 的 N 端序列。

[0148] 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、及类型置换。

[0149] 当然，技术人员会采用的氨基酸置换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置换的数量不会超过 50、40、30、25、20、15、10、5 或 3 个。

[0150] 藉由肽合成，可使用本发明的多肽的片段或部分以产制对应的全长多肽；因此，可使用该片段作为产制该全长多肽的中间体。该多肽的片段或部分亦可称为“蛋白片段”或“多肽片段”。

[0151] 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 蛋白结合的蛋白的部分或整体。于某些实施方式中，该片段对一或多种人 Wnt 蛋白具有高度亲和性。于某些实施方式中，该片段对一或多种人 FZD 蛋白具有高度亲和性。本文所描述的 Wnt 结合剂的某些片段系蛋白片段，其包含与免疫球蛋白的恒定区（例如 Fc 区）的至少一部分连接的 FZD 蛋白的胞外区的至少一部分。该蛋白片段的结合亲和性可介于约 10^{-11} 至 10^{-12}M，虽然该亲和性可依片段的不同大小而广泛地变化（介于 10^{-7} 至 10^{-13}M）。于某些实施方式中，该片段的长度为约 100 至约 200 氨基酸且包含与免疫球蛋白的恒定区的至少一部分连接的结合结构域。

[0152] 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剂缀合，诸如钥孔状帽贝血蓝素（KLH）或血清白蛋白。该抗原（不论有无载剂蛋白质）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。

[0153] 在一些实施方式中，该Wnt途径抑制剂系单克隆抗体。单克隆抗体可利用该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，淋巴细胞可于体外（in vitro）免疫。在一些实施方式中，该免疫抗原可为人蛋白质或其部分。在一些实施方式中，该免疫抗原可为小鼠蛋白质或其部分。

[0154] 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、免疫印迹及体外结合试验（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体外（in vitro）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。

[0155] 在某些实施方式中，单克隆抗体可利用该领域的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，例如藉由RT-PCR使用寡核苷酸引物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已知技术测定。该经分离的编码重链及轻链的多核苷酸接着被克隆至适当表达载体，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（见例如McCafferty et al.,1990,Nature,348:552-554;Clackson et al.,1991,Nature,352:624-628;and Marks et al.,1991,J.Mol.Biol.,222:581-597）。

[0156] 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替代以产制嵌合抗体，或以非免疫球蛋白多肽替代以产制融合抗体。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。

[0157] 在一些实施方式中，该Wnt途径抑制剂系人源化抗体。通常，人源化抗体系其中源自CDR的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CDR的残基替代的人免疫球蛋白，该替代系利用该领域的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残基替代。在一些实施方式中，该人源化抗

体可进一步藉由替代Fv框架区及/或该经替代的非人残基内的额外残基加以修饰，以精进优化抗体特异性、亲和性及/或能力。通常，该人源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共同序列的框架区。在一些实施方式中，该人源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，该等人源化抗体系用于治疗用途，因为当投予至人受试者时它们可能减少抗原性及HAMA(人抗小鼠抗体)反应。

[0158] 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，产制拮抗标靶抗原的抗体的永生化人B淋巴细胞可于体外免疫产制，或可自免疫受试者分离(见例如Cole et al.，1985，Monoclonal Antibodies and Cancer Therapy，Alan R.Liss，p.77；Boemer et al.，1991，J.Immunol.，147:86-95；及美国专利第5,750,373、5,567,610、及5,229,275号)。在一些实施方式中，该人抗体可选自噬菌体分子库，其中该噬菌体分子库表达人抗体(Vaughan et al.，1996，Nature Biotechnology，14:309-314；Sheets et al.，1998，PNAS，95:6157-6162；Hoogenboom and Winter，1991，J.Mol.Biol.，227:381；Marks et al.，1991，J.Mol.Biol.，222:581)。或者，噬菌体展示技术可被用来自未经免疫接种的捐赠者的免疫球蛋白可变区结构域基因库试管内(in vitro)产制人抗体及抗体片段。产制及使用抗体噬菌体库的技术系描述于美国专利第5,969,108、6,172,197、5,885,793、6,521,404、6,544,731、6,555,313、6,582,915、6,593,081、6,300,064、6,653,068、6,706,484及7,264,963号，及Rothe et al.，2008，J.Mol.Bio.，376:1182-1200。该领域已知的亲和性成熟策略包括但不限于链洗牌(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。

[0159] 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基因转殖小鼠中制备。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。

[0160] 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识别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源化抗体。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)及第二抗原标靶例如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素螯合剂诸如EOTUBE、DPTA、DOTA或TETA结合的臂。

[0161] 用于制备双特异性抗体的技术系该领域的技术人员所知，见例如Millstein et al.，1983，Nature，305:537-539；Brennan et al.，1985，Science，229:81；Suresh et al.，1986，Methods in Enzymol.，121:120；Traunecker et al.，1991，EMBO J.，10:3655-3659；Shalaby et al.，1992，J.Exp.Med.，175:217-225；Kostelny et

al.,1992,J.Immunol.,148:1547-1553;Gruber et al.,1994,J.Immunol.,152:5368;美国专利第5,731,168号及美国专利公开号2011/0123532。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。

[0162] 在某些实施方式中,本文描述的抗体(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,本文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)。

[0163] 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,抗体片段系自如本文讨论的抗体噬菌体库分离。举例来说,可利用方法建构Fab表达库(Huse et al.,1989,Science,246:1275-1281)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。

[0164] 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。

[0165] 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(美国专利第4,676,980号)。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体外(in vitro)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-巯基丁亚胺酸酯。

[0166] 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)可经工程化或特别修饰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源化或藉由纳入输入氨基酸序列以另行改变。

[0167] 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替代一或多个 CDR 及（若需要的话）部分框架区替代及序列修饰及 / 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 替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。

[0168] 尽管对可变区进行改变，该领域的技术人员将了解，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，例如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、删除或替代一或多个结构域中的一或多个氨基酸。本文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者 (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）替代，以提供通常由该缺少恒定区授予的一些分子柔韧性。

[0169] 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元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。

[0170] 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替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替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。

[0171] 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。

[0172] 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该领域的技术人员广为周知的生化或分子工程技术进行。

[0173] 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。

[0174] 本发明另包含实质上与本文前述的嵌合抗体、人源化抗体、人抗体或其抗体片段同源的变异体及相等物。这些可包含例如保守性替代突变，即以类似氨基酸替代一或多个氨基酸。例如，保守性替代系指以同类型的氨基酸替代另一者，像是例如以一酸性氨基酸替代另一酸性氨基酸、以一碱性氨基酸替代另一碱性氨基酸或以一中性氨基酸替代另一中性氨基酸。保守性氨基酸替代的目的系该领域广为周知并于本文描述。

[0175] 因此，本发明提供用于产制抗体的方法。在一些实施方式中，用于产制抗体的方法包含利用杂交瘤技术。在一些实施方式中，本发明提供用于产制与人 FZD 蛋白结合的抗体的方法。在一些实施方式中，本发明提供用于产制与人 Wnt 蛋白结合的抗体的方法。在一些实施方式中，用于产制抗体的方法包含筛选人噬菌体库。在一些实施方式中，该抗体系利用包含单一抗原结合部位的膜结合性异二聚体分子识别。在一些非限制性实施方式中，该抗体系利用美国专利公开号 WO 2011/0287979 所述的方法及多肽识别。

[0176] 本发明另提供识别与至少一种 FZD 蛋白结合的抗体的方法。在一些实施方式中，该抗体系藉由 FACS 筛选与 FZD 蛋白或其部分的结合加以识别。在一些实施方式中，该抗体系藉由利用 ELISA 筛选与 FZD 蛋白结合加以识别。在一些实施方式中，该抗体系藉由 FACS 筛选 FZD 蛋白与人 Wnt 蛋白结合的阻断加以识别。在一些实施方式中，该抗体系藉由筛选对 Wnt 途径信号传导的抑制或阻断加以识别。

[0177] 本发明另提供识别与至少一种 Wnt 蛋白结合的抗体的方法。在一些实施方式中，该抗体系藉由 FACS 筛选与 Wnt 蛋白或其部分的结合加以识别。在一些实施方式中，该抗体系藉由利用 ELISA 筛选与 Wnt 蛋白结合加以识别。在一些实施方式中，该抗体系藉由 FACS 筛选 Wnt 蛋白与人 FZD 蛋白结合的阻断加以识别。在一些实施方式中，该抗体系藉由筛选对 Wnt 途径信号传导的抑制或阻断加以识别。

[0178] 在一些实施方式中，产制抗至少一种人 FZD 蛋白的抗体的方法包含在抗体表达库中筛选与人 FZD 蛋白结合的抗体。在一些实施方式中，该抗体表达库系噬菌体库。在一些实施方式中，该抗体表达库系哺乳动物细胞库。在一些实施方式中，该筛选包含淘选

(panning)。在一些实施方式中,在第一次筛选中被识别的抗体,再次利用不同的FZD蛋白筛选,藉以识别与该第一FZD蛋白及第二FZD蛋白结合的抗体。在一些实施方式中,在筛选中识别的抗体与该第一FZD蛋白及至少一种其他FZD蛋白结合。在某些实施方式中,该至少一种其他FZD蛋白系选自:FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9、及FZD10。在某些实施方式中,在筛选中识别的该抗体与FZD1、FZD2、FZD5、FZD7及FZD8结合。在一些实施方式中,在筛选中被识别的抗体系FZD拮抗剂。在一些实施方式中,由本文所述的方法识别的抗体抑制Wnt途径。在一些实施方式中,在该筛选中识别的抗体抑制β-连环蛋白信号传导。

[0179] 在一些实施方式中,产制抗至少一种人Wnt蛋白的抗体的方法包含在抗体表达库中筛选与人Wnt蛋白结合的抗体。在一些实施方式中,该抗体表达库系噬菌体库。在一些实施方式中,该抗体表达库系哺乳动物细胞库。在一些实施方式中,该筛选包含淘选(panning)。在一些实施方式中,在第一次筛选中被识别的抗体,再次利用不同的Wnt蛋白筛选,藉以识别与第一Wnt蛋白及第二Wnt蛋白结合的抗体。在一些实施方式中,在筛选中识别的抗体与第一Wnt蛋白及至少一种其他Wnt蛋白结合。在某些实施方式中,该至少一种其他FZD蛋白系选自Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a、及Wnt10b。在一些实施方式中,在筛选中被识别的抗体系Wnt拮抗剂。在一些实施方式中,由本文所述的方法识别的抗体抑制Wnt途径。在一些实施方式中,在该筛选中识别的抗体抑制β-连环蛋白信号传导。

[0180] 在某些实施方式中,本文所述的抗体系经分离。在某些实施方式中,本文所述的抗体系实质上纯的。

[0181] 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其片段。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/或其他类型的替代。

[0182] 该多肽、类似物及其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。

[0183] 本文所述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。

[0184] 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。

[0185] 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定序、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，该基因必须可操作性连接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。

[0186] 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 / 或翻译调节元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，该调节元件系源自哺乳动物、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元件，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。调节元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识别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元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。

[0187] 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，例如源自大肠杆菌的质粒（包括 pCR1、pBR322、pMB9 及其衍生物），及广泛宿主范围质粒例如 M13 及其他丝状单股 DNA 噬菌体。

[0188] 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构建的细胞系。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克隆及表达载体系描述于 Pouwels et al.(1985,Cloning Vectors:A Laboratory Manual,Elsevier,New York,NY)。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。

[0189] 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迭、适当修饰且具生物功能性。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）细胞系及其变异株。哺乳动物表达载体可包含非转录元件（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、剪接供体位点及受体位点，及转录终止序列）。

[0190] 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迭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该领域的技术人员所广为周知（见例如 Luckow and Summers, 1988, Bio/Technology, 6:47）。

[0191] 因此，本发明提供包含本文所述的 FZD 结合剂或 Wnt 结合剂的细胞。在一些实施方式中，该等细胞产制本文所述的结合剂（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 OMP-54F28。

[0192] 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、流感外套序列及谷胱甘肽 -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，使用例如蛋白水解、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。

[0193] 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 超过滤装置浓缩。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，例如具有二乙基氨基乙基 (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。

[0194] 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、超音波震荡、机械破碎或使用细胞溶解剂。

[0195] 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。

[0196] 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。

[0197] 在某些实施方式中,该结合剂可以数种缀合的(即免疫缀合物或放射缀合物)或非缀合的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受试者的天然防御机制,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。

[0198] 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其片段,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)、亚胺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)、双叠氮基化合物(诸如双(对-叠氮基苯甲酰基)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)。

[0199] 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。

[0200] 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 OMP-54F28。

[0201] 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 OMP-54F28。

[0202] 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。

[0203] 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、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 OMP-54F28。

[0204] 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。

[0205] 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，对至少一种 Wnt 与至少一种 FZD 蛋白的结合的抑制为至少约 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 OMP-54F28。

[0206] 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 途径抑制剂阻断至少一种 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 OMP-54F28。

[0207] 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 OMP-54F28。

[0208] 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 - 连环蛋白活化的 Wnt 途径抑制剂系可溶性受体 OMP-54F28。

[0209] 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 (In vivo) 及活体外 (in vitro) 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

告试验测量体外的 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体外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。

[0210] 在某些实施方式中，Wnt 途径抑制剂具有一或多种下列影响 :抑制肿瘤细胞增殖、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。

[0211] 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、肝细胞肿瘤、甲状腺肿瘤、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、神经内分泌肿瘤、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，该肿瘤系肺肿瘤。在一些实施方式中，该肿瘤系卵巢肿瘤。在一些实施方式中，该肿瘤系肝肿瘤。在某些实施方式中，该肿瘤系神经内分泌肿瘤。在某些实施方式中，该肿瘤系 Wnt 依赖性肿瘤。

[0212] 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。

[0213] 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。

[0214] 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例如 PEG 化的技术。

III. 使用方法及医药组合物

[0215] 本发明提供使用 Wnt 途径抑制剂治疗疾病诸如癌，同时筛选、监测、减少、预防、减弱、及 / 或减轻不良反应及 / 或毒性的方法，该等不良反应及 / 或毒性包括但不限于与该 Wnt 途径抑制剂治疗有关的骨骼相关不良反应及 / 或毒性。与癌症治疗有关的不良反应及 / 或毒性可包括但不限于疲倦、呕吐、恶心、腹泻、疼痛、掉发、嗜中性球低下、贫血、血小板低下、心血管相关并发症、骨骼相关并发症、及任何彼等的组合。如本文中所使用的“骨骼相关并发症”（例如骨骼相关不良反应及 / 或毒性）包括但不限于骨量减少、骨质疏松症、骨折（包括无症状骨折）、及其组合。因此，在本文所述的方法的一些态样及 / 或实施方式中，筛选、监测、减少、预防、减弱、及 / 或减轻骨骼相关不良反应及 / 或毒性，系筛选、监测、减少、预防、减弱、及 / 或减轻骨密度流失及 / 或骨折风险。骨密度流失通常无征状及 / 或骨骼相关不良反应的早期征候在例如骨密度筛选时并不明显。

[0216] 骨代谢系骨形成与骨破坏的连续性双重过程。骨破坏系指骨吸收，其系由破骨细胞进行，然而骨形成系由成骨细胞进行。在成人中，骨形成与骨破坏的双重过程处于平衡状态，维持固定、恒定控制量的骨。骨代谢可藉由测量在骨形成及骨吸收期间释出的生物标志（例如酶、蛋白、及 / 或降解产物）评估及 / 或监测。这些生物标志通常被称为“骨转换标志”且包括骨形成标志及骨吸收标志。骨形成生物标志包括血清总碱性磷酸酶、血清骨骼特异性碱性磷酸酶、血清骨钙化素、血清第 1 型前胶原胺基端前肽 (P1NP) 及血清第 1 型前胶原羧基端前肽 (P1CP)。骨吸收生物标志包括尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、及抗酒石酸酸性磷酸酶 5b。

[0217] 骨的有机基质中约 90％系第 1 型胶原，即一种螺旋蛋白，其中该分子的 N 端和 C 端交联。于骨吸收期间，破骨细胞分泌酸性蛋白酶和中性蛋白酶的混合物，其能使胶原原纤维降解成包括 C- 端肽 (CTX) 的分子片段。当骨老化时，存在于 CTX 的 α 型天冬氨酸转化为 β 型（β-CTX）。于骨吸收期间，β-CTX 被释出至血流且作为成熟第 1 型胶原的降解的特定标志。

[0218] 业已使用骨代谢标志以监测停经后妇女和诊断患有骨质稀少的受试者的抗吸收治疗（例如激素取代性治疗和双磷酸盐治疗）。此外，可使用骨代谢标志以评估因使用激

素药物和非激素药物进行治疗所导致的因药物引起的骨质疏松。该等药物可包括但不限于糖皮质激素、甲状腺激素、芳香酶抑制剂、卵巢抑制剂、雄激素剥夺治疗、四氢噻唑二酮、选择性血清素再摄取抑制剂、抗痉挛剂、肝素、口服抗凝血剂、环利尿剂、钙调神经磷酸酶抑制剂、抗逆转录病毒治疗及质子泵抑制剂。先前尚未使用骨代谢标志以评估 Wnt 途径抑制剂的功效。于是，于某些实施方式中，本发明提供使用骨代谢标志以监测经 Wnt 途径抑制剂治疗的受试者的骨骼相关副作用及 / 或毒性的方法。于某些实施方式中，该方法使用骨生成生物标志以监测及 / 或侦测骨生成的减少程度。于某些实施方式中，该方法使用骨吸收生物标志以监测及 / 或侦测骨吸收的增加程度。于某些实施方式中，监测骨生成生物标志的量给予骨生成的减少程度及 / 或骨折的增加风险、骨质稀少及 / 或骨质疏松的早期指标。于某些实施方式中，监测骨吸收生物标志的量给予骨吸收的增加程度及 / 或骨折的增加风险、骨质稀少及 / 或骨质疏松的早期指标。于某些实施方式中，于骨骼功能不良的任何事件发生之前，藉由骨密度扫描，该方法侦测骨骼相关副作用及 / 或毒性。

[0219] 在某些实施方式中，该经检测、识别、监测、减少、预防、减弱、及 / 或筛选的骨骼相关不良反应及 / 或毒性，系由于投予 Wnt 途径抑制剂或使用 Wnt 途径抑制剂治疗所造成、所关联及 / 或有关。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂的活性。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂，该 Wnt 途径抑制剂系抗 FZD 抗体。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂，该 Wnt 途径抑制剂系抗 FZD 抗体 OMP-18R5。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂，该 Wnt 途径抑制剂系 FZD 可溶性受体。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂，该 Wnt 途径抑制剂系 FZD8-Fc 可溶性受体。在某些实施方式中，该骨骼相关不良反应及 / 或毒性系关于 Wnt 途径抑制剂，该 Wnt 途径抑制剂系 FZD8-Fc 可溶性受体 OMP-54F28。

[0220] 本发明提供选择受试者以使用 Wnt 途径抑制剂进行治疗的方法，其包含：测定样本中生物标志的量，及若该生物标志的量系低于预设量，则选择该受试者以使用该 Wnt 途径抑制剂进行治疗。在一些实施方式中，该选择受试者以使用 Wnt 途径抑制剂进行治疗的方法包含：自该受试者获得生物样本、测定该样本中生物标志的量、及若该生物标志的量系低于预设量则选择该受试者以使用该 Wnt 途径抑制剂进行治疗。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该骨转换标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系 β-CTX。

[0221] 在一些实施方式中，该选择受试者以使用 Wnt 途径抑制剂进行治疗的方法包含：自该受试者获得生物样本、测定该样本中骨转换标志的量、及若该骨转换标志的量系低于预设量则选择该受试者以使用该 Wnt 途径抑制剂进行治疗。在一些实施方式中，该生物样本系尿液、血液、血清、或血浆。在一些实施方式中，该骨转换标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX 或 β-CTX。因此，在一些实施方式中，该选择受试者以使用 Wnt 途径抑制剂进行治疗的方法包含：自该受试者获得生物样本、测定该样本中 β-CTX 的量、及若该

β-CTX 的量系低于预设量则选择该受试者以使用该 Wnt 途径抑制剂进行治疗。

[0222] 本发明提供识别受试者为适合使用 Wnt 途径抑制剂进行治疗的方法，其包含：测定样本中生物标志的量，及若该生物标志的量系低于预设量，则识别该受试者为适合使用该 Wnt 途径抑制剂进行治疗。在一些实施方式中，识别受试者为适合使用 Wnt 途径抑制剂进行治疗的方法包含：自该受试者获得生物样本、测定该样本中生物标志的量、及若该生物标志的量系低于预设量，则识别该受试者为适合使用该 Wnt 途径抑制剂进行治疗。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，识别受试者为适合使用 Wnt 途径抑制剂进行治疗的方法包含：自该受试者获得生物样本、测定该样本中 β-CTX 的量、及若该 β-CTX 的量系低于预设量，则识别该受试者为适合使用该 Wnt 途径抑制剂进行治疗。

[0223] 本发明亦提供监测接受 Wnt 途径抑制剂治疗的受试者是否发展骨骼相关不良反应及 / 或毒性的方法，其包含：测定样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中该生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。在一些实施方式中，监测接受 Wnt 途径抑制剂治疗的受试者是否发展骨骼相关不良反应及 / 或毒性的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中该生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系骨量减少或骨质疏松症。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，监测接受 Wnt 途径抑制剂治疗的受试者是否发展骨骼相关不良反应及 / 或毒性的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中 β-CTX 的量、及比较该样本中 β-CTX 的量与该 β-CTX 的预设量，其中 β-CTX 的量增加显示发展骨骼相关不良反应及 / 或毒性。

[0224] 本发明亦提供对接受 Wnt 途径抑制剂治疗的受试者检测骨骼相关不良反应及 / 或毒性发展的方法，其包含：测定样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中该生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。在一些实施方式中，对接受 Wnt 途径抑制剂治疗的受试者检测骨骼相关不良反应及 / 或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中该生物标志的量增加显示发展骨骼相关不良反应及 / 或毒性。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系骨量减少或骨质疏松症。在

一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉(PYD)、尿中游离脱氧吡啶啉(DPD)、尿中第1型胶原蛋白交联的N-端肽(NTX)、尿中或血清第1型胶原蛋白交联的C-端肽(CTX)、骨涎蛋白(BSP)、或抗酒石酸酸性磷酸酶5b。在一些实施方式中，该骨吸收生物标志系CTX。在一些实施方式中，该骨吸收生物标志系β-CTX。在一些实施方式中，对接受Wnt途径抑制剂治疗的受试者检测骨骼相关不良反应及/或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中β-CTX的量、及比较该样本中β-CTX的量与β-CTX的预设量，其中该β-CTX的量增加显示发展骨骼相关不良反应及/或毒性。

[0225] 本发明亦提供对接受Wnt途径抑制剂治疗的受试者识别骨骼相关不良反应及/或毒性发展的方法，其包含：测定样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则显示骨骼相关不良反应及/或毒性。在一些实施方式中，对接受Wnt途径抑制剂治疗的受试者识别骨骼相关不良反应及/或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则显示骨骼相关不良反应及/或毒性。在一些实施方式中，该骨骼相关不良反应及/或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及/或毒性系骨量减少或骨质疏松症。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉(PYD)、尿中游离脱氧吡啶啉(DPD)、尿中第1型胶原蛋白交联的N-端肽(NTX)、尿中或血清第1型胶原蛋白交联的C-端肽(CTX)、骨涎蛋白(BSP)、或抗酒石酸酸性磷酸酶5b。在一些实施方式中，该骨吸收生物标志系CTX。在一些实施方式中，该骨吸收生物标志系β-CTX。在一些实施方式中，对接受Wnt途径抑制剂治疗的受试者识别骨骼相关不良反应及/或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中β-CTX的量、及比较该样本中β-CTX的量与β-CTX的预设量，其中若该样本中β-CTX的量系高于β-CTX的预设量，则显示骨骼相关不良反应及/或毒性。

[0226] 本发明亦提供对接受Wnt途径抑制剂治疗的受试者监测骨骼相关不良反应及/或毒性发展的方法，其包含：测定样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则显示骨骼相关不良反应及/或毒性。在一些实施方式中，对接受Wnt途径抑制剂治疗的受试者监测骨骼相关不良反应及/或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则显示骨骼相关不良反应及/或毒性。在一些实施方式中，该骨骼相关不良反应及/或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及/或毒性系骨量减少或骨质疏松症。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉(PYD)、尿中游离脱氧吡啶啉(DPD)、尿中第1型胶原蛋白交联的N-端肽(NTX)、尿中或血清第1型胶原蛋白交联的C-端

肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，对接受 Wnt 途径抑制剂治疗的受试者监测心脏毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中 β-CTX 的量、及比较该样本中 β-CTX 的量与 β-CTX 的预设量，其中若该样本中 β-CTX 的量系高于 β-CTX 的预设量，则显示骨骼相关不良反应及 / 或毒性。

[0227] 本发明亦提供减少接受 Wnt 途径抑制剂治疗的受试者发生骨骼相关不良反应及 / 或毒性的方法，其包含：测定来自受试者的样本中生物标志的量、比较该样本中生物标志的量与该生物标志的预设量，以及若该样本中生物标志的量系高于该生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物诸如双膦酸盐。在一些实施方式中，减少接受 Wnt 途径抑制剂治疗的受试者发生骨骼相关不良反应及 / 或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、比较该样本中生物标志的量与该生物标志的预设量，以及若该样本中生物标志的量系高于该生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物诸如双膦酸盐。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系骨量减少或骨质疏松症。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，减少接受 Wnt 途径抑制剂治疗的受试者发生骨骼相关不良反应及 / 或毒性发展的方法包含：自该接受治疗的受试者获得生物样本、测定该样本中 β-CTX 的量、比较该样本中 β-CTX 的量与 β-CTX 的预设量、及若该样本中 β-CTX 的量系高于 β-CTX 的预设量则对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，该抗吸收药物系双膦酸盐。

[0228] 本发明亦提供使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法，其包含：测定来自受试者的样本中生物标志的量、比较该样本中生物标志的量与该生物标志的预设量、对该受试者投予治疗有效量的抗吸收药物、及对该受试者投予 Wnt 途径抑制剂。在一些实施方式中，使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法包含：在使用 Wnt 途径抑制剂治疗之前，自该接受治疗的受试者获得生物样本、测定该样本中生物标志的量、比较该样本中生物标志的量与该生物标志的预设量、对该受试者投予治疗有效量的抗吸收药物、及对该受试者投予该 Wnt 途径抑制剂。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系骨量减少或骨质疏松症。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX。在一些实施方式中，该骨吸收生物标志系 β-CTX。在一些实施方式中，使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒

性发展的方法包含：在使用 Wnt 途径抑制剂治疗之前，自该接受治疗的受试者获得生物样本、测定该样本中 β-CTX 的量、比较该样本中 β-CTX 的量与 β-CTX 的预设量；若该样本中 β-CTX 的量系高于 β-CTX 的预设量则对该受试者投予治疗有效量的抗吸收药物；及对该受试者投予该 Wnt 途径抑制剂。

[0229] 在本文所述的方法的一些实施方式中，该预设量系在血液、血清、或血浆样本中约 1500pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 1200pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 1000pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 800pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 600pg/ml 或更低。在一些实施方式中，该预设量系在血液、血清、或血浆样本中约 400pg/ml 或更低。在 β-CTX 的预设量的上下文中，用语“约”系指指称的量加或减该指称量的 10％。

[0230] 在一些实施方式中，该生物标志（例如骨吸收生物标志或 β-CTX）的预设量系该生物标志于稍早日期所获得的样本中的量。在一些实施方式中，该生物标志（例如骨吸收生物标志或 β-CTX）的预设量系该生物标志于最初筛选时所获得的样本中的量。在一些实施方式中，该生物标志（例如骨吸收生物标志或 β-CTX）的预设量系该生物标志于治疗前所获得的样本中的量。在一些实施方式中，该生物标志（例如骨吸收生物标志或 β-CTX）的预设量系该生物标志于最初筛选时所获得的样本中的量。在一些实施方式中，该生物标志（例如骨吸收生物标志或 β-CTX）的预设量系正常参考量。在一些实施方式中，该生物标志（例如骨吸收生物标志或 β-CTX）的预设量系基准量。在一些实施方式中，该基准量系在最初筛选时所测定的该生物标志的量。在一些实施方式中，该基准量系在治疗前所测定的该生物标志的量。

[0231] 在一些实施方式中，若该 β-CTX 于该样本中的量比预设量增加 2 倍或更高（即变为 2 倍或更高），则对该受试者投予治疗有效量的抗吸收药物。在一些实施方式中，若该 β-CTX 于该样本中的量比基准量增加 2 倍或更高（即变为 2 倍或更高），则对该受试者投予治疗有效量的抗吸收药物。

[0232] 在本文所述的任何方法中，生物样本系于约每周、每 2 周、每 3 周、每 4 周、每 5 周、或每 6 周获得。

[0233] 在本文所述的任何方法的一些实施方式中，受试者系利用双能量 X 光吸收仪 (DEXA) 骨密度扫描仪评估。此技术系测量骨矿物密度 (BMD) 最常用的测试。DEXA 输出包括 T 得分（比较受试者与 30 至 35 岁成人的骨密度），以及 Z 得分（比较受试者的骨密度与相符年龄性别族群的平均骨密度）。T 得分系用于根据标准评分决定受试者是否具有骨量减少或骨质疏松症。大于 -1 的 T 得分被认为是正常的骨密度；介于 -1 至 -2.5 之间的 T 得分被认为是骨量减少；小于 -2.5 的 T 得分被认为是骨质疏松症；小于 -2.5 的 T 得分且发生 1 次以上骨质疏松性骨折被认为是严重（已成立的）骨质疏松症。在一些实施方式中，若总股骨或脊椎 L1 至 L4 的 T 得分降低至小于 -2.5，表示具有骨骼相关不良反应及 / 或毒性。在一些实施方式中，若总股骨或脊椎 L1 至 L4 的 T 得分降低至小于 -2.0，表示具有骨骼相关不良反应及 / 或毒性。在一些实施方式中，若总股骨或脊椎 L1 至 L4 的 T 得分降低至小于 -1.5，表示具有骨骼相关不良反应及 / 或毒性。在一些实施方式中，若总股骨或脊椎 L1 至 L4 的 T 得分降低至小于 -1.0，表示具有骨骼相关不良反应及 / 或毒性。

[0234] 本发明亦提供使投予 Wnt 途径抑制剂的受试者改善骨骼相关不良反应及 / 或毒性发展的方法，其包含对该受试者投予治疗有效量的抗吸收药物。

[0235] 本发明亦提供筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法，其包含 :测定来自该受试者的样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则该受试者具有发生骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中，筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法包含 :在使用 Wnt 途径抑制剂治疗之前，自该受试者获得生物样本、测定该样本中生物标志的量、及比较该样本中生物标志的量与该生物标志的预设量，其中若该样本中生物标志的量系高于该生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系增加骨折风险。在一些实施方式中，该骨骼相关不良反应及 / 或毒性系骨量减少或骨质疏松症。在一些实施方式中，该生物标志系骨转换标志。在一些实施方式中，该生物标志系骨吸收生物标志。在一些实施方式中，该骨吸收生物标志系尿中羟基脯氨酸、尿中总吡啶啉 (PYD)、尿中游离脱氧吡啶啉 (DPD)、尿中第 1 型胶原蛋白交联的 N- 端肽 (NTX)、尿中或血清第 1 型胶原蛋白交联的 C- 端肽 (CTX)、骨涎蛋白 (BSP)、或抗酒石酸酸性磷酸酶 5b。在一些实施方式中，该骨吸收生物标志系 CTX。在一些实施方式中，该骨吸收生物标志系 β -CTX。在一些实施方式中，筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法包含 :在使用 Wnt 途径抑制剂治疗之前，自该受试者获得生物样本、测定该样本中 β -CTX 的量、及比较该样本中 β -CTX 的量与 β -CTX 的预设量，其中若该样本中 β -CTX 的量系高于 β -CTX 的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中，该 β -CTX 的预设量系在最初筛选时所测定的值。在一些实施方式中，β -CTX 的预设量系约 400 至 1200pg/ml。在一些实施方式中，若该受试者具有骨骼相关不良反应及 / 或毒性的风险，则在使用该 Wnt 途径抑制剂进行治疗之前，对该受试者投予治疗有效量的抗吸收药物。

[0236] 在本文所述的方法的一些实施方式中，该抗吸收药物系双膦酸盐。咸信双膦酸盐藉由“诱导”破骨细胞经历细胞凋亡，从而抑制骨消化，以预防骨质流失。在一些实施方式中，该双膦酸盐系选自 :羟乙膦酸盐 (etidronate)、氯屈膦酸盐 (clodronate)、替鲁膦酸盐 (tiludronate)、帕米膦酸盐 (pamidronate)、奈立膦酸盐 (neridronate)、奥帕膦酸盐 (olpadronate)、阿仑膦酸盐 (alendronate)(FOSAMAX)、伊班膦酸盐 (ibandronate)(BONIVA)、利塞膦酸盐 (risedronate)(ACTONEL) 及唑来膦酸 (zoledronic acid)(RECLAST)。在一些实施方式中，该双膦酸盐系唑来膦酸 (zoledronic acid)。在一些实施方式中，该抗吸收药物剂系抗 RANKL 抗体迪诺单抗 (denosumab)(PROLIA)。

[0237] 在本文所述的任何方法中，该 Wnt 途径抑制剂系抗 FZD 抗体。在本文所述的任何方法中，该 Wnt 途径抑制剂系抗 Wnt 抗体。在本文所述的任何方法中，该 Wnt 途径抑制剂系 FZD 可溶性受体。

[0238] 在本文所述的任何方法的某些实施方式中，该 Wnt 途径抑制剂系抗体，该抗体包含 :(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。

[0239] 在本文所述的任何方法的某些实施方式中，该 Wnt 途径抑制剂系包含重链可变区及轻链可变区的抗体，该重链可变区包含 SEQ ID NO:7，该轻链可变区包含 SEQ ID NO:8。

[0240] 在某些实施方式中，该 Wnt 途径抑制剂包含和 OMP-18R5 相同的重链可变区及相同的轻链可变区序列。在一些实施方式中，该 Wnt 途径抑制剂系抗体 OMP-18R5。OMP-18R5 系一种与人 FZD1、FZD2、FZD5、FZD7、及 FZD8 受体结合的 IgG2 人单克隆抗体，先前已于美国专利第 7,982,013 号中描述。

[0241] 在某些实施方式中，该 Wnt 途径抑制剂包含与保藏于美国菌种保存中心 (ATCC) 的编号 PTA-9541 的质粒所编码的抗体相同的重链及轻链氨基酸序列。在某些实施方式中，该 Wnt 途径抑制剂系由具有 ATCC 保藏编号 PTA-9541 的质粒所编码，该质粒依据布达佩斯条约的规定于 2008 年 9 月 29 日保藏于美国菌种保存中心 (ATCC)(地址 :10801University Boulevard,Manassas,VA,20110)。在某些实施方式中，该 Wnt 途径抑制剂与保藏于美国菌种保存中心 (ATCC) 的编号 PTA-9541 的质粒所编码的抗体，竞争与人 FZD 的特异性结合。

[0242] 在本文所述的任何方法的某些实施方式中，该 Wnt 途径抑制剂系 FZD 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系 FZD8 可溶性受体，其包含 SEQ ID NO:20、SEQ ID NO:30、或 SEQ ID NO:33。在一些实施方式中，该 Wnt 途径抑制剂系包含 SEQ ID NO:20 的 FZD8 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系包含 SEQ ID NO:30 的 FZD8 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系包含 SEQ ID NO:33 的 FZD8 可溶性受体。

[0243] 在本文所述的任何方法的某些实施方式中，该 Wnt 途径抑制剂系 FZD-Fc 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系 FZD8-Fc 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系 FZD8-Fc 可溶性受体，其包含 SEQ ID NO:39、SEQ ID NO:40、或 SEQ ID NO:41。在一些实施方式中，该 Wnt 途径抑制剂系包含 SEQ ID NO:39 的 FZD8-Fc 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系包含 SEQ ID NO:40 的 FZD8-Fc 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系包含 SEQ ID NO:41 的 FZD8-Fc 可溶性受体。在一些实施方式中，该 Wnt 途径抑制剂系 OMP-54F28。在一些实施方式中，该 Wnt 途径抑制剂不是 OMP-54F28。

[0244] 在一些实施方式中，该受试者罹患癌症。在一些实施方式中，该癌症系选自 :肺癌、乳癌、结肠癌、结直肠癌、黑色素瘤、胰癌、胃肠癌、肾癌 (renal cancer)、卵巢癌、肝癌、肝细胞癌 (HCC)、子宫内膜癌、肾癌 (kidney cancer)、前列腺癌、甲状腺癌、神经内分泌癌、神经胚细胞瘤、神经胶瘤、多形性神经胶质母细胞瘤、子宫颈癌、胃癌、膀胱癌、肝肿瘤、及头颈癌。如本文中所使用的“肺癌”系指所有肺癌，包括非小细胞肺癌 (NSCLC) 及小细胞肺癌 (SCLC)。在某些实施方式中，该癌系血癌诸如淋巴瘤或白血病。在一些实施方式中，该癌系乳癌。在某些实施方式中，该癌系 NSCLC。在某些实施方式中，该癌系卵巢癌。在某些实施方式中，该癌系胰癌。在一些实施方式中，该癌系肝癌。在某些实施方式中，该癌不是神经内分泌癌。

[0245] 因此，本发明亦提供治疗癌症的方法。在一些实施方式中，该等方法包含一种对有需要治疗的受试者治疗癌症的方法，其包含 :(a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;及 (b) 测定来自该受试者的样本中骨吸收生物标志的量。在一些实施方式中，治疗癌

症的方法包含 (a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;(b) 测定来自该受试者的样本中骨吸收生物标志的量 ;及 (c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量。在一些实施方式中,治疗癌症的方法包含 (a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;(b) 测定来自该受试者的样本中骨吸收生物标志的量 ;及 (c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ;其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量,则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中,治疗癌症的方法包含 (a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;(b) 测定来自该受试者的样本中骨吸收生物标志的量 ;及 (c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ;其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量,则对该受试者投予治疗有效量的抗吸收药物。

[0246] 本发明亦提供抑制肿瘤生长的方法。在一些实施方式中,该等方法包含一种对有需要抑制肿瘤生长的受试者抑制肿瘤生长的方法,其包含 :(a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;及 (b) 测定来自该受试者的样本中骨吸收生物标志的量。在一些实施方式中,抑制肿瘤生长的方法包含 (a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;(b) 测定来自该受试者的样本中骨吸收生物标志的量 ;及 (c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量。在一些实施方式中,抑制肿瘤生长的方法包含 (a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;(b) 测定来自该受试者的样本中骨吸收生物标志的量 ;及 (c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ;其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量,则该受试者具有骨骼相关不良反应及 / 或毒性的风险。在一些实施方式中,抑制肿瘤生长的方法包含 (a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ;(b) 测定来自该受试者的样本中骨吸收生物标志的量 ;及 (c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ;其中若该样本中骨吸收生物标志的量系高于该骨吸收生物标志的预设量,则对该受试者投予治疗有效量的抗吸收药物。

[0247] 在一些实施方式中,该生物样本系体液。在一些实施方式中,该生物样本系血液、血浆、血清、或尿液。在一些实施方式中,该生物样本系静脉全血样本。在一些实施方式中,该生物样本系使用 EDTA 或肝素作为抗凝剂的静脉全血样本。在一些实施方式中,该生物样本系血浆样本。在一些实施方式中,该生物样本系使用 EDTA 或肝素作为抗凝剂的血浆样本。体液样本可利用该领域已知的任何方法取得。在一些实施方式中,该生物样本系冷冻组织样本或新鲜组织样本。

[0248] 用于测量或测定样本中骨吸收生物标志 (例如 β-CTX) 的量的检测系为本领域技术人员所公知。例如,于某些实施方式中,使用能定量测量全血或血浆样本中 β-CTX 量的免疫检测。于某些实施方式中,该样本含有作为抗凝血剂的 EDTA。于某些实施方式中,该样本含有作为抗凝血剂的肝素。于某些实施方式中,该免疫检测包含针对 β-CTX 的 EKAHD-β-GGR 的氨基酸序列的 2 种高度特异性单克隆抗体,其中天冬氨酸残基系经 β 异构化。为得到免疫检测的特定信号,EKAHD-β-GGR 的 2 个链必须交联。于某些实施方式中,将样本和适当对照组置于经链霉抗生物素蛋白涂覆的微滴定孔槽中,随后加入含有针对 β-CTX 的 EKAHD-β-GGR 的氨基酸序列的生物素化单克隆抗体的溶液。经培育和冲洗后,将发色底物溶液加入至微滴定孔槽中。经培育后,令反应中止。读取该微滴定孔槽的吸亮

度并测定 β-CTX 浓度。

[0249] 在一些实施方式中，该 Wnt 途径抑制剂系以约 0.5mg/kg 的起始剂量投予。例如，抗体 OMP-18R5 系以 5％葡萄糖水 (USP) 稀释至总体积 250mL。OMP-18R5 系经由 0.22 微米滤器在 30 分钟内以静脉输注给予。在一些实施方式中，之后的剂量系以类似的方式投予。

[0250] 在本发明的另一态样中，本文中所述的方法可进一步包含投予一或多种额外的治疗剂。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。本发明亦提供包含 Wnt 途径抑制剂与额外治疗剂的医药组合物。在一些实施方式中，该一或多种额外治疗剂包含 1、2、3 或更多种额外的治疗剂。

[0251] 含有至少二种治疗剂的组合疗法通常使用具有不同作用机制的剂，虽然并非必要。使用不同作用机制的剂的组合疗法可能导致加成或协同效应。组合疗法可能允许相较于单一疗法使用较低剂量的各剂，藉以减少不良反应及 / 或毒性。组合疗法可能增加一或两种治疗剂的治疗指数。组合疗法可能减少抗药性癌细胞发生的可能性。在一些实施方式中，组合疗法包含主要影响 (例如抑制或杀灭) 非肿瘤发生性细胞的治疗剂及主要影响 (例如抑制或杀灭) 肿瘤发生性 CSC 的治疗剂。因此，在一些实施方式中，该 Wnt 途径抑制剂系与至少一种额外的治疗剂组合投予。在一些实施方式中，抗 FZD 抗体系与至少一种额外的治疗剂组合投予。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 系与至少一种额外的治疗剂组合投予。在一些实施方式中，FZD 可溶性受体系与至少一种额外的治疗剂组合投予。在一些实施方式中，该 FZD8-Fc 可溶性受体系与至少一种额外的治疗剂组合投予。

[0252] 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 (例如抗体或可溶性受体) 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调制剂中共投或利用分开的调制剂共投，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给药方案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给药方案亦描述于 The Chemotherapy Source Book, 4th Edition, 2008, M.C. Perry, Editor, Lippincott, Williams&Wilkins, Philadelphia, PA。

[0253] 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)、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)；defofamine；秋水仙胺(demecolcine)；地吖醌(diaziquone)；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)；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 RFS2000；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激素对肿瘤的作用的抗激素剂，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，该额外治疗剂系顺铂。在某些实施方式中，该额外治疗剂系卡铂。在某些实施方式中，该额外治疗剂系太平洋紫杉醇 (paclitaxel)。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卡铂 (carboplatin) 时，该经治疗的癌或肿瘤系肺癌或肺肿瘤。

[0254] 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。在某些实施方式中，该额外治疗剂系伊立替康 (irinotecan)。

[0255] 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。在某些实施方式中，该额外治疗剂系吉西他滨。在一些实施方式中，该额外治疗剂系培美曲塞。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吉西他滨时，该经治疗的癌或肿瘤系胰癌或胰肿瘤。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培美曲塞时，该经治疗的癌或肿瘤系肺癌或肺肿瘤。在一些实施方式中，该 Wnt 途径抑制剂系与培美曲塞和卡铂组合投予。在一些实施方式中，抗 FZD 抗体或 FZD 可溶性受体系与吉西他滨组合投予以治疗胰癌。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 或该 FZD8-Fc 可溶性受体 OMP-54F28 系与吉西他滨组合投予以治疗胰癌。在一些实施方式中，抗 FZD 抗体或 FZD 可溶性受体系与吉西他滨和经白蛋白结合的太平洋紫杉醇组合投予以治疗胰癌。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 或 FZD8-Fc 可溶性受体 OMP-54F28 系与吉西他滨和经白蛋白结合的太平洋紫杉醇组合投予以治疗胰癌。

在一些实施方式中，抗 FZD 抗体或 FZD 可溶性受体系与卡铂和太平洋紫杉醇或经白蛋白结合的太平洋紫杉醇组合投予以治疗卵巢癌。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 或 FZD8-Fc 可溶性受体 OMP-54F28 系与卡铂和太平洋紫杉醇或经白蛋白结合的太平洋紫杉醇组合投予以治疗卵巢癌。

[0256] 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 (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) 或其医药上可接受的盐、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。在一些实施方式中，抗 FZD 抗体或 FZD 可溶性受体系与太平洋紫杉醇或经白蛋白结合的太平洋紫杉醇组合投予以治疗乳癌。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 或 FZD8-Fc 可溶性受体 OMP-54F28 系与太平洋紫杉醇或经白蛋白结合的太平洋紫杉醇组合投予以治疗乳癌。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肺癌。在一些实施方式中，抗 FZD 抗体或 FZD 可溶性受体系与多西紫杉醇组合投予以治疗肺癌。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 或 FZD8-Fc 可溶性受体 OMP-54F28 系与多西紫杉醇组合投予以治疗肺癌。

[0257] 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（例如抗体）与作为其他肿瘤相关性蛋白质的抑制剂的小分子，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蛋白激酶的小分子。在某些实施方式中，该额外治疗剂系抑制酪氨酸蛋白激酶的小分子。在一些实施方式中，抗 FZD 抗体或 FZD 可溶性受体系与蛋白激酶抑制剂（例如索拉非尼 (sorafenib)）组合投予以治疗肝癌（例如 HCC)。在一些实施方式中，该抗 FZD 抗体 OMP-18R5 或该 FZD8-Fc 可溶性受体 OMP-54F28 系与蛋白激酶抑制剂（例如索拉非尼）组合投予以治疗肝癌（例如 HCC)。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小分子抑制剂。在一些实施方式中，该额外治疗剂系 Wnt 途径的小分子抑制剂。在一些实施方式中，该额外治疗剂系 BMP 途径的小分子抑制剂。在一些实施方式中，该额外治疗剂系抑制 β- 连环蛋白信号传导的小分子。

[0258] 在一些实施方式中，额外治疗剂包含生物性分子例如抗体。举例来说，治疗可涉及组合投予本发明的 Wnt 途径抑制剂（例如抗体）与拮抗其他肿瘤相关性蛋白质的其他抗体，包括但不限于与 EGFR、ErbB2、HER2 及 / 或 VEGF 结合的抗体。在某些实施方式中，该额外治疗剂系抗癌干细胞标志抗体的抗体。在一些实施方式中，该额外治疗剂系与缺口途径的成份结合的抗体。在一些实施方式中，该额外治疗剂系与 Wnt 途径的成份结合的抗体。在某些实施方式中，该额外治疗剂系抑制癌干细胞途径的抗体。在一些实施方式中，该额外治

疗剂系缺口途径的抗体抑制剂。在一些实施方式中，该额外治疗剂系 Wnt 途径的抗体抑制剂。在一些实施方式中，该额外治疗剂系 BMP 途径的抗体抑制剂。在一些实施方式中，该额外治疗剂系抑制 β-连环蛋白信号传导的抗体。在某些实施方式中，该额外治疗剂系血管生成抑制剂或调节剂的抗体（例如抗 VEGF 或 VEGF 受体抗体）。在某些实施方式中，该额外治疗剂系贝伐珠单抗 (bevacizumab)(AVASTIN)、曲妥珠单抗 (trastuzumab)(HERCEPTIN)、帕尼单抗 (panitumumab)(VECTIBIX) 或西妥昔单抗 (cetuximab)(ERBITUX)。组合投予可包括于单一医药调制剂中共投或利用分开的调制剂共投，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其生物活性。

[0259] 另外，以本文所述的 Wnt 途径抑制剂治疗可包括与其他生物分子组合治疗，例如一或多种细胞介素（例如淋巴介质、介白素、肿瘤坏死因子及 / 或生长因子），或可伴随手术移除肿瘤、癌细胞或任何其他医师认为必要的治疗。

[0260] 将了解的是，Wnt 途径抑制剂与额外治疗剂的组合可以任何顺序投予或同时投予。在一些实施方式中，该 Wnt 途径抑制剂系经投予至已先接受第二治疗剂治疗的受试者。在某些其他实施方式中，该 Wnt 途径抑制剂与第二治疗剂系经实质上同步或同时投予。举例来说，受试者可能在接受第二治疗剂（例如化学治疗）的疗程时被给予 Wnt 途径抑制剂（例如抗体）。在某些实施方式中，Wnt 途径抑制剂系于接受第二治疗剂治疗的一年内被投予。在某些替代性实施方式中，Wnt 途径抑制剂系于接受第二治疗剂的任何治疗的 10、8、6、4 或 2 个月内被投予。在某些其他实施方式中，Wnt 途径抑制剂系于接受第二治疗剂的任何治疗的 4、3、2、或 1 周内被投予。在一些实施方式中，Wnt 途径抑制剂系于接受第二治疗剂的任何治疗的 5、4、3、2 或 1 天内被投予。将另外了解的是，该二（或多）种剂或治疗可在数小时或数分钟内（即实质上同步）被投予至受试者。

[0261] 如该领域的技术人员所知，投予任何治疗剂可能导致不良反应及 / 或毒性。在一些情况中，不良反应及 / 或毒性非常严重以至于无法投予治疗有效剂量的特定剂。在一些情况中，药物治疗必须中断，并尝试其他剂。然而，许多相同治疗类别的剂通常展现类似的不良反应及 / 或毒性，表示受试者必须停止治疗，或可能的话忍受与该治疗剂有关的不适不良反应。

[0262] 治疗剂的不良反应可能包括但不限于麻疹、皮肤红疹、发痒、恶心、呕吐、食欲减低、下痢、畏寒、发烧、疲倦、肌肉痛、头痛、低血压、高血压、低血钾、低血球数、出血及心脏问题。

[0263] 因此，在一些实施方式中，本文所述的方法包括使用间歇性给药方案，其可能减少与投予 Wnt 途径抑制剂有关的不良反应及 / 或毒性。如本文中所使用的“间歇性投药”系指投药间隔超过每周一次的投药方案，例如每 2 周投药一次、每 3 周一次、每 4 周一次、等。在一些实施方式中，治疗受试者的方法包含根据间歇性投药方案对该受试者投予有效剂量的 Wnt 途径抑制剂（例如抗 FZD 抗体或 FZD 可溶性受体）。在一些实施方式中，该方法包含根据间歇性投药方案对该受试者投予有效剂量的 Wnt 途径抑制剂（例如抗 FZD 抗体或 FZD 可溶性受体），并增加该 Wnt 途径抑制剂的治疗指数。在一些实施方式中，该间歇性投药方案包含对该受试者投予初始剂量的 Wnt 途径抑制剂，接着以大约每 2 周一次投予该 Wnt 途径抑制剂的后续剂量。在一些实施方式中，该间歇性投药方案包含对该受试者投予初始剂量的 Wnt 途径抑制剂，接着以大约每 3 周一次投予该 Wnt 途径抑制剂的后续剂量。在一些

实施方式中，该间歇性投药方案包含对该受试者投予初始剂量的 Wnt 途径抑制剂，接着以大约每 4 周一次投予该 Wnt 途径抑制剂的后续剂量。

[0264] 在一些实施方式中，间歇性投药方案中的后续剂量系大约与初始剂量相同或少于的量。在其他实施方式中，该后续剂量的量系高于该初始剂量。如该领域的技术人员所知，使用的剂量将视所欲达成的临床目标而异。在一些实施方式中，该初始剂量系约 0.25mg/kg 至约 20mg/kg。在一些实施方式中，该初始剂量系约 0.25、0.5、1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19 或 20mg/kg。在某些实施方式中，该初始剂量系约 0.5mg/kg。在某些实施方式中，该初始剂量系约 1mg/kg。在某些实施方式中，该初始剂量系约 2.5mg/kg。在某些实施方式中，该初始剂量系约 5mg/kg。在某些实施方式中，该初始剂量系约 7.5mg/kg。在某些实施方式中，该初始剂量系约 10mg/kg。在某些实施方式中，该初始剂量系约 12.5mg/kg。在某些实施方式中，该初始剂量系约 15mg/kg。在某些实施方式中，该初始剂量系约 20mg/kg。在一些实施方式中，该后续剂量系约 0.25mg/kg 至约 20mg/kg。在某些实施方式中，该后续剂量系约 0.5、1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19 或 20mg/kg。在某些实施方式中，该后续剂量系约 0.5mg/kg。在某些实施方式中，该后续剂量系约 1mg/kg。在某些实施方式中，该后续剂量系约 2.5mg/kg。在某些实施方式中，该后续剂量系约 5mg/kg。在一些实施方式中，该后续剂量系约 7.5mg/kg。在一些实施方式中，该后续剂量系约 10mg/kg。在一些实施方式中，该后续剂量系约 12.5mg/kg。在一些实施方式中，该后续剂量系约 15mg/kg。在一些实施方式中，该后续剂量系约 20mg/kg。

[0265] 在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 2.5mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 2 周投予一次约 2.5mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 5mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 2 周投予一次约 5mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 2.5mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 3 周投予一次约 2.5mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 5mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 3 周投予一次约 5mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 10mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 3 周投予一次约 10mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 15mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 3 周投予一次约 15mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 20mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 3 周投予一次约 20mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 2.5mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 4 周投予一次约 2.5mg/kg 的后续剂量。在一些实施方式中，该间歇性投药方案包含：(a) 对受试者投予约 5mg/kg 的 Wnt 途径抑制剂的初始剂量及 (b) 每 4 周投予一次约 5mg/kg 的后续剂量。在某些实施方式中，该初始剂量与维持剂量不同，例如初始剂量系约 5mg/kg，后续剂量系约 2.5mg/kg。在某些实施方式中，间歇性投药方案可能包含负荷剂量，例如初始剂量系约 20mg/kg，后续剂量系每 2 周、每 3 周或每 4 周投予一次约 2.5mg/kg 或约 5mg/kg。

[0266] 在本文所述的方法的一些实施方式中，治疗癌的方法包含对需要治疗的受试者投予治疗有效量的 OMP-18R5，剂量为 (a) 约每一至二周至少约 0.5mg/kg，或 (b) 约每三周至

少约 1.0mg/kg。在一些实施方式中，治疗癌的方法包含对需要治疗的受试者投予治疗有效量的 OMP-18R5，剂量为约每一至二周约 0.5mg/kg 至约 1.0mg/kg。在一些实施方式中，治疗癌的方法包含对需要治疗的受试者投予治疗有效量的 OMP-18R5，剂量为约每三周约 1.0mg/kg 至约 10.0mg/kg。在一些实施方式中，治疗癌的方法包含对需要治疗的受试者投予治疗有效量的 OMP-18R5，剂量为约每三周约 10mg/kg 至约 20.0mg/kg。

[0267] 在某些实施方式中，治疗人病患的癌症的方法包含每 3 周一次对该病患投予一剂量的 Wnt 途径抑制剂，且重复此投药总共 3、4、5、6、7、8、或更多个循环。在某些实施方式中，治疗人病患的癌症的方法包含每 3 周一次对该病患投予一约 10mg/kg 剂量的 Wnt 途径抑制剂，且重复此投药总共 3、4、5、6、7、8、或更多个循环。在某些实施方式中，治疗人病患的癌症的方法包含每 3 周一次对该病患投予一约 15mg/kg 剂量的 Wnt 途径抑制剂，且重复此投药总共 3、4、5、6、7、8、或更多个循环。在某些实施方式中，治疗人病患的癌症的方法包含每 3 周一次对该病患投予一约 20mg/kg 剂量的 Wnt 途径抑制剂，且重复此投药总共 3、4、5、6、7、8、或更多个循环。在一些实施方式中，该投予系重复 4 个循环。在一些实施方式中，该投予系重复 5 个循环。在一些实施方式中，该投予系重复 6 个循环。在一些实施方式中，该投予系重复 7 个循环。在一些实施方式中，该投予系重复 8 个循环。

[0268] 本发明的另一方面关于减少 Wnt 途径抑制剂于人受试者的毒性的方法，该方法包含利用间歇性投药方案对该受试者投予 Wnt 途径抑制剂。本发明的另一方面关于减少 Wnt 途径抑制剂于人受试者的不良反应的方法，该方法包含利用间歇性投药方案对该受试者投予 Wnt 途径抑制剂。本发明的另一方面关于增加 Wnt 途径抑制剂于人受试者的治疗指数的方法，该方法包含利用间歇性投药方案对该受试者投予 Wnt 途径抑制剂。

[0269] 选择初始及后续剂量的递送方法系根据受试者耐受 Wnt 途径抑制剂导入体内的能力而定。因此，在本文所述的任何方面 / 或实施方式中，Wnt 途径抑制剂的投予可能藉由静脉注射或经静脉投予。在一些实施方式中，该投予系藉由静脉输注。在本文所述的任何方面 / 或实施方式中，Wnt 途径抑制剂的投予可能藉由非静脉途径投予。

[0270] 在某些实施方式中，该治疗涉及投予本发明的 Wnt 途径抑制剂（例如抗体）与放射治疗的组合。使用 Wnt 途径抑制剂治疗可发生于投予放射治疗之前、的同时或之后。该放射治疗的投予方案可由经验丰富的医生决定。

[0271] 本揭示内容的实施方式可进一步参照下列非限制性实例定义，其叙述使用 Wnt 途径抑制剂以治疗癌症。该领域的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

于乳房异种移植模型中间歇性投予抗 FZD 抗体 OMP-18R5 及对肿瘤生长的功效

[0272] 将 UM-PE13 乳房肿瘤细胞 (20,000 细胞）经皮下注射至 6 至 8 周大 NOD/SCID 小鼠。将动物随机分组 (n = 10 只 / 组）并经抗 FZD 抗体 OMP-18R5 与紫杉醇 (Taxol) 的组合处理及单独经紫杉醇处理。每周投予紫杉醇 (10mg/kg) 且每 3 周投予 OMP-18R5 剂量 5、10、25 或 45mg/kg 一次。该等药剂经腹膜内投予。于指定日期使用电子测径器测量肿瘤体积。

[0273] 如图 1 所示，每 3 周投予 OMP-18R5 与紫杉醇的组合于如此低剂量 5mg/kg 或 10mg/kg 下能有效地降低 PE-13 肿瘤生长。该肿瘤生长抑制作用大于每周单独投予紫杉醇所观察

到的肿瘤生长抑制作用。较高剂量的 OMP-18R5(25mg/kg 和 45mg/kg) 与紫杉醇的组合更能较大地抑制肿瘤生长且于后期时点观察到肿瘤消退。此等结果证实使用间歇性给药摄取方式能维持抗 FZD 抗体与化学治疗剂（诸如紫杉醇）组合治疗的功效。

实施例 2

间歇性投予抗 FZD 抗体 OMP-18R5 对骨生成的功效

[0274] 将 UM-PE13 乳房肿瘤细胞 (20,000 细胞）经皮下注射至 6 至 8 周大 NOD/SCID 小鼠。将动物随机分组 (n ＝ 10 只 / 组）并经抗 FZD 抗体 OMP-18R5 与紫杉醇 (Taxol) 的组合处理及单独经紫杉醇处理。每周投予紫杉醇 (15mg/kg) 一次且每 4 周、每 2 周或每周投予 OMP-18R5(25mg/kg) 一次。该等药剂经腹膜内投予。于指定日期使用电子测径器测量肿瘤体积。

[0275] 如图 2 所示，每周一次、每 2 周一次及每 4 周一次投予 OMP-18R5(25mg/kg) 与紫杉醇的组合能有效地降低 PE-13 肿瘤生长。投予 OMP-18R5 与紫杉醇的组合的肿瘤生长抑制作用大于单独投予紫杉醇所观察到的肿瘤生长抑制作用。

[0276] 于第 77 天给药结束时，对经 OMP-18R5 处理的小鼠和经紫杉醇单独处理的对照组小鼠比较评估小梁骨生成。

[0277] 自对照组小鼠和经 OMP-18R5 处理的小鼠的胫骨制备组织切片并经苏木素和伊红 (H&E) 染色。白色箭头所标记的淡粉红色染色区对应小梁骨。

[0278] 如图 3 所示，每 2 周一次投予 OMP-18R5(25mg/kg) 比每周一次投予 OMP-18R5(25mg/kg) 更能减少骨流失。重要地，每 4 周一次投予 OMP-18R5(25mg/kg) 于骨生成上并未显现可观察到的功效。

实施例 3

唑来膦酸 (zoledronic acid) 于降低 OMP-18R5 对骨生成的功效的效果

[0279] 将 NOD/SCID 小鼠随机分组 (n ＝ 5 只 / 组）并经抗 FZD 抗体 OMP-18R5 处理或经 OMP-18R5 与唑来膦酸的组合处理。小鼠仅于第 1 和 15 天经 OMP-18R5(20mg/kg) 处理或于第 1 和 15 天经 OMP-18R5(20mg/kg) 和于第 1 天经唑来膦酸（单一第 IV 剂量 100ug/kg) 组合处理。于第 29 天给药结束时，比较经单独 OMP-18R5 处理的小鼠的股骨和胫骨与经 OMP-18R5 与唑来膦酸组合处理的小鼠和经对照组抗体处理的小鼠的股骨和胫骨。

[0280] 如实施例 2 所描述的方式，制备股骨和胫骨的组织切片。

[0281] 如图 4 所示，与经对照组抗体处理的小鼠相比较，对经 OMP-18R5 处理的小鼠投予单一第 IV 剂量的唑来膦酸导致软骨下骨生成。额外的研究已证实共同投予唑来膦酸并不影响 OMP-18R5 的抗肿瘤功效。此等数据支持该假设：投予双磷酸盐可保护性对抗 Wnt 抑制作用的分解代谢作用，提供能维持骨完整性并能标靶 Wnt 路径的益处的途径。

实施例 4

OMP-18R5 对罹患实体肿瘤的病患的第 1a 期研究

[0282] 本研究系 OMP-18R5 对罹患实体肿瘤的病患的开放标记性第 1a 期剂量累增性研究，其中不继续存在标准治愈性治疗且不存在经证实具有存活益处的治疗。本研究的主要目标系测定 OMP-18R5 的安全性和最大容忍剂量。第 2 目标系测定 OMP-18R5 的致免疫速率、初期功效及药效动力学。

[0283] 令该试验的起初阶段的病患经 OMP-18R5 的给药摄取处理：每周 0.5mg/kg(n ＝ 3)

和每周 1.0mg/kg(n = 5)。于接受研究药物达约 100 天之后,接受每周一次 0.5mg/kg 的一位病患的前肋骨和腰椎发生骨折。因此,于此试验的现行阶段(研究持续进行且病患依然登录)中,使用较不频繁给药。特定地,剂量为每 2 周一次 0.5mg/kg(n = 3) 和每 3 周一次 1mg/kg(n = 4)、2.5mg/kg(n = 3)、5mg/kg(n = 3)、10mg/kg(n = 3)、15mg/kg(n = 5) 及 20mg/kg(n = 5)(2014 年 1 月 10 日)。至第 28 天,3 个受试者的多个群经处理并评估剂量限制性毒性 (DLT)。若 3 个受试者中无病患显现 DLT,则进行累增至下一个剂量群。若 3 个受试者中 1 位病患显现 DLT,则 3 个额外的受试者经处理。若 2 或多个受试者显现 DLT,则无其他受试者接受该剂量且 3 个额外的受试者加入前一剂量群,除非 6 个受试者业已经该剂量处理。于第 56 天进行肿瘤评估且随后每隔 56 天进行肿瘤评估。于第 56 天罹患稳定疾病或产生反应的病患将被允许持续接受 OMP-18R5 直至疾病进展。

[0284] 在一位病患历经骨骼相关(骨折)事件后,使用得自首 8 位病患的样本以测量 4 种骨代谢指标:骨特异性碱性磷酸酶、原胶原第 1 型 N 端前肽 (P1NP)、骨钙化素及胶原第 1 型交联性 C- 端肽(β-CTX)。虽然于治疗期间未发现骨特异性碱性磷酸酶、P1NP 及骨钙化素发生变化,但是具有至少一个随后值的所有 7 个受试者皆观察到 β-CTX 增加(表 1;增加的 β-CTX 值系标记底线)。

表 1

病患	肿瘤类型	剂量 (mg/kg)	天数	β-CTX
1	直肠	0.5 QW	第0天	570
2	直肠	0.5 QW	第0天 第28天 处理终止	196 308 217
3	神经内分泌（类癌）	0.5 QW	第0天 第28天 第56天 处理终止	219 825 896 708
4	平滑肌肉瘤	1 QW	第0天 处理终止	298 401
5	乳房	1 QW	第0天 第28天 处理终止	229 681 370
6	直肠	1 QW	第0天 第28天	162 598
7	结肠	1 QW	第0天 第28天 处理终止	144 301
8	胰	1 QW	第0天 第28天	406 551

[0285] 因此，β-CTX 似乎是 OMP-18R5 对骨的功效的早期敏感性生物标志。

[0286] 基于起初的第 1a 期研究结果，修改研究计划书以包括经由 DEXA 骨密度扫描、骨扫描及测量骨代谢生物标志骨特异性碱性磷酸酶、P1NP、骨钙化素及 β-CTX 以监测骨骼相关副作用及 / 或毒性。经修改的计划书亦包括处理骨骼相关副作用及 / 或毒性的策略。显现至少扫描值 β-CTX 值倍增或于全股骨或 L1-L4DEXA 扫描测量的 T 分数下降至低于 -2.5 的任何病患将被投予抗吸收药剂，特别是该双磷酸盐唑来膦酸。于该 β-CTX 值倍增或 T 分数下降的时点，经静脉内投予唑来膦酸（剂量 5mg）。

[0287] 表 2 显示 26 位病患的结果 (2014 年 1 月 10 日)，该等病患随后登录并经较低频率投予（即间歇性给药）OMP-18R5 处理（至少为 2 倍基础值的 β-CTX 值系经底线标记且星号表示投予唑来膦酸时）。

表 2

病患	肿瘤类型	剂量（mg/kg）	天数	β-CTX	T天数
9	黑色素瘤	0.5 Q2W	第0天	203	-0.7
			第28天	195	
			第56天	287	-0.9
10	神经内分泌（胰脏）	0.5 Q2W	第0天	306	-1.4
			第28天	286	
			第56天	304	-1.0
			第84天*	664	
			第112天	270	-0.9
			第140天	288	
			第168天	413	-1.0
			第196天	372	
			第224天	377	-0.9
			第252天	363	
			第280天	424	-1.0
			第308天	505	
			第336天	499	-1.2
			第364天	420	
			第392天	430	-1.3
			第420天	402	
			第448天	461	
				374	-1.5

11	直肠	0.5 Q2W	第0天 第42天 处理终止	308 358 327	0.9 0.9
12	神经内分泌（胰脏）	1 Q3W	第0天 第28天 第56天 第84天 第112天 第140天 第168天 第196天 第224天 第252天 第280天 第308天 第336天 第364天 第392天* 第420天 第448天 第476天 第504天 第532天 第560天	689 846 707 350 759 526 967 688 1216 1174 1223 1045 890 1380 1332 218 274 246 214 510 341	-1.4 -1.3 -1.4 -1.8 -1.7 -1.7 -1.9 -2.0 -2.3 -2.1
13	膀胱	1 Q3W	第0天 处理终止	618 876	-0.9 -1.2
14	结肠	1 Q3W	第0天 第28天 处理终止	471 760 688	+2.4 +2.2
15	结肠	1 Q3W	第0天 第28天 第56天 处理终止	340 469 586 156	-0.7 -0.8

16	乳房	2.5 Q3W	第0天 第28天* 处理终止	386 805 345	-0.7 -0.8
17	胸腺	2.5 Q3W	第0天 第28天	232 309	-1
18	硬纤维	2.5 Q3W	第0天 第28天 处理终止	607 555 824	-0.9 -1.0
19	食道	5 Q3W	第0天 第28天 处理终止 *	648 811 1336	+0.1 -0.1
20	甲状腺髓质	5 Q3W	第0天 第28天 第56天	561 665 1111	-1.0 -1.0
21	直肠	5 Q3W	第0天	629	-0.1
22	子宫颈	10 Q3W	第0天 第28天	367 697	-1.2
23	软骨肉瘤	10 Q3W	第0天 第28天* 处理终止	568 1449 199	-1.3 -1.6
24	阑尾	10 Q3W	第0天 第28天* 第56天	114 652 172	-1.3
25	神经内分泌	15 Q3W	第0天 第28天	927 ND	-0.7
26	肛门小细胞瘤	15 Q3W	第0天 第28天 第56天*	596 1171 1278	-1.3 -1.5
27	乳房	15 Q3W	第0天 第28天	492 ND	-1.9
28	直肠	15 Q3W	第0天 第28天 第56天* 第84天	385 667 898 259	+0.6 +0.4

29	直肠	15 Q3W	第0天 第28天 处理终止	964 905 907	-1.6
30	腺样囊状腺癌	20 Q3W	第0天 第28天 第56天	290 306 375	+1.5
31	直肠	20 Q3W	第0天 第28天 第56天	434 ND 848	
32	腺样囊状腺癌	20 Q3W	第0天 第28天	550 148	-2.3
33	HCC	20 Q3W	第0天 第28天	473 979	+0.8
34	小肠腺癌	20 Q3W	第0天	596	

[0288] 于 2013 年 1 月时点，首 10 位额外病患（病患 9 至 18）中仅有 2 位显现 β-CTX 值倍增（病患 10 自基础值 306 至第 84 天的 664 且病患 16 自基础值 386 至第 28 天的 805）。于 2014 年 1 月 10 日时点，26 位额外病患（病患 9 至 34）中有 9 位显现 β-CTX 值倍增。此等数据建议：于所研究的剂量下，较低频率投予 OMP-18R5 导致 β-CTX 值较少上升和较低的骨毒性。依据经修改的计划书，对病患 10 经静脉内投予唑来膦酸（剂量 5mg）。经投予唑来膦酸后，该 β-CTX 值返回至约基础值，第 112 天为 270 且于随后的测量维持于约该值。因 β-CTX 值倍增，病患 16 亦接受唑来膦酸且经治疗后其 β-CTX 值亦返回至基础值。因 β-CTX 值倍增，病患 12 接受唑来膦酸且随后其 β-CTX 值返回至基础值的约 1/3。病患 16、19、23、24、26 及 28 皆经唑来膦酸处理且随后其 β-CTX 值下降。此等数据建议：唑来膦酸阻断及 / 或抑制 OMP-18R5 的骨吸收性且可用于减轻此骨骼相关副作用。

[0289] 于 2013 年 1 月时点，本研究所登录的所有病患于经 OMP-18R5 治疗时皆未显现经 DEXA 扫描（T 分数）的骨矿物质密度（BMD）的显著变化（表 3）。于 2014 年 1 月 10 日时点，本研究所登录的所有病患于经 OMP-18R5 治疗时皆未显现经 DEXA 扫描（T 分数）的 BMD 的显著变化（表 2）。

表 3

病患	DEXA时点	位置	T分数
1	筛选	AP脊柱L1-L4	-1.6
	终止	AP脊柱L1-L4	-1.9
	筛选	AP脊柱L3	-2.0
	终止	AP脊柱L3-L4	-2.1
	筛选	双重股骨颈左侧	-1.8
	终止	双重股骨颈右侧	-1.7
	筛选	双重股骨全部平均	-1.7
	终止	双重股骨全部平均	-2.2
3	筛选	AP脊柱L1-L2	-0.1
	筛选	AP脊柱L1-L4	+0.2
	终止	AP脊柱L1-L4	+0.7
	终止	AP脊柱L3-L4	+0.5
	筛选	双重股骨颈左侧	-0.1
	终止	双重股骨颈右侧	+0.2
	筛选	双重股骨全部平均	+1.0
	终止	双重股骨全部平均	+0.7
5	筛选	股骨	-1.2
	终止	股骨	-1.0
	筛选	腰椎	-0.6
	终止	腰椎	-0.5

7	筛选	股骨	+1.2
	终止	股骨	+0.7
	筛选	腰椎	+0.9
	终止	腰椎	+0.9
9	筛选	腰椎	-0.7
	终止	腰椎	-0.9
	筛选	髋	+0.2
	终止	髋	+0.2
10	筛选	AP脊柱L1-L2	-0.9
	筛选	AP脊柱L1-L4	-0.4
	筛选	双重股骨颈左侧	-1.4
	筛选	双重股骨全部平均	-0.9
	第56天	腰椎	-0.3
	第56天	髋	-0.8
11	筛选	股骨	+1.0
	终止	髋	+0.9
	筛选	腰椎	+0.9
	终止	腰椎	+1.1
13	筛选	腰椎	+0.1
	终止	腰椎	+0.3
	筛选	髋	-0.9
	终止	髋	-1.2
14	筛选	腰椎	+3.6
	终止	腰椎	+3.9
	筛选	髋	+2.4
	终止	髋	+2.2
16	筛选	腰椎	+0.7
	终止	腰椎	+0.8

[0290] 此等数据建议：骨质疏松病患可经 OMP-18R5 治疗且未有发生骨矿物质密度进一步下降的显著风险。再者，证实 β-CTX 似乎是因使用 Wnt 途径抑制剂进行治疗所导致的骨骼相关副作用及/或毒性的早期敏感性生物标志。最后，本研究已显示伴随 OMP-18R5 治疗的骨骼相关副作用似乎是可管理且可逆转的。

实施例 5

OMP-54F28 对罹患实体肿瘤的病患的第 1a 期研究

[0291] 本研究系 OMP-54F28 对罹患实体肿瘤的病患的开放标记性第 1a 期剂量累增性研究，其中不继续存在标准治愈性治疗。本研究的主要目标系测定 OMP-54F28 的安全性和最

大容忍剂量。第 2 目标系测定 OMP-54F28 的致免疫速率、初期功效及药效动力学。

[0292] 令该试验的起初阶段的病患经 OMP-54F28 的给药摄取处理：每 3 周一次 0.5mg/kg(n = 3)、1.0mg/kg(n = 3)、2.5mg/kg(n = 3)、5mg/kg(n = 5)、10mg/kg(n = 3)、15mg/kg(n = 3) 及 20mg/kg(n = 5)。本研究持续进行且病患依然登录。至第 28 天，3 个受试者的多个群经处理并评估剂量限制性毒性 (DLT)。若 3 个受试者中无病患显现 DLT，则进行累增至下一个剂量群。若 3 个受试者中 1 位病患显现 DLT，则 3 个额外的受试者经处理。若 2 或多个受试者显现 DLT，则无其他受试者接受该剂量且 3 个额外的受试者加入前一剂量群，除非 6 个受试者业已经该剂量处理。于第 56 天进行肿瘤评估且随后每隔 56 天进行肿瘤评估。于第 56 天罹患稳定疾病或产生反应的病患将被允许持续接受 OMP-54F28 直至疾病进展。

[0293] 基于第 1 期 OMP-18R5 研究所获得的结果，显现至少扫描值 β-CTX 值倍增或于全股骨或 L1-L4DEXA 扫描测量的 T 分数下降至低于 -2.5 的任何病患将被投予唑来膦酸。于该 β-CTX 值倍增或 T 分数下降的时点，经静脉内投予唑来膦酸 (剂量 5mg)。

[0294] 表 4 显示首 6 位病患的结果 (2013 年 1 月)，该等病患经登录并经每 3 周一次 OMP-54F28 处理 (至少为 2 倍基础值的 β-CTX 值系经底线标记)。

表 4

病患	肿瘤类型	剂量 (mg/kg)	天数	β-CTX
1	卵巢	0.5 Q3W	第0天 第28天 第56天 处理终止	215 144 119 104
2	直肠	0.5 Q3W	第0天 第28天 处理终止	538 604 1122
3	胰	0.5 Q3W	第0天 第28天 第56天 第84天	497 360 414 614
4	腺囊状	1 Q3W	第0天 第28天	346 289
5	肾细胞	1 Q3W	第0天 第28天	657 346
6	神经内分泌 (子宫颈)	1 Q3W	第0天 第28天	262 238

[0295] 表 5 显示首 25 位病患的结果 (2014 年 1 月 9 日)，该等病患经登录并经每 3 周一次 OMP-54F28 处理 (至少为 2 倍基础值的 β-CTX 值系经底线标记且星号表示投予唑来膦酸时)。

表 5

病患	肿瘤类型	剂量 (mg/kg)	天数	β-CTX	T天数
1	卵巢	0.5 Q3W	第0天	215	+0.1
			第28天	144	
			第56天	119	+0.5
			处理终止	104	
2	直肠	0.5 Q3W	第0天	538	+0.5
			第28天	604	
			处理终止	1122	
3	胰	0.5 Q3W	第0天	497	-0.1
			第28天	360	
			第56天	414	+2.4
			第84天	614	
			第112天	605	+2.1
			第140天	605	
			第168天*	1009	+1.7
			第196天	251	
4	腺囊状	1 Q3W	第0天	346	+1.2
			第28天	289	
			第56天	277	+0.6
5	肾细胞	1 Q3W	第0天	657	+0.5
			第28天	346	
			第56天	344	+0.5
			第84天	359	
			第112天	359	+0.5
			第140天	300	
6	大细胞神经内分泌（子宫颈）	1 Q3W	第0天	262	+0.1
			第28天	238	
			第56天	245	+0.1

7	直肠	2.5 Q3W	第0天 第28天 处理终止	890 1450 972	-1.2 -1.4
8	NSCLC	2.5 Q3W	第0天 第28天 处理终止	396 378 497	-0.1 -0.4
9	胆管癌	2.5 Q3W	第0天 第28天	725 485	-0.5 -0.5
10	尿道上皮癌	5 Q3W	第0天 第28天 第56天	634 570 484	+3.0 +2.2
11	直肠	5 Q3W	第0天 第28天 处理终止	563 852 744	-1.3 -0.8
12	子宫颈	5 Q3W	第0天 第28天 第56天	605 479 558	-0.9 -0.5
13	肾细胞	5 Q3W	第0天 第28天 第56天	250 387 378	0.0 +0.6
14	硬纤维	5 Q3W	第0天 第28天 第56天 第84天 第112天 第140天 第168天 第196天 第224天	354 167 362 242 233 245 114 296 276	-1.3 -1.0 -1.3 -1.4
15	平滑肌肉瘤	10 Q3W	第0天 第28天	386 300	-1.8

16	硬纤维	10 Q3W	第0天 第28天 第56天* 第84天 第112天 第140天 第175天 第196天	355 435 806 180 182 192 319 222	-0.6 -0.9 -0.8
17	HCC	10 Q3W	第0天 第28天 第56天	207 148 114	+1.2 +1.1
18	直肠	15 Q3W	第0天 第28天 处理终止	796 864 529	-0.5 -0.5
19	胰	15 Q3W	第0天 第28天 处理终止	770 824 610	-1.2
20	骨癌	15 Q3W	第0天 第28天 处理终止	518 512 476	-0.8 -0.3
21	睾丸	20 Q3W	第0天 第28天 第56天 第84天*	191 239 309 482	-0.1 -0.2
22	NSCLC	20 Q3W	第0天 第28天 第56天 第84天	648 851 698 402	-0.2 -0.2
23	甲状腺	20 Q3W	第0天 第28天* 第56天	262 731 251	+0.1
24	基底细胞	20 Q3W	第0天 第28天	660 670	-0.6
25	胰	20 Q3W	第0天 第28天	511 882	

[0296] 于2013年1月时点，病患2显现β-CTX值倍增（自基础值538至第42天的1122。该病患的疾病进展且中止使用OMP-54F28进行治疗。于2014年1月9日时点，25位病患

中有 5 位显现 β-CTX 值倍增。病患 3、16、21 及 28 经唑来膦酸处理且随后其 β-CTX 值降低至基础值或低于基础值。如同经 OMP-18R5 处理所观察到的结果，此等初期数据建议：每 3 周一次经剂量 0.5mg/kg、1.0mg/kg、2.5mg/kg、5mg/kg、10mg/kg、15mg/kg 及 20mg/kg 的 OMP-54F28 处理能导致 β-CTX 值较少上升和较低的骨毒性。此等经 OMP-54F28 处理的早期结果进一步证实：伴随使用 Wnt 途径抑制剂进行治疗的骨骼相关副作用似乎是可管理且为合理的缓和策略。

[0297] 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该领域的技术人员建议且将被纳入本申请案的精神与范围内。

[0298] 本说明书所引用的所有文献、专利及专利申请案系为相同内容的所有目的全部并入本文作为参考，如同每一各别文献、专利或专利申请案被特定且个别地指明并入作为参考。

[0299] 下述系本申请案所揭露的序列：

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)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSS

OMP-18R5 轻链可变区氨基酸序列 (SEQ ID NO:8)

DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG

OMP-18R5 具有底线划记的预测的信号序列的重链氨基酸序列 (SEQ ID NO:9)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSASTKGPSVFPLeiPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

OMP-18R5 具有底线划记的预测的信号序列的轻链氨基酸序列 (SEQ ID NO:10)

MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

OMP-18R5 不具有预测的信号序列的重链氨基酸序列 (SEQ ID NO:11)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

OMP-18R5 不具有预测的信号序列的轻链氨基酸序列 (SEQ ID NO:12)

DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

不具有预测的信号序列的人 FZD1Fri 结构域氨基酸序列 (SEQ ID NO:13)

QQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERA,RQGCEALMNKFGFQWPDTLKCEKFPVHGAGELCVGQNTSDKGT

不具有预测的信号序列的人 FZD2Fri 结构域氨基酸序列 (SEQ ID NO:14)

QFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQICVGQNHSEDG

不具有预测的信号序列的人 FZD3Fri 结构域氨基酸序列 (SEQ ID NO:15)

HSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPYPRLVDL

不具有预测的信号序列的人 FZD4Fri 结构域氨基酸序列 (SEQ ID NO:16)

FGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEV

不具有预测的信号序列的人 FZD5Fri 结构域氨基酸序列 (SEQ ID NO:17)

ASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATT

不具有预测的信号序列的人 FZD6Fri 结构域氨基酸序列 (SEQ ID NO:18)

HSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFDPHTEFLG

不具有预测的信号序列的人 FZD7Fri 结构域氨基酸序列 (SEQ ID NO:19)

QPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEICVGQNTSDGSG

不具有预测的信号序列的人 FZD8Fri 结构域氨基酸序列 (SEQ ID NO:20)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY

KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTT

不具有预测的信号序列的人 FZD9Fri 结构域氨基酸序列 (SEQ ID NO:21)

LEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSL YAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALCMEAPENA

不具有预测的信号序列的人 FZD10Fri 结构域氨基酸序列 (SEQ ID NO:22)

ISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAP MCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLCMEAPNNG

人 FZD1 氨基酸 116-227(SEQ ID NO:23)

CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCR SLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELC

人 FZD2 氨基酸 39-150(SEQ ID NO:24)

CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCR SICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQIC

人 FZD3 氨基酸 28-133(SEQ ID NO:25)

CEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRVTLPCR RLCQRAYSECSKLMEMFGVPWPEDMECSRFPDC

人 FZD4 氨基酸 48-161(SEQ ID NO:26)

CDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPC GGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMC

人 FZD5 氨基酸 33-147(SEQ ID NO:27)

CQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPC RSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLC

人 FZD6 氨基酸 24-129(SEQ ID NO:28)

CEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVPTCIEQIHVVPPCR KLCEKVYSDCKKLIDTFGIRWPEELECDRLQYC

人 FZD7 氨基酸 49-160(SEQ ID NO:28)

CQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCR SLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEIC

人 FZD8 氨基酸 35-148(SEQ ID NO:30)

CQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPC RSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLC

人 FZD9 氨基酸 39-152(SEQ ID NO:31)

CQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPAC RPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALC

人 FZD10 氨基酸 34-147(SEQ ID NO:32)

CQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPAC RVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLC

不具有预测的信号序列的人 FZD8 Fri 结构域氨基酸序列 (变体)(SEQ ID NO:33)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY

KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDL

人 IgG_1Fc 区 (SEQ ID NO:34)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQTNTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK

人 IgG_1Fc 区（变体）(SEQ ID NO:35)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK

人 IgG_1Fc 区 (SEQ ID NO:36)

KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK

人 IgG_1Fc 区 (SEQ ID NO:37)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK

人 IgG_2Fc 区 (SEQ ID NO:38)

CVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREE QFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变体 54F03 氨基酸序列（不具有预测的信号序列）(SEQ ID NO:39)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGRADKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变体 54F16、54F17、54F18、54F23、54F25、54F27、54F29、54F31 及 54F34 氨基酸序列（不具有预测的信号序列）(SEQ ID NO:40)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变体 54F19、54F20、54F24、54F26、54F28、54F30、54F32、54F34 及 54F35 氨基酸

序列（不具有预测的信号序列）(SEQ ID NO:41)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

具有信号序列的 FZD8-Fc 变体 54F03 氨基酸序列 (SEQ ID NO:42)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ
FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTGRADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

具有信号序列的 FZD8-Fc 变体 54F16 氨基酸序列 (SEQ ID NO:43)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ
FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

具有信号序列的 FZD8-Fc 变体 54F26(SEQ ID NO:44)

MEWGYLLEVTSLLAALFLLQRSPIVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ
FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

具有信号序列的 FZD8-Fc 变体 54F28(SEQ ID NO:45)

MEWGYLLEVTSLLAALLLLQRSPFVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ
FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

人 Wnt1 的 C 端富含半胱氨酸的结构域 (aa 288-370)(SEQ ID NO:46)

DLVYFEKSPNFCTYSGRLGTAGTAGRACNSSSPALDGCELLCCGRGHRTRTQRVTERCNCTFHWCCHVSCRNC
THTRVLHECL

人 Wnt2 的 C 端富含半胱氨酸的结构域 (aa 267-360)(SEQ ID NO:47)

DLVYFENSPDYCIRDREAGSLGTAGRVCNLTSRGMDSCEVMCCGRGYDTSHVTRMTKCGCKFHWCCAVRCQDC

LEALDVHTCKAPKNADWTTAT

人 Wnt2b 的 C 端富含半胱氨酸的结构域 (aa 298-391)(SEQ ID NO:48)

DLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSKGTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAVRCKEC RNTVDVHTCKAPKKAEWLDQT

人 Wnt3 的 C 端富含半胱氨酸的结构域 (aa 273-355)(SEQ ID NO:49)

DLVYYENSPNFCEPNPETGSFGTRDRTCNVTSHGIDGCDLLCCGRGHNTRTEKRKEKCHCIFHWCCYVSCQEC IRIYDVHTCK

人 Wnt3a 的 C 端富含半胱氨酸的结构域 (aa 270-352)(SEQ ID NO:50)

DLVYYEASPNFCEPNPETGSFGTRDRTCNVSSHGIDGCDLLCCGRGHNARAERRREKCRCVFHWCCYVSCQEC TRVYDVHTCK

人 Wnt7a 的 C 端富含半胱氨酸的结构域 (aa 267-359)(SEQ ID NO:51)

DLVYIEKSPNYCEEDPVTGSVGTQGR4CNKTAPQASGCDLMCCGRGYNTHQYARVWQCNCKFHWCCYVKCNTC SERTEMYTCK

人 Wnt7b 的 C 端富含半胱氨酸的结构域 (aa 267-349)(SEQ ID NO:52)

DLVYIEKSPNYCEEDAATGSVGTQGRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNCKFHWCCFVKCNTC SERTEVFTCK

人 Wnt8a 的 C 端富含半胱氨酸的结构域 (aa 248-355)(SEQ ID NO:53)

ELIFLEESPDYCTCNSSLGIYGTEGRECLQNSHNTSRWERRSCGRLCTECGLQVEERKTEVISSCNCKFQWCC TVKCDQCRHVVSKYYCARSPGSAQSLGRVWFGVYI

人 Wnt8b 的 C 端富含半胱氨酸的结构域 (aa 245-351)(SEQ ID NO:54)

ELVHLEDSPDYCLENKTLGLLGTEGRECLRRGRALGRWELRSCRRLCGDCGLAVEERRAETVSSCNCKFHWCC AVRCEQCRRRVTKYFCSRAERPRGGAAHKPGRKP

人 Wnt10a 的 C 端富含半胱氨酸的结构域 (aa 335-417)(SEQ ID NO:55)

DLVYFEKSPDFCEREPRLDSAGTVGRLCNKSSAGSDGCGSMCCGRGHNILRQTRSERCHCRFHWCCFVVCEEC RITEWVSVCK

人 Wnt10b 的 C 端富含半胱氨酸的结构域 (aa 307-389)(SEQ ID NO:56)

ELVYFEKSPDFCERDPTMGSPGTRGRACNKTSRLLDGCGSLCCGRGHNVLRQTRVERCHCRFHWCCYVLCDEC KVTEWVNVCK

连接子 (SEQ ID NO:57)

ESGGGGVT

连接子 (SEQ ID NO:58)

LESGGGGVT

连接子 (SEQ ID NO:59)

GRAQVT

连接子 (SEQ ID NO:60)

WRAQVT

连接子 (SEQ ID NO:61)

ARGRAQVT

[0001]

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<110> 昂科梅德制药有限公司

<120> 使用Wnt途径抑制剂进行治疗之的方法及对该治疗之的监测

<130> 2293.106PC02/PAC/MIG

<140> 待指定
<141> 同上

<150> 61/760,523
<151> 2013-02-04

<160> 61

<170> PatentIn version 3.5

<210> 1
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<400> 3

Asn Phe Ile Lys Tyr Val Phe Ala Asn
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[0002]

1 5 10

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<400> 5

Asp Lys Ser Asn Arg Pro Ser Gly
1 5

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<400> 6

Gln Ser Tyr Ala Asn Thr Leu Ser Leu
1 5

<210> 7
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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

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[0003]

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<220>
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<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

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<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

[0004]

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

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

[0005]

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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
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Met Ala Trp Ala Leu Leu Leu Leu Thr Leu Leu Thr Gln Gly Thr Gly
1               5                   10                  15

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
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[0006]

225 230

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<220>
<223> OMP-18R5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

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

[0007]

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

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

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<223> OMP-18R5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

[0008]

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

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
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<212> PRT
<213> 智人

<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

[0009]

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> 智人

<400> 14

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
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<212> PRT
<213> 智人

<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

[0010]

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

<210> 16
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<212> PRT
<213> 智人

<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
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<213> 智人

<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

[0011]

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

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
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<213> 智人

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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
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<212> PRT
<213> 智人

<400> 19

Gln Pro Tyr His Gly Glu Lys Gly Ile Ser Val Pro Asp His Gly Phe
1 5 10 15

[0012]

Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
20 25 30

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
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<212> PRT
<213> 智人

<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

<210> 21

[0013]

<211> 137
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<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
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<213> 智人

<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

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

[0014]

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
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<213> 智人

<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
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<212> PRT
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<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

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

[0015]

100 105 110

<210> 25
<211> 106
<212> PRT
<213> 智人

<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
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<212> PRT
<213> 智人

<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

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

[0016]

<210> 27
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<213> 智人

<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
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<212> PRT
<213> 智人

<400> 28

Cys Glu Pro Ile Thr Val Pro Arg Cys Met Lys Met Ala Tyr Asn Met
1 5 10 15

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

[0017]

<211> 112
<212> PRT
<213> 智人

<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> 智人

<400> 30

Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr Asn Tyr
1 5 10 15

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

[0018]

<213> 智人

<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> 智人

<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

[0019]

<211> 129
<212> PRT
<213> 智人

<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

Leu

<210> 34
<211> 227
<212> PRT
<213> 智人

<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

[0020]

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

<210> 35
<211> 227
<212> PRT
<213> 智人

<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

[0021]

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> 智人

<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

[0022]

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> 智人

<400> 37

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

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

[0023]

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 38
<211> 224
<212> PRT
<213> 智人

<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

Thr Pro Glu Val Thr Cys Val 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变体54F03氨基酸序列

[0024]

<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

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

[0025]

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> 40
<211> 361
<212> PRT
<213> 人工序列

<220>
<223> FZD8-Fc变体54F16, 54F17, 54F18, 54F23, 54F25, 54F27, 54F29, 54F31, 及54F34氨基酸序列

<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

[0026]

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

<210> 41
<211> 363
<212> PRT
<213> 人工序列

<220>
<223> FZD8-Fc变体54F19, 54F20, 54F24, 54F26, 54F28, 54F30, 54F32, 54F34 及 54F35 氨基酸序列

<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

[0027]

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

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

[0028]

<213> 人工序列

<220>
<223> 具有信号序列的FZD8-Fc变体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

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

[0029]

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> 43
<211> 388
<212> PRT
<213> 人工序列

<220>
<223> 具有信号序列的FZD8-Fc变体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

[0030]

```
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

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> 具有信号序列的FZD8-Fc变体54F26

<400> 44

```
Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
```

[0031]

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

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

[0032]

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> 45
<211> 390
<212> PRT
<213> 人工序列

<220>
<223> 具有信号序列的FZD8-Fc变体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

[0033]

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

<210> 46
<211> 83
<212> PRT
<213> 智人

<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

[0034]

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> 智人

<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> 智人

<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

[0035]

<212> PRT
<213> 智人

<400> 49

Asp Leu Val Tyr Tyr Glu Asn 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 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> 智人

<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> 智人

<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

[0036]

35 40 45

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> 智人

<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> 智人

<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

Arg His Val Val Ser Lys Tyr Tyr Cys Ala Arg Ser Pro Gly Ser Ala
85 90 95

[0037]

Gln Ser Leu Gly Arg Val Trp Phe Gly Val Tyr Ile
100 105

<210> 54
<211> 107
<212> PRT
<213> 智人

<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> 智人

<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

Val Cys Lys

<210> 56
<211> 83
<212> PRT
<213> 智人

[0038]

<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
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 连接子

<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> 连接子

<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> 连接子

<400> 59

Gly Arg Ala Gln Val Thr
1 5

<210> 60
<211> 6
<212> PRT
<213> 人工序列

<220>
<223> 连接子

[0039]

<400> 60

Trp Arg Ala Gln Val Thr
1 5

<210> 61
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> 连接子

<400> 61

Ala Arg Gly Arg Ala Gln Val Thr
1 5

[0001]

申请人或代理人档案号 2293106PC02	国际申请号 PCT/US2014/014443

关于微生物保藏的说明

（专利合作条约实施细则 13 之 2）

微生物保藏的说明	
A.对说明书第 10 页，第 2 行 所述的已保藏的微生物或其他生物材料的说明	
B. 保藏事项	更多的保藏在附加页说明 ☐
保藏单位名称美国典型菌种保藏中心	
保藏单位地址 （包括邮政编码和国名） 美国弗吉尼亚州 20110-2209，马纳萨斯，大学路 10801	
保藏日期 2008-09-29	保藏号 ATCC PTA-9541
C.补充说明（必要时）	更多信息在附加页中 ☐
无	
D.本说明是为下列指定国作的（如果说明不是为所有指定国而作的）	
所有指定国	
E.补充说明（必要时）	
下列说明将随后向国际局提供（写出说明的类别，例如：“保藏的编号”） 无	

由受理局填写	由国际局填写
☐ 本页已经和国际申请一起收到	☐ 国际局收到本页日期
授权官员	授权官员

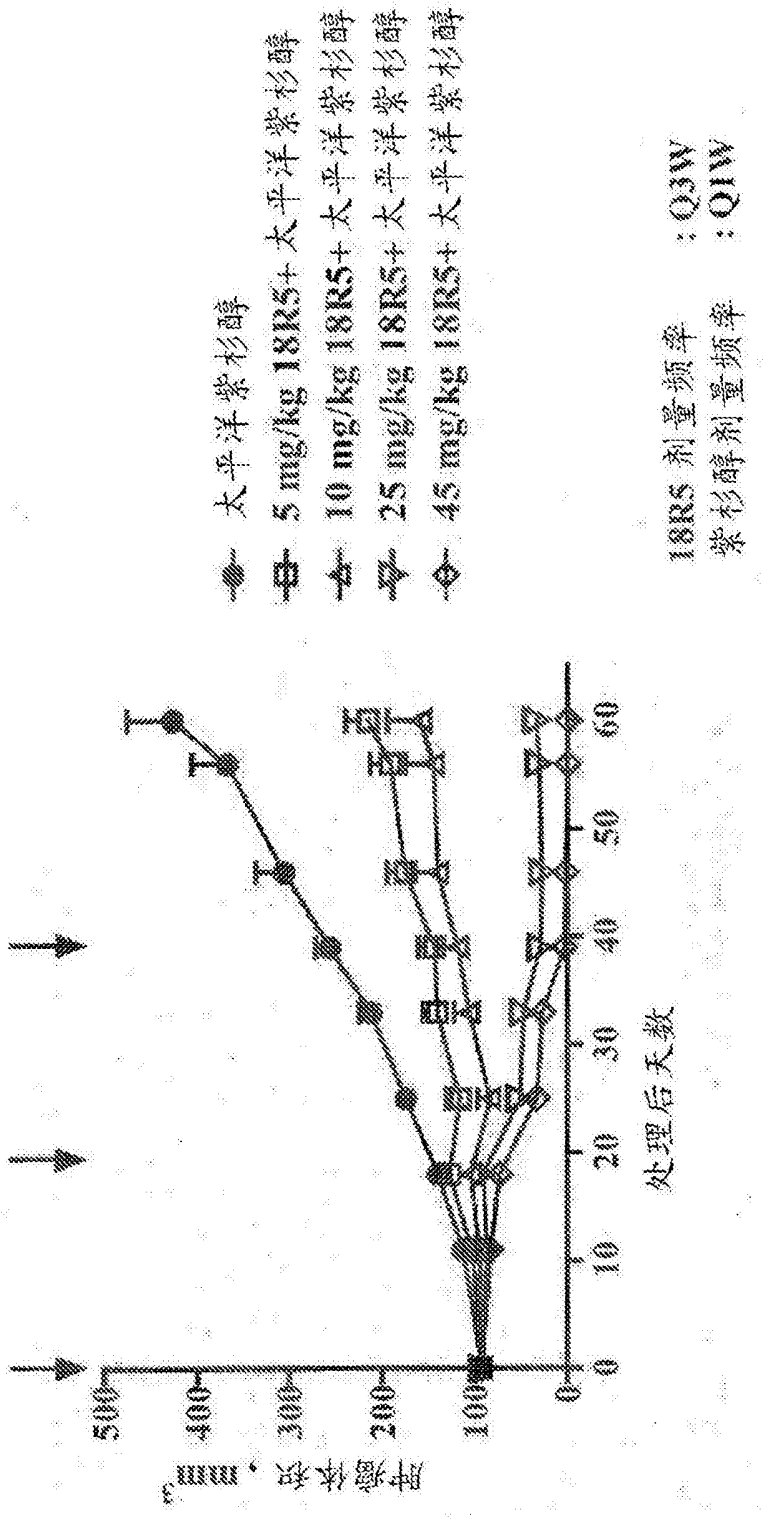
太平洋紫杉醇
5 mg/kg 18RS+ 太平洋紫杉醇
10 mg/kg 18RS+ 太平洋紫杉醇
25 mg/kg 18RS+ 太平洋紫杉醇
45 mg/kg 18RS+ 太平洋紫杉醇
18RS 剂量频率 :Q3W
紫杉醇剂量频率 :QIW
肿瘤体积，mm3
处理后天数
0
10
20
30
40
50
60
100
200
300
400
500

图 1

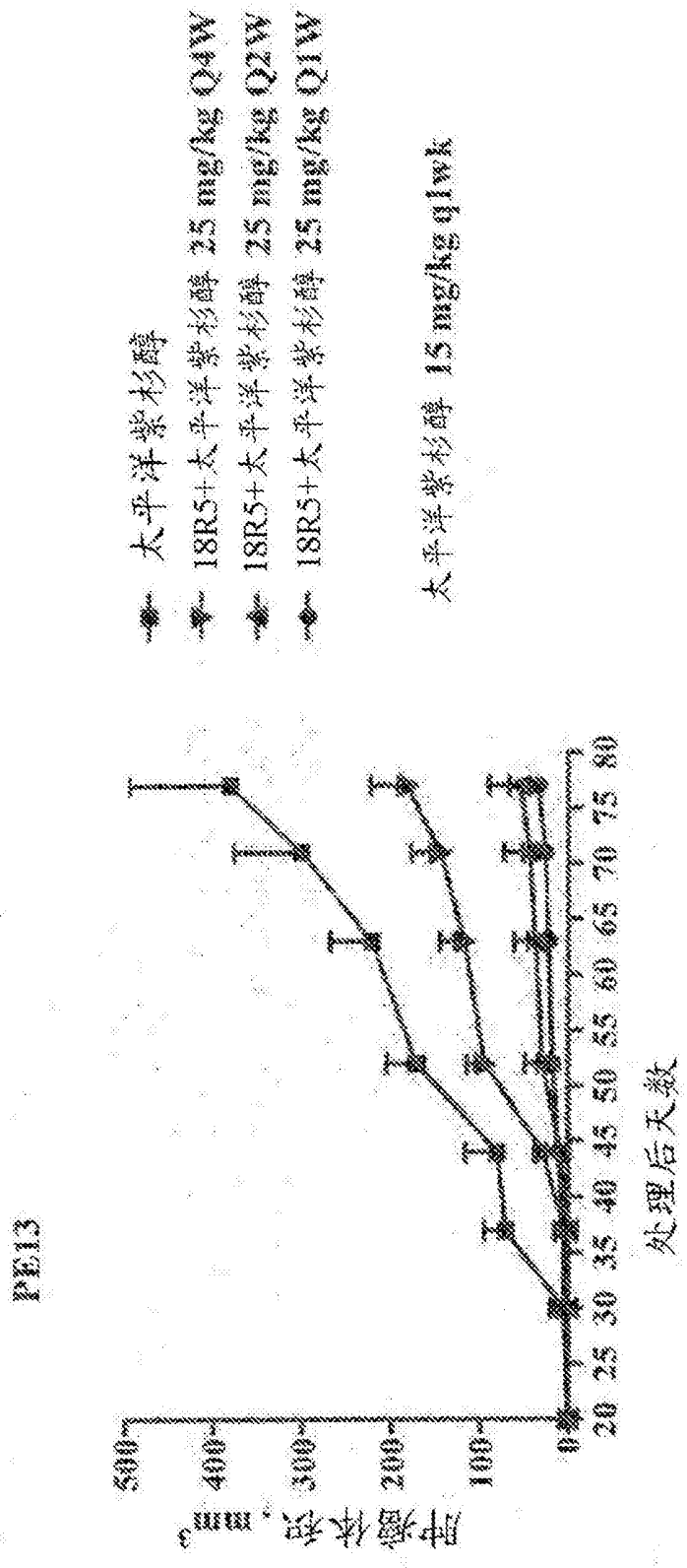
太平洋紫杉醇
18R5+太平洋紫杉醇 25 mg/kg Q4W
18R5+太平洋紫杉醇 25 mg/kg Q2W
18R5+太平洋紫杉醇 25 mg/kg Q1W
太平洋紫杉醇 15 mg/kg q1wk
PE13
肿瘤体积，mm3
500
400
300
200
100
0
20 25 30 35 40 45 50 55 60 65 70 75 80
处理后天数

图 2

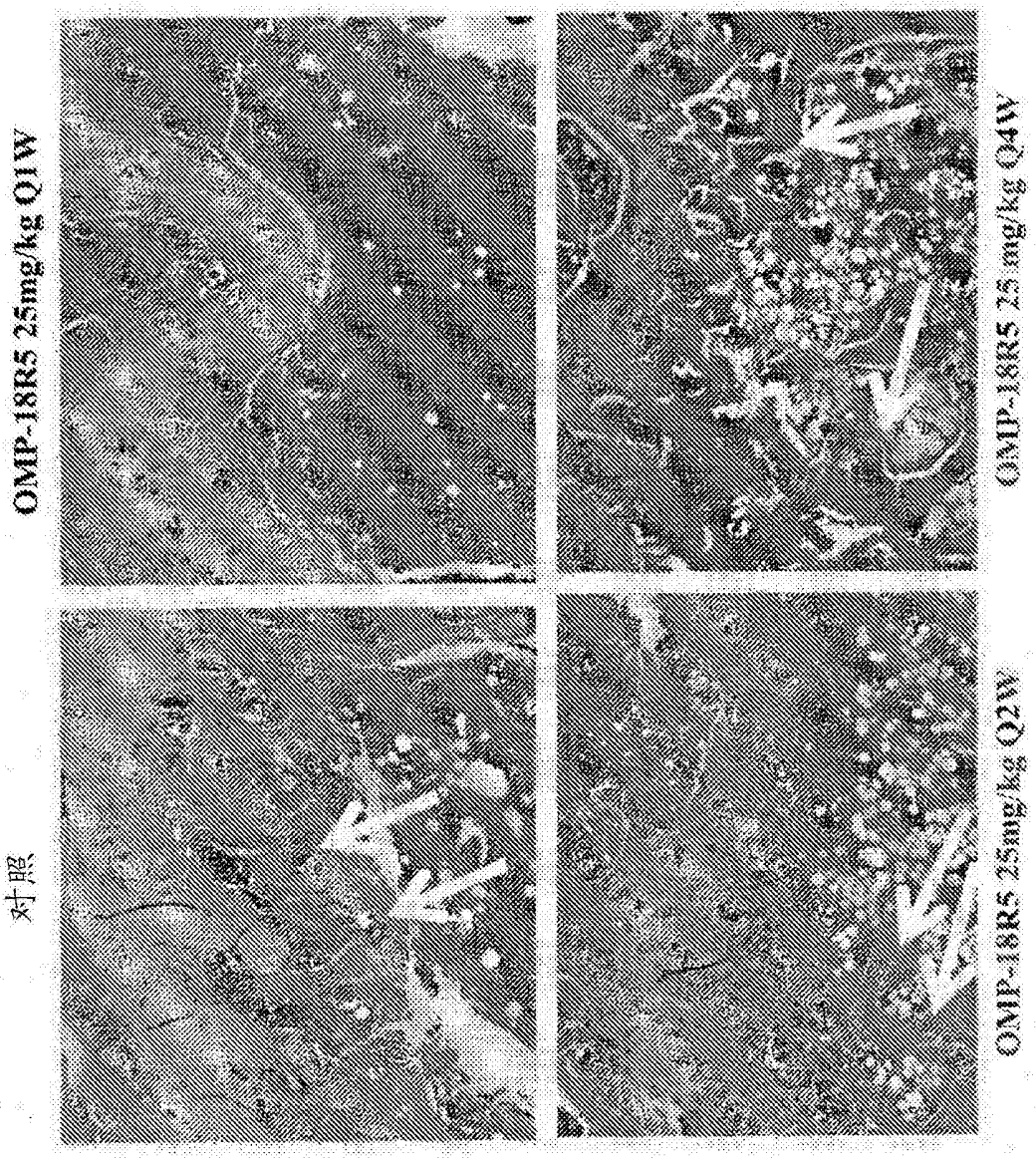
对照
OMP-18R5 25mg/kg Q1W
OMP-18R5 25mg/kg Q2W
OMP-18R5 25 mg/kg Q4W

图 3

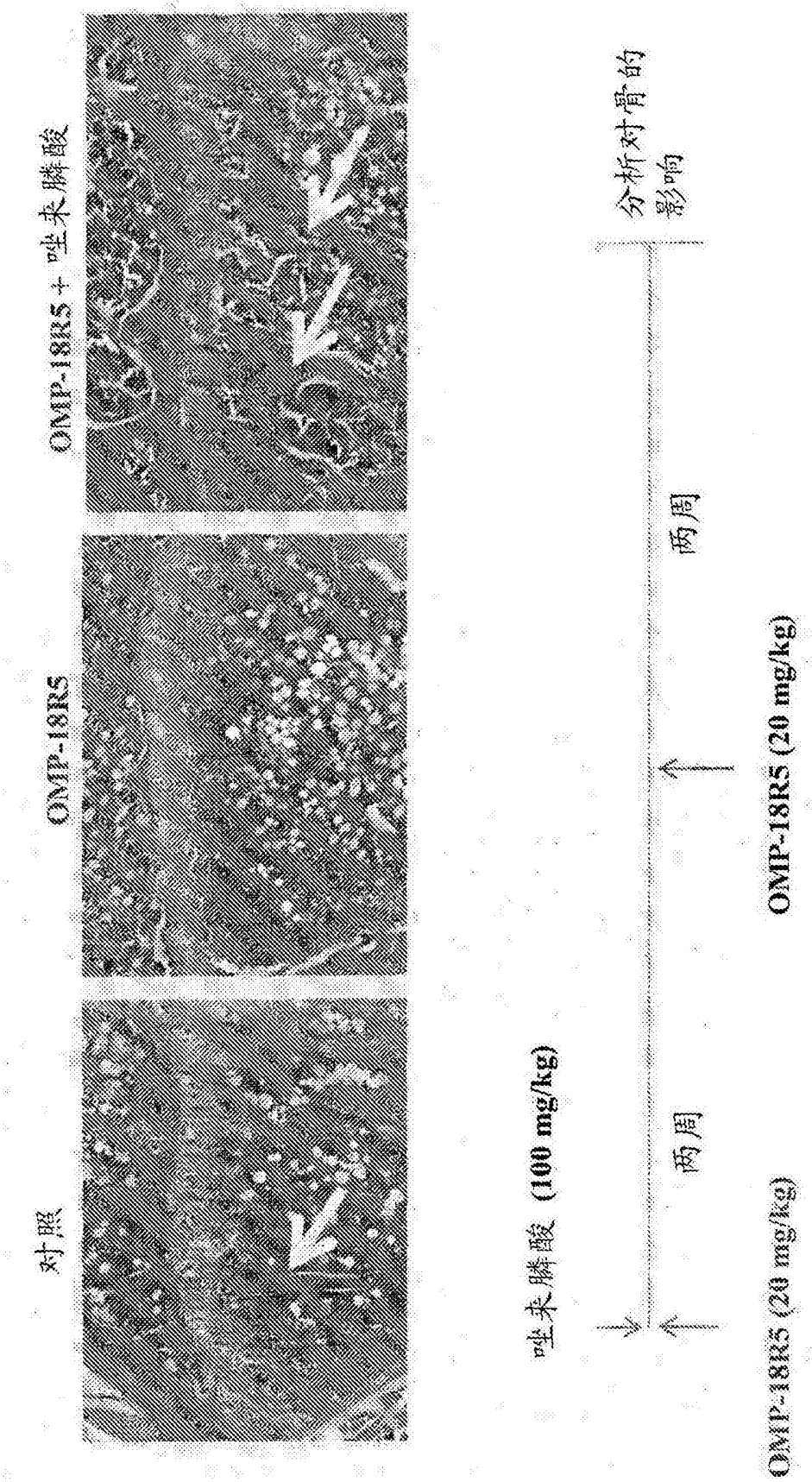
对照
OMP-18R5
OMP-18R5 + 唑来膦酸
唑来膦酸 (100 mg/kg)
两周
两周
分析对骨的影响
OMP-18R5 (20 mg/kg)
OMP-18R5 (20 mg/kg)

图 4

1.一种选择受试者以使用 Wnt 途径抑制剂进行治疗的方法,其包含 :

(a) 测定来自该受试者的样本中骨吸收生物标志的量 ;及

(b) 若该骨吸收生物标志的量低于预设量,则选择该受试者以使用该 Wnt 途径抑制剂进行治疗 ;

其中所述 Wnt 途径抑制剂是

(i)与至少一种卷曲 (FZD) 蛋白特异性结合的抗体,或

(ii)包含人 FZD8 的 Fri 结构域的可溶性受体。

2.一种识别受试者为适合使用 Wnt 途径抑制剂进行治疗的方法,其包含 :

(a) 测定来自该受试者的样本中骨吸收生物标志的量 ;及

(b) 若该骨吸收生物标志的量低于预设量,则识别该受试者为适合使用该 Wnt 途径抑制剂进行治疗 ;

其中所述 Wnt 途径抑制剂是

(i)与至少一种卷曲 (FZD) 蛋白特异性结合的抗体,或

(ii)包含人 FZD8 的 Fri 结构域的可溶性受体。

3.如权利要求 1 或 2 所述的方法,如果该骨吸收生物标志的量低于所述预设量,所述方法包括对该受试者投予该 Wnt 途径抑制剂。

4.一种对接受 Wnt 途径抑制剂治疗的受试者识别骨骼相关不良反应及 / 或毒性的方法,其包含 :

(a) 测定来自该受试者的样本中骨吸收生物标志的量 ;

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量 ;及

其中若该样本中骨吸收生物标志的量高于该预设量,则显示骨骼相关不良反应及 / 或毒性。

5.一种对接受 Wnt 途径抑制剂治疗的受试者监测骨骼相关不良反应及 / 或毒性的方法,其包含 :

(a) 测定来自该受试者的样本中骨吸收生物标志的量 ;及

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量 ;

其中若该样本中骨吸收生物标志的量高于该预设量,则显示骨骼相关不良反应及 / 或毒性。

6.如权利要求 1 至 5 中任一项所述的方法,其中该骨吸收生物标志是 β-CTX。

7.如权利要求 6 所述的方法,其中该 β-CTX 预设量是 :

(a)于稍早时间点测定的 β-CTX 的量 ;

(b)在初次筛选时测定的 β-CTX 的量 ;

(c)在治疗前测定的 β-CTX 的量 ;

(d)基准量 ;或

(e)约 1000pg/ml 或更低。

8.如权利要求 4-7 中任一项所述的方法,其中如果

(a)任一样本的所述骨吸收生物标记量高于预设量,或

(b)所述骨吸收生物标记量为预设量的 2 倍或更多,

则对该受试者投予治疗有效量的抗吸收药物。

9. 一种使接受 Wnt 途径抑制剂治疗的受试者减少骨骼相关不良反应及 / 或毒性的方法，其包含 ：

(a) 测定来自该受试者的样本中骨吸收生物标志的量 ；

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量 ；及

(c) 若该样本中骨吸收生物标志的量高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。

10. 一种使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒性发展的方法，其包含 ：

(a) 在使用 Wnt 途径抑制剂进行治疗之前，测定来自该受试者的样本中骨吸收生物标志的量 ；

(b) 比较该样本中骨吸收生物标志的量与骨吸收生物标志的预设量 ；

(c) 对该受试者投予治疗有效量的抗吸收药物 ；及

(d) 对该受试者投予该 Wnt 途径抑制剂。

11. 一种筛选受试者因 Wnt 途径抑制剂治疗所致的骨骼相关不良反应及 / 或毒性的风险的方法，其包含 ：

(a) 测定来自该受试者的样本中骨吸收生物标志的量 ；及

(b) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ；其中若该样本中骨吸收生物标志的量高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险。

12. 一种对有需要治疗的受试者治疗癌症的方法，其包含 ：

(a) 对该受试者投予治疗有效量的 Wnt 途径抑制剂 ；及

(b) 测定来自该受试者的样本中骨吸收生物标志的量。

13. 如权利要求 12 所述的方法，其另包含 ：

(c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ；其中若该样本中骨吸收生物标志的量高于该骨吸收生物标志的预设量，则该受试者具有骨骼相关不良反应及 / 或毒性的风险 ；或

(c) 比较该样本中骨吸收生物标志的量与该骨吸收生物标志的预设量 ；其中若该样本中骨吸收生物标志的量高于该骨吸收生物标志的预设量，则对该受试者投予治疗有效量的抗吸收药物。

14. 如权利要求 1-13 中任一项所述的方法，其中该生物样本系血液、血清或血浆。

15. 如权利要求 1-14 中任一项所述的方法，其中该骨吸收生物标志是 β -CTX。

16. 如权利要求 15 所述的方法，其中若该 β -CTX 的量为预设量的 2 倍或更多，则对该受试者投予治疗有效量的抗吸收药物。

17. 如权利要求 11 或 13 所述的方法，其中若该受试者具有骨骼相关不良反应及 / 或毒性的风险，则在使用该 Wnt 途径抑制剂进行治疗之前，对该受试者投予治疗有效量的抗吸收药物。

18. 一种使接受 Wnt 途径抑制剂治疗的受试者减少骨骼相关不良反应及 / 或毒性的方法，其包含对该受试者投予治疗有效量的抗吸收药物。

19. 一种使接受 Wnt 途径抑制剂治疗的受试者预防或减弱骨骼相关不良反应及 / 或毒

性发展的方法，其包含对该受试者投予治疗有效量的抗吸收药物。

20. 如权利要求 4 至 11 或 13-19 中任一项所述的方法，其中该骨骼相关不良反应及 / 或毒性是增加骨折、骨量减少或骨质疏松症的风险。

21. 如权利要求 1 至 20 中任一项所述的方法，其中该 Wnt 途径抑制剂是抗体，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)，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)。

22. 如权利要求 1 至 20 中任一项所述的方法，其中该 Wnt 途径抑制剂是抗体，其包含 ：

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。

23. 如权利要求 21 或 22 所述的方法，其中该抗体是单克隆抗体、重组抗体、嵌合抗体、人源化抗体、人抗体、双特异性抗体、IgG1 抗体、IgG2 抗体或包含抗原结合部位的抗体片段。

24. 如权利要求 1-23 中任一项所述的方法，其中该 Wnt 途径抑制剂是抗体 OMP-18R5。

25. 如权利要求 1-20 中任一项所述的方法，其中该 Wnt 途径抑制剂是包含人 FZD8 蛋白的 Fri 结构域的可溶性受体。

26. 如权利要求 25 所述的方法，其中该人 FZD 蛋白的 Fri 结构域包含 SEQ ID NO:20。

27. 如权利要求 25 或 26 所述的方法，其中所述可溶性受体包含人 Fc 区。

28. 如权利要求 1-20 或 25-27 中任一项所述的方法，其中该 Wnt 途径抑制剂包含 SEQ ID NO:41。

29. 如权利要求 1-20 或 25-28 中任一项所述的方法，其中该 Wnt 途径抑制剂是 FZD8-Fc 可溶性受体 OMP-54F28。

30. 如权利要求 8-10 或 13-29 中任一项所述的方法，其中该抗吸收药物是双膦酸盐或迪诺单抗。

31. 如权利要求 30 所述的方法，其中该双膦酸盐是选自 ：唑来膦酸、羟乙膦酸盐、氯屈膦酸盐、替鲁膦酸盐、帕米膦酸盐、奈立膦酸盐、奥帕膦酸盐、阿仑膦酸盐、伊班膦酸盐、及利塞膦酸盐。

32. 如权利要求 1 至 31 中任一项的方法，其中该受试者罹患癌症。

33. 如权利要求 32 所述的方法，其中该癌症是选自 ：肺癌、乳癌、结肠癌、结直肠癌、黑色素瘤、胰癌、胃肠癌、肾癌、卵巢癌、神经内分泌癌、肝癌、子宫内膜癌、肾癌、前列腺癌、甲状腺癌、神经胚细胞瘤、神经胶瘤、多形性神经胶质母细胞瘤、子宫颈癌、胃癌、膀胱癌、肝肿瘤及头颈癌。

34. 如权利要求 1 至 33 中任一项所述的方法，其中该受试者是经 Wnt 途径抑制剂与一或多种额外抗癌剂的组合治疗。

35. 如权利要求 4 至 11 或 13 至 34 中任一项所述的方法，其中该骨骼相关不良反应及 / 或毒性与该 Wnt 途径抑制剂有关。