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(54) **Title**: METHODS OF TREATING NEUROENDOCRINE TUMORS USING WNT PATHWAY-BINDING AGENTS

(57) **Abstract**: Novel methods of treating neuroendocrine tumors are provided. In one embodiment, the method comprises administering to a subject in need thereof a therapeutically effective dose of a Wnt antagonist. In one embodiment, the Wnt antagonist is an anti-FZD antibody. In another embodiment, the Wnt antagonist is a soluble FZD receptor polypeptide. In a further embodiment, the Wnt antagonist is an anti-Wnt antibody.

METHODS OF TREATING NEUROENDOCRINE TUMORS USING WNT PATHWAY-BINDING AGENTS

CROSS-REFERENCE TO RELATED APPLICATONS

[0001] This application claims priority benefit of U.S. Provisional Application No. 61/717,294, filed October 23, 2012 and U.S. Provisional Application No. 61/760,529, filed February 4, 2013, each of which is hereby incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The field of this invention generally relates to methods of treating neuroendocrine tumors. In one embodiment, the method comprises administering to a subject in need thereof a therapeutically effective dose of a Wnt antagonist.

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 over half of all new cases (Jemal *et al.*, 2003, *Cancer J. Clin.* 53:5-26).

[0004] 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 can alter the developmental fate of tumor cells to maintain them in an undifferentiated and proliferative state. Thus carcinogenesis can proceed by usurping homeostatic mechanisms controlling normal development and tissue repair by stem cells (reviewed in Reya & Clevers, 2005, *Nature* 434:843; Beachy *et al.*, 2004, *Nature* 432:324).

[0005] 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 (LPR5/6). 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: FZD1-10. Different Fzd CRDs have different binding affinities for specific Wnts (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 described below (Miller *et al.*, 1999, *Oncogene* 18:7860-72). To form the receptor complex that binds the FZD ligands, FZD receptors interact with LRP5/6, single pass transmembrane proteins with four extracellular EGF-like domains separated by six YWTD amino acid repeats (Johnson *et al.*, 2004, *J. Bone Mineral Res.* 19:1749).

[0006] The Wnt/β-catenin signaling pathway has been implicated in the development of gastrointestinal carcinoid tumors. Fujimori *et al.*, *Cancer Res.* 61(18): 6656-9 (2001). Nuclear translocation of β-catenin protein but absence of β-catenin and APC mutation in gastrointestinal carcinoid tumor has also been observed. Su *et al.*, *Ann. Surg. Oncol.* 13(12): 1604-9 (2006). 72 cases of gastrointestinal carcinoid tumor were investigated both immunohistochemically and by direct sequencing of β-catenin. Accumulation of β-catenin in the cytoplasm and/or nucleus was observed in 57 cases (79.2%). Mutations were also detected in exon 3 of β-catenin in 27 cases (37.5%), and in APC in one case (1.4%). Su *et al.* also reported the investigation of 91 gastrointestinal carcinoid tumors and, for comparison, 26 extragastrointestinal carcinoid tumors by immunohistochemical detection of β-catenin protein and direct sequencing of exon 3 of the β-catenin gene and exon 15 of the APC gene. Cytoplasmic accumulation and/or nuclear translocation of β-catenin were found in 27 gastrointestinal carcinoid tumors (29.7%) but not in any extragastrointestinal carcinoid tumors. Neither β-catenin nor APC gene mutation was detected in any of the cases with nuclear expression of β-catenin.

SUMMARY OF THE INVENTION

[0007] The present invention provides methods of treating a neuroendocrine tumor. Thus in one aspect, the invention provides methods of inhibiting the growth of a neuroendocrine tumor, comprising contacting the neuroendocrine tumor with an effective amount of a Wnt antagonist. In another aspect, the invention provides methods of inhibiting the proliferation of neuroendocrine tumor cells, comprising contacting the neuroendocrine tumor cells with an effective amount of a Wnt antagonist. In another aspect, the invention provides methods of reducing the tumorigenicity of neuroendocrine tumor cells, comprising contacting the neuroendocrine tumor cells with an effective amount of a Wnt antagonist. In another aspect, the invention provides methods of inducing neuroendocrine tumor cells to differentiate, comprising contacting the neuroendocrine tumor cells with an effective amount of a Wnt antagonist. In another aspect, the invention provides methods of

inhibiting the growth of a neuroendocrine tumor, comprising administering to a subject in need thereof a therapeutically effective amount of a Wnt antagonist. In another aspect, the invention provides methods of inhibiting the proliferation of neuroendocrine tumor cells, comprising administering to a subject in need thereof a therapeutically effective amount of a Wnt antagonist. In another aspect, the invention provides methods of treating neuroendocrine cancer, comprising administering to a subject in need thereof a therapeutically effective amount of a Wnt antagonist. In certain embodiments the subject is a human subject.

[0008] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the neuroendocrine tumor is a low grade, medium grade, or high grade neuroendocrine tumor. In further embodiments, the neuroendocrine tumor is a functional neuroendocrine tumor or a non-functional neuroendocrine tumor. In further embodiments, the neuroendocrine tumor is selected from the group consisting of gastroenteropancreatic neuroendocrine tumor, carcinoid tumor, pheochromocytoma, paraganglioma, medullary thyroid cancer, pulmonary neuroendocrine tumor and thymic neuroendocrine tumor. In further embodiments, the neuroendocrine tumor is a carcinoid tumor or a pancreatic neuroendocrine tumor.

[0009] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the Wnt antagonist is an antibody. In further embodiments, the Wnt antagonist is an antibody that specifically binds to at least one human Wnt. In further embodiments, the Wnt antagonist is an antibody that specifically binds to at least one human frizzled receptor (FZD). In further embodiments, the Wnt antagonist is a soluble FZD receptor.

[0010] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the Wnt antagonist is an antibody that specifically binds to at least one human frizzled receptor (FZD). In further embodiments, the antibody specifically binds to the extracellular domain of at least one human FZD. In further embodiments, the antibody specifically binds to a human FZD selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In further embodiments, the antibody specifically binds to FZD7. In further embodiments, the antibody specifically binds to more than one human FZD. In further embodiments, the antibody specifically binds to three or more human FZD selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In further embodiments, the antibody specifically binds to more than one human FZD selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In further embodiments, the antibody specifically binds to FZD1, FZD2, FZD5, FZD7, and FZD8.

[0011] In further embodiments, the antibody blocks ligand binding to FZD. In further embodiments, the antibody blocks Wnt binding to FZD. In further embodiments, the antibody blocks the activation of FZD.

[0012] In further embodiments, the antibody comprises: (1) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33); and/or (2) (a) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:34), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:35), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:36); or (b) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:41), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:42), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:43). In further embodiments, the antibody comprises: a VH comprising the amino acid sequence of SEQ ID NO:37; and/or a VL comprising the amino acid sequence of SEQ ID NO:38 or 44. In further embodiments, the antibody comprises: a heavy chain comprising the amino acid sequence of SEQ ID NO:39; and/or a light chain comprising the amino acid sequence of SEQ ID NO:40 or 45.

[0013] In further embodiments, the antibody is a monoclonal antibody. In further embodiments, the antibody is a recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, or an antibody fragment. In further embodiments, the antibody is a monospecific antibody or a bispecific antibody. In further embodiments, the antibody is an IgA, IgD, IgE, IgG or IgM antibody. In further embodiments, the antibody is an IgG1 or IgG2 antibody.

[0014] In further embodiments, the Wnt antagonist is OMP-18R5 (also known as "vantictumab").

[0015] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the Wnt antagonist is a soluble FZD receptor. In further embodiments, the soluble FZD receptor binds to Wnt. In further embodiments, the soluble receptor comprises a fragment of the extracellular domain of a human FZD receptor. In further embodiments, the fragment of the extracellular domain of the human FZD receptor comprises the Fri domain of the human FZD receptor.

[0016] In further embodiments, the human FZD receptor is selected from the group consisting of FZD4, FZD5, and FZD8. In further embodiments, the human FZD receptor is FZD8. In further embodiments, the FZD8 Fri domain comprises the amino acid sequence of SEQ ID NO:28.

[0017] In further embodiments, the soluble receptor further comprises a human Fc domain. In further embodiments, the human Fc domain comprises the amino acid sequence of SEQ ID NO:95.

[0018] In further embodiments, the Wnt antagonist is OMP-54F28.

[0019] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the methods further comprise contacting the tumor or tumor cells with a second therapeutic agent, or administering a second therapeutic agent to the subject. In further embodiments, the second therapeutic agent is a chemotherapeutic agent. In further embodiments, the second therapeutic agent is a kinase inhibitor, somatostatin analog, or a mTOR pathway inhibitor. In further embodiments, the second therapeutic

agent is sunitinib, octreotide, or everolimus. In further embodiments, the second therapeutic agent is an antibody. In further embodiments, the second therapeutic agent is an angiogenesis inhibitor.

[0020] 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 each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0021] Figure 1A. Effect of Wnt inhibitors on neuroendocrine tumor growth. The size of tumor lesions in a pancreatic neuroendocrine tumor patient was reduced following the administration of the OMP-18R5 anti-FZD7 antibody. Radiographic assessment of the size of target (T) and non-target (NT) lesions at day 56 and day 112 of OMP-18R5 anti-FZD7 antibody treatment. BL denotes the baseline size of the lesions before the administration of OMP-18R5.

[0022] Figure 1B. Effect of Wnt inhibitors on neuroendocrine tumor growth. CT image of the tumor lesions before (Baseline) and after 112 days of OMP-18R5 administration.

[0023] Figure 1C. Effect of Wnt inhibitors on neuroendocrine tumor growth. CT image of the tumor lesions before (Baseline) and after 112 days of OMP-18R5 administration. The tumor lesion at day 112 displays radiologic signs of calcification.

[0024] Figure 2A. Days on study for patients in OMP-18R5 Phase 1a study. The number of days each of the patients (n=18) enrolled in the OMP-18R5 Phase 1a study has stayed on the study as of January 25, 2013, is shown graphically in the figure. Arrows indicate the patients who remained on the study as of January 25, 2013. The vertical lines indicate dates of tumor assessments on the study. The neuroendocrine tumor patients are patients 003 (Patient 3 in Example 1), 010 (Patient 10 in Example 1), and 012 (Patient 12 in Example 1). The other patients on the study had other types of advanced solid tumors such as colorectal cancer, breast cancer, melanoma, and pancreatic cancer.

[0025] Figure 2B. Days on study for patients in OMP-18R5 Phase 1a study. The number of days each of the patients (n=29) enrolled in the OMP-18R5 Phase 1a study has stayed on the study as of October 4, 2013, is shown graphically in the figure. Arrows indicate the patients who remained on the study as of October 4, 2013. The vertical lines indicate dates of tumor assessments on the study. The neuroendocrine tumor patients are patients 003 (Patient 3 in Example 1), 010 (Patient 10 in Example 1), 012 (Patient 12 in Example 1), 025, and 026. The other patients on the study had other types of advanced solid tumors.

[0026] Figure 3A. Days on study for patients with neuroendocrine tumors in OMP-18R5 Phase 1a study were compared to days on treatment with prior regimens. Patient 10, a 69-year-old woman with neuroendocrine tumor of the pancreas, continues on study with stable disease for 279 days (as of

January 25, 2013). Patient 12, a 77-year-old woman with carcinoid tumors, continues on study with stable disease for 210 days (as of January 25, 2013).

[0027] Figure 3B. Days on study for patients with neuroendocrine tumors in OMP-18R5 Phase 1a study were compared to days on treatment with prior regimens. Patient 10 came off study at Day 448. Patient 12 continues on study with stable disease for 465 days (as of October 4, 2013). (Some numbers in regard to days on prior therapy have been corrected from the earlier data in Figure 3A).

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention provides methods of inhibiting the growth of a neuroendocrine tumor, methods of inhibiting proliferation of neuroendocrine tumor cells, methods of treating a neuroendocrine cancer, methods of inhibiting neuroendocrine tumor metastases, methods of inducing neuroendocrine tumor cell differentiation, methods of reducing tumorgenicity of neuroendocrine tumor cells and methods of reducing the frequency of cancer stem cells or tumor initiating cells in a neuroendocrine tumor. In some embodiments, the methods provided herein comprise administering a Wnt antagonist to a subject. In some embodiments, the Wnt antagonist is a FZD-binding agent that specifically binds to one or more human FZD receptors. In further embodiments, the FZD-binding agent is an antibody that specifically binds to one or binds to one or more human FZD receptors. In some embodiments, the Wnt antagonist is a Wnt binding agent that specifically binds to one or more human Wnt polypeptides. In some embodiments, the Wnt binding agent is a soluble FZD receptor. In some embodiments, the Wnt binding agent is an anti-Wnt antibody.

[0029] Human patients with late stage neuroendocrine tumors were treated with low doses of the OMP-18R5 anti-FZD antibody in the context of a Phase 1 clinical trial for patients with late stage solid tumors. (Example 1.) Surprisingly, one of the patients (a patient having a pancreatic neuroendocrine tumor) showed a reduction in tumor lesion size after 112 days of treatment with OMP-18R5 and remained on study without evidence of any progression of disease for 279 days (as of January 25, 2013). Additionally, new calcification was seen in one of the patient's lesions which may represent possible signs of tumor cell necrosis and/or differentiation. In addition, two patients with neuroendocrine tumors having carcinoid histology were also able to stay on the study for surprisingly long periods of time with stable disease during treatment with OMP-18R5. (Example 1.) Collectively, these results suggest that OMP-18R5 may be particularly useful in the treatment of a variety of neuroendocrine tumors.

1. Definitions

[0030] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0031] The term "antagonist" is used herein to include any molecule that partially or fully blocks, inhibits, or neutralizes the expression of or the biological activity of a protein, (*e.g.*, a cancer stem cell marker). The blocking, inhibiting, and/or neutralizing of biological activity includes, but is not limited to, inhibition of tumor growth. The term "antagonist" also includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of the Wnt pathway. The term "Wnt antagonist" is used herein to include any molecule that partially or fully blocks, inhibits or neutralizes the signaling of the Wnt pathway (e.g., canonical Wnt signaling), or partially or fully blocks, inhibits or neutralizes a biological activity of a component of the Wnt pathway. Wnt antagonists do not necessarily bind Wnt. For instance, in certain embodiments Wnt antagonists bind one or more other components of the Wnt pathway such as one or more FZD receptors. Suitable Wnt antagonist molecules include, but are not limited to, fragments and/or amino acid sequence variants of native FZD receptor proteins including soluble FZD receptors, as well as derivatives of soluble Frizzled-related proteins (SFRPs), and derivatives of ROR proteins. Suitable Wnt antagonist molecules further include, but are not limited to, antibodies that specifically bind to one or more FZD receptors and antibodies that specifically bind to one or more Wnt polypeptide. Soluble SFRP and ROR receptors are described in US Pat. Appl. Pub. No. 2011/0305695, which is herein incorporated by reference.

[0032] *In vivo* and *in vitro* assays for determining whether an agent (e.g., soluble FZD receptor or anti-FZD antibody) inhibits Wnt 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 canonical Wnt signaling levels *in vitro* (Gazit et al., 1999, *Oncogene* 18; 5959-66). The level of Wnt signaling in the presence of one or more Wnts (e.g., Wnt(s) expressed by transfected cells or provided by Wnt-conditioned media) with the agent present is compared to the level of signaling without the agent present. In addition to the TCF/luc reporter assay, the effect of an agent (e.g., soluble FZD receptor or anti-FZD antibody) on canonical Wnt signaling can be measured *in vitro* or *in vivo* by measuring the effect of the agent on the level of expression of beta-catenin regulated genes, such as c-myc (He et al., *Science* 281:1509-12 (1998)), cyclin D1 (Tetsu et al., *Nature* 398:422-6 (1999)) and/or fibronectin (Gradl et al. *Mol. Cell Biol.* 19:5576-87 (1999)). In certain embodiments, the effect of the agent on Wnt signaling can 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. In still further embodiments, the effect of the agent on Wnt signaling is determined by assessing the impact of the agent on the expression level of one or more genes in a Wnt signature. Non-limiting examples of the use of such assays to assess inhibition of canonical Wnt signaling are disclosed in U.S. Pat. Appl. Pub. No. 2012/0027778, which is incorporated by reference herein in its entirety.

[0033] As used herein the term "soluble receptor" refers to an amino-terminal extracellular fragment of a receptor protein preceding the transmembrane domain that can be secreted from a cell in soluble form. In some embodiments, the receptor protein is a FZD receptor. In some embodiments, the

receptor protein is the ROR1 or ROR2 receptor. In certain embodiments, the soluble receptor is linked in-frame with a polypeptide that increases the half-life of the soluble receptor. In certain embodiments, the polypeptide that increases half-life is a human Fc domain.

[0034] As used herein the term "FZD soluble receptor" refers to an amino-terminal extracellular fragment of a human FZD receptor protein preceding the transmembrane domain of the receptor that can be secreted from a cell in soluble form. FZD soluble receptors comprising the entire amino-terminal extracellular domain (ECD) (referred to herein as "FZD ECD") as well as smaller fragments of the ECD are envisioned. FZD soluble receptors comprising the Fri domain (referred to herein as "FZD Fri") are also disclosed. Soluble FZD receptors are described in US Pat. Appl. Pub. No. 2011/0305695, which is herein incorporated by reference.

[0035] FZD Fri soluble receptors can demonstrate altered biological activity, (*e.g.*, increased protein half-life) compared to soluble receptors comprising the entire FZD ECD. Protein half-life can be further increased by covalent modification with polyethylene glycol (PEG) or polyethylene oxide (PEO). FZD soluble receptors include FZD ECD or Fri domains linked in-frame to other functional and structural proteins including, but not limited to, a human Fc region (*e.g.*, human Fc derived from immunoglobulins IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM); 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 linked protein. In certain embodiments, the Fri domain of a FZD receptor is directly linked to a human Fc region. In certain embodiments, the Fri domain of a FZD receptor is linked to human IgG1 Fc (referred to herein as "FZD Fri.Fc" e.g. "FZD8 Fri.Fc"). In some embodiments, the Fri domain of a FZD receptor is linked to a human Fc region with a peptide linker. FZD soluble receptors also include variant proteins comprising amino acid insertions, deletions, substitutions, and/or conservative substitutions.

[0036] As used herein, the term "linker" or "linker region" refers to a linker inserted between a first polypeptide (*e.g.*, a FZD component) and a second polypeptide (*e.g.*, an Fc region). In some embodiments, the linker is a peptide linker. Linkers should not adversely affect the expression, secretion, or bioactivity of the polypeptides. Preferably, linkers are not antigenic and do not elicit an immune response.

[0037] The term "antibody" means an immunoglobulin molecule that recognizes and specifically binds to 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 "antibody" encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')2, and Fv fragments), single chain Fv (scFv) mutants, multispecific antibodies such as bispecific antibodies generated from at least two intact antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies

exhibit the desired biological activity. An antibody can be of any 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 such as toxins, radioisotopes, etc.

[0038] 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.

[0039] A "monoclonal antibody" 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 different antibodies directed against different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', F(ab')2, Fv), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site. Furthermore, "monoclonal antibody" refers to such antibodies made in any number of manners including but not limited to by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0040] The term "humanized antibody" 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 (*e.g.*, murine) sequences. Typically, humanized antibodies are human immunoglobulins in which residues from the complementary determining region (CDR) are replaced by residues from the CDR of a non-human species (*e.g.* mouse, rat, rabbit, hamster) that have the desired specificity, affinity, and 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 (FR) 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 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 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 CDR regions that correspond to the non-human immunoglobulin whereas all or substantially all of the FR 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. Examples of methods used to generate humanized antibodies are described in U.S. Pat. 5,225,539.

[0041] The term "human antibody" means an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide such as, for example, an antibody comprising murine light chain and human heavy chain polypeptides.

[0042] The term "epitope" or "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 are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding 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.

[0043] That a polypeptide or other agent (*e.g.*, antibody or soluble receptor) "specifically binds" to a protein means that the polypeptide or other agent reacts or associates more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the protein than with alternative substances, including unrelated proteins. In certain embodiments, "specifically binds" means, for instance, that an agent (*e.g.*, antibody or soluble receptor) binds to 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 agent (*e.g.*, antibody or soluble receptor) binds to a protein at times with a K_D of at least about 0.1μM or less, 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 agent (*e.g.*, antibody or soluble receptor) that recognizes a particular protein such as a Wnt protein or a frizzled receptor in more than one species. Likewise, because of homology between different paralogues (*e.g.*, the different human Wnt proteins or human frizzled proteins) in certain regions of their sequences, specific binding can include a polypeptide or an agent (*e.g.*, antibody or soluble receptor) that recognizes more than one paralogue (*e.g.*, more than one human Wnt protein or more than one human frizzled protein). It is understood that an agent (*e.g.*, antibody or soluble receptor) that specifically binds to a first target may or may not specifically bind to 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 agent (*e.g.*, antibody or soluble receptor) may, in certain embodiments, specifically bind to more than one target (*e.g.*, multiple different human Wnt proteins or multiple different frizzled proteins, such as FZD1, FZD2, FZD5, FZD7, and/or FZD8). In certain embodiments, the multiple targets of an antibody 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 two or more human frizzled receptors (*e.g.*, human FZD1, FZD2, FZD5, FZD7, and/or FZD8). In certain alternative embodiments, an antibody

may be bispecific 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 frizzled receptor, such as human FZD5, and further comprises a second, different antigen-binding site that recognizes a different epitope on a second frizzled receptor, such as human FZD8. Generally, but not necessarily, reference to binding means specific binding.

[0044] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. The term cancer is understood to encompass Wnt-dependent cancers. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia.

[0045] "Tumor" and "neoplasm" refer to any mass of tissue that result from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions.

[0046] The terms "cancer stem cell," "tumor stem cell," or "solid tumor stem cell" are used interchangeably herein and refer to a population of cells from a solid tumor that: (1) have extensive proliferative capacity; (2) are capable of asymmetric cell division to generate one or more kinds of differentiated progeny with reduced proliferative or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. These properties of "cancer stem cells," "tumor stem cells," or "solid tumor stem cells" confer on those cancer stem cells the ability to form palpable tumors upon serial transplantation into an immunocompromised 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.

[0047] The terms "cancer cell," "tumor cell," and grammatical equivalents refer to the total population of cells derived from a tumor or a pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the tumor cell population, and tumorigenic stem cells (cancer stem cells). As used herein, the term "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those tumor cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

[0048] The term "tumorigenic" refers to the functional features of a solid tumor 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) that allow solid tumor stem cells to form a tumor. These properties of self-renewal and proliferation to generate all other tumor cells confer on cancer stem cells the ability to form palpable tumors upon serial transplantation into an immunocompromised mouse compared to non-tumorigenic tumor cells, which are unable to form tumors upon serial transplantation. It has been observed that non-tumorigenic tumor cells may form a tumor upon primary transplantation into an immunocompromised mouse after obtaining the tumor cells from a solid tumor, but those non-tumorigenic tumor cells do not give rise to a tumor upon serial transplantation.

[0049] The term "subject" refers to any animal (*e.g.*, a mammal), including, but not limited to humans, non-human primates, 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.

[0050] The term "therapeutically effective amount" refers to an amount of an agent (*e.g.*, antibody, soluble receptor, 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 the agent can reduce the number of cancer cells; reduce the tumor size; inhibit or stop cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibit and stop tumor metastasis; inhibit and stop tumor growth; relieve to some extent one or more of the symptoms associated with the cancer; reduce morbidity and mortality; improve quality of life; decrease tumorigenicity, tumorigenic frequency, or tumorigenic capacity of a tumor; reduce the number or frequency of cancer stem cells in a tumor; differentiate tumorigenic cells to a non-tumorigenic state; or a combination of such effects. To the extent the agent prevents growth and/or kills existing cancer cells, it can be referred to as cytostatic and/or cytotoxic.

[0051] As used herein the term "inhibit tumor growth" refers to any mechanism by which tumor cell growth can be inhibited. In certain embodiments, tumor cell growth is inhibited by slowing proliferation of tumor cells. In certain embodiments, tumor cell growth is inhibited by halting proliferation of tumor cells. In certain embodiments, tumor cell growth is inhibited by killing tumor cells. In certain embodiments, tumor cell growth is inhibited by inducing apoptosis of tumor cells. In certain embodiments, tumor cell growth is inhibited by inducing differentiation of tumor cells. In certain embodiments, tumor cell growth is inhibited by depriving tumor cells of nutrients. In certain embodiments, tumor cell growth is inhibited by preventing migration of tumor cells. In certain embodiments, tumor cell growth is inhibited by preventing invasion of tumor cells.

[0052] Terms such as "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 and/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 certain embodiments, a subject is successfully "treated" for cancer 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, for example, the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity, tumorigenic frequency, or tumorigenic capacity, of a tumor; reduction in the number or frequency of

cancer stem cells in a tumor; differentiation of tumorigenic cells to a non-tumorigenic state; or some combination of effects.

[0053] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain 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 FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat *et al.* Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda Md.)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani *et al* (1997) J. *Molec. Biol.* 273:927-948)). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[0054] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to 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, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon antibodies, in certain embodiments, the polypeptides can occur as single chains or associated chains.

[0055] As used in the present disclosure and claims, the singular forms "a," "an," and "the" include plural forms unless the context clearly dictates otherwise.

[0056] 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.

[0057] 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," and "B." 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).

2. Methods of treatment

[0058] The present invention provides methods of treating neuroendocrine tumors. Neuroendocrine tumors (NETs) are tumors that arise from cells of the endocrine (hormonal) and nervous systems.

Neuroendocrine tumors (NETs) include a group of tumors with a range of morphologic, functional, and behavioral characteristics. These tumors are generally slow growing and behave in an indolent fashion. However, they have the potential to spread, primarily to the liver, and when they do, they can be life threatening and difficult to treat with current modalities.

[0059] Neuroendocrine tumors are classified by the site of their origin. In certain embodiments, the NET is selected from the group consisting of pancreatic neuroendocrine tumors (pNETs) and carcinoid tumors of the lung, stomach, duodenum, jejunum, ileum, colon and rectum. In further embodiments, the NET is selected from the group consisting neuroendocrine tumors of the ovary, thymus, thyroid medulla, adrenal glands (*e.g.*, pheocromocytoma) and paraganglia (paraganglioma). In certain embodiments, the NET treated by the methods described herein is small cell lung cancer (SCLC). In certain alternative embodiments, the NET is not small cell lung cancer. In certain embodiments, NETs are pancreatic neuroendocrine tumors (PETs) or carcinoid tumors. In certain embodiments, the NET is not small cell lung cancer, a pancreatic cancer, or a thyroid cancer.

[0060] Neuroendocrine tumors are also classified by grade and differentiation. *See*, *e.g.*, Phan *et al.*, *Pancreas*, 39(6):784-798 (2012). In certain embodiments, the neuroendocrine tumor is a well differentiated, low grade tumor. In certain embodiments, the neuroendocrine tumor is a moderately differentiated, intermediate grade tumor. In certain embodiments, the neuroendocrine tumor is a poorly differentiated, high grade tumor. In one embodiment, low grade tumors are characterized by <2 mitoses per 10 HPF (high power fields) and no necrosis. In one embodiment, intermediate grade tumors are characterized by 2-10 mitoses per 10 HPF (high power fields) or foci of necrosis. In one embodiment, high grade tumors are characterized by >10 mitoses per 10 HPF (high power fields).

[0061] Neuroendocrine tumors are also classified as functional and non-functional NETs. NETs are considered functional when a specific clinical syndrome is induced due to excessive production of hormones by the tumor cells. Examples of functional NETs include, but are not limited to, carcinoid tumors, which can result in carcinoid syndrome, and functional pNETs, for example, insulinomas, gastrinomas, vasoactive intestinal peptide (VIP)omas, glucagonomas, and somatostatinomas. Non-functional NETs are not associated with a clinical syndrome due to excessive production of hormones by the tumor cells, but can still produce symptoms related to the presence of the tumor or its metastasis (*e.g.*, abdominal pain or bloating). In certain embodiments, the neuroendocrine tumor is a functional NET. In certain embodiments, the neuroendocrine tumor is a non-functional NET. In certain embodiments, the neuroendocrine tumor is selected from the group consisting of functional carcinoid tumor, insulinoma, gastrinoma, vasoactive intestinal peptide (VIP)oma, glucagonoma, serotoninoma, histaminoma, ACTHoma, pheocromocytoma, and somatostatinoma. In certain embodiments, the neuroendocrine tumor is not SCLC.

[0062] In certain embodiments, the neuroendocrine tumor is a primary tumor. In certain embodiments, the neuroendocrine tumor is metastatic tumor. In certain embodiments, the neuroendocrine tumor has not spread outside of the wall of the primary organ. In certain

embodiments, the neuroendocrine tumor has spread through the wall of the primary organ and to nearby tissues, such as fat, muscle, or lymph nodes. In certain embodiments, the neuroendocrine tumor has spread to tissues or organs away from the primary organ, for example, to the liver, bones, or lungs.

[0063] In certain embodiments, the neuroendocrine cancer or tumor is refractory to treatment. As a non-limiting example, the cancer or tumor may be chemorefractory (i.e., resistant to one or more forms of chemotherapy). In certain embodiments, the cancer or tumor is resistant to treatment with a somatostatin analog. In certain embodiments, the cancer or tumor is resistant to treatment with a kinase inhibitor.

[0064] In certain embodiments, the neuroendocrine cancer or tumor has metastasized to the liver. By way of non-limiting example, the neuroendocrine cancer or tumor is a carcinoid or pancreatic neuroendocrine tumor that has metastasized to the liver.

[0065] In one aspect, the present invention provides the use of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) in the treatment of neuroendocrine tumor. In certain embodiments, the Wnt antagonist is useful for inhibiting Wnt signaling (*e.g.*, canonical Wnt signaling) in a neuroendocrine tumor cell, inhibiting neuroendocrine tumor growth, inducing neuroendocrine tumor differentiation, reducing neuroendocrine tumor volume, and/or reducing the tumorigenicity of a neuroendocrine tumor. The methods of use can be *in vitro, ex vivo*, or *in vivo* methods. In certain embodiments, the Wnt antagonist is the antibody OMP-18R5. In certain embodiments, the Wnt antagonist is the soluble receptor OMP-54F28.

[0066] The present invention provides for methods of treating neuroendocrine tumor comprising administering a therapeutically effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to a subject (*e.g.*, a subject in need of treatment). In certain embodiments, the neuroendocrine tumor is a pancreatic neuroendocrine tumor. In certain embodiments, the neuroendocrine tumor is a carcinoid. In certain embodiments, the neuroendocrine tumor is neuroendocrine tumor of the lung. By way of non-limiting example, the neuroendocrine tumor in the lung may be SCLC. In certain embodiments, the neuroendocrine tumor is not SCLC. In certain embodiments, the subject is a human. In certain embodiments, the Wnt antagonist is OMP-18R5. In certain embodiments, the Wnt antagonist is OMP-54F28.

[0067] The present invention further provides methods for inhibiting neuroendocrine tumor growth using the Wnt antagonists (*e.g.*, anti-FZD antibodies and soluble FZD receptors) described herein. In certain embodiments, the method of inhibiting the neuroendocrine tumor growth comprises contacting the tumor cell with a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) *in vitro*. For example, an immortalized neuroendocrine tumor cell line is cultured in medium to which is added the Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to inhibit tumor growth. In some embodiments, neuroendocrine tumor cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and cultured in medium to which is added

a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to inhibit tumor growth. In certain embodiments, the Wnt antagonist is OMP-18R5. In certain embodiments, the Wnt antagonist is OMP-54F28.

[0068] In some embodiments, the method of inhibiting neuroendocrine tumor growth comprises contacting the neuroendocrine tumor or tumor cells with the Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) *in vivo*. In certain embodiments, contacting a neuroendocrine tumor or tumor cell with a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) is undertaken in an animal model. For example, a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) may be administered to neuroendocrine tumor xenografts that have been grown in immunocompromised mice (*e.g.* NOD/SCID mice) to inhibit neuroendocrine tumor growth. In some embodiments, neuroendocrine tumor cancer stem cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and injected into immunocompromised mice that are then administered a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to inhibit neuroendocrine tumor cell growth. In some embodiments, the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) is administered at the same time or shortly after introduction of tumorigenic cells into the animal to prevent neuroendocrine tumor growth. In some embodiments, the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) is administered as a therapeutic after the tumorigenic cells have grown to a specified size. In certain embodiments, the Wnt antagonist is OMP-18R5. In certain embodiments, the Wnt antagonist is OMP-54F28.

[0069] In certain embodiments, the method of inhibiting neuroendocrine tumor growth comprises administering to a subject a therapeutically effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor). In certain embodiments, the subject is a human. In certain embodiments, the subject has a neuroendocrine tumor or has had a tumor removed.

[0070] In certain embodiments, the neuroendocrine tumor is a tumor in which Wnt signaling is active. In certain embodiment, the Wnt signaling that is active is canonical Wnt signaling. In certain embodiments, the neuroendocrine tumor is a Wnt-dependent tumor. For example, in some embodiments, the tumor is sensitive to axin over-expression. In certain embodiments, the tumor does not comprise an inactivating mutation (*e.g.*, a truncating mutation) in the adenomatous polyposis coli (APC) tumor suppressor gene or an activating mutation in the beta-catenin gene. In certain embodiments, the tumor expresses one or more genes in a Wnt gene signature, i.e., one or more genes up-regulated or down-regulated by the Wnt signaling pathway. In certain embodiments, the neuroendocrine tumor for which a subject is being treated involves such a tumor.

[0071] In certain embodiments, the neuroendocrine tumor expresses one or more human frizzled receptors to which the Wnt antagonist FZD-binding antibody described herein binds. In certain embodiments, the neuroendocrine tumor over-expresses the human frizzled receptor(s). In certain embodiments, the Wnt antagonist is OMP-18R5.

[0072] In certain embodiments, the neuroendocrine tumor expresses one or more human Wnt polypeptides to which the Wnt antagonist soluble FZD receptor described herein binds. In certain embodiments, the neuroendocrine tumor over-expresses the human Wnt polypeptide(s). In certain embodiments, the Wnt antagonist is OMP-54F28.

[0073] In certain embodiments, the neuroendocrine tumor expresses one or more human Wnt polypeptides to which the Wnt antagonist anti-Wnt antibody described herein binds. In certain embodiments, the neuroendocrine tumor over-expresses the human Wnt polypeptide(s).

[0074] In certain embodiments, the neuroendocrine tumor is a pancreatic neuroendocrine tumor. In certain embodiments, the neuroendocrine tumor is a carcinoid. In certain embodiments, the neuroendocrine tumor is neuroendocrine tumor of the lung. In certain embodiments, the neuroendocrine tumor is not SCLC.

[0075] The invention also provides a method of inhibiting Wnt signaling in a neuroendocrine tumor cell comprising contacting the cell with an effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor). In certain embodiments, the method is an *in vivo* method wherein the step of contacting the cell with the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) comprises administering a therapeutically effective amount of the Wnt antagonist to the subject. In some alternative embodiments, the method is an *in vitro* or ex vivo method. In certain embodiments, the Wnt signaling that is inhibited is canonical Wnt signaling. In certain embodiments, the Wnt signaling is signaling by Wnt1, Wnt2, Wnt3, Wnt3A, Wnt7a, Wnt7b, and/or Wnt10B. In certain embodiments, the Wnt signaling is signaling by Wnt1, Wnt3A, Wnt7b, and/or Wnt10B.

[0076] In addition, the invention provides a method of reducing the tumorigenicity of a neuroendocrine tumor in a subject, comprising administering a therapeutically effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to the subject. In certain embodiments, the neuroendocrine tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the neuroendocrine tumor is reduced by administration of the agent. In certain embodiments, the Wnt antagonist is OMP-18R5. In certain embodiments, the Wnt antagonist is OMP-54F28.

[0077] Thus, the invention also provides a method of reducing the frequency of cancer stem cells in a neuroendocrine tumor, comprising contacting the tumor with an effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor).

[0078] The invention further provides methods of differentiating tumorigenic neuroendocrine tumor cells into non-tumorigenic cells comprising contacting the tumorigenic neuroendocrine tumor cells with a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) by administering the Wnt antagonist to a subject that has a neuroendocrine tumor comprising the tumorigenic cells or that has had such a neuroendocrine tumor removed.

[0079] The use of the Wnt antagonists (*e.g.*, an anti-FZD antibodies and soluble FZD receptors) described herein to induce the differentiation of neuroendocrine tumor cells is also provided. For

example, methods of inducing cells to differentiate comprising contacting the cells with an effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) described herein are envisioned. Methods of inducing cells in a neuroendocrine tumor in a subject to differentiate comprising administering a therapeutically effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to the subject are also provided. In certain embodiments, the differentiation of neuroendocrine tumor cells is associated with changes in the radiographic image of the tumor lesion. In certain embodiments, the differentiation of neuroendocrine tumor cells is associated with calcification in the tumor lesion. In certain embodiments, the Wnt antagonist is OMP-18R5. In certain embodiments, the Wnt antagonist is OMP-54F28.

[0080] Methods of treating a neuroendocrine tumor in a subject, wherein the neuroendocrine tumor is associated with Wnt signaling activation and/or is characterized by an increased level of stem cells and/or progenitor cells are further provided. In some embodiments, the treatment methods comprise administering a therapeutically effective amount of a Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) to the subject. In certain embodiments, the Wnt signaling is canonical Wnt signaling.

[0081] In certain embodiments, in addition to administering the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) described herein, the method or treatment further comprises administering a second anti-cancer agent (prior to, concurrently with, and/or subsequently to administration of the Wnt antagonist). Pharmaceutical compositions comprising the Wnt antagonist and the second anti-cancer agent are also provided. In certain embodiments, the administration of the combination of the Wnt antagonist and a second anti-cancer agent has a synergistic effect, such as a synergistic effect on the frequency of cancer stem cells.

[0082] It will be appreciated that the combination of a Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) and a second anti-cancer agent may be administered in any order or concurrently. In selected embodiments, the Wnt antagonist will be administered to patients that have previously undergone treatment with the second anti-cancer agent. In certain other embodiments, the Wnt antagonist and the second anti-cancer agent will be administered substantially simultaneously or concurrently. For example, a subject may be given the Wnt antagonist while undergoing a course of treatment with the second anti-cancer agent (*e.g.*, chemotherapy). In certain embodiments, the Wnt antagonist will be administered within 1 year of the treatment with the second anti-cancer agent. In certain alternative embodiments, the Wnt antagonist will be administered within 10, 8, 6, 4, or 2 months of any treatment with the second anti-cancer agent. In certain other embodiments, the Wnt antagonist will be administered within 4, 3, 2, or 1 week of any treatment with the second anti-cancer agent. In some embodiments, the Wnt antagonist will be administered within 5, 4, 3, 2, or 1 days of any treatment with the second anti-cancer agent. It will further be appreciated that the two agents or treatment may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[0083] Useful classes of anti-cancer agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (*e.g.*, platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, performing compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second anti-cancer agent is an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[0084] Anticancer agents that may be administered in combination with the Wnt antagonists (*e.g.*, anti-FZD antibodies or soluble FZD receptors) include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the combined administration of a Wnt antagonist and a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. Treatment with a Wnt antagonist can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Chemotherapies contemplated by the invention include chemical substances or drugs which are known in the art and are commercially available, such as gemcitabine, irinotecan, doxorubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, TAXOL, methotrexate, cisplatin, melphalan, vinblastine and carboplatin. 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 Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992).

[0085] Chemotherapeutic agents useful in the instant invention also include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin,

streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; 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"); cyclophosphamide; thiotepa; taxoids, *e.g.* paclitaxel (TAXOL, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; 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 antiandrogens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0086] In certain embodiments, the chemotherapeutic agent is a kinase inhibitor. In certain embodiments, the kinase inhibitor is a multi-targeted receptor tyrosine kinase inhibitor. Kinase inhibitors include, but are not limited to, sunitinib (marketed as SUTENT by Pfizer), pazopanib, crizotinib, dasatinib. In certain embodiments, the second anticancer agent is sunitinib.

[0087] In certain embodiments, the chemotherapeutic agent is an inhibitor of mammalian target of rapamycin (mTOR). mTOR inhibitors include, but are not limited to, temsirolimus, sirolimus, deforolimus and everolimus. In certain embodiments, the second anticancer agent is everolimus.

[0088] In certain embodiments, the chemotherapeutic agent is a somatostatin analog. Somatostatin analogs act through interaction with specific, high affinity membrane receptors for somatostatin. Somatostatin analogs include, but are not limited to, octreotide, somatulin, and RC 160 (octastatin). In certain embodiments, the second anticancer agent is octreotide.

[0089] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (*e.g.*, topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HCl, daunorubicin citrate, mitoxantrone HCL, actinomycin D, etoposide, topotecan HCl, teniposide (VM-26), and irinotecan. In certain embodiments, the second anticancer agent is irinotecan.

[0090] In certain embodiments, the chemotherapeutic agent is an alkylating agent. In certain embodiments, the chemotherapeutic agent is temozolomide.

[0091] 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 (GlaxoSmithKline), 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 second anticancer agent is gemcitabine. In certain embodiments, the tumor to be treated is a pancreatic neuroendocrine tumor and the second anticancer agent is an anti-metabolite (*e.g.*, gemcitabine).

[0092] In certain embodiments, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. By way of non-limiting example, the agent comprises a taxane. In certain embodiments, the agent comprises 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 (*e.g.*, 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 Eg5 kinesin or an inhibitor of a mitotic kinase such as Aurora A or Plk1.

[0093] In certain embodiments, the treatment involves the combined administration of a Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) described herein and radiation therapy. Treatment with the Wnt antagonist can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Any dosing schedule for such radiation therapy can be used as determined by the skilled practitioner.

[0094] In some embodiments, the second anti-cancer agent comprises an antibody. Thus, treatment can involve the combined administration of a Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) with antibodies against tumor-associated antigens including, but not limited to, antibodies that bind to EGFR, ErbB2, HER2, DLL4, NOTCH, and/or VEGF. Exemplary anti-DLL4 antibodies, are described, for example, in U.S. Patent Application Publication No. US 2008/0187532,

incorporated by reference herein in its entirety. In certain embodiments, the second anti-cancer agent is an antibody that is an angiogenesis inhibitor (*e.g.*, an anti-VEGF antibody). Additional anti-DLL4 antibodies are described in, *e.g.*, International Patent Publication Nos. WO 2008/091222 and WO 2008/0793326, and U.S. Patent Application Publication Nos. US 2008/0014196, US 2008/0175847, US 2008/0181899, and US 2008/0107648, each of which is incorporated by reference herein in its entirety. Exemplary anti-NOTCH antibodies are described, for example, in U.S. Patent Application Publication No. US 2008/0131434, incorporated by reference herein in its entirety. In certain embodiments, the second anti-cancer agent is an antibody that is an angiogenesis inhibitor (*e.g.*, an anti-VEGF antibody). In certain embodiments, the second anti-cancer agent is an inhibitor of NOTCH signaling. In certain embodiments, the second anti-cancer agent is AVASTIN (bevacizumab), HERCEPTIN (trastuzumab), VECTIBIX (panitumumab), or ERBITUX (cetuximab). 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.

[0095] Furthermore, treatment can include administration of one or more cytokines (*e.g.*, lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of cancer cells or any other therapy deemed necessary by a treating physician.

[0096] For the treatment of the disease, the appropriate dosage of a Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) described herein depends on the type of neuroendocrine tumor to be treated, the severity and course of the neuroendocrine tumor, the responsiveness of the neuroendocrine tumor, whether the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) is administered for therapeutic or preventative purposes, previous therapy, patient's clinical history, and so on all at the discretion of the treating physician. The Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the neuroendocrine tumor is achieved (*e.g.* reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor). The administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. In certain embodiments, dosage is from 0.01μg to 100mg per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain embodiments, the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) is given once every two weeks or once every three weeks. In certain embodiments, the dosage of the Wnt antagonist (*e.g.*, anti-FZD antibody or soluble FZD receptor) is from about 0.1mg to about 20mg per kg of body weight. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. In certain embodiments, the Wnt antagonist is OMP-18R5. In certain embodiments, the Wnt antagonist is OMP-54F28.

[0097] In certain embodiments, OMP-18R5 is administered intravenously at a dose of about 0.1mg/kg to about 20mg/kg or a dose of about 0.5mg/kg to about 10mg/kg. Such doses may, in some embodiments, be given about every week, every two weeks, every three weeks or every four weeks. In certain embodiments, OMP-18R5 is administered intravenously at a dosage of about 0.5mg/kg to about 10mg/kg about every two to four weeks. In certain embodiments, OMP-18R5 is administered intravenously at a dosage of about 1.0mg/kg to about 10mg/kg approximately about every three weeks. In certain embodiments, OMP-18R5 is administered intravenously 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 certain embodiments, the antibody is administered at a dosage of about 0.5mg/kg to about 1.0mg/kg about every one to two weeks. In some alternative embodiments, the antibody is administered at a dosage of about 1.0mg/kg to about 5.0mg/kg about every three weeks.

[0098] By way of non-limiting example, OMP-54F28 may be administered intravenously at a dose of about 0.1mg/kg to about 20mg/kg. This dose may, in some embodiments, be given every week, every two weeks, every three weeks or every four weeks. In certain embodiments, OMP-54F28 is administered intravenously at a dosage of about 0.5mg/kg to about 10mg/kg every two to four weeks. In certain embodiments, OMP-54F28 is administered intravenously at a dosage of about 0.5mg/kg to about 10mg/kg about every three weeks.

3. FZD-binding agents

[0099] Another aspect of the methods of the invention is the use of a FZD-binding agent (*e.g.*, anti-FZD antibody) in the treatment of neuroendocrine tumors. In certain embodiments, the FZD-binding agents (*e.g.*, anti-FZD antibodies) that are useful in the methods of the invention specifically bind one or more human frizzled receptors (FZDs). In certain embodiments, the agents specifically bind two, three, four, five, six, seven, eight, nine, or ten frizzled receptors. The human frizzled receptor or receptors bound by the agent can be selected from the group consisting of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In certain embodiments, the one or more human frizzled receptors comprise FZD1, FZD2, FZD5, FZD7, and/or FZD8. In certain embodiments, the one or more human frizzled receptors comprise FZD7. In certain embodiments, the one or more human frizzled receptors comprise FZD5 and/or FZD8. In certain embodiments, the agent specifically binds FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding agent specifically binds FZD7. In certain embodiments, the FZD-binding agent specifically binds FZD5. The full-length amino acid (aa) and nucleotide (nt) sequences for FZD1-10 are known in the art and also provided herein as SEQ ID NO:1 (FZD1 aa), SEQ ID NO:2 (FZD2 aa), SEQ ID NO:3 (FZD3 aa), SEQ ID NO:4 (FZD4 aa), SEQ ID NO:5 (FZD5 aa), SEQ ID NO:6 (FZD6 aa), SEQ ID NO:7 (FZD7 aa), SEQ ID NO:8 (FZD8 aa), SEQ ID NO:9 (FZD9 aa), SEQ ID NO:10 (FZD10 aa).

[00100] In certain embodiments, a FZD-binding agent (*e.g.*, anti-FZD antibody) that is useful in the methods of the invention specifically binds to two or more human frizzled receptors. In certain embodiments, the two or more human frizzled receptors are selected from the group consisting of FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the two or more frizzled receptors comprise FZD1 and a second frizzled receptor selected from the group consisting of FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the two or more frizzled receptors comprise FZD2 and a second frizzled receptor selected from the group consisting of FZD1, FZD5, FZD7, and FZD8. In certain embodiments, the two or more frizzled receptors comprise FZD5 and a second frizzled receptor selected from the group consisting of FZD1, FZD2, FZD7, and FZD8. In certain embodiments, the two or more frizzled receptors comprise both FZD5 and FZD8. In certain embodiments, the two or more frizzled receptors comprise FZD7 and a second frizzled receptor selected from the group consisting of FZD1, FZD2, FZD5, and FZD8. In certain embodiments, the agent specifically binds to three or more human frizzled receptors. In certain embodiments, the three or more human frizzled receptors comprise three or more frizzled receptors selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the agent further specifically binds to one or more additional human frizzled receptors.

[00101] In certain embodiments, a FZD-binding agent (*e.g.*, anti-FZD antibody) that is useful in the methods of the invention specifically binds to the extracellular domain (ECD) within the one or more human frizzled receptors to which it binds. Sequences of the extracellular domain of each of the human frizzled receptors are known in the art and are also provided as SEQ ID NO:11 (FZD1 ECD), SEQ ID NO:12 (FZD2 ECD), SEQ ID NO:13 (FZD3 ECD), SEQ ID NO:14 (FZD4 ECD), SEQ ID NO:15 (FZD5 ECD), SEQ ID NO:16 (FZD6 ECD), SEQ ID NO:17 (FZD7 ECD), SEQ ID NO:18 (FZD8 ECD), SEQ ID NO:19 (FZD9 ECD), and SEQ ID NO:20 (FZD10 ECD). Particularly useful antibodies are described in U.S. Patent 7,982,013 and U.S. Pat. Appl. Pub. No. 2012/0027778, which are herein incorporated by reference in their entirety.

[00102] In certain embodiments, a FZD-binding agent (*e.g.*, anti-FZD antibody) that is useful in the methods of the invention specifically binds to the Fri domain (FRI) (also known as the cysteine-rich domain (CRD)) within the human frizzled receptor(s) to which it binds. Sequences of the Fri domain of each of the human frizzled receptors are known in the art and are also provided herein. The Fri domain of FZD1 includes approximately amino acids 87-237 of SEQ ID NO:11. The Fri domain of FZD2 includes approximately amino acids 24-159 of SEQ ID NO:12. The Fri domain of FZD3 includes approximately amino acids 23-143 of SEQ ID NO:13. The Fri domain of FZD4 includes approximately amino acids 40-170 of SEQ ID NO:14. The Fri domain of FZD5 includes approximately amino acids 27-157 of SEQ ID NO:15. The Fri domain of FZD6 includes approximately amino acids 19-146 of SEQ ID NO:16. The Fri domain of FZD7 includes approximately amino acids 33-170 of SEQ ID NO:17. The Fri domain of FZD8 includes approximately amino acids 28-158 of SEQ ID NO:18. The Fri domain of FZD9 includes

approximately amino acids 23-159 of SEQ ID NO:19. The Fri domain of FZD10 includes approximately amino acids 21-154 of SEQ ID NO:20. The corresponding, predicted Fri domains for each of the human FZD receptors are provided as SEQ ID NOs:21-30. The minimal, core Fri domain sequences for each of the human FZD receptors (FZD1-10) are provided as SEQ ID NOs:73-82. Those of skill in the art may differ in their understanding of the exact amino acids corresponding to the various Fri domains. Thus in specific embodiments, the N-terminus or C-terminus of the domains outlined above and herein can extend or be shortened by 1, 2, 3, 4, 5, 6, 7, 8, 9, or even 10 amino acids.

[00103] In certain embodiments, an individual antigen-binding site of a FZD-binding antibody is capable of binding (or binds) the one, two, three, four, or five (or more) human frizzled receptors. In certain embodiments, an individual antigen-binding site of the FZD-binding antibody is capable of specifically binding one, two, three, four, or five human frizzled receptors selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, an individual binding site of the antibody specifically binds to at least FZD5 and FZD8.

[00104] In certain embodiments, a FZD-binding agent (*e.g.*, anti-FZD antibody) that is useful in the methods of the invention binds to one or more (for example, two or more, three or more, or four or more) human frizzled receptors with a dissociation constant (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 FZD-binding agent or antibody that binds to more than one FZD, binds to those FZDs with a K_D of about 100nM or less, about 20nM or less, or about 10nM or less. In certain embodiments, the FZD-binding agent or antibody binds to each of one or more (*e.g.*, 1, 2, 3, 4, or 5) of the following FZDs with a dissociation constant of about 40nM or less: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding agent or antibody binds to each of one or more of the following FZDs with a dissociation constant of about 10nM or less: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding agent or antibody binds to each of the following FZDs with a dissociation constant of about 10nM or less: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the dissociation constant of the agent or antibody to a particular FZD is the dissociation constant determined using an FZD-Fc fusion protein comprising the FZD extracellular domain or Fri domain immobilized on a Biacore chip.

[00105] In certain embodiments, a FZD-binding agent (*e.g.*, anti-FZD antibody) that is useful in the methods of the invention is an antagonist of at least one human frizzled receptor (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 FZDs) bound by the agent. In certain embodiments, the agent 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 one or more activity of the bound human frizzled receptor.

[00106] In certain embodiments, the FZD-binding agent (*e.g.*, anti-FZD antibody) inhibits binding of a ligand to the at least one human frizzled receptor. In certain embodiments, the ligand is a human Wnt protein. Nineteen human Wnt proteins have been identified: Wnt1, Wnt2, Wnt2B/13, Wnt3,

Wnt3A, Wnt4, Wnt5A, Wnt5B, Wnt6, Wnt7A, Wnt7B, Wnt8A, Wnt8B, Wnt9A (previously Wnt14), Wnt9B (previously Wnt15), Wnt10A, Wnt10B, Wnt11, and Wnt16. In certain embodiments, the agent inhibits binding of Wnt3A to FZD8. In certain embodiments, the inhibition of binding of a particular ligand to a particular human frizzled protein provided by the FZD-binding agent 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 ligand such as a Wnt to a FZD, further inhibits Wnt signaling (*e.g.*, inhibits canonical Wnt signaling).

[00107] In certain embodiments, the FZD-binding agent (*e.g.*, anti-FZD antibody) inhibits Wnt signaling. It is understood that a FZD-binding agent that inhibits Wnt signaling may, in certain embodiments, inhibit signaling by one or more Wnts, but not necessarily by all Wnts. In certain alternative embodiments, signaling by all human Wnts may be inhibited. In certain embodiments, signaling by one or more Wnts selected from the group consisting of Wnt1, Wnt2, Wnt2B/13, Wnt3, Wnt3A, Wnt4, Wnt5A, Wnt5B, Wnt6, Wnt7A, Wnt7B, Wnt8A, Wnt8B, Wnt9A (previously Wnt14), Wnt9B (previously Wnt15), Wnt10A, Wnt10B, Wnt11, and Wnt16 is inhibited. In certain embodiments, the Wnt signaling that is inhibited is signaling by Wnt1, Wnt2, Wnt3, Wnt3A, Wnt7a, Wnt7b, and/or Wnt10B. In certain embodiments, the agent inhibits signaling by (at least) Wnt1, Wnt3A, Wnt7b, and Wnt10B. In particular embodiments, the agent inhibits signaling by (at least) Wnt3A. In certain embodiments, the inhibition of signaling by a Wnt provided by the FZD-binding agent is a reduction in the level of signaling by the Wnt of 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, the Wnt signaling that is inhibited is canonical Wnt signaling.

[00108] *In vivo* and *in vitro* assays for determining whether a FZD-binding agent (or candidate FZD-binding agent) inhibits Wnt signaling are known in the art. See, *e.g.*, U.S. Pat. Appl. Pub. No. 2012/0027778, which is incorporated by reference herein in its entirety.

[00109] In certain embodiments, the FZD-binding agents (*e.g.*, anti-FZD antibodies) useful in the methods of the invention have one or more of the following effects: inhibit proliferation of neuroendocrine tumor cells, reduce the tumorigenicity of a neuroendocrine tumor by reducing the frequency of cancer stem cells in the neuroendocrine tumor, inhibit neuroendocrine tumor growth, increase survival, trigger cell death of neuroendocrine tumor cells, differentiate tumorigenic neuroendocrine tumor cells to a non-tumorigenic state, or prevent metastasis of tumor cells.

[00110] In certain embodiments, the FZD-binding agents useful in the methods of the invention are capable of inhibiting neuroendocrine tumor growth. In certain embodiments, the FZD-binding agents are capable of inhibiting neuroendocrine tumor growth *in vivo* (*e.g.*, in a xenograft mouse model and/or in a human having cancer).

[00111] In certain embodiments, the FZD-binding agents useful in the methods of the invention are capable of reducing the tumorigenicity of a neuroendocrine tumor. In certain embodiments, the agent or antibody is capable of reducing the tumorigenicity of a neuroendocrine 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. An example of a limiting dilution assay used to test the efficacy of an anti-FZD antibody is provided in Example 8 of US 2012/0027778, which is incorporated by reference herein in its entirety. 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 Number WO 2008/042236, U.S. Patent Application Publication No. 2008/0064049, and U.S. Patent Application Publication No. 2008/0178305, each of which is incorporated by reference herein in its entirety.

[00112] In certain embodiments, the FZD-binding agent (*e.g.*, antibody) useful in the methods of the invention is a polypeptide. In certain embodiments, the agent or polypeptide is an antibody. In certain embodiments, the antibody is an IgG1 antibody or an IgG2 antibody. In certain embodiments, the antibody is a monoclonal antibody. In certain embodiments, the antibody is a human antibody or a humanized antibody. In certain embodiments, the antibody is an antibody fragment.

[00113] In certain embodiments, an anti-FZD antibody for the methods of the invention comprise one, two, three, four, five and/or six of the CDRs of the 18R5, 18R8, and/or 44R24 human antibodies (see Table 1 below) with up to four (i.e., 0, 1, 2, 3, or 4) conservative amino acid substitutions per CDR. In certain embodiments, the heavy chain CDR(s) are contained within a heavy chain variable region and/or the light chain CDR(s) are contained within a light chain variable region.

Table 1. CDRs of 18R8, 18R5, and 44R24 human antibodies

Ab(s)	Heavy Chain		
	CDR1	CDR2	CDR3
18R8	GFTFSHYTLS (SEQ ID NO:31)	VISGDGSYTYYADSVKG (SEQ ID NO:32)	NFIKYVFAN (SEQ ID NO:33)
18R5	GFTFSHYTLS (SEQ ID NO:31)	VISGDGSYTYYADSVKG (SEQ ID NO:32)	NFIKYVFAN (SEQ ID NO:33)
44R24	GFTFSSYYIT (SEQ ID NO:46)	TISYSSSNTYYADSVKG (SEQ ID NO:47)	SIVFDY (SEQ ID NO:48)

Ab(s)	Light Chain		
	CDR1	CDR2	CDR3
18R8	SGDKLGKKYAS (SEQ ID NO:41)	EKDNRPSG (SEQ ID NO:42)	SSFAGNSLE (SEQ ID NO:43)

18R5	SGDNIGSFYVH (SEQ ID NO:34)	DKSNRPSG (SEQ ID NO:35)	QSYANTLSL (SEQ ID NO:36)
44R24	SGDALGNRYVY (SEQ ID NO:49)	SG (SEQ ID NO:50)	GSWDTRPYPKY (SEQ ID NO:51)

* Site directed change introduced to CDR1 to remove N-linked glycosylation site is underlined.

[00114] In one embodiment, an anti-FZD antibody that is useful in the methods of the invention comprises a heavy chain variable region comprising: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and/or (c) a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain embodiments, the anti-FZD antibody further comprises a light chain variable region comprising: (a) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:41), or a variant of thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:42), or a variant of thereof comprising 1, 2, 3, or 4 amino acid substitutions; and/or (c) a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:43), or a variant of thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain embodiments, the amino acid substitutions are conservative substitutions. In a further embodiment, an anti-FZD antibody that is useful in the methods of the invention comprises (a) a heavy chain variable region comprising a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33); and/or (b) a light chain variable region comprising a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:41), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:42), and/or a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:43).

[00115] In one embodiment, an anti-FZD antibody that is useful in the methods of the invention comprises a heavy chain variable region comprising: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and/or (c) a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain embodiments, the anti-FZD antibody further comprises a light chain variable region comprising: (a) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:34), or a variant of thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:35), or a variant of thereof comprising 1, 2, 3, or 4 amino acid substitutions; and/or (c) a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:36), or a variant of thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain embodiments, the amino acid

substitutions are conservative substitutions. In a further embodiment, an anti-FZD antibody that is useful in the methods of the invention comprises (a) a heavy chain variable region comprising a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33); and/or (b) light chain variable region comprising a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:34), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:35), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:36).

[00116] In one embodiment, an anti-FZD antibody that is useful in the methods of the invention comprises a heavy chain variable region comprising: (a) a heavy chain CDR1 comprising GFTFSSYYIT (SEQ ID NO:46), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; (b) a heavy chain CDR2 comprising TISYSSSNTYYADSVKG (SEQ ID NO:47), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (c) a heavy chain CDR3 comprising SIVFDY (SEQ ID NO:48), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions. In certain embodiments, the anti-FZD antibody further comprises a light chain variable region comprising: (a) a light chain CDR1 comprising SGDALGNRYVY (SEQ ID NO:49), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; (b) a light chain CDR2 comprising SG (SEQ ID NO:50), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and (c) a light chain CDR3 comprising GSWDTRPYPKY (SEQ ID NO:51), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions. In certain embodiments, the antibody comprises: (a) a heavy chain CDR1 comprising GFTFSSYYIT (SEQ ID NO:46), a heavy chain CDR2 comprising TISYSSSNTYYADSVKG (SEQ ID NO:47), and a heavy chain CDR3 comprising SIVFDY (SEQ ID NO:48); and/or (b) a light chain CDR1 comprising SGDALGNRYVY (SEQ ID NO:49), a light chain CDR2 comprising SG (SEQ ID NO:50), and a light chain CDR3 comprising GSWDTRPYPKY (SEQ ID NO:51).

[00117] In certain embodiments, an anti-FZD antibody useful for the methods of the invention comprise: (a) a heavy chain variable region having at least about 80%, 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:37 or SEQ ID NO:52; and/or (b) a light chain variable region having at least about 80%, 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:44, SEQ ID NO:38 or SEQ ID NO:53. In certain embodiments, an anti-FZD antibody useful for the methods of the invention comprise: (a) a heavy chain variable region having at least about 80%, 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:37; and (b) a light chain variable region having at least about 80%, 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:38. In certain embodiments, the anti-FZD antibody useful for the methods of the invention comprises (a) a heavy chain variable region having the amino

acid sequence of SEQ ID NO:37 or SEQ ID NO:52; and/or (b) a light chain variable region having the amino acid sequence of SEQ ID NO:44, SEQ ID NO:38 or SEQ ID NO:53. In certain embodiments, the anti-FZD antibody comprises (a) a heavy chain variable region having the amino acid sequence of SEQ ID NO:37; and/or (b) a light chain variable region having the amino acid sequence of SEQ ID NO:44. In certain embodiments, the anti-FZD antibody comprises (a) a heavy chain variable region having the amino acid sequence of SEQ ID NO:37; and/or (b) a light chain variable region having the amino acid sequence of SEQ ID NO:38. In certain embodiments, the anti-FZD antibody comprises (a) a heavy chain variable region having the amino acid sequence of SEQ ID NO:52; and/or (b) a light chain variable region having the amino acid sequence of SEQ ID NO:53.

Table 2. VH and VL of selected human anti-FZD antibodies

Ab(s)	Heavy Chain Variable Region (VH) amino acid sequence	Light Chain Variable Region (VL) amino acid sequence
18R8	SEQ ID NO:37	SEQ ID NO:44
18R5	SEQ ID NO:37	SEQ ID NO:38
44R24	SEQ ID NO:52	SEQ ID NO:53

[00118] In certain embodiments, an anti-FZD antibody useful for the methods of the invention comprises (a) a heavy chain of SEQ ID NO:39 and light chain of SEQ ID NO:45; or (b) a heavy chain of SEQ ID NO:39 and light chain of SEQ ID NO:40.

Table 3. The heavy chain and light chain of selected human anti-FZD antibodies

Ab(s)	Heavy Chain Variable Region (VH) amino acid sequence	Light Chain Variable Region (VL) amino acid sequence
18R8	SEQ ID NO:39	SEQ ID NO:45
18R5	SEQ ID NO:39	SEQ ID NO:40

[00119] In certain embodiments, the FZD-binding agent useful in the methods of the invention comprises, consists essentially of, or consists of an anti-FZD antibody selected from the group consisting of 18R8, 18R5, and 44R24 IgG antibodies.

[00120] In certain embodiments, the FZD-binding agent useful in the methods of the invention comprises the heavy chains and light chains of the 18R8 IgG2 antibody (with or without the leader sequence). In certain embodiments, the FZD-binding agent is the 18R8 IgG2 antibody. DNA encoding the heavy chains and light chains of the 18R8 IgG2 antibody was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on September 29, 2008, and assigned ATCC deposit designation number PTA-9540. In certain embodiments, the FZD-binding agent useful in the

methods of the invention comprises the heavy chains and light chains of the 18R5 IgG2 antibody (with or without the leader sequence). In certain embodiments, the FZD-binding agent is the 18R5 IgG2 antibody. The 18R5 IgG2 antibody is also referred to herein as OMP-18R5. DNA encoding the heavy chains and light chains of the 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. Additional information regarding the OMP-18R5 antibody can be found, for example, in U.S. Patent No. 7,982,013, which is incorporated by reference herein in its entirety. In U.S. Patent No. 7,982,013, the OMP-18R5 antibody is generally referred to as "18R5" or the "18R5 IgG2 antibody."

[00121] In certain embodiments, the FZD-binding agent useful in the methods of the invention is an IgG antibody encoded by the plasmid deposited with the ATCC on August 26, 2009, and assigned deposit designation number PTA-10307, PTA-10309, or PTA-10311.

[00122] In certain embodiments, the FZD-binding agent useful in the methods of the invention is an agent that competes for specific binding to FZD1, FZD2, FZD5, FZD7, and/or FZD8 with an antibody encoded by the plasmid having ATCC deposit designation number PTA-9540, PTA-9541, PTA-10307, or PTA-10309 (*e.g.*, in a competitive binding assay). In certain alternative embodiments, the FZD-binding agent is an agent that competes for specific binding to FZD5 and/or FZD8 with an antibody encoded by the plasmid having ATCC deposit designation number PTA-10311.

[00123] In certain embodiments, the FZD-binding agent (*e.g.*, antibody) useful in the methods of the invention binds to the same epitope as or binds to an epitope that overlaps with the epitope of the 18R5, 18R8, or 44R24 antibody.

[00124] In certain embodiments, the FZD-binding agent FZD-binding agent (*e.g.*, antibody) useful in the methods of the invention competes for specific binding to a human frizzled receptor with the 18R5, 18R8, or 44R24 antibody.

[00125] Further examples of FZD-binding agents useful in the methods of the invention are disclosed in U.S. Pat. Appl. Pub. No. 2012/0027778, which is incorporated by reference herein in its entirety.

[00126] In certain embodiments, the FZD-binding agent useful in the methods of the invention has a circulating half-life in mice, cynomolgous monkeys, or humans of at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the FZD-binding agent is an IgG (*e.g.*, IgG1 or IgG2) antibody that has a circulating half-life in mice, cynomolgous monkeys, or humans of at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. Methods of increasing 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. Pat. Pub. 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.

[00127] In certain embodiments, an anti-FZD antibody useful for the methods of the invention is a bispecific antibody that specifically recognizes a human frizzled receptor. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. In one embodiment, the bispecific anti-FZD antibody specifically recognizes different epitopes within the same human frizzled receptor. In another embodiment, the bispecific anti-FZD antibody specifically recognizes different epitopes within a human frizzled receptor or on different human frizzled receptors.

[00128] Alternatively, in certain alternative embodiments, an anti-FZD antibody useful for the methods of the invention is not a bispecific antibody.

[00129] In certain embodiments, an anti-FZD antibody useful for the methods of the invention is 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) the same one or more human FZD receptors (*e.g.*, FZD1, FZD2, FZD5, FZD7, or FZD8, or a homologous epitope on some combination of the FZDs). In certain embodiments, an antigen-binding site of the monospecific anti-FZD antibody is capable of binding (or binds) one, two, three, four, or five (or more) human frizzled receptors.

[00130] In certain embodiments, the FZD-binding agent useful for the methods of the invention 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, *Curr. Opin. Biotechnol.*, 18:295-304 (2007), Hosse *et al.*, *Protein Science*, 15:14-27 (2006), Gill *et al.*, *Curr. Opin. Biotechnol.*, 17:653-658 (2006), Nygren, *FEBS J.*, 275:2668-76 (2008), and Skerra, *FEBS J.*, 275:2677-83 (2008), each of which is incorporated by reference herein in its entirety.

[00131] In certain embodiments, the FZD-binding agent useful for the methods of the invention comprises a protein scaffold of a type selected from the group consisting of protein A, a lipocalin, a fibronectin domain, an ankyrin consensus repeat domain, and thioredoxin.

[00132] In certain embodiments, the FZD-binding agent useful for the methods of the invention has been naturally or unnaturally modified. By way of non-limiting example, the polypeptide may be labeled. In certain embodiments, the polypeptide is glycosylated, pegylated, phosphorylated, or acetylated, amidated. In certain embodiments, the modifications increase stability and/or the *in vivo* half-life of the polypeptide. In certain embodiments, the polypeptides are cyclic. In certain further embodiments, the polypeptides comprise one or more N-methyl amino acids.

[00133] In certain embodiments, the FZD-binding agent useful for the methods of the invention is (or comprises) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:54-72, or (b) an amino acid sequence having at least about 80%, at least about 85%, at least about 88%, or at least about 90% amino acid sequence identity to a sequence selected from the group consisting of SEQ ID NOs:54-67 or 69-72. In certain embodiments, the polypeptides comprise,

consist essentially of, or consist of a cyclic peptide selected from the group consisting of SEQ ID NOs:54-72. In certain embodiments, the amino acid sequence is SEQ ID NO:64. In certain alternative embodiments, the amino acid sequence is SEQ ID NO:68.

[00134] In certain embodiments, the FZD-binding polypeptide useful for the methods of the invention is less than about 500 amino acids in length, less than about 200 amino acids in length, less than about 100 amino acids in length, less than about 50 amino acids in length, less than about 20 amino acids amino acids in length, or less than about 15 amino acids in length. In certain embodiments, the FZD-binding polypeptide is at least about 3, at least about 5, or at least about 7 amino acids in length. Accordingly, in certain embodiments the polypeptide is between about 5 and about 20 amino acids in length. In some embodiments, the polypeptide is between about 7 and about 15 amino acids in length.

4. Soluble receptors

[00135] An additional aspect of the methods of the invention is the use of Wnt antagonist soluble receptors in the treatment of neuroendocrine tumors. In certain embodiments, the soluble receptor useful in the methods of the invention comprises the extracellular domain of a FZD receptor. In some embodiments, the soluble receptor useful in the methods of the invention comprises a Fri domain of a FZD receptor. In certain embodiments, the FZD receptor is a human FZD receptor. In certain embodiments, the human FZD receptor is FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, or FZD10. In certain embodiments, the FZD receptor is FZD8. In certain embodiments, the Wnt antagonist used in the methods described herein comprises a human FZD8 Fri domain and a human Fc region.

[00136] In some alternative embodiments, the soluble receptor useful in the methods of the invention comprises a portion of a SFRP. In some embodiments, the soluble receptor useful in the methods of the invention comprises a Fri domain of a SFRP. In certain embodiments, the SFRP is a human SFRP. In some embodiments, the human SFRP is SFRP1, SFRP2, SFRP3, SFRP4, or SFRP5. The minimal, core Fri domain sequences for each of the human SFRPs (SFRP1-5) are provided as SEQ ID NOs:83-87.

[00137] In other alternative embodiments, the soluble receptor useful in the methods of the invention comprises the extracellular domain of a ROR protein. In some embodiments, the soluble receptor useful in the methods of the invention comprises a Fri domain of a ROR protein. In certain embodiments, the ROR is a human ROR. In some embodiments, the human ROR is ROR1 or ROR2. The minimal, core Fri domain sequences of human ROR1 and ROR2 are provided as SEQ ID NO:88 and SEQ ID NO:89.

[00138] In certain embodiments, the soluble receptors (*e.g.*, FZD8 Fri.Fc) that are useful in the methods of the invention specifically bind one, two, three, four, five, six, seven, eight, nine, ten, or more Wnt proteins. By way of non-limiting example, the Wnt-binding agent may bind Wnt1, Wnt2,

Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and/or Wnt10b. In certain embodiments, the Wnt-binding agent binds Wnt1, Wnt2, Wnt3, Wnt3a, and Wnt7b. In certain embodiments, the soluble receptor is a Wnt antagonist. In certain embodiments, the soluble receptor inhibits Wnt-signaling. In some embodiments, the soluble receptor inhibits canonical Wnt signaling.

[00139] Nonlimiting examples of soluble FZD receptors useful in the methods of the invention can be found in U.S. Patent No. 7,723,477, which is incorporated by reference herein in its entirety. Additional soluble receptors (*e.g.*, soluble FZD receptors) are disclosed in US 2011/0305695, which is incorporated by reference herein in its entirety.

[00140] In certain embodiments, a soluble receptor useful in the methods of the invention comprises a Fri domain of a human FZD receptor, or a fragment or variant of the Fri domain that binds one or more human Wnt proteins. In certain embodiments, the human FZD receptor is FZD4. In certain alternative embodiments, the human FZD receptor is FZD5. In certain additional alternative embodiments, the human FZD receptor is FZD8. In certain embodiments, the FZD is FZD4 and the soluble receptor comprises SEQ ID NO:76 or comprises approximately amino acids 40 to 170 of SEQ ID NO:90. In certain embodiments, the FZD is FZD5 and the soluble receptor comprises SEQ ID NO:77 or comprises approximately amino acids 27-157 of SEQ ID NO:91. In certain embodiments, the FZD is FZD8 and the soluble receptor comprises SEQ ID NO:80 or comprises approximately amino acids 28-158 of SEQ ID NO:92.

[00141] In certain embodiments, the soluble receptor useful in the methods of the invention comprises a minimal Fri domain sequence selected from the group consisting of SEQ ID NOs:73-89. In certain embodiments, the soluble receptor useful in the methods of the invention comprises a variant of any one of the aforementioned 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(s).

[00142] In certain embodiments, the soluble receptor useful in the methods of the invention, such as a soluble receptor comprising a minimum Fri domain of a human FZD receptor, further comprises a human Fc region (*e.g.*, a human IgG1 Fc region). Soluble receptors comprising the Fri domain of a FZD receptor and human IgG1 Fc are referred to herein as "FZD Fri.Fc" (e.g. FZD8 Fri.Fc). 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 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 undesirable disulfide bond formation. In certain embodiments, the Fc region comprises or consists of SEQ ID NO:93, SEQ ID NO:94, or SEQ ID NO:95.

[00143] In certain embodiments, a soluble receptor useful in the methods of the invention is a fusion protein comprising at least a minimum Fri domain (*e.g.*, a minimum Fri domain of a FZD receptor) and an 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 peptide linker). In some embodiments, the first polypeptide is linked to the Fc region via a peptide linker.

[00144] 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.*, an 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:96), LESGGGGVT (SEQ ID NO:97), GRAQVT (SEQ ID NO:98), WRAQVT (SEQ ID NO:99), and ARGRAQVT (SEQ ID NO:100). 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).

[00145] In certain embodiments, soluble receptors useful for the methods of the invention 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's 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 soluble receptors useful for the methods of the invention may comprise a mixture of polypeptides with different N-termini. In some embodiments, the N-termini differ in length by 1, 2, 3, 4, or 5 amino acids. In some embodiments, the

soluble receptor polypeptide is substantially homogeneous, i.e., the polypeptides have the same N-terminus. 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. In some embodiments, the signal sequence of the polypeptide comprises or consists of a sequence selected from the group listed in Table 3. In some embodiments, the signal sequence is SEQ ID NO:101. In some embodiments, the signal sequence is SEQ ID NO:104. In some embodiments, the signal sequence is SEQ ID NO:106.

Table 3. Signal sequences.

MEWGYLLEVTSLLAALALLQRSSGAAA	SEQ ID NO:101
MEWGYLLEVTSLLAALALLQRSSGALA	SEQ ID NO:102
MEWGYLLEVTSLLAALALLQRSSGVLA	SEQ ID NO:103
MEWGYLLEVTSLLAALLLLQRSPIVHA	SEQ ID NO:104
MEWGYLLEVTSLLAALFLLQRSPIVHA	SEQ ID NO:105
MEWGYLLEVTSLLAALLLLQRSPFVHA	SEQ ID NO:106
MEWGYLLEVTSLLAALLLLQRSPIIYA	SEQ ID NO:107
MEWGYLLEVTSLLAALLLLQRSPIAHA	SEQ ID NO:108

[00146] In certain embodiments, a soluble receptor useful in the methods of the invention comprises a first polypeptide comprising a FZD domain component and an Fc region. In some embodiments, the FZD domain component is from FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, or FZD10. In some embodiments, the Fc region is from an IgG1 immunoglobulin. In some embodiments, the soluble receptor comprises: (a) a first polypeptide consisting essentially of amino acids selected from the group consisting of: X1 to Y1 of SEQ ID NO:11, X2 to Y2 of SEQ ID NO:12, X3 to Y3 of SEQ ID NO:13, X4 to Y4 of SEQ ID NO:14, X5 to Y5 of SEQ ID NO:15, X6 to Y6 of SEQ ID NO:16, X7 to Y7 of SEQ ID NO:17, X8 to Y8 of SEQ ID NO:18, X9 to Y9 of SEQ ID NO:19, and X10 to Y10 of SEQ ID NO:20; and

(b) a second polypeptide consisting essentially of amino acids A to B of SEQ ID NO:95;

wherein X1 = amino acid 69, 70, 71,72, 73, 74, 75, or 76

Y1 = amino acid 236, 237, 238, 239, 240, 241, 242, or 243

X2 = amino acid 22, 23, 24, 25, 26, 27 or 28

Y2 = amino acid 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171 or 172

X3 = amino acid 18, 19, 20, 21, 22, 23, 24, or 25

Y3 = amino acid 141, 142, 143, 144, 145, 146, 147, 148, or 149

X4 = amino acid 38, 39, 40, 41, or 42

Y4 = amino acid 168, 169, 170, 171, 172, 173, 174, 175 or 176

X5 = amino acid 25, 26, 27, 28 or 29

Y5 = amino acid 155, 156, 157, 158, 159, 160, 161, 162, 163, or 164

X6 = amino acid 19, 20, 21, 22, 23, or 24

Y6 = amino acid 144, 145, 146, 147, 148, 149, 150, 151 or 152

X7 = amino acid 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34

Y7 = amino acid 178, 179, 180, 181, 182, 183, 184, 185, or 186

X8 = amino acid 25, 26, 27, 28, 29, 30, or 31

Y8 = amino acid 156, 157, 158, 159, 160, 161, 162, 163, or 164

X9 = amino acid 21, 22, 23, or 24

Y9 = amino acid 137, 138, 139, 140, 141, 142, 143, 144, 145, or 146

X10 = amino acid 20, 21, 22, 23, 24, or 25

Y10 = amino acid 152, 153, 154, 155, 156, 157, 158, 159, or 160

A = amino acid 1, 2, 3, 4, 5, or 6

B = amino acid 231 or 232.

In some embodiments, the first polypeptide is directly linked to the second polypeptide. In some embodiments, the first polypeptide is linked to the second polypeptide via a peptide linker. In some embodiments, the first polypeptide is linked to the second polypeptide via the peptide linker GRA. A polypeptide (*e.g.*, a first or second polypeptide) that "consists essentially of" certain amino acids or is "consisting essentially of" certain amino acids may, in some embodiments, include one or more (*e.g.*, one, two, three, four or more) additional amino acids at one or both ends, so long as the additional amino acids do not materially affect the function of the Wnt-binding agent.

[00147] In certain embodiments, a soluble receptor useful in the methods of the invention comprises: (a) a first polypeptide consisting essentially of amino acids X to Y of SEQ ID NO:18; and (b) a second polypeptide consisting essentially of amino acids A to B of SEQ ID NO:95; wherein the first polypeptide is directly linked to the second polypeptide; and wherein

X = amino acid 25, 26, 27, 28, 29, 30, or 31

Y = amino acid 156, 157, 158, 159, 160, 161, 162, 163, or 164

A = amino acid 1, 2, 3, 4, 5, or 6

B = amino acid 231 or 232.

In some embodiments, the first polypeptide consists essentially of amino acids 25-158 of SEQ ID NO:18. In other embodiments, the first polypeptide consists of amino acids 25-158 of SEQ ID NO:18. In some embodiments, the first polypeptide consists essentially of amino acids 28-158 of SEQ ID NO:18. In other embodiments, the first polypeptide consists of amino acids 28-158 of SEQ ID NO:18. In some embodiments, the first polypeptide consists of amino acids 31-158 of SEQ ID NO:18. In some embodiments, the second polypeptide consists of amino acids 1-232 of SEQ ID NO:95. In some embodiments, the second polypeptide consists of amino acids 3-232 of SEQ ID NO:95. In some embodiments, the second polypeptide consists of amino acids 6-232 of SEQ ID

NO:95. In some embodiments, the first polypeptide is SEQ ID NO:28 and the second polypeptide is SEQ ID NO:95. In some embodiments, the first polypeptide is SEQ ID NO:28 and the second polypeptide is SEQ ID NO:94. In some embodiments, the first polypeptide is SEQ ID NO:28 and the second polypeptide is SEQ ID NO:93.

[00148] In some embodiments, the soluble receptor useful in the methods of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO:109-121. In certain alternative embodiments, the soluble receptor comprises an amino acid sequence selected from the group consisting of SEQ ID NO:109-121, comprising one or more (*e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, etc.) conservative substitutions. In certain embodiments, soluble receptor comprises a sequence having at least about 90%, about 95%, or about 98% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO:109-121. In certain embodiments, the variant soluble receptor maintains its ability to bind one or more human Wnts.

[00149] In certain embodiments, the soluble receptor useful in the methods of the invention comprises the sequence of SEQ ID NO:109. In certain embodiments, the soluble receptor comprises the sequence of SEQ ID NO:115. In some embodiments, the soluble receptor consists of a homodimer formed by polypeptides consisting of SEQ ID NO:115. In certain embodiments, the soluble receptor comprises the sequence of SEQ ID NO:117. In some embodiments, the soluble receptor consists of a homodimer formed by polypeptides consisting of SEQ ID NO:117.

[00150] In some embodiments, the soluble receptors (*e.g.*, FZD8 Fri.Fc) useful in the methods of the invention inhibit the growth of a neuroendocrine tumor or tumor cells. In some embodiments, the soluble receptors induce neuroendocrine tumor cells to differentiate. In some embodiments, the soluble receptors induce the expression of differentiation markers on a neuroendocrine tumor or tumor cell. In certain embodiments, the soluble receptors reduce the frequency of cancer stem cells in a neuroendocrine tumor. In certain embodiments, the soluble receptors inhibit the growth of a Wnt-dependent neuroendocrine tumor. In some embodiments, a soluble receptor comprising SEQ ID NO:115 inhibits neuroendocrine tumor growth to a greater extent than a soluble receptor comprising SEQ ID NO:109. In some embodiments, a soluble receptor comprising SEQ ID NO:117 inhibits neuroendocrine tumor growth to a greater extent than a soluble receptor comprising SEQ ID NO:109. In some embodiments, a soluble receptor inhibits tumor growth to a greater extent than a soluble receptor comprising a FZD domain component, an Fc domain and a linker component connecting the FZD domain component and the Fc domain. In some embodiments, the linker component is an intervening peptide linker.

[00151] In certain embodiments, the soluble receptor useful in the methods of the invention (before signal sequence cleavage) comprises SEQ ID NO:115 and a signal sequence selected from the group consisting of SEQ ID NO: 104-108. In some embodiments, the soluble receptor (before signal sequence cleavage) comprises SEQ ID NO:117 and a signal sequence selected from the group consisting of SEQ ID NO: 104-108. In some embodiments, the soluble receptor comprises SEQ ID

NO:105 and SEQ ID NO:115. In some embodiments, the soluble receptor comprises SEQ ID NO:105 and SEQ ID NO:117. In some embodiments, the soluble receptor comprises SEQ ID NO:106 and SEQ ID NO:115. In some embodiments, the soluble receptor comprises SEQ ID NO:106 and SEQ ID NO:117. In some embodiments, the soluble receptor comprises SEQ ID NO:133.

[00152] In some embodiments, the soluble receptor (*e.g.*, FZD8 Fri.Fc) is a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, and SEQ ID NO:117. In certain embodiments, the substantially purified soluble receptor polypeptide comprises at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% polypeptide that has an N-terminal sequence of ASA. In certain embodiments, the substantially purified soluble receptor polypeptide consists of a polypeptide that has an N-terminal sequence of ASA. In some embodiments, the nascent soluble receptor polypeptide comprises a signal sequence selected from the group consisting of SEQ ID NOs: 101-108. In some embodiments, the nascent soluble receptor polypeptide comprises a signal sequence of SEQ ID NO:106. In some embodiments, the nascent soluble receptor polypeptide comprises a signal sequence that results in a substantially homogeneous polypeptide product with one N-terminal sequence.

[00153] In certain embodiments, the soluble FZD receptor polypeptide is OMP-54F28. OMP-54F28 is a homodimer formed by two polypeptide chains that each consists of SEQ ID NO:117. Additional information regarding OMP-54F28 can be found in U.S. Pat. Appl. Pub. No. 2011/0305695, which is incorporated by reference herein in its entirety. OMP-54F28 is generally referred to as "54F28" in U.S. Pat. Appl. Pub. No. 2011/0305695.

[00154] In certain embodiments, a soluble receptor (*e.g.*, FZD8 Fri.Fc) useful in the methods of the invention comprises an Fc region of an immunoglobulin. In certain embodiments, at least a portion of the Fc region has been deleted or otherwise altered so as to provide desired biochemical or biological characteristics, such as increased cancer cell localization, increased tumor penetration, reduced serum half-life, or increased serum half-life, reduced or no ADCC activity, reduced or no complement-dependent cytotoxicity (CDC) when compared with a soluble receptor of approximately the same immunogenicity comprising a native or unaltered Fc constant region. Modifications to the Fc region may include additions, deletions, or substitutions of one or more amino acids in one or more domains. Additional soluble receptors (*e.g.*, soluble FZD receptors) comprising a modified Fc region are disclosed in US 2011/0305695, which is incorporated by reference herein in its entirety.

[00155] In certain embodiments, the soluble receptors (*e.g.*, FZD8 Fri.Fc) useful in the methods of the invention bind to at least one Wnt with a dissociation constant (K_D) of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, or about 10nM or less. The soluble receptors can be assayed for specific binding by any method known in the art. Such assays are routine and well known in the art (see, *e.g.*, Ausubel *et al*, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety).

[00156] In certain embodiments, the soluble receptor (*e.g.*, FZD8 Fri.Fc) useful in the methods of the invention (*e.g.*, a FZD8 Fri.Fc) is an antagonist of at least one Wnt (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Wnts) bound by the soluble receptor. In certain embodiments, the soluble receptor 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 one or more activity of the bound human Wnt(s). *In vivo* and *in vitro* assays for determining whether a soluble receptor inhibits Wnt signaling are known in the art. Suitable methods are disclosed in US 2011/0305695, which is incorporated by reference herein in its entirety.

[00157] In certain embodiments, a soluble receptor (*e.g.*, FZD8 Fri.Fc) useful in the methods of the invention is derivatized with a water soluble polymer. Suitable water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), dextran, poly(n-vinyl pyrrolidone)-polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. In certain embodiments, the water soluble polymer is polyethylene glycol (PEG).

[00158] In certain embodiments, the soluble receptor (*e.g.*, FZD8 Fri.Fc) useful in the methods of the invention has a circulating half-life in mice, cynomolgous monkeys, or humans of at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the soluble receptors have a half-life of at least about 50 hours in a rat when administered via the tail vein at a dose ranging from about 2mg/kg to about 10mg/kg. In certain embodiments, the soluble receptor is a soluble FZD receptor that comprises a Fri domain of a human FZD receptor (or a fragment or variant of the Fri domain that binds one or more Wnts) and a human Fc region and has a half-life *in vivo* (*e.g.*, in a mouse or rat) that is longer than a soluble FZD receptor comprising the extracellular domain of the FZD receptor and a human Fc region.

5. Anti-Wnt antibodies

[00159] A further aspect of the methods of the invention is the use of anti-Wnt antibodies in the treatment of neuroendocrine tumors. In certain embodiments, the anti-Wnt antibodies that are useful in the methods of the invention specifically bind one or more Wnt polypeptides. In certain embodiments, the antibodies specifically bind two, three, four, five, six, seven, eight, nine, ten or more Wnts. The human Wnt(s) bound by the antibody may be 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, the one or more (or

two or more, three or more, four or more, five or more, etc.) Wnts bound by the antibody or other antibody comprise 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.) Wnts comprise Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, and Wnt10b.

[00160] In certain embodiments, an individual antigen-binding site of a Wnt-binding antibody useful in the methods of the invention is capable of binding (or binds) the one, two, three, four, or five (or more) human Wnts. In certain embodiments, an individual antigen-binding site of the Wnt-binding antibody is capable of specifically binding one, two, three, four, or five human Wnts selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b.

[00161] In certain embodiments, the Wnt-binding antibody useful in the methods of the invention binds to the C-terminal cysteine rich domain of a human Wnt. In certain embodiments, the antibody binds to a domain (within the one or more Wnt proteins to which the antibody binds) that is selected from the group consisting of SEQ ID NOs:122-132. In some embodiments, the Wnt-binding antibody binds within SEQ ID NO:122. In some embodiments, the Wnt-binding antibody binds within amino acids 288-370 of Wnt1.

[00162] In certain embodiments, the Wnt-binding antibody useful in the methods of the invention binds to one or more (for example, two or more, three or more, or four or more) Wnts with a dissociation constant (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 antibody useful in the methods of the invention that binds to more than one Wnt, binds to those Wnts with a K_D of about 100nM or less, about 20nM or less, or about 10nM or less. In certain embodiments, the Wnt -binding antibody binds to each of one or more (*e.g.*, 1, 2, 3, 4, or 5) of the following Wnts with a dissociation constant of about 40nM or less: Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt10a, and Wnt10b.

[00163] In certain embodiments, the anti-Wnt antibody useful in the methods of the invention is an IgG1 antibody or an IgG2 antibody. In certain embodiments, the antibody is a monoclonal antibody. In certain embodiments, the antibody is a human antibody or a humanized antibody. In certain embodiments, the antibody is an antibody fragment.

[00164] The antibodies or other antibodies of the present invention can be assayed for specific binding by any method known in the art. Such assays are routine and well known in the art (see, *e.g.*, Ausubel *et al*, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety).

[00165] In certain embodiments, the Wnt-binding antibody useful in the methods of the invention is an antagonist of at least one Wnt (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Wnts) bound by the antibody. In certain embodiments, the antibody 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 one or more activity of the bound human Wnt(s).

[00166] In certain embodiments, the Wnt-binding antibody useful in the methods of the invention inhibits binding of a ligand to the at least one human Wnt. In certain embodiments, the Wnt-binding antibody inhibits binding of a human Wnt protein to one or more of its ligands. Nineteen human Wnt proteins have been identified: Wnt1, Wnt2, Wnt2B/13, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a (previously Wnt14), Wnt9b (previously Wnt15), Wnt10a, Wnt10b, Wnt11, and Wnt16. Ten human FZD receptors proteins have been identified (FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10). In certain embodiments, the Wnt-binding antibody inhibits binding of FZD4, FZD5, and/or FZD8 to one or more Wnts (*e.g.*, Wnt3a). In certain embodiments, the inhibition of binding of a particular ligand to a Wnt provided by the Wnt-binding antibody 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 antibody that inhibits binding of a Wnt to a ligand such as a FZD, further inhibits Wnt signaling (*e.g.*, inhibits canonical Wnt signaling).

[00167] In certain embodiments, the Wnt-binding antibody useful in the methods of the invention inhibits Wnt signaling. It is understood that a Wnt-binding antibody that inhibits Wnt signaling can, in certain embodiments, inhibit signaling by one or more Wnts, but not necessarily by all Wnts. In certain alternative embodiments, signaling by all human Wnts can be inhibited. In certain embodiments, signaling by one or more Wnts selected from the group consisting of Wnt1, Wnt2, Wnt2b/13, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a (previously Wnt14), Wnt9b (previously Wnt15), Wnt10a, Wnt10b, Wnt11, and Wnt16 is inhibited. In certain embodiments, the Wnt signaling that is inhibited is signaling by Wnt1, Wnt2, Wnt3, Wnt3a, Wnt7a, Wnt7b, and/or Wnt10b. In certain embodiments, the antibody inhibits signaling by (at least) Wnt1, Wnt3a, Wnt7b, and Wnt10b. In particular embodiments, the antibody inhibits signaling by (at least) Wnt3a. In certain embodiments, the inhibition of signaling by a Wnt provided by the Wnt-binding antibody is a reduction in the level of signaling by the Wnt of 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, the Wnt signaling that is inhibited is canonical Wnt signaling.

[00168] *In vivo* and *in vitro* assays for determining whether a Wnt-binding antibody inhibits Wnt 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 canonical Wnt signaling levels *in vitro* (Gazit *et al.*, 1999, *Oncogene*, 18; 5959-66). The level of Wnt signaling in the presence of one or more Wnts (*e.g.*, Wnt(s) expressed by transfected cells or provided by Wnt-conditioned media) with the Wnt-binding antibody present is compared to the level of signaling without the Wnt-binding antibody present. In addition to the TCF/Luc reporter assay, the effect of a Wnt-binding antibody (or candidate antibody) on canonical Wnt signaling may be measured *in vitro* or *in vivo* by measuring the effect of the

antibody 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 an antibody on Wnt signaling may also be assessed by measuring the effect of the antibody on the phosphorylation state of Dishevelled-1, Dishevelled-2, Dishevelled-3, LRP5, LRP6, and/or β-catenin.

[00169] In certain embodiments, the Wnt-binding antibodies useful in the methods of the invention have one or more of the following effects: inhibit proliferation of neuroendocrine tumor cells, reduce the tumorigenicity of a neuroendocrine tumor by reducing the frequency of cancer stem cells in the tumor, inhibit neuroendocrine tumor growth, trigger cell death of neuroendocrine tumor cells, differentiate neuroendocrine tumorigenic cells to a non-tumorigenic state, prevent metastasis of neuroendocrine tumor cells or decrease survival.

[00170] In certain embodiments, the Wnt-binding antibodies useful in the methods of the invention are capable of inhibiting neuroendocrine tumor growth. In certain embodiments, the Wnt-binding antibodies are capable of inhibiting neuroendocrine tumor growth *in vivo* (*e.g.*, in a xenograft mouse model, and/or in a human having cancer).

[00171] In certain embodiments, the Wnt-binding antibodies useful in the methods of the invention are capable of reducing the tumorigenicity of a neuroendocrine tumor. In certain embodiments, the antibody is capable of reducing the tumorigenicity of a neuroendocrine 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 neuroendocrine 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 Number WO 2008/042236, U.S. Patent Application Publication No. 2008/0064049, and U.S. Patent Application Publication No. 2008/0178305, each of which is incorporated by reference herein in its entirety.

[00172] In certain embodiments, the Wnt-binding antibody useful in the methods of the invention has a circulating half-life in mice, cynomolgous monkeys, or humans of at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the Wnt-binding antibody is an IgG (*e.g.*, IgG1 or IgG2) antibody that has a circulating half-life in mice, cynomolgous monkeys, or humans of at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks.

[00173] In certain embodiments, an anti-Wnt antibody useful for the methods of the invention is a bispecific antibody that specifically recognizes a human Wnt. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. In one

embodiment, the bispecific anti-Wnt antibody specifically recognizes different epitopes within the same human Wnt. In another embodiment, the bispecific anti-Wnt antibody specifically recognizes different epitopes within different human Wnts or on different Wnts.

[00174] Alternatively, in certain alternative embodiments, an anti-Wnt antibody useful for the methods of the invention is not a bispecific antibody.

[00175] In certain embodiments, an anti-Wnt antibody useful for the methods of the invention is monospecific. In certain embodiments, each of the one or more antigen-binding sites that an antibody contains is capable of binding (or binds) the same one or more human Wnts. In certain embodiments, an antigen-binding site of the monospecific antibody is capable of binding (or binds) one, two, three, four, or five (or more) human Wnts.

[00176] Anti-Wnt antibodies useful for the methods of the invention are disclosed in International Publication Number WO 2011/088127, which is incorporated by reference in its entirety.

6. Antibodies and production thereof

[00177] The antibodies (*e.g.*, anti-FZD and anti-Wnt antibodies) useful in the methods of the invention can be produced by any suitable method known in the art. Polyclonal antibodies can be prepared by any known method. Polyclonal antibodies are raised by immunizing an animal (*e.g.* a rabbit, rat, mouse, donkey, etc.) by multiple subcutaneous or intraperitoneal injections of the relevant antigen (a purified peptide fragment, full-length recombinant protein, fusion protein, etc.) optionally conjugated to keyhole limpet hemocyanin (KLH), serum albumin, etc. diluted in sterile saline and combined with an adjuvant (*e.g.* Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. The polyclonal antibody is then recovered from blood, ascites and the like, of an animal so immunized. Collected blood is clotted, and the serum decanted, clarified by centrifugation, and assayed for antibody titer. The polyclonal antibodies can be purified from serum or ascites according to standard methods in the art including affinity chromatography, ion-exchange chromatography, gel electrophoresis, dialysis, etc.

[00178] Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein (1975) *Nature* 256:495. Using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit the production by lymphocytes of antibodies that will specifically bind to an immunizing antigen. Lymphocytes can also be immunized *in vitro*. Following immunization, the 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 as determined by immunoprecipitation, immunoblotting, or by an *in vitro* binding assay (*e.g.* radioimmunoassay (RIA); enzyme-linked immunosorbent assay (ELISA)) can then be propagated either *in vitro* culture using

standard methods (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, 1986) or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can then be purified from the culture medium or ascites fluid as described for polyclonal antibodies above.

[00179] Alternatively monoclonal antibodies can also be made using recombinant DNA methods as described in U.S. Patent 4,816,567. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cell, 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 procedures. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors, which when transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, monoclonal antibodies are generated by the host cells. Also, recombinant monoclonal antibodies or fragments thereof of the desired species can be isolated from phage display libraries expressing CDRs of the desired species as described (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).

[00180] 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 1) for those regions of, for example, a human antibody to generate a chimeric antibody or 2) 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.

[00181] In some embodiments, the monoclonal antibody useful in the methods of the invention is a humanized antibody. In certain embodiments, such antibodies are used therapeutically to reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. Humanized antibodies can be produced using various techniques known in the art. In certain alternative embodiments, the antibody useful in the methods of the invention is a human antibody.

[00182] Human antibodies can be directly prepared using various techniques known in the art. Immortalized human B lymphocytes immunized *in vitro* or isolated from an immunized individual that produce an antibody directed against a target antigen can be generated (See, *e.g.*, Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boemer *et al.*, 1991, *J. Immunol.*, 147 (1):86-95; and U.S. Patent 5,750,373). Also, the human antibody can be selected from a phage library, where that phage library expresses human antibodies, as described, for example, in Vaughan *et al.*, 1996, *Nat. Biotech.*, 14:309-314, Sheets *et al.*, 1998, *Proc. Nat'l. Acad. Sci.*, 95:6157-6162, Hoogenboom and Winter, 1991, *J. Mol. Biol.*, 227:381, and Marks *et al.*, 1991, *J. Mol. Biol.*, 222:581). Techniques for the generation and use of antibody phage libraries are also 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.*, 2007, *J. Mol. Bio.*, doi:10.1016/j.jmb.2007.12.018 (each of which is incorporated by reference in its entirety). Affinity maturation strategies and chain shuffling strategies (Marks *et al.*, 1992, *Bio/Technology* 10:779-783, incorporated by reference in its entirety) are known in the art and may be employed to generate high affinity human antibodies.

[00183] Humanized antibodies can also be made in transgenic mice containing human immunoglobulin loci that 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. Patents 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016.

[00184] In certain embodiments, the antibody useful in the methods of the invention is a bispecific antibody that specifically recognizes a human frizzled receptor or a human Wnt polypeptide. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. The different epitopes can either be within the same molecule (*e.g.* the same human frizzled receptor or same human Wnt polypeptide) or on different molecules. Bispecific antibodies can be intact antibodies or antibody fragments.

[00185] Alternatively, in certain alternative embodiments, antibodies useful for the invention are not bispecific antibodies.

[00186] In certain embodiments, the antibodies useful for the invention are 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) the same human FZD receptor or the same human Wnt polypeptide. In certain embodiments, an antigen-binding site of a monospecific antibody is capable of binding (or binds) one, two, three, four, or five (or more) human frizzled receptors or human Wnt polypeptide.

[00187] In certain embodiments, an antibody useful for the methods of the invention is an antibody fragment. Antibody fragments can display increased tumor penetration relative to a full antibody. Various techniques are known for the production of antibody fragments. Traditionally, these fragments are derived via proteolytic digestion of intact antibodies (for example Morimoto *et al.*, 1993, *Journal of Biochemical and Biophysical Methods* 24:107-117; Brennan *et al.*, 1985, *Science*, 229:81). In certain embodiments, antibody fragments are produced recombinantly. Fab, Fv, and scFv antibody fragments can all be expressed in and secreted from E. coli or other host cells, thus allowing the production of large amounts of these fragments. Such antibody fragments can also be isolated from the antibody phage libraries discussed above. The antibody fragment can also be linear antibodies as described in U.S. Patent 5,641,870, for example, and can be monospecific or bispecific. Single-chain antibodies useful in the methods of the invention can be prepared as described, for example, in U.S. Pat. No. 4,946,778. In addition, methods can be adapted for the construction of Fab expression libraries (Huse, *et al.*, *Science* 246:1275-1281 (1989)) to allow rapid and effective

identification of monoclonal Fab fragments with the desired specificity for a FZD receptor or a Wnt polypeptide. Antibody fragments may be produced by techniques in the art including, but not limited to: (a) a F(ab')2 fragment produced by pepsin digestion of an antibody molecule; (b) a Fab fragment generated by reducing the disulfide bridges of an F(ab')2 fragment, (c) a Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent, and (d) Fv fragments. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

[00188] 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).

[00189] In certain embodiments, an antibody useful for the methods of the invention is a heteroconjugate antibody. 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. Pat. No. 4,676,980). It is contemplated that the 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.

[00190] It is known in the art that the constant Fc region mediates several effector functions. For example, binding of the C1 component of complement to antibodies 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. Further, antibodies or soluble receptors can bind to cells via the Fc region, with a Fc receptor site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. 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 (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production.

[00191] In certain embodiments, the Wnt antagonist polypeptides (antibodies and Fc comprising soluble receptors) useful for the methods of the invention provide for altered effector functions that, in turn, affect the biological profile of the administered polypeptides. For example, 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 thereby increasing tumor localization. In other

cases it may be that constant region modifications moderate complement binding and thus reduce the serum half-life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region may be used to eliminate disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. Similarly, modifications to the constant region may easily be made using well known biochemical or molecular engineering techniques well within the purview of the skilled artisan.

[00192] In certain embodiments, a Wnt antagonist polypeptide comprising an Fc region (antibodies and Fc comprising soluble receptors) useful for the methods of the invention does not have one or more effector functions. For instance, in some embodiments, the polypeptide has no antibody-dependent cellular cytotoxicity (ADCC) activity and/or no complement-dependent cytotoxicity (CDC) activity. In certain embodiments, the polypeptide does not bind to an Fc receptor and/or complement factors. In certain embodiments, the antibody has no effector function.

[00193] The invention also pertains to the use of immunoconjugates comprising a Wnt antagonist polypeptide (*e.g.*, anti-FZD and anti-Wnt antibody) conjugated to a cytotoxic agent. Cytotoxic agents include chemotherapeutic agents, growth inhibitory agents, toxins (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), radioactive isotopes (i.e., a radioconjugate), etc. Chemotherapeutic agents useful in the generation of such immunoconjugates include, for example, methotrexate, adriamycin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents. Enzymatically active toxins and fragments thereof that can be used include 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. A variety of radionuclides are available for the production of radioconjugated antibodies including ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Conjugates of the antibody and 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 tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). 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 also be used.

[00194] Conjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells (U.S. Pat. No. 4,676,980). It is contemplated that the 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.

[00195] Regardless of how useful quantities are obtained, the Wnt antagonists polypeptides (*e.g.*, antibodies and soluble receptors) useful in the methods of the invention can be used in any one of a number of conjugated (i.e. an immunoconjugate) or unconjugated forms. Alternatively, the polypeptides can be used in a nonconjugated or "naked" form. In certain embodiments, the polypeptides are used in nonconjugated form to harness the subject's natural defense mechanisms including complement-dependent cytotoxicity (CDC) and antibody dependent cellular toxicity (ADCC) to eliminate the malignant cells. In some embodiments, the polypeptides can be conjugated to radioisotopes, such as ^{90}Y, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re and ^{188}Re using anyone of a number of well-known chelators or direct labeling. In other embodiments, the compositions can comprise Wnt antagonist polypeptides coupled to drugs, prodrugs or biological response modifiers such as methotrexate, adriamycin, and lymphokines such as interferon. Still other embodiments comprise the use of Wnt antagonist polypeptides conjugated to specific biotoxins such as ricin or diptheria toxin. In yet other embodiments, the Wnt antagonist polypeptides can be complexed with other immunologically active ligands (*e.g.* antibodies or fragments thereof) wherein the resulting molecule binds to both the neoplastic cell and an effector cell such as a T cell. The selection of which conjugated or unconjugated Wnt antagonist polypeptides to use will depend of the type and stage of neuroendocrine tumor, use of adjunct treatment (*e.g.*, chemotherapy or external radiation) and patient condition. It will be appreciated that one skilled in the art could readily make such a selection in view of the teachings herein.

[00196] The polypeptides and analogs can be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half-life or absorption of the protein. The moieties can also reduce or eliminate any desirable side effects of the proteins and the like. An overview for those moieties can be found in Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing Co., Easton, PA (2000).

[00197] The chemical moieties most suitable for derivatization include water soluble polymers. A water soluble polymer is desirable because the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. In some embodiments, the polymer will be pharmaceutically acceptable for the preparation of a therapeutic product or composition. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and other considerations. The effectiveness of the derivatization can be ascertained by administering the derivative, in the desired form (i.e., by osmotic pump, or by injection or infusion, or, further formulated for oral, pulmonary or other delivery routes), and determining its effectiveness. Suitable water soluble polymers include, but are not limited to,

polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), dextran, poly(n-vinyl pyrrolidone)-polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde can have advantages in manufacturing due to its stability in water.

[00198] The isolated polypeptides (*e.g.*, antibodies and soluble receptors) useful in the methods of the invention can be produced by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed 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. See, *e.g.* Zoeller *et al.*, *Proc. Nat'l. Acad. Sci. USA* 81:5662-5066 (1984) and U.S. Pat. No. 4,588,585.

[00199] In some embodiments a DNA sequence encoding a polypeptide of interest would be constructed by chemical synthesis using an oligonucleotide synthesizer. Such 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 an isolated 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.

[00200] Once assembled (by synthesis, site-directed mutagenesis or another method), the polynucleotide sequences encoding a particular isolated polypeptide of interest will 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 mapping, and 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.

[00201] In certain embodiments, recombinant expression vectors are used to amplify and express Wnt antagonist polypeptides (*e.g.*, antibodies or soluble receptors). Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide of interest operatively linked to suitable transcriptional 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, as described in detail below. Such 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. Structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, 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.

[00202] The choice of expression control sequence and expression vector will depend 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, adenovims and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from Esherichia coli, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

[00203] Suitable host cells for expression of a Wnt antagonist polypeptide (*e.g.*, antibody or soluble receptor) include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example E. coli or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels *et al.* (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985), the relevant disclosure of which is hereby incorporated by reference. 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 04009823, each of which is hereby incorporated by reference herein in its entirety.

[00204] Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells can be

performed because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa, and BHK cell lines. Mammalian expression vectors can comprise nontranscribed 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 nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

[00205] The proteins produced by a transformed host can be purified according to any suitable method. Such 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 hexahistidine, 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, nuclear magnetic resonance and x-ray crystallography.

[00206] For example, supernatants from 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. Alternatively, 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. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Finally, one or more reversed-phase high performance liquid chromatography (RP-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 Wnt antagonist polypeptide (*e.g.*, antibody or soluble receptor). Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

[00207] 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. High performance liquid chromatography (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.

[00208] Methods known in the art for purifying a Wnt antagonist polypeptide (*e.g.*, antibody or soluble receptor) also include, for example, those described in U.S. Patent Publication No. 2008/0312425, 2008/0177048, and 2009/0187005, each of which is hereby incorporated by reference herein in its entirety.

7. Pharmaceutical compositions

[00209] The Wnt antagonist polypeptides (*e.g.*, antibodies and soluble receptors) can be formulated into a pharmaceutical composition by any suitable method known in the art. In certain embodiments, the pharmaceutical compositions comprise a pharmaceutically acceptable vehicle. The pharmaceutical compositions find use in inhibiting neuroendocrine tumor growth and treating neuroendocrine tumor in human patients.

[00210] In certain embodiments, formulations are prepared for storage and use by combining a purified Wnt antagonist (*e.g.*, an anti-FZD antibody or soluble FZD receptor) with a pharmaceutically acceptable vehicle (*e.g.* carrier, excipient) (Remington, The Science and Practice of Pharmacy 20th Edition Mack Publishing, 2000). Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (*e.g.* octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight polypeptides (*e.g.* less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG).

[00211] In certain embodiments, the pharmaceutical composition is frozen. In certain alternative embodiments, the pharmaceutical composition is lyophilized.

[00212] The pharmaceutical compositions of the present invention can be administered in any number of ways for either local or systemic treatment. Administration can be topical (such as to mucous membranes including vaginal and rectal delivery) such as transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal); oral; or parenteral including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial (*e.g.*, intrathecal or intraventricular) administration.

[00213] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories for oral, parenteral, or rectal administration or for administration by inhalation. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other diluents (*e.g.* water) to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of the type described above. The tablets, pills, etc. of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

[00214] The Wnt antagonists (*e.g.*, anti-FZD antibodies or soluble FZD receptors) can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions as described in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

[00215] In certain embodiments, pharmaceutical formulations include the Wnt antagonists (*e.g.*, anti-FZD antibodies or soluble FZD receptors) complexed with liposomes (Epstein, *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688; Hwang, *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:4030; and U.S. Patent 4,485,045 and 4,544,545). Liposomes with enhanced circulation time are disclosed in U.S. Patent 5,013,556. Some liposomes can be generated by the reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[00216] In addition sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles (*e.g.* films, or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(v nylalcohol), polylactides (U.S. Patent 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid

copolymers such as the LUPRON DEPOT TM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

EXAMPLES

[00217] 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.

Example 1

Neuroendocrine tumor response to OMP-18R5 in a Phase 1a clinical study

[00218] In the context of a Phase I clinical trial for the OMP-18R5 human anti-FZD antibody in patients with advanced solid tumors, three patients with late stage neuroendocrine tumors that had previously undergone multiple other therapies were treated with low, periodic doses of OMP-18R5 as a single agent. The prolonged stable disease of all three of these neuroendocrine patients suggests that even as a single agent at low dosages OMP-18R5 may have a surprising level of efficacy against neuroendocrine tumors, including both neuroendocrine tumors having carcinoid histology and pancreatic neuroendocrine tumors.

[00219] At the time of her enrollment in the OMP-18R5 trial, Patient 3 was a 59 years old female. She was diagnosed with neuroendocrine tumor (carcinoid) in 2004. She underwent a small bowel resection and was treated with radiofrequency ablation of liver lesions. Prior to enrollment in the OMP-18R5 trial, she received prior systemic treatment with a combination of trametinib (MEK1/2 MAP kinase inhibitor) and GSK2141795 Akt inhibitor but her disease progressed after 1 month of treatment. In the OMP-18R5 trial Patient 3 received a weekly dose of 0.5mg/kg OMP-18R5 for 112 days. Her disease remained stable during OMP-18R5 treatment, but she was removed from the trial after suffering a bone fracture on day 112. Especially in light of her rapid disease progression while on a previous therapy, this patient's extended period of disease control while being treated with OMP-18R5 suggests that the antibody may have a surprising level of clinical efficacy even as a single agent at a low dose.

[00220] At the time of her enrollment in the OMP-18R5 trial, Patient 10 was a 69 year old female with pancreatic neuroendocrine tumor. She was diagnosed in 2001 and treated with surgery comprising 80% distal pancreatectomy, splenectomy, and wedge resection of posterior wall of stomach. Prior to enrollment in the OMP-18R5 trial, she received systemic treatments with (1) regorafenib (partial response: 3 years); (2) anti-LOXL2 antibody (stable disease: 5.5 months); and (3) anti-CSFR1

antibody (progressive disease after 6 weeks on study). As of January 25, 2013, Patient 10 in the OMP-18R5 trial had received 0.5mg/kg OMP-18R5 every other week for 279 days. Patient 10 continued to receive 0.5mg/kg OMP-18R5 every other week for 448 days. After 112 days of OMP-18R5 treatment, a 21% reduction in Patient 10's target tumor liver metastasis was determined by the investigator. Tumor reduction was confirmed by an independent radiographic assessment (shown in Table 4). See Figures 1A-1C. The control non-target disease lesion showed no change during the same treatment period. Radiographic examination further revealed signs of calcification in the tumor lesion of Patient 10 following 112 days of OMP-18R5 treatment (Figure 1C). The observed calcification of the tumor lesion may indicate that OMP-18R5 induced differentiation of the tumor cells and/or tumor necrosis. Subsequent computed tomography (CT) scans on days 168, 224, 280, 336, and 392 indicated that the patient still did not have progressive disease. Patient 10 came off treatment at Day 448 after signs of progressive disease. This patient's extended period of disease control while being treated with OMP-18R5 was additonal evidence that the antibody may have a surprising level of clinical efficacy as a single agent at a low dose even when given every other week.

Table 4. Patient 10: Independent Radiographic Assessment RECIST 1.1

Lesion (mm)	**Mar 27, 2012 (BASELINE)**	**Jun 11, 2012**	**Aug 6, 2012**
1. Liver: Rt Lobe (Ant-Lat) (TARGET)	13.6 x 22.9	17.3 x 23.9	13.3 x 20.5
2. Liver: IVC (TARGET)	16.2 x 16.2	15.9 x 15.9	10.8 x 13.1
3. Liver: Rt Dome (TARGET)	7.9 x 11.6	7.1 x 11.4	4.9 x 8.5
4. Porto-caval Node (TARGET)*	16.6 x 23.9	14.1 x 15.5	9.1 x 14.2
5. Porto-caval Node (NON-TARGET)	11.1 x 14.5	10.5 x 12.6	9.5 x 14.8
TOTAL: Target (mm, %Δ) **TOTAL: Non-Target**	67.3 Non-PD	65.3 **(-3%)** Non-PD	51.2 **(-24%)** Normal**

* Per RECIST 1.1: LN ≥15mm in shortest diameter are measurable

** Per RECIST 1.1: LN <10mm in shortest diameter considered 'normal'

Non-PD: non-progressive disease

[00221] At the time of her enrollment in the OMP-18R5 trial, Patient 12 was a 77 year old female with a neuroendocrine tumor (carcinoid). She was diagnosed in 2006. Prior to enrollment in the OMP-18R5 trial, she received systemic treatments with (1) sandostatin (stable disease: 20 months); (2) inhibitor of heat shock protein 90 (stable disease: 23 months); and (3) a combination of sandostatin and anti-angiopoietin-2 antibody (stable disease: 4 months). As of January 25, 2013, Patient 12 in the OMP-18R5 trial had received 1mg/kg OMP-18R5 every third week for 210 days. As of October 4,

2013, Patient 12 had received 1mg/kg OMP-18R5 every third week for 465 days. This patient was assessed to have stable disease on days 56, 112, 168, 224, 280, 336, 392, and 448. The extended period of time during which this patient has remained on the clinical trial without disease progression further supports the clinical efficacy of OMP-18R5 against neuroendocrine tumors.

[00222] Figure 2A shows the number of days that each of the patients (n=18) enrolled in the OMP-18R5 Phase 1a study as of January 25, 2013, stayed on the OMP-18R5 Phase 1a study. Figure 2B shows the number of days that each of the patients (n=29) enrolled in the OMP-18R5 Phase 1a study as of October 4, 2013, stayed on the OMP-18R5 Phase 1a study. The patients with neuroendocrine tumors that had been treated with OMP-18R5 remained on study for surprisingly long periods of times relative to the other Phase 1a patients having other tumor types (including colorectal cancer, breast cancer, melanoma and pancreatic cancer).

[00223] Also, as of January 25, 2013, the three patients with neuroendocrine tumors had had stable disease ~2- to 7-fold longer on OMP-18R5 treatment than when they were on the prior therapies on which they previously progressed. Using Growth Modulation Index as a tool to gauge the observed activity (time on current therapy divided by time on prior therapy before progressive disease; GMI $\geq$1.33 considered excellent; *Von Hoff; Clinical Cancer Research 4:1079-1086, 1998*), all three neuroendocrine (NET) patients significantly surpassed this mark (Patient 12: 1.8; Patient 10: 6.3; Patient 3, off study: 3.8). For Patient 10, the final GMI was 10.7 and as of October 4, 2013 the GMI for Patient 12 was 1.4. A comparison of the time each of the three neuroendocrine tumor patients remained on the OMP-18R5 study as of January 25, 2013 versus their time on prior therapies is shown in Figure 3A. A comparison of the time each of the three neuroendocrine tumor patients remained on the OMP-18R5 study as of October 4, 2013 versus their time on prior therapies is shown in Figure 3B.

Example 2

In vivo prevention of neuroendocrine tumor growth using a Wnt antagonist

[00224] This example describes a use of a Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) to prevent neuroendocrine tumor growth in a xenograft model. In certain embodiments, neuroendocrine tumor cells from a patient sample (solid tumor biopsy or pleural effusion) that have been passaged as a xenograft in mice are prepared for repassaging into experimental animals. Neuroendocrine tumor tissue is removed under sterile conditions, cut up into small pieces, minced completely using sterile blades, and single cell suspensions obtained by enzymatic digestion and mechanical disruption. Specifically, pleural effusion cells or the resulting tumor pieces are mixed with ultra-pure collagenase III in culture medium (200-250 units of collagenase per mL) and incubated at 37°C for 3-4 hours with pipetting up and down through a 10 mL pipette every 15-20 minutes. Digested cells are filtered through a 45μM nylon mesh, washed with RPMI/20% FBS, and washed twice with HBSS.

Dissociated neuroendocrine tumor cells are then injected subcutaneously into the mammary fat pads of NOD/SCID mice to elicit tumor growth.

[00225] In certain embodiments, dissociated neuroendocrine tumor cells are first sorted into tumorigenic and non-tumorigenic cells based on cell surface markers before injection into experimental animals. Specifically, neuroendocrine tumor cells dissociated as described above are washed twice with HEPES-buffered saline solution (HBSS) containing 2% heat-inactivated calf serum (HICS) and resuspended at 10^6 cells per 100µl. Antibodies are added and the cells incubated for 20 minutes on ice followed by two washes with HBSS/2% HICS. Antibodies include anti-ESA (Biomeda, Foster City, CA), anti-CD44, anti-CD24, and Lineage markers anti-CD2, -CD3, -CD10, -CD16, -CD18, -CD31, -CD64, and -CD140b (collectively referred to as Lin; PharMingen, San Jose, CA). Antibodies are directly conjugated to fluorochromes to positively or negatively select cells expressing these markers. Mouse cells are eliminated by selecting against $H2K^{d+}$ cells, and dead cells are eliminated by using the viability dye 7AAD. Flow cytometry is performed on a FACSVantage (Becton Dickinson, Franklin Lakes, NJ). Side scatter and forward scatter profiles are used to eliminate cell clumps. Isolated ESA+, CD44+, CD24-/low, Lin- tumorigenic cells are then injected subcutaneously into NOD/SCID mice to elicit tumor growth.

[00226] By way of example, Wnt antagonists (*e.g.*, OMP-18R5 or OMP-54F28) are analyzed for their ability to reduce the growth of neuroendocrine tumor cells. Dissociated neuroendocrine tumor cells (10,000 per animal) are injected subcutaneously into the flank region of 6-8 week old NOD/SCID mice. Two days after tumor cell injection, animals are injected intraperitoneal (i.p.) with 10mg/kg anti-FZD antibody or soluble FZD receptor two times per week. Tumor growth is monitored weekly until growth is detected, after which point tumor growth is measured twice weekly for a total of 8 weeks. FZD-binding antibodies which significantly reduce tumor growth as compared to PBS injected controls are thus identified.

Example 3

In vivo treatment of neuroendocrine tumors using a Wnt antagonist

[00227] This example describes the use of a Wnt antagonists (*e.g.*, OMP-18R5 or OMP-54F28) to treat neuroendocrine cancer in a xenograft model. In certain embodiments, neuroendocrine tumor cells from a patient sample (solid tumor biopsy or pleural effusion) that have been passaged as a xenograft in mice are prepared for repassaging into experimental animals. Neuroendocrine tumor tissue is removed, cut up into small pieces, minced completely using sterile blades, and single cell suspensions obtained by enzymatic digestion and mechanical disruption. Dissociated neuroendocrine tumor cells are then injected subcutaneously either into the mammary fat pads, for breast tumors, or into the flank, for non-breast tumors, of NOD/SCID mice to elicit tumor growth. Alternatively, ESA+, CD44+, CD24-/low, Lin- tumorigenic tumor cells are isolated as described above and injected.

[00228] Following tumor cell injection, animals are monitored for tumor growth. Once neuroendocrine tumors reach an average size of approximately 150 to 200mm, Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treatment begins. Each animal receives 100μg Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) or control agents i.p. two to five times per week for a total of 6 weeks. Tumor size is assessed twice a week during these 6 weeks. The ability of Wnt antagonists (*e.g.*, OMP-18R5 or OMP-54F28) to prevent further neuroendocrine tumor growth or to reduce neuroendocrine tumor size compared to control agents is thus determined.

[00229] At the end point of antibody treatment, tumors are harvested for further analysis. In some embodiments a portion of the neuroendocrine tumor is analyzed by immunofluorescence to assess Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) penetration into the tumor and tumor response. A portion of each harvested neuroendocrine tumor from Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treated and control mice is fresh-frozen in liquid nitrogen, embedded in O.C.T., and cut on a cryostat as 10μm sections onto glass slides. In some embodiments, a portion of each neuroendocrine tumor is formalin-fixed, paraffin-embedded, and cut on a microtome as 10μm section onto glass slides. Sections are post-fixed and incubated with chromophore labeled antibodies that specifically recognize the injected Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) to detect Wnt antagonists (*e.g.*, OMP-18R5 or OMP-54F28) or control agents present in the tumor biopsy. Furthermore antibodies that detect different tumor and tumor-recruited cell types such as, for example, anti-VE cadherin (CD144) or anti-PECAM-1 (CD31) antibodies to detect vascular endothelial cells, anti-smooth muscle alpha-actin antibodies to detect vascular smooth muscle cells, anti-Ki67 antibodies to detect proliferating cells, TUNEL assays to detect dying cells, anti-β-catenin antibodies to detect Wnt signaling, and anti-intracellular domain (ICD) Notch fragment antibodies to detect Notch signaling can be used to assess the effects of Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treatment on, for example, angiogenesis, tumor growth and tumor morphology.

[00230] In certain embodiments, the effect of Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treatment on neuroendocrine tumor cell gene expression is also assessed. Total RNA is extracted from a portion of each harvested neuroendocrine tumor from Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treated and control antibody treated mice and used for quantitative RT-PCR. Expression levels of FZD receptors, components of Wnt signaling pathway including, for example, Wnt1 and β-catenin, as well as additional cancer stem cell markers previously identified (*e.g.* CD44) are analyzed relative to the housekeeping gene GAPDH as an internal control. Changes in neuroendocrine tumor cell gene expression upon treatment with Wnt antagonists (*e.g.*, OMP-18R5 or OMP-54F28) are thus determined.

[00231] In addition, the effect of Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treatment on the frequency of cancer stem cells in a neuroendocrine tumor is assessed. Neuroendocrine tumor samples from Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treated versus control agent treated mice are cut up into small pieces, minced completely using sterile blades, and single cell suspensions obtained

by enzymatic digestion and mechanical disruption. Dissociated neuroendocrine tumor cells are then analyzed by FACS analysis for the presence of tumorigenic cancer stem cells based on ESA+, CD44+, CD24-/low, Lin- surface cell marker expression as described in detail above.

[00232] The tumorigenicity of cells isolated based on ESA+, CD44+, CD24-/low, Lin- expression following Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treatment can then assessed. ESA+, CD44+, CD24-/low, Lin- cancer stem cells isolated from Wnt antagonist (*e.g.*, OMP-18R5 or OMP-54F28) treated versus control agent treated mice are re-injected subcutaneously into the mammary fat pads of NOD/SCID mice. The tumorigenicity of cancer stem cells based on the number of injected cells required for consistent neuroendocrine tumor formation is then determined.

Example 4

Treatment of human neuroendocrine tumor using anti-FZD receptor antibodies or soluble FZD receptors

[00233] This example describes certain methods for treating neuroendocrine tumor using antibodies against a FZD receptor to target neuroendocrine tumors comprising cancer stem cells and/or tumor cells in which FZD receptor expression has been detected and/or tumor cells having a Wnt gene signature indicating that they are responsive to inhibition of Wnt signaling.

[00234] In some embodiments, the presence of cancer stem cell marker or FZD receptor or the expression of one or more genes in a Wnt gene signature can first be determined from a tumor biopsy. Tumor cells from a biopsy from a patient diagnosed with neuroendocrine tumor are removed under sterile conditions. In some embodiments the tissue biopsy is fresh-frozen in liquid nitrogen, embedded in O.C.T., and cut on a cryostat as 10μm sections onto glass slides. In some embodiments, the tissue biopsy is formalin-fixed, paraffin-embedded, and cut on a microtome as 10μm section onto glass slides.

[00235] Sections are incubated with antibodies against a FZD receptor to detect FZD protein expression. Alternatively, sections can be analyzed for the presence of one or more genes in the Wnt gene signature.

[00236] The presence of cancer stem cells also may be determined. Tissue biopsy samples are cut up into small pieces, minced completely using sterile blades, and cells subject to enzymatic digestion and mechanical disruption to obtain a single cell suspension. Dissociated neuroendocrine tumor cells are then incubated with anti-ESA, -CD44, -CD24, –Lin, and –FZD antibodies to detect cancer stem cells, and the presence of ESA+, CD44+, CD24-/low, Lin-, FZD+ tumor stem cells is determined by flow cytometry as described in detail above.

[00237] Cancer patients whose neuroendocrine tumors are diagnosed as expressing a FZD receptor and/or one or more genes in the Wnt gene signature are treated with anti-FZD receptor antibodies or soluble FZD receptors. In certain embodiments, humanized or human monoclonal anti-FZD receptor

antibodies or soluble FZD receptors are purified and formulated with a suitable pharmaceutical vehicle for injection. In some embodiments, patients are treated with the FZD antibodies or soluble FZD receptors at least once a month for at least 10 weeks. In some embodiments, patients are treated with the FZD antibodies or soluble FZD receptors at least once a week for at least about 14 weeks. Each administration of the antibody or soluble FZD receptors should be a pharmaceutically effective dose. In some embodiments, between about 2 to about 100mg/ml of an anti-FZD antibody or soluble FZD receptors is administered. In some embodiments, between about 5 to about 40mg/ml of an anti-FZD antibody or soluble FZD receptors is administered. The antibody or soluble FZD receptors can be administered prior to, concurrently with, or after standard radiotherapy regimens or chemotherapy regimens using one or more chemotherapeutic agent. Patients are monitored to determine whether such treatment has resulted in an anti-tumor response, for example, based on tumor regression, reduction in the incidences of new tumors, lower tumor antigen expression, decreased numbers of cancer stem cells, or other means of evaluating disease prognosis.

[00238] All publications, patents, patent applications, internet sites, and accession numbers/database sequences (including both polynucleotide and polypeptide sequences) cited herein, as well as U.S. Serial No. 61/717,294, filed October 23, 2012, are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

SEQUENCES

Human FZD1 full length amino acid sequence (SEQ ID NO:1; underlining indicates ECD)
MAEEEAPKKSRAAGGGASWELCAGALSARLAEEGSGDAGGRRRPPVDPRRLARQLLLLLW
LLEAPLLLGVRAQAAGQGPGQGPGPGQQPPPPPQQQQSGQQYNGERGISVPDHGYCQPIS
IPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVL
EQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELCVGQNTSDKGTPTP
SLLPEFWTSNPQHGGGGHRGGFPGGAGASERGKFSCPRALKVPSYLNYHFLGEKDCGAPC
EPTKVYGLMYFGPEELRFSRTWIGIWSVLCCASTLFTVLTYLVDMRRFSYPERPIIFLSG
CYTAVAVAYIAGFLLEDRVVCNDKFAEDGARTVAQGTKKEGCTILFMMLYFFSMASSIWW
VILSLTWFLAAGMKWGHEAIEANSQYFHLAAWAVPAIKTITILALGQVDGDVLSGVCFVG
LNNVDALRGFVLAPLFVYLFIGTSFLLAGFVSLFRIRTIMKHDGTKTEKLEKLMVRIGVF
SVLYTVPATIVIACYFYEQAFRDQWERSWVAQSCKSYAIPCPHLQAGGGAPPHPPMSPDF
TVFMIKYLMTLIVGITSGFWIWSGKTLNSWRKFYTRLTNSKQGETTV

Human FZD2 full length amino acid sequence (SEQ ID NO: 2; underlining indicates ECD)
MRPRSALPRLLLPLLLLPAAGPAQFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNL
LGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQG
CEALMNKFGFQWPERLRCEHFPRHGAEQICVGQNHSEDGAPALLTTAPPPGLQPGAGGTP
GGPGGGGAPPRYATLEHPFHCPRVLKVPSYLSYKFLGERDCAAPCEPARPDGSMFFSQEE
TRFARLWILTWSVLCCASTFFTVTTYLVDMQRFRYPERPIIFLSGCYTMVSVAYIAGFVL
QERVVCNERFSEDGYRTVVQGTKKEGCTILFMMLYFFSMASSIWWVILSLTWFLAAGMKW
GHEAIEANSQYFHLAAWAVPAVKTITILAMGQIDGDLLSGVCFVGLNSLDPLRGFVLAPL

FVYLFIGTSFLLAGFVSLFRIRTIMKHDGTKTEKLERLMVRIGVFSVLYTVPATIVIACY
FYEQAFREHWERSWVSQHCKSLAIPCPAHYTPRMSPDFTVYMIKYLMTLIVGITSGFWIW
SGKTLHSWRKFYTRLTNSRHGETTV

Human FZD3 full length amino acid sequence (SEQ ID NO:3; underlining indicates ECD)
MAMTWIVFSLWPLTVFMGHIGGHSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAAL
AMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVP
WPEDMECSRFPDCDEPYPRLVDLNLAGEPTEGAPVAVQRDYGFWCPRELKIDPDLGYSFL
HVRDCSPPCPNMYFRREELSFARYFIGLISIICLSATLFTFLTFLIDVTRFRYPERPIIF
YAVCYMMVSLIFFIGFLLEDRVACNASIPAQYKASTVTQGSHNKACTMLFMILYFFTMAG
SVWWVILTITWFLAAVPKWGSEAIEKKALLFHASAWGIPGTLTIILLAMNKIEGDNISGV
CFVGLYDVDALRYFVLAPLCLYVVVGVSLLLAGIISLNRVRIEIPLEKENQDKLVKFMIR
IGVFSILYLVPLLVVIGCYFYEQAYRGIWETTWIQERCREYHIPCPYQVTQMSRPDLILF
LMKYLMALIVGIPSVFWVGSKKTCFEWASFFHGRRKKEIVNESRQVLQEPDFAQSLLRDP
NTPIIRKSRGTSTQGTSTHASSTQLAMVDDQRSKAGSIHSKVSSYHGSLHRSRDGRYTPC
SYRGMEERLPHGSMSRLTDHSRHSSSHRLNEQSRHSSIRDLSNNPMTHITHGTSMNRVIE
EDGTSA

Human FZD4 full length amino acid sequence (SEQ ID NO:4; underlining indicates ECD)
MLAMAWRGAGPSVPGAPGGVGLSLGLLLQLLLLLGPARGFGDEEERRCDPIRISMCQNLG
YNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPC
GGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEVPLPHKTPIQP
GEECHSVGTNSDQYIWVKRSLNCVLKCGYDAGLYSRSAKEFTDIWMAVWASLCFISTAFT
VLTFLIDSSRFSYPERPIIFLSMCYNIYSIAYIVRLTVGRERISCDFEEAAEPVLIQEGL
KNTGCAIIFLLMYFFGMASSIWWVILTLTWFLAAGLKWGHEAIEMHSSYFHIAAWAIPAV
KTIVILIMRLVDADELTGLCYVGNQNLDALTGFVVAPLFTYLVIGTLFIAAGLVALFKIR
SNLQKDGTKTDKLERLMVKIGVFSVLYTVPATCVIACYFYEISNWALFRYSADDSNMAVE
MLKIFMSLLVGITSGMWIWSAKTLHTWQKCSNRLVNSGKVKREKRGNGWVKPGKGSETVV

Human FZD5 full length amino acid sequence (SEQ ID NO:5; underlining indicates ECD)
MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQ
DEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLM
RQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPTLPGPPGAPASGG
ECPAGGPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQPSFSADERTFATFWIG
LWSVLCFISTSTTVATFLIDMERFRYPERPIIFLSACYLCVSLGFLVRLVVGHASVACSR
ERNHIHYETTGPALCTIVFLLVYFFGMASSIWWVILSLTWFLAAGMKWGNEAIAGYAQYF
HLAAWLIPSVKSITALALSSVDGDPVAGICYVGNQNLNSLRGFVLGPLVLYLLVGTLFLL
AGFVSLFRIRSVIKQGGTKTDKLEKLMIRIGIFTLLYTVPASIVVACYLYEQHYRESWEA
ALTCACPGHDTGQPRAKPEYWVLMLKYFMCLVVGITSGVWIWSGKTVESWRRFTSRCCCR
PRRGHKSGGAMAAGDYPEASAALTGRTGPPGPAATYHKQVSLSHV

Human FZD6 full length amino acid sequence (SEQ ID NO:6; underlining indicates ECD)
MEMFTFLLTCIFLPLLRGHSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEH
FLPLANLECSPNIETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEE
LECDRLQYCDETVPVTFDPHTEFLGPQKKTEQVQRDIGFWCPRHLKTSGGQGYKFLGIDQ
CAPPCPNMYFKSDELEFAKSFIGTVSIFCLCATLFTFLTFLIDVRRFRYPERPIIYYSVC
YSIVSLMYFIGFLLGDSTACNKADEKLELGDTVVLGSQNKACTVLFMLLYFFTMAGTVWW
VILTITWFLAAGRKWSCEAIEQKAVWFHAVAWGTPGFLTVMLLAMNKVEGDNISGVCFVG
LYDLDASRYFVLLPLCLCVFVGLSLLLAGIISLNHVRQVIQHDGRNQEKLKKFMIRIGVF
SGLYLVPLVTLLGCYVYEQVNRITWEITWVSDHCRQYHIPCPYQAKAKARPELALFMIKY
LMTLIVGISAVFWVGSKKTCTEWAGFFKRNRKRDPISESRRVLQESCEFFLKHNSKVKHK
KKHYKPSSHKLKVISKSMGTSTGATANHGTSAVAITSHDYLGQETLTEIQTSPETSMREV
KADGASTPRLREQDCGEPASPAASISRLSGEQVDGKGQAGSVSESARSEGRISPKSDITD
TGLAQSNNLQVPSSSEPSSLKGSTSLLVHPVSGVRKEQGGGCHSDT

Human FZD7 full length amino acid sequence (SEQ ID NO:7; ECD is underlined)

MRDPGAAAPLSSLGLCALVLALLGALSAGAGAQPYHGEKGISVPDHGFCQPISIPLCTDI
AYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPC
RSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEICVGQNTSDGSGGPGGGPTAYP
TAPYLPDLPFTALPPGASDGRGRPAFPFSCPRQLKVPPYLGYRFLGERDCGAPCEPGRAN
GLMYFKEEERRFARLWVGVWSVLCCASTLFTVLTYLVDMRRFSYPERPIIFLSGCYFMVA
VAHVAGFLLEDRAVCVERFSDDGYRTVAQGTKKEGCTILFMVLYFFGMASSIWWVILSLT
WFLAAGMKWGHEAIEANSQYFHLAAWAVPAVKTITILAMGQVDGDLLSGVCYVGLSSVDA
LRGFVLAPLFVYLFIGTSFLLAGFVSLFRIRTIMKHDGTKTEKLEKLMVRIGVFSVLYTV
PATIVLACYFYEQAFREHWERTWLLQTCKSYAVPCPPGHFPPMSPDFTVFMIKYLMTMIV
GITTGFWIWSGKTLQSWRRFYHRLSHSSKGETAV

Human FZD8 full length amino acid sequence (SEQ ID NO:8; ECD is underlined):

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPSPPRRLPPPPPGEQPPSGS
GHGRPPGARPPHRGGGRGGGGGDAAAPPARGGGGGGKARPPGGGAAPCEPGCQCRAPMVS
VSSERHPLYNRVKTGQIANCALPCHNPFFSQDERAFTVFWIGLWSVLCFVSTFATVSTFL
IDMERFKYPERPIIFLSACYLFVSVGYLVRLVAGHEKVACSGGAPGAGGAGGAGGAAAGA
GAAGAGAGGPGGRGEYEELGAVEQHVRYETTGPALCTVVFLLVYFFGMASSIWWVILSLT
WFLAAGMKWGNEAIAGYSQYFHLAAWLVPSVKSIAVLALSSVDGDPVAGICYVGNQSLDN
LRGFVLAPLVIYLFIGTMFLLAGFVSLFRIRSVIKQQDGPTKTHKLEKLMIRLGLFTVLY
TVPAAVVVACLFYEQHNRPRWEATHNCPCLRDLQPDQARRPDYAVFMLKYFMCLVVGITS
GVWVWSGKTLESWRSLCTRCCWASKGAAVGGGAGATAAGGGGGPGGGGGGGPGGGGGPGG
GGGSLYSDVSTGLTWRSGTASSVSYPKQMPLSQV

Human FZD9 full length amino acid sequence (SEQ ID NO:9; underlining indicates ECD)

MAVAPLRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNL
LGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARL
RCAPIMEQFNFGWPDSLDCARLPTRNDPHALCMEAPENATAGPAEPHKGLGMLPVAPRPA
RPPGDLGPGAGGSGTCENPEKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSA
LCFFSTAFTVLTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAGA
LYVIQEGLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHEAIEAHGSYFHM
AAWGLPALKTIVILTLRKVAGDELTGLCYVASTDAAALTGFVLVPLSGYLVLGSSFLLTG
FVALFHIRKIMKTGGTNTEKLEKLMVKIGVFSILYTVPATCVIVCYVYERLNMDFWRLRA
TEQPCAAAAGPGGRRDCSLPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSL
CYRKIAAGRARAKACRAPGSYGRGTHCHYKAPTVVLHMTNTDPSLENPTHL

Human FZD10 full length amino acid sequence (SEQ ID NO:10; ECD is underlined)

MQRPGPRLWLVLQVMGSCAAISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHEN
QREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPI
MEQFNFKWPDSLDCRKLPNKNDPNYLCMEAPNNGSDEPTRGSGLFPPLFRPQRPESAQEH
PLKDGGPGRGGCDNPGKFHHVEKSASCAPLCTPGVDVYWSREDKRFAVVWLAIWAVLCFF
SSAFTVLTFLIDPARFRYPERPIIFLSMCYCVYSVGYLIRLFAGAESIACDRDSGQLYVI
QEGLESTGCTLVFLVLYYFGMASSLWWVVLTLTWFLAAGKKWGHEAIEANSSYFHLAAWA
IPAVKTILILVMRRVAGDELTGVCYVGSMDVNALTGFVLIPLACYLVIGTSFILSGFVAL
FHIRRVMKTGGENTDKLEKLMVRIGLFSVLYTVPATCVIACYFYEMLNMDYWKILAAQHK
CKMNNQTKTLDCLMAASIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQQVCSRRLK
KKSRRKPASVITSGGIYKKAQHPQKTHHGKYEIPAQSPTCV

Human FZD1 ECD with signal sequence (SEQ ID NO:11)

MAEEEAPKKSRAAGGGASWELCAGALSARLAEEGSGDAGGRRRPPVDPRRLARQLLLLLW
LLEAPLLLGVRAQAAGQGPGQGPGPGQQPPPPPQQQQSGQQYNGERGISVPDHGYCQPIS

IPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVL
EQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELCVGQNTSDKGTPTP
SLLPEFWTSNPQHGGGGHRGGFPGGAGASERGKFSCPRALKVPSYLNYHFLGEKDCGAPC
EPTKVYGLMYFGPEELRFSRT

Human FZD2 ECD with signal sequence (SEQ ID NO:12)
MRPRSALPRLLLPLLLLPAAGPAQFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNL
LGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQG
CEALMNKFGFQWPERLRCEHFPRHGAEQICVGQNHSEDGAPALLTTAPPPGLQPGAGGTP
GGPGGGGAPPRYATLEHPFHCPRVLKVPSYLSYKFLGERDCAAPCEPARPDGSMFFSQEE
TRFARLWILT

Human FZD3 ECD with signal sequence (SEQ ID NO:13)
MAMTWIVFSLWPLTVFMGHIGGHSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAAL
AMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVP
WPEDMECSRFPDCDEPYPRLVDLNLAGEPTEGAPVAVQRDYGFWCPRELKIDPDLGYSFL
HVRDCSPPCPNMYFRREELSFARY

Human FZD4 ECD with signal sequence (SEQ ID NO:14)
MLAMAWRGAGPSVPGAPGGVGLSLGLLLQLLLLLGPARGFGDEEERRCDPIRISMCQNLG
YNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPC
GGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEVPLPHKTPIQP
GEECHSVGTNSDQYIWVKRSLNCVLKCGYDAGLYSRSAKEFTDI

Human FZD5 ECD with signal sequence (SEQ ID NO:15)
MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQ
DEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLM
RQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPTLPGPPGAPASGG
ECPAGGPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQPSFSADERT

Human FZD6 ECD with signal sequence (SEQ ID NO:16)
MEMFTFLLTCIFLPLLRGHSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEH
FLPLANLECSPNIETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEE
LECDRLQYCDETVPVTFDPHTEFLGPQKKTEQVQRDIGFWCPRHLKTSGGQGYKFLGIDQ
CAPPCPNMYFKSDELEFAKSFIGTVSI

Human FZD7 ECD with signal sequence (SEQ ID NO:17)
MRDPGAAAPLSSLGLCALVLALLGALSAGAGAQPYHGEKGISVPDHGFCQPISIPLCTDI
AYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPC
RSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEICVGQNTSDGSGGPGGGPTAYP
TAPYLPDLPFTALPPGASDGRGRPAFPFSCPRQLKVPPYLGYRFLGERDCGAPCEPGRAN
GLMYFKEEERRFARL

Human FZD8 ECD with signal sequence (SEQ ID NO:18)
MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPSPPRRLPPPPPGEQPPSGS
GHGRPPGARPPHRGGGRGGGGGDAAAPPARGGGGGGKARPPGGGAAPCEPGCQCRAPMVS
VSSERHPLYNRVKTGQIANCALPCHNPFFSQDERAFT

Human FZD9 ECD with signal sequence (SEQ ID NO:19)
MAVAPLRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNL
LGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARL
RCAPIMEQFNFGWPDSLDCARLPTRNDPHALCMEAPENATAGPAEPHKGLGMLPVAPRPA
RPPGDLGPGAGGSGTCENPEKFQYVEKSRSCAPRCGPGVEVFWSRRDKDF

Human FZD10 ECD with signal sequence (SEQ ID NO:20)
MQRPGPRLWLVLQVMGSCAAISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHEN
QREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPI
MEQFNFKWPDSLDCRKLPNKNDPNYLCMEAPNNGSDEPTRGSGLFPPLFRPQRPHSAQEH
PLKDGGPGRGGCDNPGKFHHVEKSASCAPLCTPGVDVYWSREDKRFA

Human FZD1 Fri domain amino acid sequence (SEQ ID NO:21; amino acids 87-237 of SEQ ID NO:1)
QQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDA
GLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFG
FQWPDTLKCEKFPVHGAGELCVGQNTSDKGT

Human FZD2 Fri domain amino acid sequence (SEQ ID NO:22; amino acids 24-159 of SEQ ID NO:2)
QFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQ
CSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPR
HGAEQICVGQNHSEDG

Human FZD3 Fri domain amino acid sequence (SEQ ID NO:23; amino acids 23-143 of SEQ ID NO:3)
HSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDF
RPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPY
PRLVDL

Human FZD4 Fri domain amino acid sequence (SEQ ID NO:24; amino acids 40-170 of SEQ ID NO:4)
FGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQF
FLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNH
MCMEGPGDEEV

Human FZD5 Fri domain amino acid sequence (SEQ ID NO:25; amino acids 27-157 of SEQ ID NO:5)
ASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFL
CSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVL
CMDYNRSEATT

Human FZD6 Fri domain amino acid sequence (SEQ ID NO:26; amino acids 19-146 of SEQ ID NO:6)
HSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLC
KAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFD
PHTEFLG

Human FZD7 Fri domain amino acid sequence (SEQ ID NO:27; amino acids 33-170 of SEQ ID NO:7)
QPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVK
VQCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENF
PVHGAGEICVGQNTSDGSG

Human FZD8 Fri domain amino acid sequence (SEQ ID NO:28; amino acids 28-158 of SEQ ID NO:8)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFF
LCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL
CMDYNRTDLTT

Human FZD9 Fri domain amino acid sequence (SEQ ID NO:29; amino acids 23-159 of SEQ ID NO:9)
LEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQY
GCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARL
PTRNDPHALCMEAPENA

Human FZD10 Fri domain amino acid sequence (SEQ ID NO:30; amino acids 21-154 of SEQ ID NO:10)
ISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCH
GHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNK
NDPNYLCMEAPNNG

18R5 VH CDR1 (SEQ ID NO:31)
GFTFSHYTLS

18R5 VH CDR2 (SEQ ID NO:32)
VISGDGSYTYYADSVKG

18R5 VH CDR3 (SEQ ID NO:33)
NFIKYVFAN

18R5 VL CDR1 (SEQ ID NO:34)
SGDNIGSFYVH

18R5 VL CDR2 (SEQ ID NO:35)
DKSNRPSG

18R5 VL CDR3 (SEQ ID NO:36)
QSYANTLSL

18R5 VH (SEQ ID NO:37)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRF
TISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSS

18R5 VL (SEQ ID NO:38)
DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGN
TATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG

18R5 heavy chain (IgG2) amino acid sequence, underlining indicates VH (SEQ ID NO:39)
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVS
VISGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

18R5 LIGHT CHAIN light chain (lambda) amino acid sequence, underlining indicates VL (SEQ ID NO:40)
MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYD
KSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ
WKSHRSYSCQVTHEGSTVEKTVAPTECS
18R8 VL CDR1 (SEQ ID NO:41)
SGDKLGKKYAS

18R8 VL CDR2 (SEQ ID NO:42)
EKDNRPSG

18R8 VL CDR3 (SEQ ID NO:43)
SSFAGNSLE

18R8 VL (SEQ ID NO:44)
DIELTQPPSVSVAPGQTARISCSGDKLGKKYASWYQQKPGQAPVLVIYEKDNRPSGIPERFSGSNSGN
TATLTISGTQAEDEADYYCSSFAGNSLEVFGGGTKLTVLG

18R8 18R8 light chain (lambda) amino acid sequence, underlining indicates VL (SEQ ID NO:45)
MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDKLGKKYASWYQQKPGQAPVLVIYE
KDNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCSSFAGNSLEVFGGGTKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ
WKSHRSYSCQVTHEGSTVEKTVAPTECS

44R24 VH CDR1 (SEQ ID NO:46)
GFTFSSYYIT

44R24 VH CDR2 (SEQ ID NO:47)
TISYSSSNTYYADSVKG

44R24 VH CDR3 (SEQ ID NO:48)
SIVFDY

44R24 VL CDR1 (SEQ ID NO:49)
SGDALGNRYVY

44R24 VL CDR2 (SEQ ID NO:50)
SG

44R24 VL CDR3 (SEQ ID NO:51)
GSWDTRPYPKY

44R24 VH (SEQ ID NO:52)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYITWVRQAPGKGLEWVSTISYSSSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSIVFDYWGQGTLVTVSS

44R24 VL (SEQ ID NO:53)
DIELTQPPSVSVAPGQTARISCSGDALGNRYVYWYQQKPGQAPVLVIPSGIPERFSGSNS
GNTATLTISGTQAEDEADYYCGSWDTRPYPKYVFGGGTKLTVLG

SEQ ID NO:54
CPLYFPLYC

SEQ ID NO:55
CPLVWPLIC

SEQ ID NO:56
CPLAWPLIC

SEQ ID NO:57
CPVKYPLVC

SEQ ID NO:58
CPLRFPLFC

SEQ ID NO:59
CPLAWPLIC

SEQ ID NO:60
CPVAFPLYC

SEQ ID NO:61
CPVNYPLYC

SEQ ID NO:62
CPVKFPLYC

SEQ ID NO:63
CPLTYPLYC

SEQ ID NO:64
CPLRWPLMC

SEQ ID NO:65
CPLQYPLMC

SEQ ID NO:66
CPLSFPLYC

SEQ ID NO:67
CPLNWPLMC

SEQ ID NO:68
CP(L/V)X(Y/F/W)PL(Y/F/I/V/M)C

SEQ ID NO:69
DTLSALIERGLM

SEQ ID NO:70
DVWWLGSTWLKR

SEQ ID NO:71
FGNYLNDVRFLI

SEQ ID NO:72
TNLADIAHWISG

Minimum FZD and SFRP Fri domain sequences
h-FZD1 amino acids 116-227 (SEQ ID NO:73)
CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAP
VCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELC

h-FZD2 amino acids 39-150 (SEQ ID NO:74)
CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAP
VCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQIC

h-FZD3 amino acids 28-133 (SEQ ID NO:75)

CEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAP
ICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDC

h-FZD4 amino acids 48-161 (SEQ ID NO:76)
CDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVP
MCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMC

h-FZD5 amino acids 33-147 (SEQ ID NO:77)
CQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTP
ICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLC

h-FZD6 amino acids 24-129 (SEQ ID NO:78)
CEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVP
TCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYC

h-FZD7 amino acids 49-160 (SEQ ID NO:79)
CQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAP
VCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEIC

h-FZD8 amino acids 35-148 (SEQ ID NO:80)
CQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTP
ICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLC

h-FZD9 amino acids 39-152 (SEQ ID NO:81)
CQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAP
MCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALC

h-FZD10 amino acids 34-147 (SEQ ID NO:82)
CQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAP
MCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLC

h-SFRP1 amino acids 57-165 (SEQ ID NO:83)
CVDIPADLRLCHNVGYKKMVLPNLLEHETMAEVKQQASSWVPLLNKNCHAGTQVFLCSLF
APVCLDRPIYPCRWLCEAVRDSCEPVMQFFGFYWPEMLKCDKFPEGDVC

h-SFRP2 amino acids 40-152 (SEQ ID NO:84)
CKPIPANLQLCHGIEYQNMRLPNLLGHETMKEVLEQAGAWIPLVMKQCHPDTKKFLCSLF
APVCLDDLDETIQPCHSLCVQVKDRCAPVMSAFGFPWPDMLECDRFPQDNDLC

h-SFRP3 amino acids 35-147 (SEQ ID NO:85)
CEPVRIPLCKSLPWNMTKMPNHLHHSTQANAILAIEQFEGLLGTHCSPDLLFFLCAMYAP
ICTIDFQHEPIKPCKSVCERARQGCEPILIKYRHSWPENLACEELPVYDRGVC

h-SFRP4 amino acids 24-136 (SEQ ID NO:86)
CEAVRIPMCRHMPWNITRMPNHLHHSTQENAILAIEQYEELVDVNCSAVLRFFFCAMYAP
ICTLEFLHDPIKPCKSVCQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVC

h-SFRP5 amino acids 53-162 (SEQ ID NO:87)
CLDIPADLPLCHTVGYKRMRLPNLLEHESLAEVKQQASSWLPLLAKRCHSDTQVFLCSLF
APVCLDRPIYPCRSLCEAVRAGCAPLMEAYGFPWPEMLHCHKFPLDNDLC

h-ROR1 minimal Fri domain (SEQ ID NO:88)
CQPYRGIACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCH
YAFPYCDETSSVPKPRDLCRDECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPE
SPEAANC

h-ROR2 minimal Fri domain (SEQ ID NO:89)
CQPYPGIACARFIGNRTIYVDSLQMQGEIENRITAAFTMIGTSTHLSDQCSQFAIPSFCH
FVFPLCDARSRTPKPRELCRDECEVLESDLCRQEYTIARSNPLILMRLQLPKCEALPMPE
SPDAANC

Human FZD4 Fri domain (predicted signal sequence underlined) (SEQ ID NO:90)
MLAMAWRGAGPSVPGAPGGVGLSLGLLLQLLLLLGPARGFGDEEERRCDPIRISMCQNLG
YNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPC
GGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEV

Human FZD5 Fri domain (predicted signal sequence underlined) (SEQ ID NO:91)
MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQ
DEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLM
RQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATT

Human FZD8 Fri domain (predicted signal sequence underlined) (SEQ ID NO:92)
MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHD
TQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAP
LMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTT

Human IgG1 Fc region (SEQ ID NO:93)
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG1 Fc region (SEQ ID NO:94)
KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG1 Fc region (SEQ ID NO:95)
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Linker (SEQ ID NO:96)
ESGGGGVT

Linker (SEQ ID NO:97)
LESGGGGVT

Linker (SEQ ID NO:98)
GRAQVT

Linker (SEQ ID NO:99)
WRAQVT

Linker (SEQ ID NO:100)
ARGRAQVT

Signal Sequence (SEQ ID NO:101)

MEWGYLLEVTSLLAALALLQRSSGAAA

Signal Sequence (SEQ ID NO:102)
MEWGYLLEVTSLLAALALLQRSSGALA

Signal Sequence (SEQ ID NO:103)
MEWGYLLEVTSLLAALALLQRSSGVLA

Signal Sequence (SEQ ID NO:104)
MEWGYLLEVTSLLAALLLLQRSPIVHA

Signal Sequence (SEQ ID NO:105)
MEWGYLLEVTSLLAALFLLQRSPIVHA

Signal Sequence (SEQ ID NO:106)
MEWGYLLEVTSLLAALLLLQRSPFVHA

Signal Sequence (SEQ ID NO:107)
MEWGYLLEVTSLLAALLLLQRSPIIYA

Signal Sequence (SEQ ID NO:108)
MEWGYLLEVTSLLAALLLLQRSPIAHA

FZD8-Fc amino acid sequence-variant 54F03 (without predicted signal sequence; the "GRA" linker sequence between the FZD8 sequence and the Fc sequence of the fusion protein is underlined) (SEQ ID NO:109)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGRADK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

FZD8-Fc variants
FZD8-Fc variant 54F03 amino acid sequence (without predicted signal sequence; alternative cleavage) (SEQ ID NO:110)
AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMY
TPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGR
ADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F09 amino acid sequence (without predicted signal sequence) (SEQ ID NO:111)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPSP
PDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F09 amino acid sequence (without predicted signal sequence; alternative cleavage) (SEQ ID NO:112)
AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMY
TPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAA

PSPPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F15 amino acid sequence (without predicted signal sequence) (SEQ ID NO:113)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F15 amino acid sequence (without predicted signal sequence; alternative cleavage) (SEQ ID NO:114)
AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMY
TPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAA
PDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F16, 54F17, 54F18, 54F23, 54F25, 54F27, 54F29, 54F31, and 54F34 amino acid sequence (without predicted signal sequence) (SEQ ID NO:115)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F16 amino acid sequence (without predicted signal sequence; alternative cleavage)
(SEQ ID NO:116)
AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMY
TPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPG

FZD8-Fc variant 54F19, 54F20, 54F24, 54F26, 54F28, 54F30, 54F32, 54F34 and 54F35 amino acid sequence (without predicted signal sequence) (SEQ ID NO:117)
ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSS
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F19 amino acid sequence (without predicted signal sequence; alternative cleavage) (SEQ ID NO:118)
ALAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMY
TPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEP
KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH

NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F20 amino acid sequence (without predicted signal sequence; alternative cleavage) (SEQ ID NO:119)
VLAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMY
TPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEP
KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F34 amino acid sequence (without predicted signal sequence) (SEQ ID NO:120)
KELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

FZD8-Fc variant 54F33 amino acid sequence (without predicted signal sequence) (SEQ ID NO:121)
KELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

h-Wnt1 C-terminal cysteine rich domain (aa 288-370) (SEQ ID NO:122)
DLVYFEKSPNFCTYSGRLGTAGTAGRACNSSSPALDGCELLCCGRGHRTRTQRVTERCNCTFHWCCHV
SCRNCTHTRVLHECL

h-Wnt2 C-terminal cysteine rich domain (aa 267-360) (SEQ ID NO:123)
DLVYFENSPDYCIRDREAGSLGTAGRVCNLTSRGMDSCEVMCCGRGYDTSHVTRMTKCGCKFHWCCAV
RCQDCLEALDVHTCKAPKNADWTTAT

h-Wnt2b C-terminal cysteine rich domain (aa 298-391) (SEQ ID NO:124)
DLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSKGTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAV
RCKECRNTVDVHTCKAPKKAEWLDQT

h-Wnt3 C-terminal cysteine rich domain (aa 273-355) (SEQ ID NO:125)
DLVYYENSPNFCEPNPETGSFGTRDRTCNVTSHGIDGCDLLCCGRGHNTRTEKRKEKCHCIFHWCCYV
SCQECIRIYDVHTCK

h-Wnt3a C-terminal cysteine rich domain (aa 270-352) (SEQ ID NO:126)
DLVYYEASPNFCEPNPETGSFGTRDRTCNVSSHGIDGCDLLCCGRGHNARAERREKCRCVFHWCCYV
SCQECTRVYDVHTCK

h-Wnt7a C-terminal cysteine rich domain (aa 267-359) (SEQ ID NO:127)
DLVYIEKSPNYCEEDPVTGSVGTQGRACNKTAPQASGCDLMCCGRGYNTHQYARVWQCNCKFHWCCYV
KCNTCSERTEMYTCK

h-Wnt7b C-terminal cysteine rich domain (aa 267-349) (SEQ ID NO:128)

DLVYIEKSPNYCEEDAATGSVGTQGRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNCKFHWCCFV
KCNTCSERTEVFTCK

h-Wnt8a C-terminal cysteine rich domain (aa 248-355) (SEQ ID NO:129)
ELIFLEESPDYCTCNSSLGIYGTEGRECLQNSHNTSRWERRSCGRLCTECGLQVEERKTEVISSCNCK
FQWCCTVKCDQCRHVVSKYYCARSPGSAQSLGRVWFGVYI

h-Wnt8b C-terminal cysteine rich domain (aa 245-351) (SEQ ID NO:130)
ELVHLEDSPDYCLENKTLGLLGTEGRECLRRGRALGRWELRSCRRLCGDCGLAVEERRAETVSSCNCK
FHWCCAVRCEQCRRRVTKYFCSRAERPRGGAAHKPGRKP

h-Wnt10a C-terminal cysteine rich domain (aa 335-417) (SEQ ID NO:131)
DLVYFEKSPDFCEREPRLDSAGTVGRLCNKSSAGSDGCGSMCCGRGHNILRQTRSERCHCRFHWCCFV
VCEECRITEWVSVCK

h-Wnt10b C-terminal cysteine rich domain (aa 307-389) (SEQ ID NO:132)
ELVYFEKSPDFCERDPTMGSPGTRGRACNKTSRLLDGCGSLCCGRGHNVLRQTRVEERCHCRFHWCCYV
LCDECKVTEWVNVCK

FZD8-Fc variant 54F28 with predicted signal sequence underlined (SEQ ID NO:133)
MEWGYLLEVTSLLAALLLLQRSPFVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLE
VHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCD
RLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

What we claim is:

1. A method of inhibiting the growth of a neuroendocrine tumor, comprising contacting the neuroendocrine tumor with an effective amount of a Wnt antagonist.

2. A method of inhibiting the proliferation of neuroendocrine tumor cells, comprising contacting the neuroendocrine tumor cells with an effective amount of a Wnt antagonist.

3. A method of reducing the tumorigenicity of neuroendocrine tumor cells, comprising contacting the neuroendocrine tumor cells with an effective amount of a Wnt antagonist.

4. A method of inducing neuroendocrine tumor cells to differentiate, comprising contacting the neuroendocrine tumor cells with an effective amount of a Wnt antagonist.

5. A method of inhibiting the growth of a neuroendocrine tumor, comprising administering to a subject in need thereof a therapeutically effective amount of a Wnt antagonist.

6. A method of inhibiting the proliferation of neuroendocrine tumor cells, comprising administering to a subject in need thereof a therapeutically effective amount of a Wnt antagonist.

7. A method of treating neuroendocrine cancer, comprising administering to a subject in need thereof a therapeutically effective amount of a Wnt antagonist.

8. The method of any one of claims 5 to 7, wherein the subject is a human subject.

9. The method of any one of claims 1 to 8, wherein the neuroendocrine tumor is a low grade, medium grade, or high grade neuroendocrine tumor.

10. The method of any one of claims 1 to 8, wherein the neuroendocrine tumor is a functional neuroendocrine tumor or a non-functional neuroendocrine tumor.

11. The method of any one of claims 1 to 8, wherein the neuroendocrine tumor is selected from the group consisting of gastroenteropancreatic neuroendocrine tumor, pancreatic neuroendocrine tumor, carcinoid tumor, pheochromocytoma, paraganglioma, medullary thyroid cancer, pulmonary neuroendocrine tumor, and thymic neuroendocrine tumor.

12. The method of claim 11, wherein the neuroendocrine tumor is a carcinoid tumor or a pancreatic neuroendocrine tumor.

13. The method of any one of claims 1 to 12, wherein the Wnt antagonist is an antibody.

14. The method of claim 13, wherein the Wnt antagonist is an antibody that specifically binds to at least one human Wnt.

15. The method of claim 13, wherein the Wnt antagonist is an antibody that specifically binds to at least one human frizzled receptor (FZD).

16. The method of claim 15, wherein the antibody specifically binds to the extracellular domain of at least one human FZD.

17. The method of claim 15 or 16, wherein the antibody specifically binds to a human FZD selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8.

18. The method of any one of claims 15 to 17, wherein the antibody specifically binds to FZD7.

19. The method of any one of claims 15 to 18, wherein the antibody specifically binds to more than one human FZD.

20. The method of any one of claims 15 to 19, wherein the antibody specifically binds to more than one human FZD selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8.

21. The method of any one of claims 15 to 20, wherein the antibody specifically binds to three or more human FZD selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8.

22. The method of any one of claims 15 to 21, wherein the antibody specifically binds to FZD1, FZD2, FZD5, FZD7, and FZD8.

23. The method of any one of claims 15 to 22, wherein the antibody blocks ligand binding to FZD.

24. The method of claim 23, wherein the antibody blocks Wnt binding to FZD.

25. The method of any one of claims 15 to 22, wherein the antibody blocks the activation of FZD.

26. The method of any one of claims 15 to 25, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33); and/or

(b) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:34), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:35), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:36); or
a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:41), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:42), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:43).

27. The method of claim 26, wherein the antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37; and/or

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:38 or SEQ I D NO:44.

28. The method of claim 26, wherein the antibody comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO:39; and/or

(b) a light chain comprising the amino acid sequence of SEQ ID NO:40 or SEQ ID NO:45.

29. The method according to any one of claims 13-28, wherein the antibody is a monoclonal antibody.

30. The method according to any one of claims 13-29, wherein the antibody is a recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, or an antibody fragment.

31. The method according to any one of claims 13-30, wherein the antibody is a monospecific antibody or a bispecific antibody.

32. The method according to any one of claims 13-31, wherein the antibody is an IgA, IgD, IgE, IgG or IgM antibody.

33. The method of claim 32, wherein the antibody is an IgG1 or IgG2 antibody.

34. The method of any one of claims 1 to 12, wherein the Wnt antagonist is a soluble FZD receptor.

35. The method of claim 34, wherein the soluble FZD receptor binds to Wnt.

36. The method of any one of claims 34 or 35, wherein the soluble receptor comprises a fragment of the extracellular domain of a human FZD receptor.

37. The method of claim 36, wherein the fragment of the extracellular domain of the human FZD receptor comprises the Fri domain of the human FZD receptor.

38. The method of any one of claims 36 or 37, wherein the human FZD receptor is selected from the group consisting of FZD4, FZD5, and FZD8.

39. The method of claim 38, wherein the human FZD receptor is FZD8.

40. The method of claim 39, wherein the FZD8 Fri domain comprises the amino acid sequence of SEQ ID NO: 28.

41. The method of any one of claims 34 to 40, wherein the soluble receptor further comprises a human Fc domain.

42. The method of claim 41, wherein the human Fc domain comprises the amino acid sequence of SEQ ID NO: 95.

43. The method of any one of claims 1-42, which further comprises contacting the tumor or tumor cells with a second therapeutic agent, or administering a second therapeutic agent to the subject.

44. The method of claim 43, wherein the second therapeutic agent is a chemotherapeutic agent.

45. The method of claim 44, wherein the second therapeutic agent is a kinase inhibitor, somatostatin analog, or a mTOR pathway inhibitor.

46. The method of claim 45, wherein the second therapeutic agent is sunitinib, octreotide, or everolimus.

47. The method of claim 43, wherein the second therapeutic agent is an antibody.

48. The method of claim 43, wherein the second therapeutic agent is an angiogenesis inhibitor.

49. The method of any one of claims 1 to 13 or claims 43-48, wherein the Wnt antagonist is OMP-18R5.

50. The method of claim 49, wherein OMP-18R5 is administered intravenously to the 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.

51. The method of claim 50, wherein OMP-18R5 is administered at a dosage of about 0.5mg/kg to about 1.0mg/kg about every one to two weeks.

52. The method of claim 50, wherein OMP-18R5 is administered at a dosage of about 1.0mg/kg to about 5.0mg/kg about every three weeks.

53. The method of any one of claims 1 to 12 or claims 43-48, wherein the Wnt antagonist is OMP-54F28.

54. The method of any one of claims 1-10 or claims 13-53, wherein the neuroendocrine tumor is not small cell lung tumor (SCLC).

55. The method of any one of claims 1-53, wherein the neuroendocrine tumor is a carcinoid tumor.

56. The method of any one of claims 1-53, wherein the neuroendocrine tumor is a pancreatic neuroendocrine tumor.

AMENDED CLAIMS
received by the International Bureau on 13 March 2014 (13.03.2014)

What is claimed is:

1. A Wnt antagonist for use in the treatment of neuroendocrine cancer, wherein the Wnt antagonist is (i) an antibody that specifically binds at least one frizzled (FZD) receptor or (ii) a soluble FZD receptor.

2. A Wnt antagonist for use in inhibiting growth of a neuroendocrine cancer, wherein the Wnt antagonist is (i) an antibody that specifically binds at least one frizzled (FZD) receptor or (ii) a soluble FZD receptor.

3. The Wnt antagonist for use according to claim 1 or claim 2, wherein the antibody specifically binds one or more human FZD receptors selected from the group consisting of: FZD1, FZD2, FZD5, FZD7, and FZD8.

4. The Wnt antagonist for use according to any one of claims 1 to 3, wherein the antibody comprises:
(a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33), and a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:34), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:35), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:36); or
(b) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:31), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:32), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:33), and a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:41), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:42), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:43).

5. The Wnt antagonist for use according to any one of claim 1 to 4, wherein the antibody comprises:
(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:37; and/or
(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:38 or SEQ ID NO:44.

6. The Wnt antagonist for use according to any one of claims 1 to 5, wherein the antibody comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO:39; and/or

(b) a light chain comprising the amino acid sequence of SEQ ID NO:40 or SEQ ID NO:45.

7. The Wnt antagonist for use according to any one of claims 3 to 6, wherein the antibody is a monoclonal antibody, recombinant antibody, chimeric antibody, humanized antibody, human antibody, bispecific antibody, IgG1 antibody, IgG2 antibody, and/or antibody fragment.

8. The Wnt antagonist for use according to any one of claims 1 to 6, wherein the antibody is OMP-18R5.

9. The Wnt antagonist for use according to claim 1 or claim 2, wherein the soluble FZD receptor comprises the Fri domain of human FZD8.

10. The Wnt antagonist for use according to claim 9, wherein the soluble FZD receptor further comprises a human Fc domain.

11. The Wnt antagonist for use according to claim 9 or claim 10, wherein the FZD8 Fri domain comprises the amino acid sequence of SEQ ID NO:28.

12. The Wnt antagonist for use according to claim 10 or claim 11, wherein the human Fc domain comprises the amino acid sequence of SEQ ID NO:95.

13. The Wnt antagonist for use according to claim 1 or claim 2, wherein the soluble FZD receptor comprises the amino acid sequence of SEQ ID NO:117.

14. The Wnt antagonist for use according to claim 1 or claim 2, wherein the soluble FZD receptor is OMP-54F28.

15. The Wnt antagonist for use according to any one of claims 1 to 14, wherein the neuroendocrine cancer is selected from the group consisting of pancreatic neuroendocrine cancer, carcinoid cancer, gastroenteropancreatic neuroendocrine cancer, pheochromocytoma, paraganglioma, medullary thyroid cancer, pulmonary neuroendocrine cancer, and thymic neuroendocrine cancer.

16. The Wnt antagonist for use according to claim 15, wherein the neuroendocrine cancer is a pancreatic neuroendocrine cancer.

17. The Wnt antagonist for use according to claim 15, wherein the neuroendocrine cancer is a carcinoid.

18. The Wnt antagonist for use according to any one of claims 1 to 17, which further comprises using at least one additional therapeutic agent in combination with the Wnt antagonist.

19. The Wnt antagonist for use according to claim 18, wherein the additional therapeutic agent is a chemotherapeutic agent.

20. The Wnt antagonist for use according to claim 18 or claim 19, wherein the additional therapeutic agent is:
    (a) albumin-bound paclitaxel (ABRAXANE);
    (b) gemcitabine; or
    (c) albumin-bound paclitaxel and gemcitabine.

21. A pharmaceutical composition comprising a Wnt antagonist for the treatment of neuroendocrine cancer, wherein the Wnt antagonist is (i) an antibody that specifically binds at least one frizzled (FZD) receptor or (ii) a soluble FZD receptor.

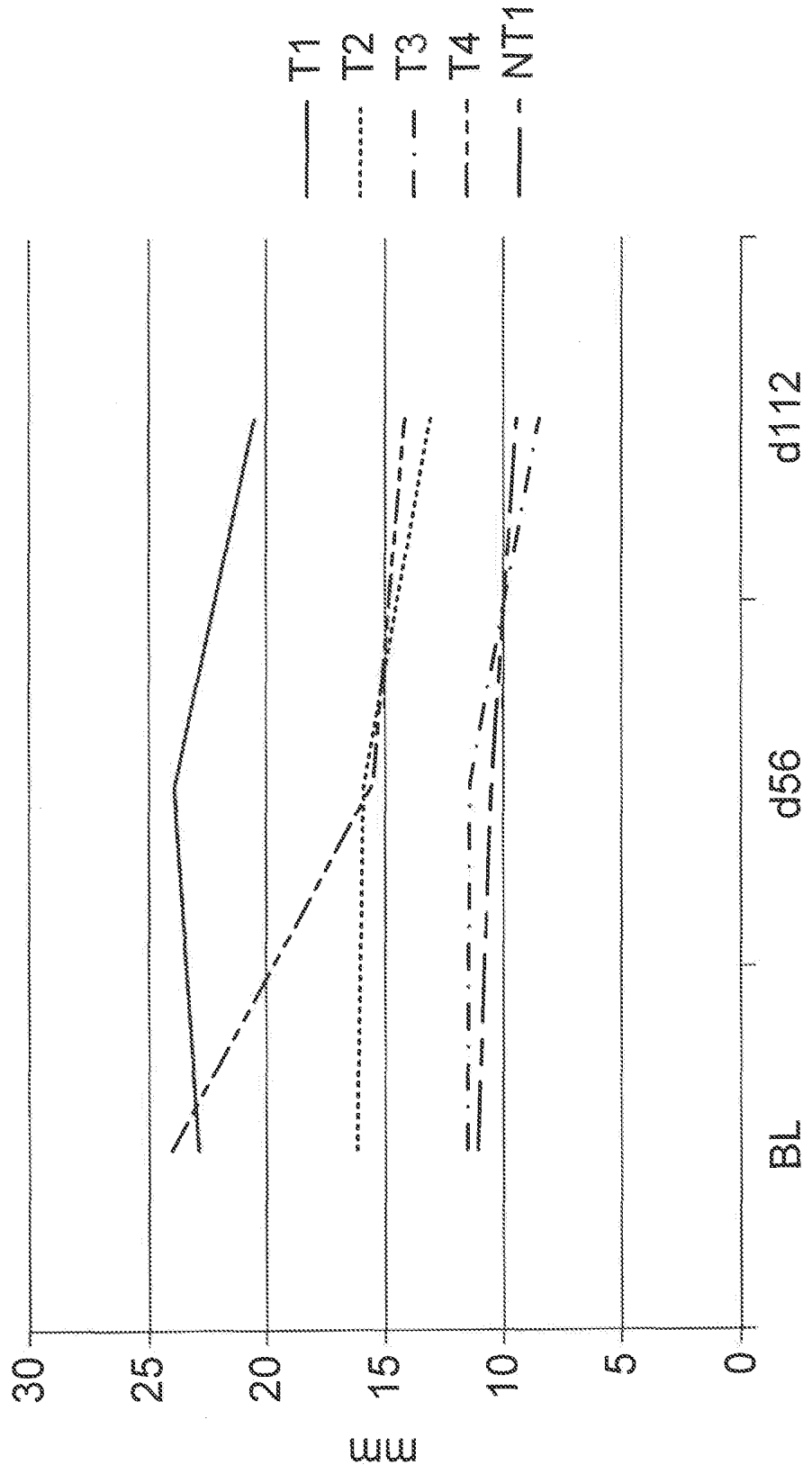


Figure 1A

Figure 1B

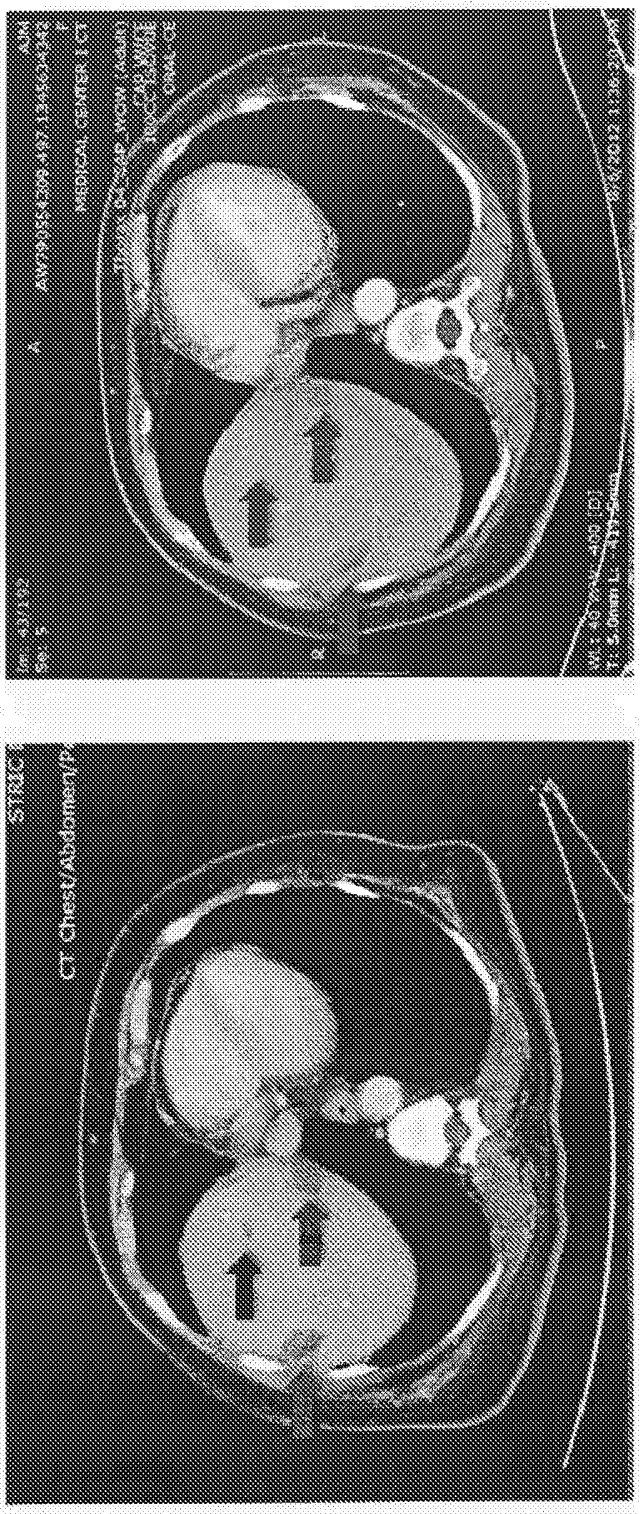


Figure 1C

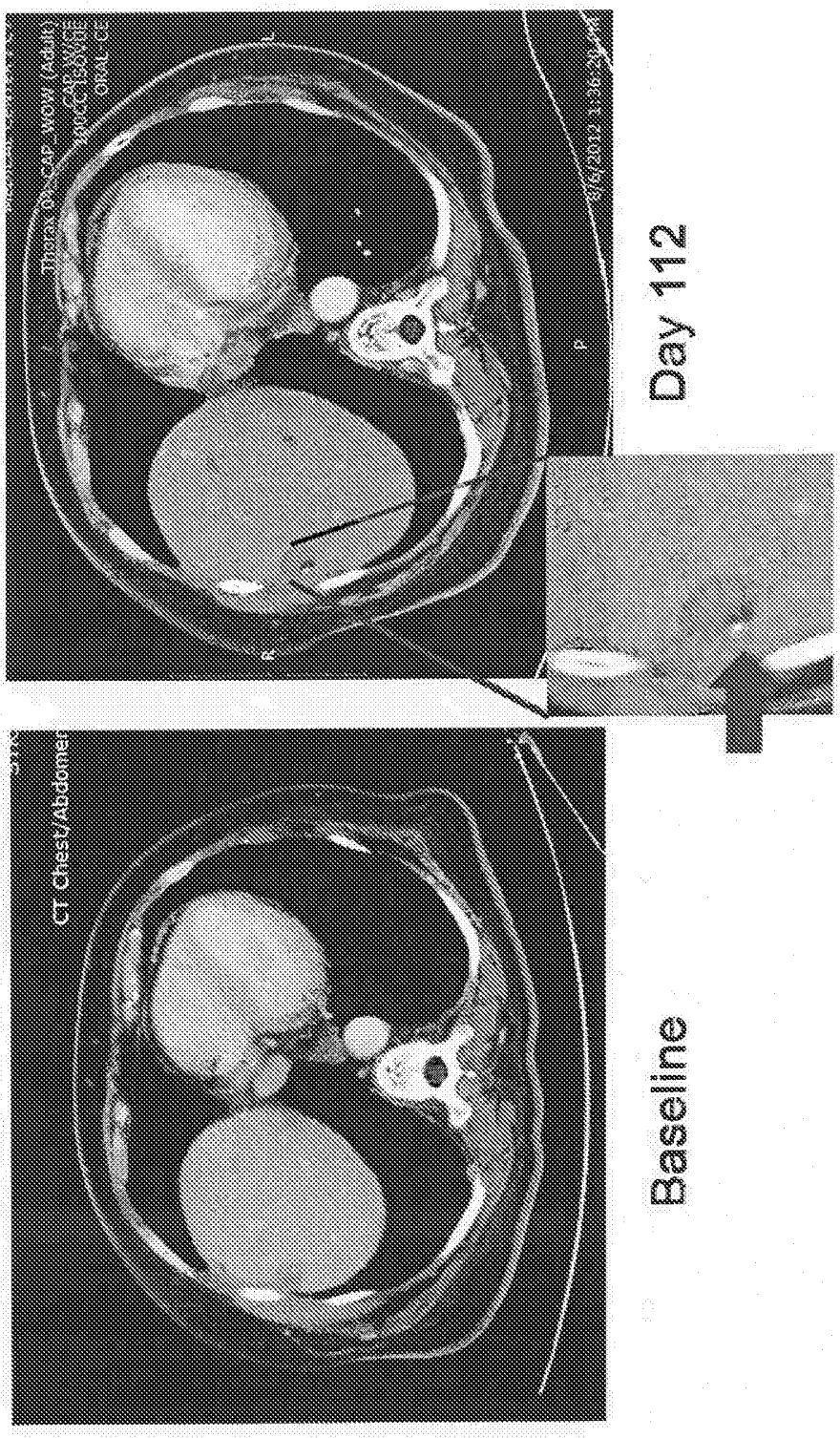
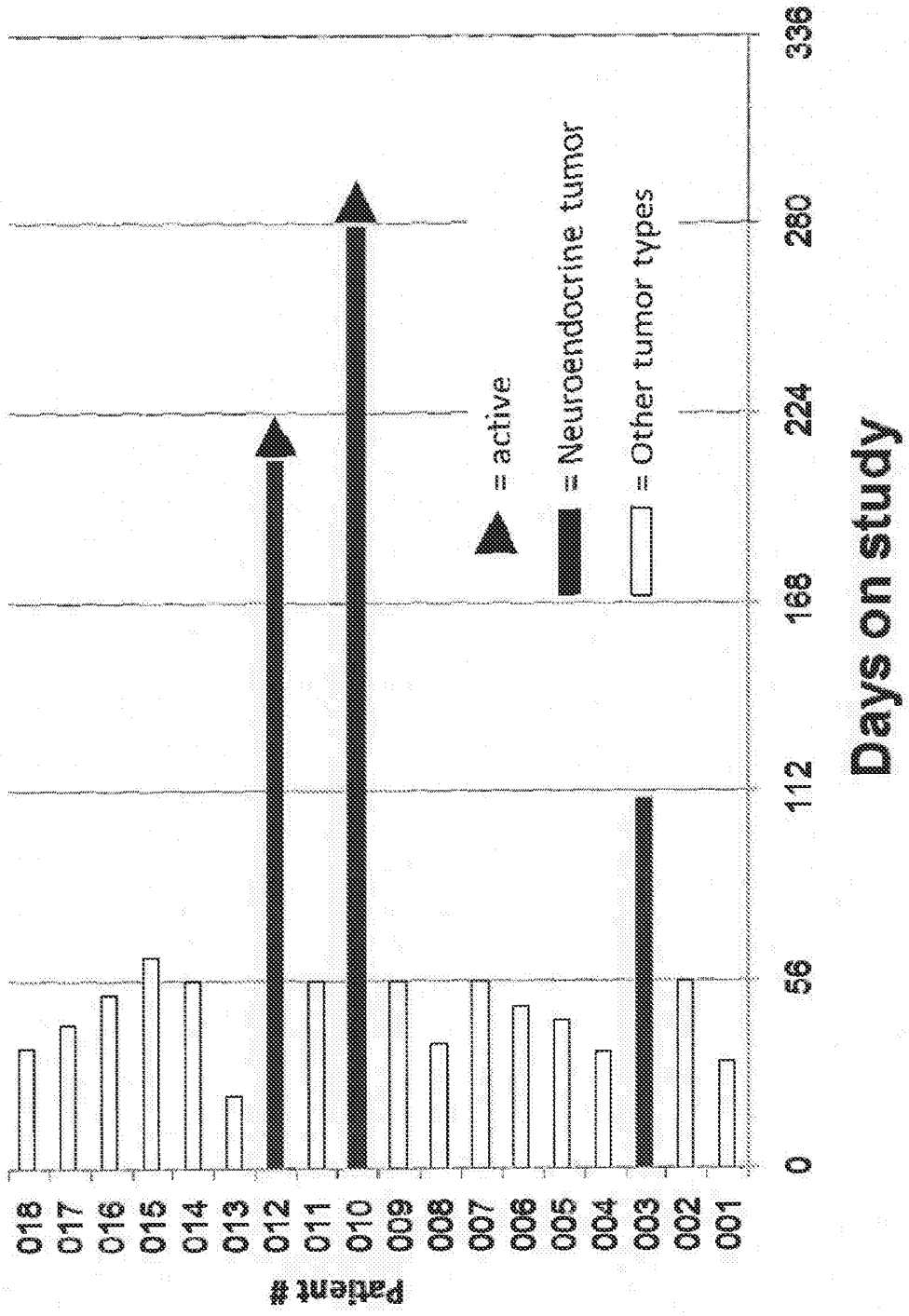


Figure 2A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013
012
011
010
009
008
007
006
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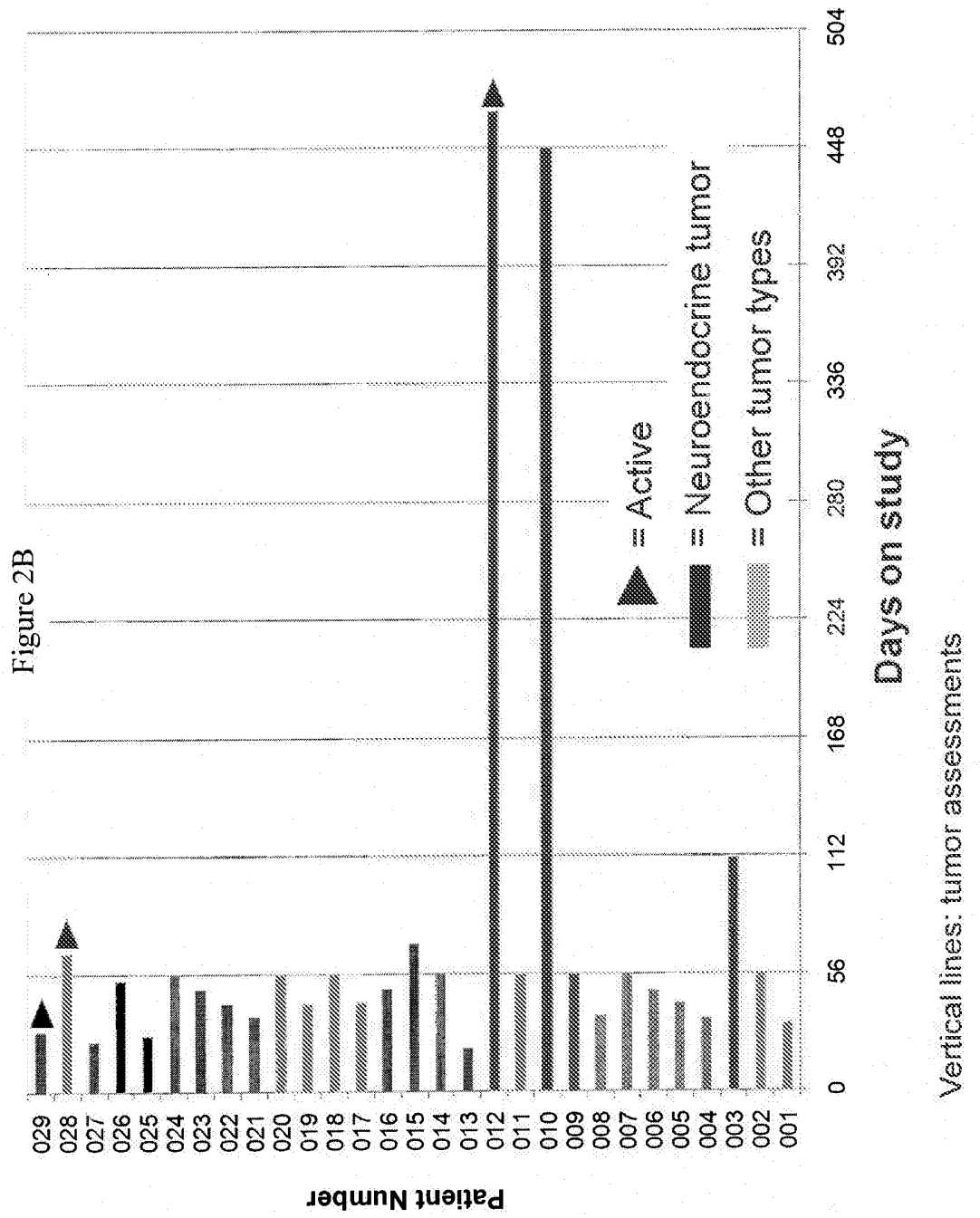
004
003
002
001
0
56
112
168
224
280
336
Days on study
= active
= Neuroendocrine tumor
= Other tumor types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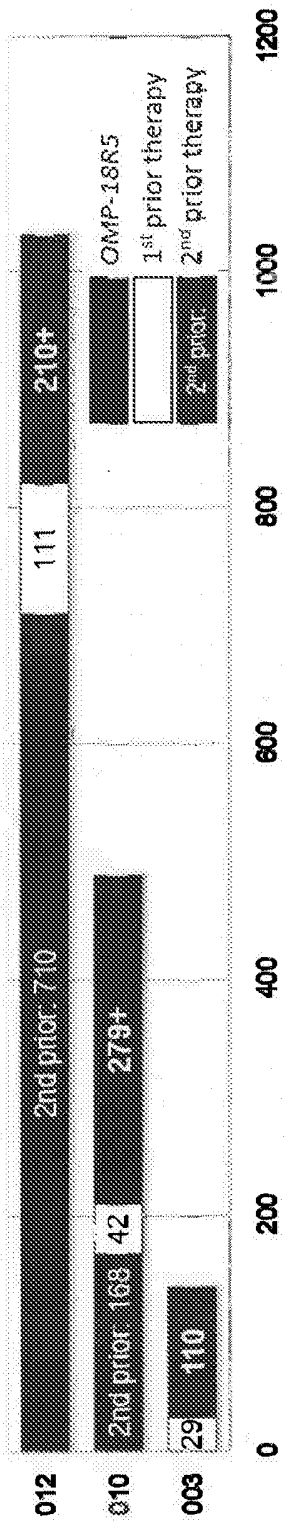
010
009
008
007
006
005
004
003
002
001
0
56
112
168
224
280
336
392
448
504
Days on study
= Active
= Neuroendocrine tumor
= Other tumor types
Vertical lines: tumor assessments

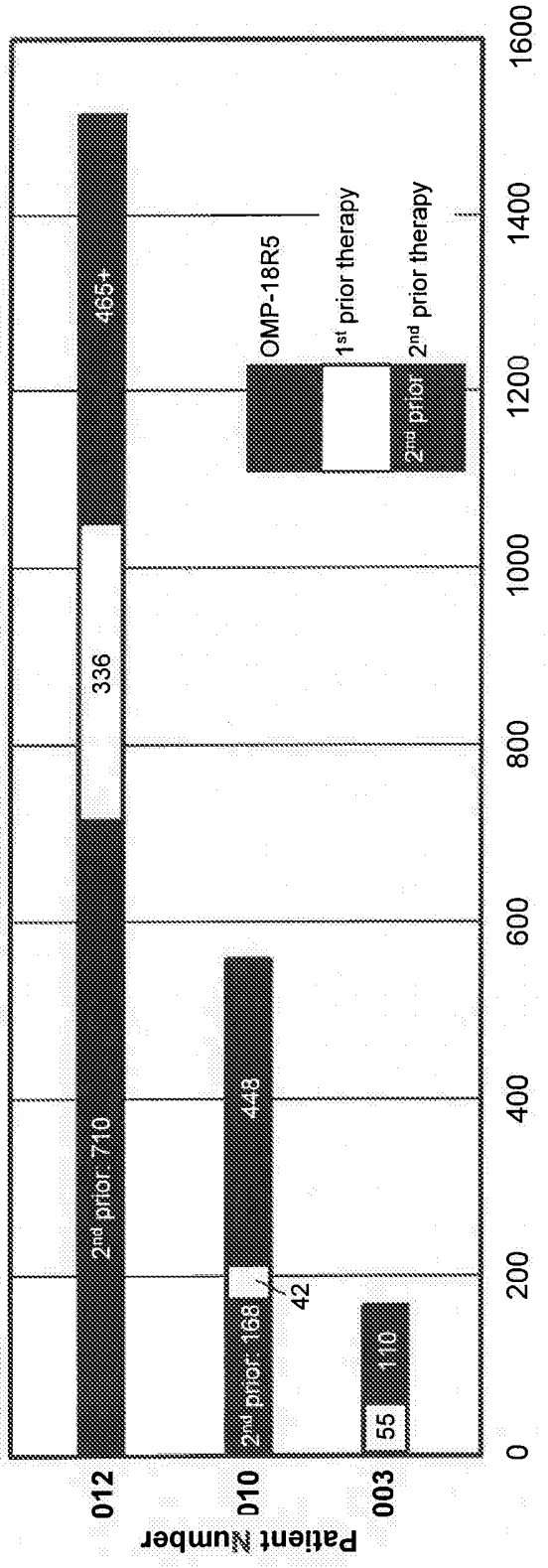
Figure 2B

Figure 3A



Patient No.	Type	Age	Gender	Diagnosis	Systemic Therapy
003	Carcinoid	59	female	2004: resection (curative intent)	1. GSK 1120212 (MEK inhibitor) + GSK 2141795 (AKT inhibitor) * 2. OMP-18R5*
010	Pancreatic neuroendocrine tumor (PNET)	69	female	2001: resection (curative intent) 2006: liver mets	1. Regorafenib (1093 days) 2. AB0024 (αLOXL2)* 3. AMG820 (αCSFR1)* 4. OMP-18R5*
012	Carcinoid	77	female	2006: liver mets	1. Sandostatin (569 days) 2. XL888 (HSP90 Inhibitor)* 3. Sand + REGN910 (αAng2)* 4. OMP-18R5*

* Days on study for these therapies are shown in graph above


Patient Number
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010
003
0
200
400
600
800
1000
1200
1400
1600
465+
336
2nd prior: 710
448
42
2nd prior: 168
110
55
OMP-18R5
1st prior therapy
2nd prior therapy
2nd prior

Figure 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/66087

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; C12N 15/11 (2013.01)
USPC - 424/172.1, 514/44A
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)
USPC: 424/172.1, 514/44A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/172.1; 514/44A; 536/24.5; 530/389.1 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Scholar, Patents and Web
Search terms: neuroendocrine tumor (e.g. pheochromocytoma, neurofibroma, pituitary adenoma), Wnt antagonist, antisense, antibody, small molecule, Frizzled (FZD), FZD 1 ,4 or 7, cancer, growth, proliferation, differentiation administer

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 2012/003189 A1 (CHENG et al.) 5 January 2012 (05.01.2012). Especially para [0009], [0028], [0051], [0083], [0084]. [0096].	1-3, 5-8 ---------- 4
X	US 2011/0020368 A1 (HYNES et al.) 27 January 2011 (27.01.2011). Especially para [0002], [0014], [0018], [0065], [0137].	1, 2, 5-8
Y	US 2012/0027778 A1 (GURNEY) 2 February 2012 (02.02.2012). Especially para [0039]	4

☐ 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
19 December 2013 (19.12.2013)	16 JAN 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: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/66087

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.: 9-56
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

(12)发明专利申请

(10)申请公布号 CN 104768579 A
(43)申请公布日 2015.07.08

(21)申请号 201380055630.2
(22)申请日 2013.10.22
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61/760,529 2013.02.04 US
(85)PCT国际申请进入国家阶段日
2015.04.23
(86)PCT国际申请的申请数据
PCT/US2013/066087 2013.10.22
(87)PCT国际申请的公布数据
WO2014/066328 EN 2014.05.01
(83)生物保藏信息
PTA-10311 2009.08.26
PTA-9541 2008.09.29
PTA-10309 2009.08.26
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(54)发明名称

使用Wnt途径结合剂来治疗神经内分泌肿瘤的方法

(57)摘要

本发明提供治疗神经内分泌肿瘤的新颖方法。在一实施方式中，该方法包含对需要治疗神经内分泌肿瘤的个体投予治疗有效剂量的Wnt拮抗剂。在一实施方式中，该Wnt拮抗剂系抗FZD抗体。在另一实施方式中，该Wnt拮抗剂系可溶性FZD受体多肽。在又一实施方式中，该Wnt拮抗剂系抗Wnt抗体。

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1. 一种抑制神经内分泌肿瘤生长的方法，其包含使该神经内分泌肿瘤与有效量的 Wnt 拮抗剂接触。

2. 一种抑制神经内分泌肿瘤细胞增生的方法，其包含使该神经内分泌肿瘤细胞与有效量的 Wnt 拮抗剂接触。

3. 一种减少神经内分泌肿瘤细胞的肿瘤发生性的方法，其包含使该神经内分泌肿瘤细胞与有效量的 Wnt 拮抗剂接触。

4. 一种诱导神经内分泌肿瘤细胞分化的方法，其包含使该神经内分泌肿瘤细胞与有效量的 Wnt 拮抗剂接触。

5. 一种抑制神经内分泌肿瘤生长的方法，其包含对需要抑制神经内分泌肿瘤生长的个体投予治疗有效量的 Wnt 拮抗剂。

6. 一种抑制神经内分泌肿瘤细胞增生的方法，其包含对需要抑制神经内分泌肿瘤细胞增生的个体投予治疗有效量的 Wnt 拮抗剂。

7. 一种治疗神经内分泌癌的方法，其包含对需要治疗神经内分泌癌的个体投予治疗有效量的 Wnt 拮抗剂。

8. 如权利要求第 5 至 7 项中任一项的方法，其中该个体系人个体。

9. 如权利要求第 1 至 8 项中任一项的方法，其中该神经内分泌肿瘤是低恶性度、中恶性度或高恶性度神经内分泌肿瘤。

10. 如权利要求第 1 至 8 项中任一项的方法，其中该神经内分泌肿瘤是功能性神经内分泌肿瘤或非功能性神经内分泌肿瘤。

11. 如权利要求第 1 至 8 项中任一项的方法，其中该神经内分泌肿瘤系选自胃肠胰腺神经内分泌瘤、胰腺神经内分泌瘤、类癌瘤、嗜铬细胞瘤、副神经节瘤、甲状腺髓质癌、肺神经内分泌瘤及胸腺神经内分泌瘤。

12. 如权利要求第 11 项的方法，其中该神经内分泌肿瘤是类癌瘤或胰腺神经内分泌瘤。

13. 如权利要求第 1 至 12 项中任一项的方法，其中该 Wnt 拮抗剂是抗体。

14. 如权利要求第 13 项的方法，其中该 Wnt 拮抗剂是与至少一种人 Wnt 特异性结合的抗体。

15. 如权利要求第 13 项的方法，其中该 Wnt 拮抗剂是与至少一种人卷曲受体 (FZD) 特异性结合的抗体。

16. 如权利要求第 15 项的方法，其中该抗体与至少一种人 FZD 的胞外域特异性结合。

17. 如权利要求第 15 或 16 项的方法，其中该抗体与选自 FZD1、FZD2、FZD5、FZD7 及 FZD8 的人 FZD 特异性结合。

18. 如权利要求第 15 至 17 项中任一项的方法，其中该抗体与 FZD7 特异性结合。

19. 如权利要求第 15 至 18 项中任一项的方法，其中该抗体与超过一种人 FZD 特异性结合。

20. 如权利要求第 15 至 19 项中任一项的方法，其中该抗体与选自 FZD1、FZD2、FZD5、FZD7 及 FZD8 中超过一种人 FZD 特异性结合。

21. 如权利要求第 15 至 20 项中任一项的方法，其中该抗体与选自 FZD1、FZD2、FZD5、FZD7 及 FZD8 中三种或超过三种人 FZD 特异性结合。

22. 如权利要求第 15 至 21 项中任一项的方法，其中该抗体与 FZD1、FZD2、FZD5、FZD7 及 FZD8 特异性结合。

23. 如权利要求第 15 至 22 项中任一项的方法，其中该抗体阻断配体与 FZD 结合。

24. 如权利要求第 23 项的方法，其中该抗体阻断 Wnt 与 FZD 结合。

25. 如权利要求第 15 至 22 项中任一项的方法，其中该抗体阻断 FZD 的活化。

26. 如权利要求第 15 至 25 项中任一项的方法，其中该抗体包含 :(a) 重链 CDR1、重链 CDR2 及重链 CDR3，该重链 CDR1 包含 GFTFSHYTLS(SEQ ID NO :31)，该重链 CDR2 包含 VISGDGSYTYYADSVKG(SEQ ID NO :32) 且该重链 CDR3 包含 NFIKYVFAN(SEQ ID NO :33) ;及 / 或 (b) 轻链 CDR1、轻链 CDR2 及轻链 CDR3，该轻链 CDR1 包含 SGDNIGSFYVH(SEQ ID NO :34)，该轻链 CDR2 包含 DKSNRPSG(SEQ ID NO :35) 且该轻链 CDR3 包含 QSYANTLSL(SEQ ID NO : 36) ;或该轻链 CDR1 包含 SGDKLGKKYAS(SEQ ID NO :41)，该轻链 CDR2 包含 EKDNRPSG(SEQ ID NO :42) 且该轻链 CDR3 包含 SSFAGNSLE(SEQ ID NO :43)。

27. 如权利要求第 26 项的方法，其中该抗体包含 :(a) 包含 SEQ ID NO :37 的氨基酸序列的重链可变区 ;及 / 或 (b) 包含 SEQ ID NO :38 或 SEQ ID NO :44 的氨基酸序列的轻链可变区。

28. 如权利要求第 26 项的方法，其中该抗体包含 :(a) 包含 SEQ ID NO :39 的氨基酸序列的重链 ;及 / 或 (b) 包含 SEQ ID NO :40 或 SEQ ID NO :45 的氨基酸序列的轻链。

29. 如权利要求第 13 至 28 项中任一项的方法，其中该抗体是单克隆抗体。

30. 如权利要求第 13 至 29 项中任一项的方法，其中该抗体是重组抗体、嵌合抗体、人源化抗体、人抗体或抗体片段。

31. 如权利要求第 13 至 30 项中任一项的方法，其中该抗体说单特异性抗体或双特异性抗体。

32. 如权利要求第 13 至 31 项中任一项的方法，其中该抗体是 IgA、IgD、IgE、IgG 或 IgM 抗体。

33. 如权利要求第 32 项的方法，其中该抗体是 IgG1 或 IgG2 抗体。

34. 如权利要求第 1 至 12 项中任一项的方法，其中该 Wnt 拮抗剂是可溶性 FZD 受体。

35. 如权利要求第 34 项的方法，其中该可溶性 FZD 受体与 Wnt 结合。

36. 如权利要求第 34 或 35 项中任一项的方法，其中该可溶性受体包含人 FZD 受体的胞外域的片段。

37. 如权利要求第 36 项的方法，其中该人 FZD 受体的胞外域的片段包含该人 FZD 受体的 Fri 结构域。

38. 如权利要求第 36 或 37 项中任一项的方法，其中该人 FZD 受体系选自 FZD4、FZD5 及 FZD8。

39. 如权利要求第 38 项的方法，其中该人 FZD 受体是 FZD8。

40. 如权利要求第 39 项的方法，其中该 FZD8Fri 结构域包含 SEQ ID NO :28 的氨基酸序列。

41. 如权利要求第 34 至 40 项中任一项的方法，其中该可溶性受体另包含人 Fc 结构域。

42. 如权利要求第 41 项的方法，其中该人 Fc 结构域包含 SEQ ID NO :95 的氨基酸序列。

43. 如权利要求第 1 至 42 项中任一项的方法，其另包含使该肿瘤或肿瘤细胞与第二治

疗剂接触，或对该个体投予第二治疗剂。

44. 如权利要求第 43 项的方法，其中该第二治疗剂是化学治疗剂。

45. 如权利要求第 44 项的方法，其中该第二治疗剂是激酶抑制剂、生长抑素类似物或 mTOR 途径抑制剂。

46. 如权利要求第 45 项的方法，其中该第二治疗剂是舒尼替尼、奥曲肽或依维莫司。

47. 如权利要求第 43 项的方法，其中该第二治疗剂是抗体。

48. 如权利要求第 43 项的方法，其中该第二治疗剂为血管生成抑制剂。

49. 如权利要求第 1 至 13 或 43 至 48 项中任一项的方法，其中该 Wnt 拮抗剂是 OMP-18R5。

50. 如权利要求第 49 项的方法，其中 OMP-18R5 是经静脉投予至需要该剂的个体，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。

51. 如权利要求第 50 项的方法，其中 OMP-18R5 是以约每一至二周约 0.5mg/kg 至约 1.0mg/kg 的剂量投予。

52. 如权利要求第 50 项的方法，其中 OMP-18R5 系以约每三周约 1.0mg/kg 至约 5.0mg/kg 的剂量投予。

53. 如权利要求第 1 至 12 或 43 至 48 项中任一项的方法，其中该 Wnt 拮抗剂是 OMP-54F28。

54. 如权利要求第 1 至 10 或 13 至 53 项中任一项的方法，其中该神经内分泌肿瘤不是小细胞肺肿瘤 (SCLC)。

55. 如权利要求第 1 至 53 项中任一项的方法，其中该神经内分泌肿瘤是类癌瘤。

56. 如权利要求第 1 至 53 项中任一项的方法，其中该神经内分泌肿瘤是胰腺神经内分泌瘤。

使用 Wnt 途径结合剂来治疗神经内分泌肿瘤的方法

相关申请的交叉引用

[0001] 本申请案主张于 2012 年 10 月 23 日提出的美国临时申请案第 61/717,294 号及 2013 年 2 月 4 日提出的美国临时申请案第 61/760,529 号的优先权，各案以引用方式整体纳入此处。

技术领域

[0002] 本发明的领域大抵关于治疗神经内分泌肿瘤的方法。在一实施方式中，该方法包含对需要治疗神经内分泌肿瘤的个体投予治疗有效剂量的 Wnt 拮抗剂。

背景技术

[0003] 癌症是发达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。一般预期每 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，其中四种一乳癌、肺癌、结直肠癌及前列腺癌一占所有新病例的一半以上 (Jemal et al., 2003, Cancer J. Clin. 53 :5-26)。

[0004] 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。未经调节的 Wnt 途径活化与多种人癌症有关，其可改变肿瘤细胞的发育命运以使彼等维持在未经分化及增生状态。因此癌的发生可藉由篡夺控制干细胞正常发育及组织修复的稳态机制进行 (于 Reya&Clevers, 2005, Nature, 434 :843 ;Beachy et al., 2004, Nature, 432 :324 中回顾)。

[0005] 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(LPR5/6) 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 个保守性半胱胺酸的大型胞外 N 端配体结合结构域，称为多半胱氨酸区 (CRD) 或 Fri 结构域。共有十种人 FZD 受体 :FZD1 至 10。不同的 FZD CRD 对特定 Wnt 有不同的结合亲和性 (Wu&Nusse, 2002, J. Biol. Chem. 277 :41762-9)，Fzd 受体已被分成活化下述的典型 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)。为了形成与该 FZD 配体结合的受体复合物，FZD 受体与 LRP5/6 交互作用，LRP5/6 为具有被六个 YWTD 氨基酸重复分开的四个 EGF 样胞外域的单次穿膜跨膜蛋白 (Johnson et al., 2004, J. Bone Mineral Res. 19 :1749)。

[0006] Wnt/β- 连环蛋白信号传导途径已知与胃肠类癌瘤的发生有关。Fujimori et al., Cancer Res. 61(18) :6656-9(2001)。也在胃肠类癌瘤中观察到 β- 连环蛋白的核转位但不见 β- 连环蛋白及 APC 突变。Su et al., Ann. Surg. Oncol. 13(12) :1604-9(2006)。以免

疫组织化学及直接定序两种方法研究 72 例胃肠类癌瘤的 β-连环蛋白。在 57 例 (79.2%) 中观察到 β-连环蛋白累积于细胞质及 / 或细胞核。亦于 27 例 (37.5%) 检测到 β-连环蛋白的外显子 3 突变，及 1 例 (1.4%) 的 APC 突变。Su 等亦报告对于 91 例胃肠类癌瘤的调查，为了比较起见，26 例胃肠外类癌瘤藉由免疫组织化学检测 β-连环蛋白及直接定序该 β-连环蛋白基因的外显子 3 及该 APC 基因的外显子 15。β-连环蛋白的细胞质累积及 / 或核转位被发现于 27 例胃肠类癌瘤 (29.7%) 但不见于任何胃肠外类癌瘤。不论是 β-连环蛋白或 APC 基因突变皆未于 β-连环蛋白的核表达的任何病例中检测到。

发明内容

[0007] 本发明提供治疗神经内分泌肿瘤的方法。因此在一态样中，本发明提供抑制神经内分泌肿瘤生长的方法，其包含使该神经内分泌肿瘤与有效量的 Wnt 拮抗剂接触。在另一态样中，本发明提供抑制神经内分泌肿瘤细胞增生的方法，其包含使该神经内分泌肿瘤细胞与有效量的 Wnt 拮抗剂接触。在另一态样中，本发明提供减少神经内分泌肿瘤细胞的肿瘤发生性的方法，其包含使该神经内分泌肿瘤细胞与有效量的 Wnt 拮抗剂接触。在另一态样中，本发明提供诱导神经内分泌肿瘤细胞分化的方法，其包含使该神经内分泌肿瘤细胞与有效量的 Wnt 拮抗剂接触。在另一态样中，本发明提供抑制神经内分泌肿瘤生长的方法，其包含对需要抑制神经内分泌肿瘤生长的个体投予治疗有效量的 Wnt 拮抗剂。在另一态样中，本发明提供抑制神经内分泌肿瘤细胞增生的方法，其包含对需要抑制神经内分泌肿瘤细胞增生的个体投予治疗有效量的 Wnt 拮抗剂。在另一态样中，本发明提供治疗神经内分泌癌的方法，其包含对需要治疗神经内分泌癌的个体投予治疗有效量的 Wnt 拮抗剂。在某些实施方式中，该个体系人个体。

[0008] 在前述各种态样或实施方式的特定实施方式以及本说明书中他处所述的其他态样及 / 或实施方式中，该神经内分泌肿瘤系低恶性度、中恶性度或高恶性度神经内分泌肿瘤。在其他实施方式中，该神经内分泌肿瘤系功能性神经内分泌肿瘤或非功能性神经内分泌肿瘤。在其他实施方式中，该神经内分泌肿瘤系选自胃肠胰腺神经内分泌瘤、类癌瘤、嗜铬细胞瘤、副神经节瘤、甲状腺髓质癌、肺神经内分泌瘤及胸腺神经内分泌瘤。在其他实施方式中，该神经内分泌肿瘤系类癌瘤或胰腺神经内分泌瘤。

[0009] 在前述各种态样或实施方式的特定实施方式以及本说明书中他处所述的其他态样及 / 或实施方式中，该 Wnt 拮抗剂系抗体。在其他实施方式中，该 Wnt 拮抗剂系与至少一种人 Wnt 特异性结合的抗体。在其他实施方式中，该 Wnt 拮抗剂系与至少一种人卷曲受体 (FZD) 特异性结合的抗体。在其他实施方式中，该 Wnt 拮抗剂系可溶性 FZD 受体。

[0010] 在前述各种态样或实施方式的特定实施方式以及本说明书中他处所述的其他态样及 / 或实施方式中，该 Wnt 拮抗剂系与至少一种人卷曲受体 (FZD) 特异性结合的抗体。在其他实施方式中，该抗体与至少一种人 FZD 的胞外域特异性结合。在其他实施方式中，该抗体与选自 FZD1、FZD2、FZD5、FZD7 或 FZD8 的人 FZD 特异性结合。在其他实施方式中，该抗体与 FZD7 特异性结合。在其他实施方式中，该抗体与超过一种人 FZD 特异性结合。在其他实施方式中，该抗体与选自 FZD1、FZD2、FZD5、FZD7 或 FZD8 中三种或超过三种人 FZD 特异性结合。在其他实施方式中，该抗体与选自 FZD1、FZD2、FZD5、FZD7 或 FZD8 中超过一种人 FZD 特异性结合。在其他实施方式中，该抗体与 FZD1、FZD2、FZD5、FZD7 及 FZD8 特异性结合。

[0011] 在其他实施方式中，该抗体阻断配体与 FZD 结合。在其他实施方式中，该抗体阻断 Wnt 与 FZD 结合。在其他实施方式中，该抗体阻断 FZD 的活化。

[0012] 在其他实施方式中，该抗体包含：(1) 包含 GFTFSHYTLS(SEQ ID NO:31) 的重链 CDR1、包含 VISGDGSYTYYADSVKG(SEQ ID NO:32) 的重链 CDR2 及包含 NFIKYVFAN(SEQ ID NO:33) 的重链 CDR3，及 / 或 (2)(a) 包含 SGDNIGSFYVH(SEQ ID NO:34) 的轻链 CDR1、包含 DKSNRPSG(SEQ ID NO:35) 的轻链 CDR2 及包含 QSYANTLSL(SEQ ID NO:36) 的轻链 CDR3；或 (b) 包含 SGDKLGKKYAS(SEQ ID NO:41) 的轻链 CDR1、包含 EKDNRPSG(SEQ ID NO:42) 的轻链 CDR2 及包含 SSFAGNSLE(SEQ ID NO:43) 的轻链 CDR3。在其他实施方式中，该抗体包含：包含 SEQ ID NO:37 的氨基酸序列的 VH；及 / 或包含 SEQ ID NO:38 或 44 的氨基酸序列的 VL。在其他实施方式中，该抗体包含：包含 SEQ ID NO:39 的氨基酸序列的重链；及 / 或包含 SEQ ID NO:40 或 45 的氨基酸序列的轻链。

[0013] 在其他实施方式中，该抗体系单克隆抗体。在其他实施方式中，该抗体系重组抗体、嵌合抗体、人源化抗体、人抗体或抗体片段。在其他实施方式中，该抗体系单特异性抗体或双特异性抗体。在其他实施方式中，该抗体系 IgA、IgD、IgE、IgG 或 IgM 抗体。在其他实施方式中，该抗体系 IgG1 或 IgG2 抗体。

[0014] 在其他实施方式中，该 Wnt 拮抗剂系 OMP-18R5(又称为 vantictumab)。

[0015] 在前述各种态样或实施方式的特定实施方式以及本说明书中他处所述的其他态样及 / 或实施方式中，该 Wnt 拮抗剂系可溶性 FZD 受体。在其他实施方式中，该可溶性 FZD 受体与 Wnt 结合。在其他实施方式中，该可溶性受体包含人 FZD 受体的胞外域的片段。在其他实施方式中，该人 FZD 受体的胞外域的片段包含该人 FZD 受体的 Fri 结构域。

[0016] 在其他实施方式中，该人 FZD 受体系选自 FZD4、FZD5 或 FZD8。在其他实施方式中，该人 FZD 受体系 FZD8。在其他实施方式中，该 FZD8Fri 结构域包含 SEQ ID NO:28 的氨基酸序列。

[0017] 在其他实施方式中，该可溶性受体另包含人 Fc 结构域。在其他实施方式中，该人 Fc 结构域包含 SEQ ID NO:95 的氨基酸序列。

[0018] 在其他实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0019] 在前述各种态样或实施方式的特定实施方式以及本说明书中他处所述的其他态样及 / 或实施方式中，该方法另包含使该肿瘤或肿瘤细胞与第二治疗剂接触，或对该个体投予第二治疗剂。在其他实施方式中，该第二治疗剂系化学治疗剂。在其他实施方式中，该第二治疗剂系激酶抑制剂、生长抑素类似物或 mTOR 途径抑制剂。在其他实施方式中，该第二治疗剂系舒尼替尼 (sunitinib)、奥曲肽 (octreotide) 或依维莫司 (everolimus)。在其他实施方式中，该第二治疗剂系抗体。在其他实施方式中，该第二治疗剂系血管生成抑制剂。

[0020] 本发明的态样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

[0021] 图 1A。Wnt 抑制剂对神经内分泌肿瘤生长的影响。在投予 OMP-18R5 抗 FZD7 抗体

之后，胰腺神经内分泌肿瘤病患的肿瘤病灶大小减少。目标 (T) 及非目标 (NT) 病灶大小在 OMP-18R5 抗 FZD7 抗体治疗第 56 天及第 112 天经放射线学评估。BL 表示在投予 OMP-18R5 之前的病灶基准期大小。

[0022] 图 1B。Wnt 抑制剂对神经内分泌肿瘤生长的影响。在 OMP-18R5 投予之前（基准期）与 112 天后的肿瘤病灶 CT 影像。

[0023] 图 1C。Wnt 抑制剂对神经内分泌肿瘤生长的影响。在 OMP-18R5 投予之前（基准期）与 112 天后的肿瘤病灶 CT 影像。第 112 天的肿瘤病灶显示钙化的放射线征候。

[0024] 图 2A。病患参与 OMP-18R5 第 1a 期试验的天数。到 2013 年 1 月 25 日止，每位参与 OMP-18R5 第 1a 期试验的病患 (n = 18) 参加试验的天数，如该图所示。箭头显示在 2013 年 1 月 25 日时仍参加试验的病患。直线表示试验中的肿瘤评估日期。神经内分泌肿瘤病患为病患 003（实施例 1 中的病患 3）、010（实施例 1 中的病患 10）及 012（实施例 1 中的病患 12）。其他参与试验的病患有其他类型的末期实质肿瘤，如结直肠癌、乳癌、黑色素瘤及胰腺癌。

[0025] 图 2B。病患参与 OMP-18R5 第 1a 期试验的天数。到 2013 年 10 月 4 日止，每位参与 OMP-18R5 第 1a 期试验的病患 (n = 29) 参加试验的天数，如该图所示。箭头显示在 2013 年 10 月 4 日时仍参加试验的病患。直线表示试验中的肿瘤评估日期。神经内分泌肿瘤病患为病患 003（实施例 1 中的病患 3）、010（实施例 1 中的病患 10）、012（实施例 1 中的病患 12）、025 及 026。其他参与试验的病患有其他类型的末期实质肿瘤。

[0026] 图 3A。神经内分泌肿瘤病患参与 OMP-18R5 第 1a 期试验的天数与先前治疗的天数比较。病患 10(69 岁女性罹患胰腺神经内分泌肿瘤）的疾病稳定持续参与试验共 279 天（到 2013 年 1 月 25 日）。病患 12(77 岁女性罹患类癌瘤）的疾病稳定持续参与试验共 210 天（到 2013 年 1 月 25 日）。

[0027] 图 3B。神经内分泌肿瘤病患参与 OMP-18R5 第 1a 期试验的天数与先前治疗的天数比较。病患 10 在第 448 天退出试验。病患 12 的疾病稳定持续参与试验共 465 天（到 2013 年 10 月 4 日）。（一些先前治疗的天数数据已经校正图 3A 的稍早数据）。

具体实施方式

[0028] 本发明提供抑制神经内分泌肿瘤生长的方法、抑制神经内分泌肿瘤细胞增生的方法、治疗神经内分泌癌的方法、抑制神经内分泌肿瘤转移的方法、诱导神经内分泌肿瘤细胞分化的方法、减少神经内分泌肿瘤细胞的肿瘤发生性的方法及减少癌干细胞或肿瘤起始细胞于神经内分泌肿瘤中的频率的方法。在一些实施方式中，此处提供的方法包含对个体投予 Wnt 拮抗剂。在一些实施方式中，该 Wnt 拮抗剂系与一或多种人 FZD 受体特异性结合的 FZD 结合剂。在其他实施方式中，该 FZD 结合剂系与一种人 FZD 受体特异性结合或与一或多种人 FZD 受体结合的抗体。在一些实施方式中，该 Wnt 拮抗剂系与一或多种人 Wnt 多肽特异性结合的 Wnt 结合剂。在一些实施方式中，该 Wnt 结合剂系可溶性 FZD 受体。在一些实施方式中，该 Wnt 结合剂系抗 Wnt 抗体。

[0029] 具有末期神经内分泌肿瘤的人病患在末期实质肿瘤病患的第一期临床试验中经低剂量的 OMP-18R5 抗 FZD 抗体治疗。（实施例 1。）令人意外的是，其中一名病患（罹患胰腺神经内分泌瘤的病患）在以 OMP-18R5 治疗 112 天之后显示肿瘤病灶大小减少，且维持接

受治疗 279 天（至 2013 年 1 月 25 日）而无任何疾病恶化的证据。此外，在一名病患的病灶中见到新的钙化，这可能代表肿瘤细胞坏死及 / 或分化的可能征候。另外，二名具有类癌瘤组织学的神经内分泌肿瘤病患也能持续接受治疗，在 OMP-18R5 的治疗期间令人意外地维持长期稳定疾病状态。（实施例 1。）总结来说，这些结果显示 OMP-18R5 可能特别适用于治疗多种神经内分泌肿瘤。

1. 定义

[0030] 为了促进对本发明的了解，以下定义一些用语及用词。

[0031] 此处所使用的用语“拮抗剂”包括部分或完全阻断、抑制或中和蛋白（例如癌干细胞标志）的表达或生物活性的任何分子。该阻断、抑制及 / 或中和生物活性包括但不限于抑制肿瘤生长。用语“拮抗剂”亦包括部分或完全阻断、抑制或中和 Wnt 途径的生物活性的任何分子。此处使用的用语“Wnt 拮抗剂”包括部分或完全阻断、抑制或中和该 Wnt 途径的信号传导（例如典型 Wnt 信号传导）的任何分子，或部分或完全阻断、抑制或中和该 Wnt 途径的成分的生物活性的任何分子。Wnt 拮抗剂不一定与 Wnt 结合。举例来说，在某些实施方式中，Wnt 拮抗剂与该 Wnt 途径之一或多种其他成分诸如一或多种 FZD 受体结合。适当的 Wnt 拮抗剂分子包括但不限于天然 FZD 受体蛋白，天然 FZD 受体蛋白包括可溶性 FZD 受体的片段及 / 或氨基酸序列变异体，以及可溶性卷曲相关蛋白 (SFRP) 的衍生物及 ROR 蛋白的衍生物。适当的 Wnt 拮抗剂分子另包括但不限于与一或多种 FZD 受体特异性结合的抗体及与一或多种 Wnt 多肽特异性结合的抗体。可溶性 SFRP 及 ROR 受体系描述于美国专利申请案公开号 2011/030569，其以引用方式纳入此处。

[0032] 用于测定剂（例如可溶性 FZD 受体或抗 FZD 抗体）是否抑制 Wnt 信号传导的体内及体外测定系该领域所知。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典型 Wnt 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)。在一或多种 Wnt（例如由转染细胞表达或由 Wnt 条件培养基提供的 Wnt）存在且有该剂存在时的 Wnt 信号传导量系与无该剂存在时的信号传导量比较。除了 TCF/luc 报告子测定之外，剂（例如可溶性 FZD 受体或抗 FZD 抗体）对典型 Wnt 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.,Science,281 :1509-12(1998))、细胞周期素 D1(Tetsu et al.,Nature,398 :422-6(1999)) 及 / 或纤维黏连蛋白 (Gradl et al.Mol.Cell Biol.19 :5576-87(1999))。在某些实施方式中，该剂对 Wnt 信号传导的影响亦可藉由测量该剂对 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。在仍其他实施方式中，该剂对 Wnt 信号传导的影响藉由检测该剂对 Wnt 标签基因中的一或多种基因的表达量的影响测定。使用该些试验检测典型 Wnt 信号传导的抑制的非限制性实例系揭示于美国专利申请案公开号 2012/0027778，其系以引用方式整体纳入此处。

[0033] 此处使用的用语“可溶性受体”系指受体蛋白在跨膜结构域之前的氨基端胞外片段，其可以可溶形式自细胞分泌。在一些实施方式中，该受体蛋白系 FZD 受体。在一些实施方式中，该受体蛋白系 ROR1 或 ROR2 受体。在某些实施方式中，该可溶性受体系与增加该可溶性受体的半衰期的多肽符合读框地连接。在某些实施方式中，该增加半衰期的多肽系人 Fc 结构域。

[0034] 此处使用的用语“FZD 可溶性受体”系指人 FZD 受体蛋白在该受体的跨膜结构域之前的氨基端胞外片段，其可以可溶形式自细胞分泌。包含完整氨基端胞外域 (ECD)（此处称为“FZD ECD”）的 FZD 可溶性受体以及包含该 ECD 的较小片段的 FZD 可溶性受体被设想。包含 Fri 结构域的 FZD 可溶性受体（此处称为“FZD Fri”）亦被揭示。可溶性 FZD 受体系描述于美国专利申请案公开号 2011/030569，其以引用方式纳入此处。

[0035] FZD Fri 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。FZD 可溶性受体包括与其他功能性及结构性蛋白符合读框地连接的 FZD ECD 或 Fri 结构域，该等功能性及结构性蛋白包括但不限于人 Fc 区（例如源自免疫球蛋白 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM 的人 Fc）；蛋白标签（例如 myc、FLAG、GST）；其他内源性蛋白或蛋白片段；或任何其他有用的蛋白序列包括在 FZD ECD 或 Fri 结构域与连接蛋白之间的任何连接子区。在某些实施方式中，FZD 受体的 Fri 结构域系与人 Fc 区直接相连。在某些实施方式中，FZD 受体的 Fri 结构域系与人 IgG1Fc 相连（此处称为“FZD Fri.Fc”，例如“FZD8Fri.Fc”）。在一些实施方式中，FZD 受体的 Fri 结构域系与人 Fc 区经由肽连接子相连。FZD 可溶性受体亦包括含有氨基酸插入、删除、取代及 / 或保守性取代的变异体蛋白。

[0036] 此处使用的用语“连接子”或“连接子区”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。较佳地，连接子不具抗原性且不诱发免疫反应。

[0037] 用语“抗体”系指免疫球蛋白分子，该免疫球蛋白分子藉由其的可变区内的至少一个抗原辨识区辨识标靶且与之特异性结合，像是蛋白质、多肽、肽、碳水化合物、多核苷酸、脂质或前述的组合。此处所使用的用语“抗体”包含完整多克隆抗体、完整单克隆抗体、抗体片段（诸如 Fab、Fab’、F(ab’)2 及 Fv 片段）、单链 Fv(scFv) 突变体、多特异性抗体诸如由至少二种完整抗体生成的双特异性抗体、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位点的经修饰的免疫球蛋白分子只要该抗体展现所期望的生物活性。抗体可为下列五种主要免疫球蛋白中的任一者：IgA、IgD、IgE、IgG 及 IgM 或彼等的亚型（同型）（例如 IgG1、IgG2、IgG3、IgG4、IgA1 及 IgA2），此系根据彼等分别被称为 α、δ、ε、γ 及 μ 的重链恒定结构域命名。不同类型的免疫球蛋白具有不同且广为周知的次单位结构及三维构型。抗体可为未经修饰或与其他分子如毒素、放射性同位素等共轭。

[0038] 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。

[0039] 用语“单克隆抗体”系指涉及高度特异性辨认及结合单一抗原性决定簇或表位的同源性抗体群。此与多克隆抗体相反，多克隆抗体通常包括以不同的抗原决定簇为目标的不同抗体。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) 突变体、包含抗体部分的融合蛋白及任何其他包含抗原识别位点的经修饰的免疫球蛋白分子。另外，“单克隆抗体”系指由任何数量的方式包括但不

限于杂交瘤、噬菌体选择、重组表达及转基因动物制备的该等抗体。

[0040] 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（例如鼠）序列的特定免疫球蛋白链、嵌合性免疫球蛋白或彼等的片段。通常，人源化抗体系其中互补决定区 (CDR) 的残基经具有所期望的特异性、亲和性及能力的非人物种（例如小鼠、大鼠、兔、仓鼠）的 CDR 残基取代的人免疫球蛋白 (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 架构区 (FR) 残基系由具有所期望的特异性、亲和性及能力的非人物种的抗体的对应残基取代。该人源化抗体可进一步藉由取代 Fv 架构区及 / 或该经取代的非人残基内的额外残基加以修饰，以精进优化抗体的特异性、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 区，然而所有或实质上所有的 FR 区系具有人免疫球蛋白共同序列的该区。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)，通常包含人免疫球蛋白的该部分。用于生成人源化抗体的方法的实例系描述于美国专利第 5,225,539 号。

[0041] 用语“人抗体”系指由人体产生的抗体或利用该领域已知的任何技术制备的具有对应由人体产生的抗体的氨基酸序列的抗体。此定义的人抗体包括完整或全长抗体、彼等的片段及 / 或包含至少一个人重链及 / 或轻链多肽的抗体，诸如包含鼠轻链及人重链多肽的抗体。

[0042] 用语“表位”或“抗原决定簇”在此处可交换使用，系指可被特定抗体辨识且特异性结合的抗原部分。当抗原系多肽时，表位可自连续氨基酸或藉由蛋白质的三级折叠并列的非连续氨基酸形成。自连续氨基酸形成的表位在蛋白变性时通常仍被保留，然而藉由三级折叠形成的表位通常在蛋白变性时消失。表位通常包括至少 3 个及更常地至少 5 或 8 至 10 个呈独特空间构形的氨基酸。

[0043] 多肽或其他剂（例如抗体或可溶性受体）与蛋白“特异性结合”系指该多肽或其他剂相较于选择性物质（包括不相关的蛋白）以更频繁、更快速、更长期、更高亲和性或以上述某些特性的组合与该蛋白反应或相连。在某些实施方式中，“特异性结合”系指例如剂（如抗体或可溶性受体）以大约 0.1mM 或更低，但通常低于大约 1μM 的 K_D 与蛋白结合。在某些实施方式中，“特异性结合”系指剂（例如抗体或可溶性受体）有时以至少约 0.1μM 或更低，至少约 0.01μM 或更低，且有时以至少约 1nM 或更低的 K_D 与蛋白结合。由于不同物种的同源性蛋白之间具有序列一致性，特异性结合可包括辨识超过一种物种中的特定蛋白诸如 Wnt 蛋白或卷曲受体的剂（例如抗体或可溶性受体）。同样地，由于不同的旁系同源基因（例如不同的人 Wnt 蛋白或人卷曲蛋白）在彼等的序列的特定区域内具有同源性，特异性结合可包括辨识超过一种旁系同源基因（例如超过一种人 Wnt 蛋白或超过一种人卷曲蛋白）的多肽或剂（例如抗体或可溶性受体）。应了解的是，与第一标靶特异性结合的剂（例如抗体或可溶性受体）可能与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，剂（例如抗体或可溶性受体）在某些实施方式中可能特异性地与超过一种标靶（例如多种不同的人 Wnt 蛋白或多种不同的卷曲蛋白，诸如 FZD1、FZD2、FZD5、FZD7 及 / 或 FZD8）结合。在某些实施方式中，抗体的多

重标靶可能由该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种人卷曲受体（例如人 FZD1、FZD2、FZD5、FZD7 及 / 或 FZD8）特异性结合。在某些可供选择的实施方式中，抗体可能为双特异性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一个辨识第一卷曲受体（例如人 FZD5）上的表位的抗原结合部位，还包含第二个辨识第二卷曲受体（例如人 FZD8）上的不同表位的不同抗原结合部位。一般来说（但不必然），所谓的结合系指特异性结合。

[0044] 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。一般了解用语“癌”包含 Wnt 依赖性癌。癌的实例包括但不限于癌、淋巴瘤、胚细胞瘤、肉瘤及白血病。

[0045] 用语“肿瘤”及“瘤 (neoplasm)”系指任何由过度细胞生长或增生所导致的组织团块，不论是良性（非癌性）或包括癌前性病灶的恶性（癌性）。

[0046] 用语“癌干细胞”、“肿瘤干细胞”或“实质肿瘤干细胞”在此处可交换使用，系指具有下列特性的实质肿瘤的细胞群：(1) 具有广泛增生能力，(2) 能进行不对称细胞分裂以产生一或多种类型的经分化的细胞后代，该经分化的细胞具有减少的增生或发育能力，及 (3) 能进行对称细胞分裂以自我更新或自我维持。“癌干细胞”、“肿瘤干细胞”或“实质肿瘤干细胞”的这些特性授予这些癌干细胞在连续移植至免疫不全小鼠时形成明显可见的肿瘤的能力，相较之下大部分肿瘤细胞则无法形成肿瘤。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且可在将来突变发生时改变的细胞类型的肿瘤。

[0047] 用语“癌细胞”、“肿瘤细胞”及文法相同用语系指源自肿瘤或癌前病灶的整体细胞族群，包括非肿瘤发生性细胞（其包含大部分的肿瘤细胞族群）及肿瘤发生性干细胞（癌干细胞）。此处使用的用语“肿瘤细胞”仅用于指称该些缺乏更新及分化能力的肿瘤细胞时，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。

[0048] 用语“肿瘤发生性”系指实质肿瘤干细胞的功能特性，包括自我更新（导致额外的肿瘤发生性癌干细胞）及增生以产生所有其他肿瘤细胞（导致经分化及因此非肿瘤发生性肿瘤细胞）以允许实质肿瘤干细胞形成肿瘤的特性。这些自我更新及增生以产生所有其他肿瘤细胞的特性，授予癌干细胞在连续移植至免疫不全小鼠时形成明显可见的肿瘤的能力，相较之下非肿瘤发生性肿瘤细胞在连续移植时无法形成肿瘤。已经观察到，自实质肿瘤获得的非肿瘤发生性肿瘤细胞，在经初代移植至免疫不全小鼠时可能形成肿瘤，但该些非肿瘤发生性肿瘤细胞在经连续移植后不会形成肿瘤。

[0049] 用语“个体”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、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，用语“个体”及“病患”在此处可交换使用以指称人个体。

[0050] 用语“治疗有效量”系指有效“治疗”个体或哺乳动物的疾病或疾患的剂（例如抗体、可溶性受体、多肽、多核苷酸、小型有机分子或其他药物）的量。以癌而言，治疗有效量的剂可减少癌细胞的数量；减少肿瘤大小；抑制或停止癌细胞浸润至周边器官包括例如癌扩散至软组织及骨骼；抑制及停止肿瘤转移；抑制及停止肿瘤生长；缓解与癌有关之一或多种征状至某种程度；减少发病率及死亡率；改善生活质量；降低肿瘤的肿瘤发生性、肿瘤发生频率或肿瘤发生能力；减少肿瘤中的癌干细胞的数量或频率；使肿瘤发生细胞分化成

非肿瘤发生状态；或该些效应的组合。以该剂预防现存癌细胞生长及/或杀死现存癌细胞的程度，其可被称为细胞静止性及/或细胞毒性。

[0051] 此处使用的用语“抑制肿瘤生长”系指肿瘤细胞生长可藉以被抑制的任何机制。在某些实施方式中，肿瘤细胞生长系藉由延缓肿瘤细胞增生而被抑制。在某些实施方式中，肿瘤细胞生长系藉由停止肿瘤细胞增生而被抑制。在某些实施方式中，肿瘤细胞生长系藉由杀死肿瘤细胞而被抑制。在某些实施方式中，肿瘤细胞生长系藉由诱导肿瘤细胞细胞凋亡而被抑制。在某些实施方式中，肿瘤细胞生长系藉由诱导肿瘤细胞分化而被抑制。在某些实施方式中，肿瘤细胞生长系藉由剥夺肿瘤细胞养分而被抑制。在某些实施方式中，肿瘤细胞生长系藉由预防肿瘤细胞移动而被抑制。在某些实施方式中，肿瘤细胞生长系藉由预防肿瘤细胞入侵而被抑制。

[0052] 用语诸如“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，该措施预防及/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。在某些实施方式中，个体系经本发明的方法成功“治疗”癌，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例如癌扩散至软组织及骨；抑制或缺乏肿瘤转移；抑制或缺乏肿瘤生长；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；减少肿瘤的肿瘤发生性、肿瘤发生频率或肿瘤发生能力；减少肿瘤中的癌干细胞的数量或频率；使肿瘤发生细胞分化成非肿瘤发生状态；或一些效应的组合。

[0053] 抗体的“可变区”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架构区(FR)及连接该四个架构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FR拉靠近，并与来自其他链的CDR一起形成抗体的抗原结合部位。至少有两种技术用于测定CDR：(1)根据跨种序列变异性的方法(即Kabat et al.Sequences of Proteins of Immunological Interest,(5th ed.,1991,National Institutes of Health,Bethesda Md))；及(2)根据抗原-抗体复合体的结晶学研究的方法(Al-lazikani et al(1997)J.Molec.Biol.273:927-948))。此外，有时该领域使用这两种技术的组合以测定CDR。

[0054] 用语“多肽”、“肽”及“蛋白”在此处可交换使用，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，诸如与标记成份共轭。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等）的多肽，以及包含该领域习知的其他修饰的多肽。应了解的是，由于本发明的多肽以抗体为基础，因此在某些实施方式中，该多肽可能为单链或相连的多个链。

[0055] 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。

[0056] 应了解的是，只要此处的实施方式系以用语“包含”描述，其亦提供其他以“由…组成”及/或“实质上由…组成”的用语所描述的类似实施方式。

[0057] 用语“及/或”使用于此处诸如“A及/或B”的词组中，系意图包括“A及B”两者、“A或B”、“A”及“B”。同样地，用语“及/或”使用于词组诸如“A、B及/或C”时，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）。

2. 治疗方法

[0058] 本发明提供治疗神经内分泌肿瘤的方法。神经内分泌肿瘤（NET）系源自内分泌（荷尔蒙）及神经系统的细胞的肿瘤。神经内分泌肿瘤（NET）包括型态、功能及行为特征各异的肿瘤。这些肿瘤通常生长缓慢，并以缓慢的方式进展。然而，它们有可能扩散，通常扩散至肝脏，而且当扩散时可危及生命，难以用目前的治疗方案治疗。

[0059] 神经内分泌肿瘤系依据来源部位分类。在某些实施方式中，NET系选自胰腺神经内分泌瘤（pNET）、肺类癌瘤、胃类癌瘤、十二指肠类癌瘤、空肠类癌瘤、回肠类癌瘤、结肠类癌瘤及直肠类癌瘤。在其他实施方式中，NET系选自卵巢、胸腺、甲状腺髓质、肾上腺（例如嗜铬细胞瘤）及副神经节（副神经节瘤）的神经内分泌肿瘤。在某些实施方式中，利用此处所述的方法治疗的NET系小细胞肺癌（SCLC）。在某些可供选择的实施方式中，该NET不是小细胞肺癌。在某些实施方式中，NET是胰腺神经内分泌瘤（PET）或类癌瘤。在某些实施方式中，该NET不是小细胞肺癌、胰腺癌或甲状腺癌。

[0060] 神经内分泌肿瘤也可依照分级及分化分类。见例如Phan et al.，Pancreas，39(6)：784-798(2012)。在某些实施方式中，该神经内分泌肿瘤系分化良好的低恶性度肿瘤。在某些实施方式中，该神经内分泌肿瘤系分化中度的中恶性度肿瘤。在某些实施方式中，该神经内分泌肿瘤系分化不良的高恶性度肿瘤。在一实施方式中，低恶性度肿瘤的特征为每10个高倍数视野（HPF）<2个有丝分裂且无坏死。在一实施方式中，中恶性度肿瘤的特征为每10个高倍数视野（HPF）有2至10个有丝分裂或有坏死中心。在一实施方式中，高恶性度肿瘤的特征为每10个高倍数视野（HPF）>10个有丝分裂。

[0061] 神经内分泌肿瘤亦可分为功能性及非功能性NET。当肿瘤细胞产生过多荷尔蒙而引发特定临床症候群时，该NET被视为功能性。功能性NET的实例包括但不限于类癌瘤，其可导致类癌瘤症候群，及功能性pNET，例如胰岛瘤、胃泌素瘤、血管活性肠肽（VIP）瘤、升糖素瘤及生长抑素瘤。非功能性NET不会因为肿瘤细胞产生过多的荷尔蒙导致临床症候群，但仍可产生与肿瘤存在或其转移有关的症状（例如腹痛或腹胀）。在某些实施方式中，该神经内分泌肿瘤系功能性NET。在某些实施方式中，该神经内分泌肿瘤系非功能性NET。在某些实施方式中，该神经内分泌肿瘤系选自功能性类癌瘤、胰岛瘤、胃泌素瘤、血管活性肠肽（VIP）瘤、升糖素瘤、血清素瘤、组织胺瘤、ACTH瘤、嗜铬细胞瘤及生长抑素瘤。在某些实施方式中，该神经内分泌肿瘤不是SCLC。

[0062] 在某些实施方式中，该神经内分泌肿瘤系原发性肿瘤。在某些实施方式中，该神经内分泌肿瘤系转移性肿瘤。在某些实施方式中，该神经内分泌肿瘤并未扩散至原发性器官的壁以外。在某些实施方式中，该神经内分泌肿瘤扩散出原发性器官的壁到达邻近组织，诸如脂肪、肌肉或淋巴结。在某些实施方式中，该神经内分泌肿瘤扩散至远离原发性器官的组织或器官，例如肝脏、骨骼或肺脏。

[0063] 在某些实施方式中，该神经内分泌癌或肿瘤对治疗有耐性。以非限制性实例来说，该癌或肿瘤可能具有化学疗法耐性（即对一或多种形式的化学疗法具抗药性）。在某些实

施方式中，该癌或肿瘤对生长抑素类似物的治疗具有抗药性。在某些实施方式中，该癌或肿瘤对激酶抑制剂的治疗具有抗药性。

[0064] 在某些实施方式中，该神经内分泌癌或肿瘤已转移至肝脏。举非限制性实例说明，该神经内分泌癌或肿瘤系已转移至肝脏的类癌或胰腺神经内分泌肿瘤。

[0065] 在一态样中，本发明提供 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）于治疗神经内分泌肿瘤的用途。在某些实施方式中，该 Wnt 拮抗剂可用于抑制神经内分泌肿瘤细胞中的 Wnt 信号传导（例如典型 Wnt 信号传导）、抑制神经内分泌肿瘤生长、诱导神经内分泌肿瘤分化、减少神经内分泌肿瘤体积及 / 或减少神经内分泌肿瘤的肿瘤发生性。使用方法可为体外、间接体内或体内方法。在某些实施方式中，该 Wnt 拮抗剂系抗体 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系可溶性受体 OMP-54F28。

[0066] 本发明提供治疗神经内分泌肿瘤的方法，该方法包含对个体（例如需要治疗的个体）投予治疗有效量的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）。在某些实施方式中，该神经内分泌肿瘤系胰腺神经内分泌瘤。在某些实施方式中，该神经内分泌肿瘤系类癌瘤。在某些实施方式中，该神经内分泌肿瘤系肺脏的神经内分泌肿瘤。以非限制性实例说明，该肺脏的神经内分泌肿瘤可能是 SCLC。在某些实施方式中，该神经内分泌肿瘤不是 SCLC。在某些实施方式中，该个体系人。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0067] 本发明另提供抑制神经内分泌肿瘤生长的方法，该方法使用如此处所述的 Wnt 拮抗剂（例如抗 FZD 抗体及可溶性 FZD 受体）。在某些实施方式中，抑制神经内分泌肿瘤生长的方法包含使该肿瘤细胞与 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）于体外接触。举例来说，永生化神经内分泌肿瘤细胞系系于添加该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）的培养基中培养以抑制肿瘤生长。在一些实施方式中，神经内分泌肿瘤细胞系自病患样本诸如像是组织活体检查、胸膜渗液或血液样本分离，并于其中添加 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）以抑制肿瘤生长的培养基中培养。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0068] 在一些实施方式中，抑制神经内分泌肿瘤生长的方法包含使该神经内分泌肿瘤或肿瘤细胞与 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）于活体内接触。在某些实施方式中，使神经内分泌肿瘤或肿瘤细胞与 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）接触系于动物模型中进行。举例来说，Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）可被投予至生长于免疫不全小鼠（例如 NOD/SCID 小鼠）的神经内分泌异种肿瘤移植以抑制神经内分泌肿瘤生长。在一些实施方式中，神经内分泌肿瘤癌干细胞系自病患样本诸如像是组织活体检查、胸膜渗液或血液样本分离，并注射至免疫不全小鼠，接着对该小鼠投予 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）以抑制神经内分泌肿瘤细胞生长。在一些实施方式中，该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）系于导入肿瘤发生细胞至该动物体内时同时投予或随即于导入后投予，以抑制神经内分泌肿瘤生长。在一些实施方式中，该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）系于该肿瘤发生细胞已生长至特定大小后作为治疗剂投予。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0069] 在某些实施方式中，抑制神经内分泌肿瘤生长的方法包含对个体投予治疗有效量

的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）。在某些实施方式中，该个体系人。在某些实施方式中，该个体具有神经内分泌肿瘤或肿瘤已被移除。

[0070] 在某些实施方式中，该神经内分泌肿瘤系其中 Wnt 信号传导活跃的肿瘤。在某些实施方式中，该活跃的 Wnt 信号传导系典型 Wnt 信号传导。在某些实施方式中，该神经内分泌肿瘤系 Wnt 依赖性肿瘤。举例来说，在一些实施方式中，该肿瘤对 axin 过度表达敏感。在某些实施方式中，该肿瘤不包含大肠腺瘤息肉 (APC) 肿瘤抑瘤基因的失活突变（例如截短突变）或 β－连环蛋白基因的活化突变。在某些实施方式中，该肿瘤表达 Wnt 基因标签中之一或多种基因，即一或多种基因藉由 Wnt 信号传导途径的上调或下调。在某些实施方式中，个体所被治疗的神经内分泌肿瘤涉及该肿瘤。

[0071] 在某些实施方式中，该神经内分泌肿瘤表达一或多种被此处所述的 Wnt 拮抗剂 FZD 结合抗体结合的人卷曲受体。在某些实施方式中，该神经内分泌肿瘤过度表达人卷曲受体。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。

[0072] 在某些实施方式中，该神经内分泌肿瘤表达一或多种被此处所述的 Wnt 拮抗剂可溶性 FZD 受体结合的人 Wnt 多肽。在某些实施方式中，该神经内分泌肿瘤过度表达人 Wnt 多肽。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0073] 在某些实施方式中，该神经内分泌肿瘤表达一或多种被此处所述的 Wnt 拮抗剂抗 Wnt 抗体结合的人 Wnt 多肽。在某些实施方式中，该神经内分泌肿瘤过度表达人 Wnt 多肽。

[0074] 在某些实施方式中，该神经内分泌肿瘤系胰腺神经内分泌瘤。在某些实施方式中，该神经内分泌肿瘤系类癌瘤。在某些实施方式中，该神经内分泌肿瘤系肺脏的神经内分泌肿瘤。在某些实施方式中，该神经内分泌肿瘤不是 SCLC。

[0075] 本发明亦提供抑制神经内分泌肿瘤细胞中 Wnt 信号传导的方法，该方法包含使该细胞与有效量的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）接触。在某些实施方式中，该方法系活体内方法，其中使该细胞与该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）接触的步骤包含对该个体投予治疗有效量的该 Wnt 拮抗剂。在一些可供选择的实施方式中，该方法系体外或间接体内方法。在某些实施方式中，该被抑制的 Wnt 信号传导系典型 Wnt 信号传导。在某些实施方式中，该 Wnt 信号传导系由 Wnt1、Wnt2、Wnt3、Wnt3A、Wnt7a、Wnt7b 及 / 或 Wnt10B 信号传导。在某些实施方式中，该 Wnt 信号传导系由 Wnt1、Wnt3A、Wnt7b 及 / 或 Wnt10B 信号传导。

[0076] 此外，本发明提供减少个体体内的神经内分泌肿瘤的肿瘤发生性的方法，该方法包含对该个体投予治疗有效量的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）。在某些实施方式中，该神经内分泌肿瘤包含癌干细胞。在某些实施方式中，该神经内分泌肿瘤中癌干细胞的频率系藉由投予该剂而减少。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0077] 因此，本发明亦提供减少神经内分泌肿瘤中的癌干细胞的频率的方法，该方法包含使该肿瘤与有效量的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）接触。

[0078] 本发明另提供使肿瘤发生性神经内分泌肿瘤细胞分化成非肿瘤发生性细胞的方法，该方法包含藉由投予该 Wnt 拮抗剂至具有包含该肿瘤发生性细胞的神经内分泌肿瘤的个体，或该神经内分泌肿瘤已移除的个体，以使该肿瘤发生性神经内分泌肿瘤细胞与 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）接触。

[0079] 本发明亦提供此处所述的 Wnt 拮抗剂（例如抗 FZD 抗体及可溶性 FZD 受体）于诱导神经内分泌肿瘤细胞分化的用途。举例来说，本发明考虑诱导细胞分化的方法，其包含使该细胞与有效量的如此处所述的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）接触。本发明亦提供诱导个体体内的神经内分泌肿瘤中的细胞分化的方法，其包含对该个体投予治疗有效量的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）。在某些实施方式中，该神经内分泌肿瘤细胞的分化系与该肿瘤病灶的放射影像的改变有关。在某些实施方式中，该神经内分泌肿瘤细胞的分化系与该肿瘤病灶的钙化有关。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0080] 本发明另提供治疗个体的神经内分泌肿瘤的方法，其中该神经内分泌肿瘤与 Wnt 信号传导活化有关及 / 或具有干细胞及 / 或祖细胞的量增加的特征。在一些实施方式中，该治疗方法包含对该个体投予治疗有效量的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）。在某些实施方式中，该 Wnt 信号传导系典型 Wnt 信号传导。

[0081] 在某些实施方式中，除了投予此处所述的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）以外，该方法或治疗另包含（在投予该 Wnt 拮抗剂之前、的同时及 / 或之后）投予第二抗癌剂。本发明亦提供包含 Wnt 拮抗剂与该第二抗癌剂的医药组合物。在某些实施方式中，投予该 Wnt 拮抗剂与第二抗癌剂的组合具有协同效应，诸如对癌干细胞的频率的协同效应。

[0082] 将了解的是，Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）与第二抗癌剂的组合可能以任何顺序或同时投予。在选择的实施方式中，该 Wnt 拮抗剂将被投予至已先接受第二抗癌剂治疗的病患。在某些其他实施方式中，该 Wnt 拮抗剂与第二抗癌剂将被实质上同步或同时投予。举例来说，个体可能在接受该第二抗癌剂（例如化学治疗）的疗程时被给予 Wnt 拮抗剂。在某些实施方式中，该 Wnt 拮抗剂将于接受该第二抗癌剂治疗的一年内被投予。在某些替代性实施方式中，该 Wnt 拮抗剂将于接受该第二抗癌剂的任何治疗的 10、8、6、4 或 2 个月内被投予。在某些其他实施方式中，该 Wnt 拮抗剂将于接受该第二抗癌剂的任何治疗的 4、3、2 或 1 周内被投予。在一些实施方式中，该 Wnt 拮抗剂将于接受该第二抗癌剂的任何治疗的 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）被投予至个体。

[0083] 有用类别的抗癌剂包括例如抗微管蛋白剂、阿里他汀类 (auristatins)、DNA 次要凹槽结合剂、DNA 复制抑制剂、烷化剂（例如铂复合物诸如顺铂 (cisplatin)、单（铂）、二（铂）及三核铂复合物及卡铂 (carboplatin))、蒽环类 (anthracycline)、抗生素、抗叶酸剂、抗代谢物、化学疗法致敏剂、双联霉素 (duocarmycin)、依托泊苷 (etoposide)、氟化嘧啶、离子载体、莱克西托素 (lexitropsin)、亚硝基尿素、普拉汀诺 (platinol)、表现化合物 (performing compounds)、嘌呤抗代谢物、嘌呤霉素 (puromycin)、放射线致敏剂、类固醇、紫杉烷、拓扑异构酶抑制剂、长春花生物碱或该类似物。在某些实施方式中，第二抗癌剂系抗代谢物、抗有丝分裂剂、拓扑异构酶抑制剂或血管生成抑制剂。

[0084] 可能与该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）组合投予的抗癌剂包括化学治疗剂。因此，在一些实施方式中，该方法或治疗牵涉组合投予 Wnt 拮抗剂与化学治疗剂或多种不同化学治疗剂的鸡尾酒组合。Wnt 拮抗剂的治疗可发生于投予化学治疗剂之前、的同时或之后。本发明考虑的化学治疗剂包括该领域已知且市售的化学物质或药

物，诸如吉西他滨(gemcitabine)、伊立替康(irinotecan)、多柔比星(doxorubicin)、5-氟尿嘧啶(5-fluorouracil)、胞嘧啶阿拉伯糖苷(Ara-C)或环磷酰胺(cyclophosphamide)、噻替派(thiotepa)、白消安(busulfan)、细胞毒素(cytoxin)、汰癌胜(TAXOL)、甲胺喋呤(methotrexate)、顺铂(cisplatin)、霉法兰(melphalan)、长春碱(vinblastine)及卡铂(carboplatin)。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计划可根据制造商的说明使用或由经验丰富的医生凭经验决定。该等化学治疗剂的准备及给药计划亦描述于Chemotherapy Service Ed.,M.C.Perry,Williams&Wilkins,Baltimore,Md.(1992)。

[0085] 可用于本发明的化学治疗剂亦包括但不限于：烷化剂诸如噻替派(thiotepa)及环磷酰胺(cyclophosphamide)(CYTOXAN)；烷基磺酸盐诸如白消安(busulfan)、英丙舒凡(improsulfan)及哌泊舒凡(piposulfan)；氮丙啶诸如苯多巴(benzodopa)、卡波醌(carboquone)、甲基优瑞多巴(meturedopa)及优瑞多巴(uredopa)；伸乙亚胺(ethylenimines)及甲基三聚氰胺(methylmelamines)包括阿草特胺(altretamine)、三亚乙基三聚氰胺(triethylenemelamine)、三乙烯磷酰胺(trietylenephosphoramide)、三乙烯硫磷酰胺(triethylenethiophosphoramide)及三羟甲基三聚氰胺(trimethylolmelamime)；氮芥子气诸如氯芥苯丁酸(chlorambucil)、萘氮芥(chlornaphazine)、氯磷酰胺(cholophosphamide)、雌二醇氮芥(estramustine)、异环磷酸胺(ifosfamide)、双氯乙基甲胺(mechlorethamine)、盐酸氧氮芥(mechlorethamine oxide hydrochloride)、霉法兰(melphalan)、新氮芥(novembichin)、胆甾醇苯乙酸氮芥(phenesterine)、松龙苯芥(prednimustine)、氯乙环磷酰胺(trofosfamide)、尿嘧啶芥(uracil mustard)；亚硝基脲(nitrosourea)诸如卡氮芥(carmustine)、吡葡亚硝脲(chlorozotocin)、福莫司汀(fotemustine)、罗氮芥(lomustine)、尼氮芥(nimustine)、雷诺氮芥(ranimustine)；抗生素诸如阿克拉霉素(aclacinomycin)、放线菌素(actinomycin)、安曲霉素(anthramycin)、氮丝胺酸(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-正白胺酸、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)、阿糖胞苷 (cytarabine)、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)；氨基酮戊酸 (aminolevulinic acid)；安吖啶 (amsacrine)；贝斯特氮芥 (bestrabucil)；比生群 (bisantrene)；依达曲沙 (edatrexate)；地弗胺 (defofamine)；秋水仙胺 (demecolcine)；地吖醌 (diaziquone)；艾弗米啶 (elformithine)；依利醋铵 (elliptinium acetate)；依托格鲁 (etoglucid)；硝酸镓 (gallium nitrate)；羟基脲；香菇糖 (lentinan)；氯尼达明 (lonidamine)；米托胍腙 (mitoguazone)；米托蒽醌 (mitoxantrone)；莫哌达醇 (mopidamol)；二胺硝吖啶 (nitracrine)；喷司他丁 (pentostatin)；蛋胺氮芥 (phenamet)；吡柔比星 (pirarubicin)；鬼臼酸 (podophyllinic acid)；2-乙基酰肼 (2-ethylhydrazide)；丙卡巴肼 (procarbazine)；PSK；雷佐生 (razoxane)；西佐喃 (sizofuran)；锗螺胺 (spirogermanium)；细交链孢菌酮酸 (tenuazonic acid)；三亚胺醌 (triaziquone)；2,2',2"-三氯三乙胺 (2,2',2"-trichlorotriethylamine)；乌拉坦 (urethan)；长春地辛 (vindesine)；达卡巴嗪 (dacarbazine)；甘露莫司汀 (mannomustine)；二溴甘露醇 (mitobronitol)；二溴卫矛醇 (mitolactol)；哌泊溴烷 (pipobroman)；加壬唑素 (gacytosine)；阿拉伯糖苷 (Ara-C)；环磷酰胺 (cyclophosphamide)；噻替派；类紫杉醇 (taxoids) 例如太平洋紫杉醇 (TAXOL,Bristol-Myers Squibb Oncology,Princeton,N.J.) 及多西紫杉醇 (TAXOTERE,Rhone-Poulenc Rorer,Antony,France)；苯丁酸氮芥 (chlorambucil)；吉西他滨 (gemcitabine)；6-硫鸟嘌呤；巯嘌呤 (mercaptopurine)；甲胺喋呤 (methotrexate)；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)；截瘤达 (xeloda)；伊班膦酸盐 (ibandronate)；CPT11；拓扑异构酶抑制剂 RFS 2000；二氟甲基鸟胺酸 (DMFO)；视黄酸；埃斯培拉霉素 (esperamicin)；卡培他滨 (capecitabine) 及上述任一剂的医药上可接受的盐、酸或衍生物。化学治疗剂亦包括用来调节或抑制荷尔蒙对肿瘤的作用的抗荷尔蒙剂，诸如抗雌激素剂包括例如它莫西芬 (tamoxifen)、雷洛昔芬 (raloxifene)、芳香酶抑制剂 4(5)-咪唑、4-羟基它莫西芬、曲沃昔芬 (trioxifene)、雷洛昔芬 (keoxifene)、LY117018、奥那司酮 (onapristone) 及托瑞米芬 (toremifene)(Fareston)；及抗雄性素剂诸如氟他胺 (flutamide)、尼鲁米特 (nilutamide)、比卡鲁胺 (bicalutamide)、柳普林 (leuprolide) 及戈舍瑞林 (goserelin)；及上述任一剂的医药上可接受的盐、酸或衍生物。

[0086] 在某些实施方式中，该化学治疗剂系激酶抑制剂。在某些实施方式中，该激酶抑制剂系多标靶受体酪胺酸激酶抑制剂。激酶抑制剂包括但不限于舒尼替尼 (sunitinib)(由 Pfizer 以 SUTENT 的名称贩卖)、帕唑帕尼 (pazopanib)、克唑替尼 (crizotinib)、达沙替尼

(dasatinib)。在某些实施方式中，该第二抗癌剂系舒尼替尼。

[0087] 在某些实施方式中，该化学治疗剂系雷帕霉素 (rapamycin) 的哺乳动物标靶 (mTOR) 的抑制剂。mTOR 抑制剂包括但不限于替西罗莫司 (temsirolimus)、西罗莫司 (sirolimus)、蒂佛洛利莫司 (deforolimus) 及依维莫司 (everolimus)。在某些实施方式中，该第二抗癌剂系依维莫司。

[0088] 在某些实施方式中，该化学治疗剂系生长抑素类似物。生长抑素类似物经由与生长抑素的特定高亲和性膜受体交互作用发挥作用。生长抑素类似物包括但不限于奥曲肽 (octreotide)、索马杜林 (somatulin) 及 RC160(octastatin)。在某些实施方式中，该第二抗癌剂系奥曲肽。

[0089] 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)。在某些实施方式中，该第二抗癌剂系伊立替康。

[0090] 在某些实施方式中，该化学治疗剂系烷化剂。在某些实施方式中，该化学治疗剂系替莫唑胺 (temozolomide)。

[0091] 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，但仍有足够之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 (GlaxoSmithKline)、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，该第二抗癌剂系吉西他滨。在某些实施方式中，该欲被治疗的肿瘤系胰腺神经内分泌肿瘤且该第二抗癌剂系抗代谢剂 (例如吉西他滨)。

[0092] 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。藉由非限制性实例说明，该剂包含紫杉烷 (taxane)。在某些实施方式中，该剂包含太平洋紫杉醇 (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 (例如 ABRAXANE)、DHA- 太平洋紫杉醇或 PG- 太平洋紫杉醇。在某些替代性实施方式中，该抗有丝分裂剂包含长春花生物碱，诸如长春新碱 (vincristine)、长春碱 (vinblastine)、长春瑞滨 (vinorelbine) 或长春地辛 (vindesine) 或彼等的医药上可接受的盐、酸或衍生物。在一些实施方式中，该抗有丝分裂剂系 Eg5 驱动蛋白 (kinesin) 的抑制剂或有丝分裂激酶诸如 Aurora A 或 Plk1 的抑制剂。

[0093] 在某些实施方式中，该治疗涉及组合投予此处所述的 Wnt 拮抗剂 (例如抗 FZD 抗体或可溶性 FZD 受体) 与放射疗法。Wnt 拮抗剂的治疗可发生于投予放射治疗之前、的同时或之后。可使用任何由本领域技术人员决定的放射治疗投予计划。

[0094] 在一些实施方式中，该第二抗癌剂包含抗体。因此，治疗可涉及组合投予 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）与拮抗肿瘤相关性抗原的抗体，包括但不限于与 EGFR、ErbB2、HER2、DLL4、NOTCH 及 / 或 VEGF 结合的抗体。示范性抗 DLL4 抗体系描述于例如美国专利申请公开案 US 2008/0187532，其以引用方式整体纳入此处。在某些实施方式中，该第二抗癌剂系血管生成抑制剂的抗体（例如抗 VEGF 抗体）。其他抗 DLL4 抗体系描述于例如国际专利公开案 WO 2008/091222 及 WO 2008/0793326，美国专利申请公开案 US 2008/0014196、US 2008/0175847、US 2008/0181899 及 US2008/0107648，各以引用方式整体纳入此处。示范性抗缺口抗体系描述于例如美国专利申请公开案 US 2008/0131434，其以引用方式整体纳入此处。在某些实施方式中，该第二抗癌剂系属于血管生成抑制剂的抗体（例如抗 VEGF 抗体）。在某些实施方式中，该第二抗癌剂系缺口信号传导的抑制剂。在某些实施方式中，该第二抗癌剂系 AVASTIN（贝伐珠单抗 (bevacizumab)）、HERCEPTIN（曲妥珠单抗 (trastuzumab)）、VECTIBIX（帕尼单抗 (panitumumab)）或 ERBITUX（西妥昔单抗 (cetuximab)）。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。

[0095] 另外，治疗可包括投予一或多种细胞介素（例如淋巴介质、介白素、肿瘤坏死因子及 / 或生长因子），或可伴随手术移除癌细胞或任何其他医师认为必要的治疗。

[0096] 以疾病的治疗而言，此处所述的 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）的适当剂量取决于所欲治疗的神经内分泌肿瘤类型、神经内分泌肿瘤的严重性及病程、神经内分泌肿瘤的反应性、该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）系经投予以达治疗或预防目的、先前治疗、该病患的临床病史等，所有皆由主治医师斟酌。该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）可经投予一次或在数天至数月期间的治疗系列投予，或直到达成疗效或达到减少神经内分泌肿瘤（例如肿瘤大小减少）。理想投药计划可自药物累积于病患体内的测量值加以计算，且将视个别 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）的相对强度而异。主治医师可轻易地决定理想剂量、投药方法及重复频率。在某些实施方式中，剂量系介于每公斤体重 0.01 微克至 100 毫克，给药频率可为每天、每周、每月或每年一次或多次。在某些实施方式中，该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）系每二周给予一次或每三周给予一次。在某些实施方式中，该 Wnt 拮抗剂（例如抗 FZD 抗体或可溶性 FZD 受体）的剂量系约 0.1mg 至约 20mg 每公斤体重。主治医师可根据在体液或组织中测量的药物的停留时间及浓度以预估投药的重复频率。在某些实施方式中，该 Wnt 拮抗剂系 OMP-18R5。在某些实施方式中，该 Wnt 拮抗剂系 OMP-54F28。

[0097] 在某些实施方式中，OMP-18R5 系以约 0.1mg/kg 至约 20mg/kg 的剂量或约 0.5mg/kg 至约 10mg/kg 的剂量经静脉投予。该等剂量在一些实施方式中可能约每周、每二周、每三周或每四周给予。在某些实施方式中，OMP-18R5 系以约每二至四周约 0.5mg/kg 至约 10mg/kg 的剂量经静脉投予。在某些实施方式中，OMP-18R5 系以约每三周约 1.0mg/kg 至约 10mg/kg 的剂量经静脉投予。在某些实施方式中，OMP-18R5 系以下列剂量经静脉投予：(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。在某些实施方式中，该抗体系以约每一至二周约 0.5mg/kg 至约 1.0mg/kg 的剂量投予。在一些可供选择的实施方式中，该抗体系以约每三周约 1.0mg/kg 至约 5.0mg/kg 的剂量投予。

[0098] 以非限制性实例说明，OMP-54F28 可能以约 0.1mg/kg 至约 20mg/kg 的剂量经静脉

投予。此剂量在一些实施方式中可能每周、每二周、每三周或每四周给予。在某些实施方式中，OMP-54F28 系以每二至四周约 0.5mg/kg 至约 10mg/kg 的剂量经静脉投予。在某些实施方式中，OMP-54F28 系以约每三周约 0.5mg/kg 至约 10mg/kg 的剂量经静脉投予。

3.FZD- 结合剂

[0099] 本发明的方法的另一态样系 FZD 结合剂（例如抗 FZD 抗体）于治疗神经内分泌肿瘤的用途。在某些实施方式中，该可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）与一或多种人卷曲受体 (FZD) 特异性结合。在某些实施方式中，该剂与二、三、四、五、六、七、八、九或十种卷曲受体特异性结合。被该剂结合的人卷曲受体可选自 FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9 及 FZD10。在某些实施方式中，该一或多种人卷曲受体包含 FZD1、FZD2、FZD5、FZD7 及 / 或 FZD8。在某些实施方式中，该一或多种人卷曲受体包含 FZD7。在某些实施方式中，该一或多种人卷曲受体包含 FZD5 及 / 或 FZD8。在某些实施方式中，该剂与 FZD1、FZD2、FZD5、FZD7 及 FZD8 特异性结合。在某些实施方式中，该 FZD 结合剂与 FZD7 特异性结合。在某些实施方式中，该 FZD 结合剂与 FZD5 特异性结合。FZD1 至 10 的全长氨基酸 (aa) 及核苷酸 (nt) 序列系该领域已知，且亦提供于此处为 SEQ ID NO：1(FZD1aa)、SEQ ID NO:2(FZD2aa)、SEQ ID NO:3(FZD3aa)、SEQ ID NO:4(FZD4aa)、SEQ ID NO:5(FZD5aa)、SEQ ID NO:6(FZD6aa)、SEQ ID NO:7(FZD7aa)、SEQ ID NO:8(FZD8aa)、SEQ ID NO:9(FZD9aa)、SEQ ID NO:10(FZD10aa)。

[0100] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）与二或多种人卷曲受体特异性结合。在某些实施方式中，该二或多种人卷曲受体系选自 FZD2、FZD5、FZD7 及 FZD8。在某些实施方式中，该二或多种卷曲受体包含 FZD1 及选自 FZD2、FZD5、FZD7 或 FZD8 的第二卷曲受体。在某些实施方式中，该二或多种卷曲受体包含 FZD2 及选自 FZD1、FZD5、FZD7 或 FZD8 的第二卷曲受体。在某些实施方式中，该二或多种卷曲受体包含 FZD5 及选自 FZD1、FZD2、FZD7 或 FZD8 的第二卷曲受体。在某些实施方式中，该二或多种卷曲受体包含 FZD5 及 FZD8。在某些实施方式中，该二或多种卷曲受体包含 FZD7 及选自 FZD1、FZD2、FZD5 或 FZD8 的第二卷曲受体。在某些实施方式中，该剂与三或多种人卷曲受体特异性结合。在某些实施方式中，该三或多种人卷曲受体包含选自 FZD1、FZD2、FZD5、FZD7 及 FZD8 的三或多种人卷曲受体。在某些实施方式中，该剂另与一或多种额外的人卷曲受体特异性结合。

[0101] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）与其所结合之一或多种人卷曲受体内的胞外域 (ECD) 特异性结合。各人卷曲受体的胞外域的序列系该领域已知，且亦提供于此处为 SEQ ID NO:11(FZD1ECD)、SEQ ID NO:12(FZD2ECD)、SEQ ID NO:13(FZD3ECD)、SEQ ID NO:14(FZD4ECD)、SEQ ID NO:15(FZD5ECD)、SEQ ID NO:16(FZD6ECD)、SEQ ID NO:17(FZD7ECD)、SEQ ID NO:18(FZD8ECD)、SEQ ID NO:19(FZD9ECD)、及 SEQ ID NO:20(FZD10ECD)。特别有用的抗体系描述于美国专利第 7,982,013 号及美国专利申请案公开号 2012/0027778，彼等以引用方式整体纳入此处。

[0102] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）与其所结合的人卷曲受体内的 Fri 结构域 (FRI)（亦称为多半胱氨酸区 (CRD)）特异性结合。各人卷曲受体的 Fri 结构域的序列系该领域所知，亦提供于下。FZD1 的 Fri 结构域包括 SEQ ID NO:11 的大约氨基酸 87 至 237。FZD2 的 Fri 结构域包括 SEQ ID NO:12 的大约氨基酸

24 至 159。FZD3 的 Fri 结构域包括 SEQ ID NO :13 的大约氨基酸 23 至 143。FZD4 的 Fri 结构域包括 SEQ ID NO :14 的大约氨基酸 40 至 170。FZD5 的 Fri 结构域包括 SEQ ID NO :15 的大约氨基酸 27 至 157。FZD6 的 Fri 结构域包括 SEQ ID NO :16 的大约氨基酸 19 至 146。FZD7 的 Fri 结构域包括 SEQ ID NO :17 的大约氨基酸 33 至 170。FZD8 的 Fri 结构域包括 SEQ ID NO :18 的大约氨基酸 28 至 158。FZD9 的 Fri 结构域包括 SEQ ID NO :19 的大约氨基酸 23 至 159。FZD10 的 Fri 结构域包括 SEQ ID NO :20 的大约氨基酸 21 至 154。各人 FZD 受体的对应预测 Fri 结构域系提供为 SEQ ID NO :21 至 30。各人 FZD 受体 (FZD1 至 10) 的最小核心 Fri 结构域序列系提供为 SEQ ID NO :73 至 82。该领域的技术人员对于对应各种 Fri 结构域的确切氨基酸的了解可能互异。因此在特定实施方式中，上述及此处所述的结构域的 N 端或 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。

[0103] 在某些实施方式中，FZD 结合抗体的个别抗原结合部位能与一、二、三、四或五（或更多）种人卷曲受体结合。在某些实施方式中，该 FZD 结合抗体的个别抗原结合部位能与选自 FZD1、FZD2、FZD5、FZD7 及 FZD8 的一、二、三、四或五种人卷曲受体特异性结合。在某些实施方式中，该抗体的个别结合部位与至少 FZD5 及 FZD8 特异性结合。

[0104] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）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 或更低的解离常数 (K_D) 与一或多种（例如二或多种、三或多种或四或多种）人卷曲受体结合。举例来说，在某些实施方式中，与超过一种 FZD 结合的 FZD 结合剂或抗体以约 100nM 或更低、约 20nM 或更低或约 10nM 或更低的 K_D 与该些 FZD 结合。在某些实施方式中，该 FZD 结合剂或抗体以约 40nM 或更低的解离常数与下列一或多种（例如 1、2、3、4 或 5 种）FZD 的各者结合 :FZD1、FZD2、FZD5、FZD7 及 FZD8。在某些实施方式中，该 FZD 结合剂或抗体以约 10nM 或更低的解离常数与下列一或多种 FZD 的各者结合 :FZD1、FZD2、FZD5、FZD7 及 FZD8。在某些实施方式中，该 FZD 结合剂或抗体以约 10nM 或更低的解离常数与下列 FZD 的各者结合 :FZD1、FZD2、FZD5、FZD7 及 FZD8。在某些实施方式中，该剂或抗体对特定 FZD 的解离常数系利用固定于 Biacore 芯片上的包含 FZD 胞外域或 Fri 结构域的 FZD-Fc 融合蛋白所测得的解离常数。

[0105] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）系该剂所结合的至少一种人卷曲受体（即 1、2、3、4、5、6、7、8、9 或 10 种 FZD) 的拮抗剂。在某些实施方式中，该剂抑制至少约 10％、至少约 20％、至少约 30％、至少约 50％、至少约 75％、至少约 90％或约 100％的该经结合的人卷曲受体的一或多种活性。

[0106] 在某些实施方式中，该 FZD 结合剂（例如抗 FZD 抗体）抑制配体与该至少一种人卷曲受体的结合。在某些实施方式中，该配体系人 Wnt 蛋白。十九种人 Wnt 蛋白已被识别 :Wnt1、Wnt2、Wnt2B/13、Wnt3、Wnt3A、Wnt4、Wnt5A、Wnt5B、Wnt6、Wnt7A、Wnt7B、Wnt8A、Wnt8B、Wnt9A（前称 Wnt14)、Wnt9B（前称 Wnt15)、Wnt10A、Wnt10B、Wnt11 及 Wnt16。在某些实施方式中，该剂抑制 Wnt3A 与 FZD8 的结合。在某些实施方式中，由该 FZD 结合剂所提供的对特定配体与特定人卷曲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，抑制配体诸如 Wnt 与 FZD 结合的剂另抑制 Wnt 信号传导（例如抑制典型 Wnt 信号传导）。

[0107] 在某些实施方式中，该 FZD 结合剂（例如抗 FZD 抗体）抑制 Wnt 信号传导。应了解的是，抑制 Wnt 信号传导的 FZD 结合剂在某些实施方式中可能抑制藉由一或多种 Wnt 的

信号传导，但不一定抑制藉由所有 Wnt 的信号传导。在某些选择性实施方式中，藉由所有人 Wnt 的信号传导可能皆被抑制。在某些实施方式中，藉由选自 Wnt1、Wnt2、Wnt2B/13、Wnt3、Wnt3A、Wnt4、Wnt5A、Wnt5B、Wnt6、Wnt7A、Wnt7B、Wnt8A、Wnt8B、Wnt9A（前称 Wnt14）、Wnt9B（前称 Wnt15）、Wnt10A、Wnt10B、Wnt11 及 Wnt16 之一或多种 Wnt 的信号传导系经抑制。在某些实施方式中，该经抑制的 Wnt 信号传导系由 Wnt1、Wnt2、Wnt3、Wnt3A、Wnt7a、Wnt7b 及 / 或 Wnt10B 信号传导。在某些实施方式中，该剂抑制藉由（至少）Wnt1、Wnt3A、Wnt7b 及 Wnt10B 的信号传导。在特定实施方式中，该剂抑制藉由（至少）Wnt3A 的信号传导。在某些实施方式中，由该 FZD 结合剂所提供的对藉由 Wnt 信号传导的抑制系减少该 Wnt 信号传导量至少约 10％、至少约 25％、至少约 50％、至少约 75％、至少约 90％或至少约 95％。在某些实施方式中，该被抑制的 Wnt 信号传导系典型 Wnt 信号传导。

[0108] 用于测定 FZD 结合剂（或候选 FZD 结合剂）是否抑制 Wnt 信号传导的活体内及体外测定系该领域所知。见例如美国专利申请案公开号 2012/0027778，其系以引用方式整体纳入此处。

[0109] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂（例如抗 FZD 抗体）具有一或多种下列效应：抑制神经内分泌肿瘤细胞增生、藉由减少神经内分泌肿瘤中的癌干细胞的频率以减少该神经内分泌肿瘤的肿瘤发生性、抑制神经内分泌肿瘤生长、增加存活、诱发神经内分泌肿瘤细胞的细胞死亡、使肿瘤发生性神经内分泌肿瘤细胞分化成非肿瘤发生性状态或预防肿瘤细胞的转移。

[0110] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂能抑制神经内分泌肿瘤生长。在某些实施方式中，该 FZD 结合剂可抑制活体内的神经内分泌肿瘤生长（例如在异种移植小鼠模型及 / 或于罹患癌的人体内）。

[0111] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂能减少神经内分泌肿瘤的肿瘤发生性。在某些实施方式中，该剂或抗体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神经内分泌肿瘤的肿瘤发生性。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。用于检测抗 FZD 抗体的疗效的限制稀释试验实例系提供于 US2012/0027778 的实施例 8，其系以引用方式整体纳入此处。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、美国专利申请案公开号 2008/0064049 及美国专利申请案公开号 2008/0178305，彼等各自以引用方式整体纳入此处。

[0112] 在某些实施方式中，该可用于本发明的方法中的 FZD 结合剂（例如抗体）系多肽。在某些实施方式中，该剂或多肽系抗体。在某些实施方式中，该抗体系 IgG1 抗体或 IgG2 抗体。在某些实施方式中，该抗体系单克隆抗体。在某些实施方式中，该抗体系人抗体或人源化抗体。在某些实施方式中，该抗体系抗体片段。

[0113] 在某些实施方式中，用于本发明的方法中的抗 FZD 抗体包含 18R5、18R8 及 / 或 44R24 人抗体的一、二、三、四、五及 / 或六个 CDR（见下表 1），每个 CDR 具有最多四个（即 0、1、2、3 或 4 个）保守性氨基酸取代。在某些实施方式中，该重链 CDR 系包含于重链可变区之内，及 / 或该轻链 CDR 系包含于轻链可变区之内。

表 1. 18R8、18R5 和 44R24 人抗体的 CDR

抗体	重链		
	CDR1	CDR2	CDR3
18R8	GFTFSHYTLS (SEQ ID NO:31)	VISGDGSYTYYADSVKG (SEQ ID NO:32)	NFIKYVFAN (SEQ ID NO:33)
18R5	GFTFSHYTLS (SEQ ID NO:31)	VISGDGSYTYYADSVKG (SEQ ID NO:32)	NFIKYVFAN (SEQ ID NO:33)
44R24	GFTFSSYYIT (SEQ ID NO:46)	TISYSSSNTYYADSVKG (SEQ ID NO:47)	SIVFDY (SEQ ID NO:48)

抗体	轻链		
	CDR1	CDR2	CDR3
18R8	SGDKLGKKYAS (SEQ ID NO:41)	EKDNRPSG (SEQ ID NO:42)	SSFAGNSLE (SEQ ID NO:43)
18R5	SGDNIGSFYVH (SEQ ID NO:34)	DKSNRPSG (SEQ ID NO:35)	QSYANTLSL (SEQ ID NO:36)
44R24	SGDALGNRYVY (SEQ ID NO:49)	SG (SEQ ID NO:50)	GSWDTRPYPKY (SEQ ID NO:51)

* 为了移除 N- 连接糖基化位点所导入的 CDR1 定点改变是以划线标示。

[0114] 在一实施方式中，可用于本发明的方法中的抗 FZD 抗体包含重链可变区，该重链可变区包含 :(a) 包含 GFTFSHYTLS(SEQ ID NO :31) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的重链 CDR1 ;(b) 包含 VISGDGSYTYYADSVKG(SEQ ID NO :32) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的重链 CDR2 ;及 / 或 (c) 包含 NFIKYVFAN(SEQ ID NO :33) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的重链 CDR3。在某些实施方式中，该抗 FZD 抗体另包含轻链可变区，该轻链可变区包含 :(a) 包含 SGDKLGKKYAS(SEQ ID NO :41) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的轻链 CDR1 ;(b) 包含 EKDNRPSG(SEQ ID NO :42) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的轻链 CDR2 ;及 / 或 (c) 包含 SSFAGNSLE(SEQ ID NO :43) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的轻链 CDR3。在某些实施方式中，该氨基酸取代系保守性取代。在其他实施方式中，可用于本发明的方法中的抗 FZD 抗体包含 (a) 包含下列的重链可变区 :包含 GFTFSHYTLS(SEQ ID NO :31) 的重链 CDR1、包含 VISGDGSYTYYADSVKG(SEQ ID NO :32) 的重链 CDR2 及包含 NFIKYVFAN(SEQ ID NO :33) 的重链 CDR3，及 / 或 (b) 包含下列的轻链可变区 :包含 SGDKLGKKYAS(SEQ ID NO :41) 的轻链 CDR1、包含 EKDNRPSG(SEQ ID NO : 42) 的轻链 CDR2 及 / 或包含 SSFAGNSLE(SEQ ID NO :43) 的轻链 CDR3。

[0115] 在一实施方式中，可用于本发明的方法中的抗 FZD 抗体包含重链可变区，该重链可变区包含 :(a) 包含 GFTFSHYTLS(SEQ ID NO :31) 或其包含 1、2、3 或 4 个氨基酸取代的变

异体的重链 CDR1；(b) 包含 VISGDGSYTYYADSVKG(SEQ ID NO:32) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的重链 CDR2；及 / 或 (c) 包含 NFIKYVFAN(SEQ ID NO:33) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的重链 CDR3。在某些实施方式中，该抗 FZD 抗体另包含轻链可变区，该轻链可变区包含：(a) 包含 SGDNIGSFYVH(SEQ ID NO:34) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的轻链 CDR1；(b) 包含 DKSNRPSG(SEQ ID NO:35) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的轻链 CDR2；及 / 或 (c) 包含 QSYANTLSL(SEQ ID NO:36) 或其包含 1、2、3 或 4 个氨基酸取代的变异体的轻链 CDR3。在某些实施方式中，该氨基酸取代系保守性取代。在其他实施方式中，可用于本发明的方法中的抗 FZD 抗体包含 (a) 包含下列的重链可变区：包含 GFTFSHYTLS(SEQ ID NO:31) 的重链 CDR1、包含 VISGDGSYTYYADSVKG(SEQ ID NO:32) 的重链 CDR2 及包含 NFIKYVFAN(SEQ ID NO:33) 的重链 CDR3，及 / 或 (b) 包含下列的轻链可变区：包含 SGDNIGSFYVH(SEQ ID NO:34) 的轻链 CDR1、包含 DKSNRPSG(SEQ ID NO:35) 的轻链 CDR2 及包含 QSYANTLSL(SEQ ID NO:36) 的轻链 CDR3。

[0116] 在一实施方式中，可用于本发明的方法中的抗 FZD 抗体包含重链可变区，该重链可变区包含：(a) 包含 GFTFSSYYIT(SEQ ID NO:46) 或其包含 1、2、3 或 4 个保守性氨基酸取代的变异体的重链 CDR1；(b) 包含 TISYSSSNTYYADSVKG(SEQ ID NO:47) 或其包含 1、2、3 或 4 个保守性氨基酸取代的变异体的重链 CDR2；及 / 或 (c) 包含 SIVFDY(SEQ ID NO:48) 或其包含 1、2、3 或 4 个保守性氨基酸取代的变异体的重链 CDR3。在某些实施方式中，该抗 FZD 抗体另包含轻链可变区，该轻链可变区包含：(a) 包含 SGDALGNRYVY(SEQ ID NO:49) 或其包含 1、2、3 或 4 个保守性氨基酸取代的变异体的轻链 CDR1；(b) 包含 SG(SEQ ID NO:50) 或彼的包含 1、2、3 或 4 个保守性氨基酸取代的变异体的轻链 CDR2；及 (c) 包含 GSWDTRPYPKY(SEQ ID NO:51) 或其包含 1、2、3 或 4 个保守性氨基酸取代的变异体的轻链 CDR3。在某些实施方式中，该抗体包含：(a) 包含 GFTFSSYYIT(SEQ ID NO:46) 的重链 CDR1，包含 TISYSSSNTYYADSVKG(SEQ ID NO:47) 的重链 CDR2 及包含 SIVFDY(SEQ ID NO:48) 的重链 CDR3，及 / 或 (b) 包含 SGDALGNRYVY(SEQ ID NO:49) 的轻链 CDR1，包含 SG(SEQ ID NO:50) 的轻链 CDR2 及包含 GSWDTRPYPKY(SEQ ID NO:51) 的轻链 CDR3。

[0117] 在某些实施方式中，可用于本发明的方法中的抗 FZD 抗体包含：(a) 与 SEQ ID NO:37 或 SEQ ID NO:52 具有至少约 80%、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；及 / 或 (b) 与 SEQ ID NO:44、SEQ ID NO:38 或 SEQ ID NO:53 具有至少约 80%、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，可用于本发明的方法中的抗 FZD 抗体包含：(a) 与 SEQ ID NO:37 具有至少约 80%、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；及 (b) 与 SEQ ID NO:38 具有至少约 80%、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，该可用于本发明的方法中的抗 FZD 抗体包含 (a) 具有 SEQ ID NO:37 或 SEQ ID NO:52 的氨基酸序列的重链可变区；及 / 或 (b) 具有 SEQ ID NO:44、SEQ ID NO:38 或 SEQ ID NO:53 的氨基酸序列的轻链可变区。在某些实施方式中，该抗 FZD 抗体包括 (a) 具有 SEQ ID NO:37 的氨基酸序列的重链可变区；及 / 或 (b) 具有 SEQ ID NO:44 的氨基酸序列的轻链可变区。在某些实施方式中，该抗 FZD 抗体包括 (a) 具有 SEQ ID NO:37 的氨基酸序列的重链可变区；及 / 或 (b) 具有 SEQ ID NO:38 的氨基酸序列的轻链可变

区。在某些实施方式中，该抗 FZD 抗体包括 (a) 具有 SEQ ID NO :52 的氨基酸序列的重链可变区 ;及 / 或 (b) 具有 SEQ ID NO :53 的氨基酸序列的轻链可变区。

表 2. 经选择的人抗 FZD 抗体的 VH 及 VL

抗体	重链可变区 (VH) 氨基酸序列	轻链可变区 (VL) 氨基酸序列
18R8	SEQ ID NO:37	SEQ ID NO:44
18R5	SEQ ID NO:37	SEQ ID NO:38
44R24	SEQ ID NO:52	SEQ ID NO:53

[0118] 在某些实施方式中，可用于本发明的方法中的抗 FZD 抗体包含 (a)SEQ ID NO :39 的重链及 SEQ ID NO :45 的轻链 ;或 (b)SEQ ID NO :39 的重链及 SEQ ID NO :40 的轻链。

表 3. 经选择的人抗 FZD 抗体的重链及轻链

抗体	重链可变区 (VH) 氨基酸序列	轻链可变区 (VL) 氨基酸序列
18R8	SEQ ID NO:39	SEQ ID NO:45
18R5	SEQ ID NO:39	SEQ ID NO:40

[0119] 在某些实施方式中，该可用于本发明的方法中的 FZD 结合剂包含下列、实质上由下列组成或由下列组成 :选自 18R8、18R5 及 44R24IgG 抗体的抗 FZD 抗体。

[0120] 在某些实施方式中，该可用于本发明的方法中的 FZD 结合剂包含 (有或无前导序列的)18R8 IgG2 抗体的重链及轻链。在某些实施方式中，该 FZD 结合剂系 18R8 IgG2 抗体。编码该 18R8 IgG2 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-9540 保藏于美国菌种保存中心 (10801 University Boulevard, Manassas, VA, USA)。在某些实施方式中，该可用于本发明的方法中的 FZD 结合剂包含 (有或无前导序列的)18R5 IgG2 抗体的重链及轻链。在某些实施方式中，该 FZD 结合剂系 18R5 IgG2 抗体。该 18R5 IgG2 抗体在此处亦被称为 OMP-18R5。编码该 18R5 IgG2 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。有关 OMP-18R5 抗体的其他信息可见于例如美国专利第 7,982,013 号，其以引用方式整体纳入此处。在美国专利第 7,982,013 号中，该 OMP-18R5 抗体通常被称为“18R5”或“18R5 IgG2 抗体”。

[0121] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂系由 2009 年 8 月 26 日以保藏编号 PTA-10307、PTA-10309 或 PTA-10311 保藏于 ATCC 的质粒所编码的 IgG 抗体。

[0122] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂系 (于例如竞争性结合试验中) 与抗体竞争与 FZD1、FZD2、FZD5、FZD7 及 / 或 FZD8 的特异性结合的剂，该抗体系由具有 ATCC 保藏编号 PTA-9540、PTA-9541、PTA-10307 或 PTA-10309 的质粒所编码。在某些可供选择的实施方式中，该 FZD 结合剂系与抗体竞争与 FZD5 及 / 或 FZD8 的特异性结合的剂，该抗体系由具有 ATCC 保藏编号 PTA-10311 的质粒所编码。

[0123] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂 (例如抗体) 与和 18R5、18R8 或 44R24 抗体的表位相同的表位结合，或与 18R5、18R8 或 44R24 抗体的表位重迭的表

位结合。

[0124] 在某些实施方式中，可用于本发明的方法中的FZD结合剂（例如抗体）与该18R5、18R8或44R24抗体竞争与人卷曲受体的特异性结合。

[0125] 可用于本发明的方法中的其他FZD结合剂的实例系揭示于美国专利申请案公开号2012/0027778，其系以引用方式整体纳入此处。

[0126] 在某些实施方式中，该可用于本发明的方法中的FZD结合剂于小鼠、食蟹猴或人体内具有至少约10小时、至少约24小时、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FZD结合剂系于小鼠、食蟹猴或人体内具有至少约10小时、至少约24小时、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。增加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pH6.0时抗体对新生儿Fc受体（FcRn）的pH依赖性结合（见例如美国专利公开号2005/0276799、2007/0148164及2007/0122403）。增加缺乏Fc区的抗体片段的循环半衰期的已知方法包括像是PEG化的技术。

[0127] 在某些实施方式中，可用于本发明的方法中的抗FZD抗体系特异性辨识人卷曲受体的双特异性抗体。双特异性抗体系可特异性辨识及结合至少二种不同表位的抗体。在一实施方式中，该双特异性抗FZD抗体特异性辨识相同人卷曲受体内的不同表位。在另一实施方式中，该双特异性抗FZD抗体特异性辨识人卷曲受体内或在不同人卷曲受体上的不同表位。

[0128] 另外，在某些可供选择的实施方式中，可用于本发明的方法中的抗FZD抗体不是双特异性抗体。

[0129] 在某些实施方式中，可用于本发明的方法中的抗FZD抗体是单特异性。举例来说，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能与该相同之一或多个人FZD受体（例如FZD1、FZD2、FZD5、FZD7或FZD8，或一些FZD组合的同源性表位）结合。在某些实施方式中，该单特异性抗FZD抗体的抗原结合部位能与一、二、三、四或五（或更多）种人卷曲受体结合。

[0130] 在某些实施方式中，该可用于本发明的方法中的FZD结合剂系不是抗体的多肽。用于识别及产生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, Curr. Opin. Biotechnol., 18:295-304(2007)、Hosse et al., Protein Science, 15:14-27(2006)、Gill et al., Curr. Opin. Biotechnol., 17:653-658(2006)、Nygren, FEBS J., 275:2668-76(2008)及Skerra, FEBS J., 275:2677-83(2008)，各参考文献以引用方式整体纳入此处。

[0131] 在某些实施方式中，可用于本发明的方法中的FZD结合剂包含选自蛋白A、脂质运载蛋白(lipocalin)、纤维黏连蛋白结构域、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。

[0132] 在某些实施方式中，该可用于本发明的方法中的FZD结合剂已经天然或非天然修饰。藉由非限制性实例说明，该多肽可能经标记。在某些实施方式中，该多肽系经糖基化、PEG化、磷酸化或乙酰化酰胺化。在某些实施方式中，该修饰增加该多肽的稳定性及/或活体内半衰期。在某些实施方式中，该多肽系环状。在某些其他实施方式中，该多肽包含一或

多种 N- 甲基氨基酸。

[0133] 在某些实施方式中，可用于本发明的方法中的 FZD 结合剂系（或包含）多肽，该多肽包含选自 SEQ ID NO :54 至 72 的氨基酸序列，或 (b) 与选自 SEQ ID NO :54 至 67 或 69 至 72 的序列具有至少约 80％、至少约 85％、至少约 88％或至少约 90％氨基酸序列一致性的氨基酸序列。在某些实施方式中，该多肽包含下列、实质上由下列组成或由下列组成 :选自 SEQ ID NO :54 至 72 的环状肽。在某些实施方式中，该氨基酸序列系 SEQ ID NO :64。在某些可供选择的实施方式中，该氨基酸序列系 SEQ ID NO :68。

[0134] 在某些实施方式中，可用于本发明的方法中的 FZD 结合多肽小于约 500 个氨基酸长度、小于约 200 个氨基酸长度、小于约 100 个氨基酸长度、小于约 50 个氨基酸长度、小于约 20 个氨基酸长度或小于约 15 个氨基酸长度。在某些实施方式中，该 FZD 结合多肽系至少约 3 个、至少约 5 个或至少约 7 个氨基酸长度。因此，在某些实施方式中，该多肽系介于约 5 至约 20 个氨基酸长度。在一些实施方式中，该多肽系介于约 7 至约 15 个氨基酸长度。

4. 可溶性受体

[0135] 本发明的方法的另一态样系 Wnt 拮抗剂可溶性受体于治疗神经内分泌肿瘤的用途。在某些实施方式中，该可用于本发明的方法中的可溶性受体包含 FZD 受体的胞外域。在一些实施方式中，该可用于本发明的方法中的可溶性受体包含 FZD 受体的 Fri 结构域。在某些实施方式中，该 FZD 受体系人 FZD 受体。在某些实施方式中，该人 FZD 受体系 FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9 或 FZD10。在某些实施方式中，该 FZD 受体系 FZD8。在某些实施方式中，于此处所述的方法中使用的 Wnt 拮抗剂包含人 FZD8Fri 结构域及人 Fc 区。

[0136] 在一些可供选择的实施方式中，该可用于本发明的方法中的可溶性受体包含 SFRP 的一部分。在一些实施方式中，该可用于本发明的方法中的可溶性受体包含 SFRP 的 Fri 结构域。在某些实施方式中，该 SFRP 系人 SFRP。在一些实施方式中，该人 SFRP 系 SFRP1、SFRP2、SFRP3、SFRP4 或 SFRP5。各人 SFRP(SFRP1 至 5) 的最小核心 Fri 结构域序列系提供为 SEQ ID NO :83 至 87。

[0137] 在其他可供选择的实施方式中，该可用于本发明的方法中的可溶性受体包含 ROR 蛋白的胞外域。在一些实施方式中，该可用于本发明的方法中的可溶性受体包含 ROR 蛋白的 Fri 结构域。在某些实施方式中，该 ROR 系人 ROR。在一些实施方式中，该人 ROR 系 ROR1 或 ROR2。该人 ROR1 及 ROR2 的最小核心 Fri 结构域序列系提供为 SEQ ID NO :88 及 SEQ ID NO :89。

[0138] 在某些实施方式中，该可用于本发明的方法中的可溶性受体（例如 FZD8Fri.Fc）与一、二、三、四、五、六、七、八、九、十或更多种 Wnt 蛋白特异性结合。举非限制性实例说明，该 Wnt 结合剂可能与 Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a 及 / 或 Wnt10b 结合。在某些实施方式中，该 Wnt 结合剂与 Wnt1、Wnt2、Wnt3、Wnt3a 及 Wnt7b 结合。在某些实施方式中，该可溶性受体系 Wnt 拮抗剂。在某些实施方式中，该可溶性受体抑制 Wnt 信号传导。在一些实施方式中，该可溶性受体抑制典型 Wnt 信号传导。

[0139] 可用于本发明的方法中的可溶性 FZD 受体的非限制性实例可见于美国专利第 7,723,477 号，其以引用方式整体纳入此处。其他可溶性受体（例如可溶性 FZD 受体）系揭示于 US 2011/0305695，其系以引用方式整体纳入此处。

[0140] 在某些实施方式中，可用于本发明的方法中的可溶性受体包含人 FZD 受体的 Fri 结构域，或与一或多种人 Wnt 蛋白结合的该 Fri 结构域的片段或变异体。在某些实施方式中，该人 FZD 受体系 FZD4。在某些可供选择的实施方式中，该人 FZD 受体系 FZD5。在某些其他可供选择的实施方式中，该人 FZD 受体系 FZD8。在某些实施方式中，该 FZD 系 FZD4 且该可溶性受体包含 SEQ ID NO :76 或包含 SEQ ID NO :90 的大约氨基酸 40 至 170。在某些实施方式中，该 FZD 系 FZD5 且该可溶性受体包含 SEQ ID NO :77 或包含 SEQ ID NO :91 的大约氨基酸 27 至 157。在某些实施方式中，该 FZD 系 FZD8 且该可溶性受体包含 SEQ ID NO :80 或包含 SEQ ID NO :92 的大约氨基酸 28 至 158。

[0141] 在某些实施方式中，该可用于本发明的方法中的可溶性受体包含选自 SEQ ID NO :73 至 89 的最小 Fri 结构域序列。在某些实施方式中，该可用于本发明的方法中的可溶性受体包含前述 Fri 结构域序列的任一者的变异体，其包含一或多个（例如一、二、三、四、五、六、七、八、九、十个等）保守性取代且能与 Wnt 结合。

[0142] 在某些实施方式中，该可用于本发明的方法中的可溶性受体，诸如包含人 FZD 受体的最小 Fri 结构域的可溶性受体，另包含人 Fc 区（例如人 IgG1Fc 区）。包含 FZD 受体的 Fri 结构域及人 IgG1Fc 的可溶性受体在此处称为“FZD Fri.Fc”（例如 FZD8Fri.Fc）。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（例如在绞链结构域）。在一些实施方式中，在绞链结构域的氨基酸系经改变以阻止非所欲的双硫键形成。在一些实施方式中，半胱氨酸系经丝氨酸取代以阻止非所欲的双硫键形成。在某些实施方式中，该 Fc 区包含或由 SEQ ID NO :93、SEQ ID NO :94 或 SEQ ID NO :95 组成。

[0143] 在某些实施方式中，可用于本发明的方法中的可溶性受体系包含至少最小 Fri 结构域（例如 FZD 受体的最小 Fri 结构域）及 Fc 区的融合蛋白。此处所使用的“融合蛋白”系由包含至少二种基因的核苷酸序列的核酸分子表达的杂合蛋白。在一些实施方式中，该第一多肽的 C 端系与该免疫球蛋白 Fc 区的 N 端连接。在一些实施方式中，该第一多肽（例如 FZD Fri 结构域）系与该 Fc 区（即不含插入肽连接子）直接相连。在一些实施方式中，该第一多肽系与该 Fc 区经由肽连接子相连。

[0144] 此处使用的用语“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的技术人员所知，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 :96)、LESGGGGVT(SEQ ID NO :97)、GRAQVT(SEQ ID NO :98)、WRAQVT(SEQ ID NO :99) 及 ARGRAQVT(SEQ ID NO :100)。此处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 端的氨基酸残基的插入肽序列。

[0145] 在某些实施方式中，可用于本发明的方法中的可溶性受体包含引导蛋白运输的信

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，该可用于本发明的方法中的可溶性受体可能包含具有不同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个氨基酸。在一些实施方式中，该可溶性受体多肽为实质上相同，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让一个切割位点变成主要切割位点的氨基酸取代及/或删除，藉此导致具有一种N端的实质上相同的多肽。在一些实施方式中，该多肽的信号序列包含或由选自表3所列的序列组成。在一些实施方式中，该信号序列系SEQ ID NO:101。在一些实施方式中，该信号序列系SEQ ID NO:104。在一些实施方式中，该信号序列系SEQ ID NO:106。

表3. 信号序列。

MEWGYLLEVTSLLAALALLQRSSGAAA	SEQ ID NO:101
MEWGYLLEVTSLLAALALLQRSSGALA	SEQ ID NO:102
MEWGYLLEVTSLLAALALLQRSSGVLA	SEQ ID NO:103
MEWGYLLEVTSLLAALLLLQRSPIVHA	SEQ ID NO:104
MEWGYLLEVTSLLAALFLLQRSPIVHA	SEQ ID NO:105
MEWGYLLEVTSLLAALLLLQRSPFVHA	SEQ ID NO:106
MEWGYLLEVTSLLAALLLLQRSPIIYA	SEQ ID NO:107
MEWGYLLEVTSLLAALLLLQRSPIAHA	SEQ ID NO:108

[0146] 在某些实施方式中，可用于本发明的方法中的可溶性受体包含第一多肽，该第一多肽包含FZD结构域成分及Fc区。在一些实施方式中，该FZD结构域成分系来自FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9或FZD10。在一些实施方式中，该Fc区系来自IgG1免疫球蛋白。在一些实施方式中，该可溶性受体包含：(a)实质上由选自下列的氨基酸组成的第一多肽：SEQ ID NO:11的X1至Y1、SEQ ID NO:12的X2至Y2、SEQ ID NO:13的X3至Y3、SEQ ID NO:14的X4至Y4、SEQ ID NO:15的X5至Y5、SEQ ID NO:16的X6至Y6、SEQ ID NO:17的X7至Y7、SEQ ID NO:18的X8至Y8、SEQ ID NO:19的X9至Y9及SEQ ID NO:20的X10至Y10；及(b)实质上由SEQ ID NO:95的氨基酸A至B组成的第二多肽；其中

X1＝氨基酸69、70、71、72、73、74、75或76

Y1＝氨基酸236、237、238、239、240、241、242或243

X2 ＝氨基酸 22、23、24、25、26、27 或 28

Y2 ＝氨基酸 158、159、160、161、162、163、164、165、166、167、168、169、170、171 或 172

X3 ＝氨基酸 18、19、20、21、22、23、24 或 25

Y3 ＝氨基酸 141、142、143、144、145、146、147、148 或 149

X4 ＝氨基酸 38、39、40、41 或 42

Y4 ＝氨基酸 168、169、170、171、172、173、174、175 或 176

X5 ＝氨基酸 25、26、27、28 或 29

Y5 ＝氨基酸 155、156、157、158、159、160、161、162、163 或 164

X6 ＝氨基酸 19、20、21、22、23 或 24

Y6 ＝氨基酸 144、145、146、147、148、149、150、151 或 152

X7 ＝氨基酸 22、23、24、25、26、27、28、29、30、31、32、33 或 34

Y7 ＝氨基酸 178、179、180、181、182、183、184、185 或 186

X8 ＝氨基酸 25、26、27、28、29、30 或 31

Y8 ＝氨基酸 156、157、158、159、160、161、162、163 或 164

X9 ＝氨基酸 21、22、23 或 24

Y9 ＝氨基酸 137、138、139、140、141、142、143、144、145 或 146

X10 ＝氨基酸 20、21、22、23、24 或 25

Y10 ＝氨基酸 152、153、154、155、156、157、158、159 或 160

A ＝氨基酸 1、2、3、4、5 或 6

B ＝氨基酸 231 或 232。

在一些实施方式中，该第一多肽系与该第二多肽直接相连。在一些实施方式中，该第一多肽系与该第二多肽经由肽连接子相连。在一些实施方式中，该第一多肽系与该第二多肽经由肽连接子 GRA 相连。“实质上由”某些氨基酸“组成”的多肽（例如第一或第二多肽），在一些实施方式中可能在一或两端包括一或多个（例如一、二、三、四或更多个）额外的氨基酸，只要该额外的氨基酸不实质影响该 Wnt 结合剂的功能。

[0147] 在某些实施方式中，可用于本发明的方法中的可溶性受体包含：(a) 实质上由 SEQ ID NO：18 的氨基酸 X 至 Y 组成的第一多肽；及 (b) 实质上由 SEQ ID NO：95 的氨基酸 A 至 B 组成的第二多肽；其中该第一多肽系与该第二多肽直接相连；且其中

X ＝氨基酸 25、26、27、28、29、30 或 31

Y ＝氨基酸 156、157、158、159、160、161、162、163 或 164

A ＝氨基酸 1、2、3、4、5 或 6

B ＝氨基酸 231 或 232。

在一些实施方式中，该第一多肽实质上由 SEQ ID NO：18 的氨基酸 25 至 158 组成。在其他实施方式中，该第一多肽由 SEQ ID NO：18 的氨基酸 25 至 158 组成。在一些实施方式中，该第一多肽实质上由 SEQ ID NO：18 的氨基酸 28 至 158 组成。在其他实施方式中，该第一多肽由 SEQ ID NO：18 的氨基酸 28 至 158 组成。在一些实施方式中，该第一多肽由 SEQ ID NO：18 的氨基酸 31 至 158 组成。在一些实施方式中，该第二多肽由 SEQ ID NO：95 的氨基酸 1 至 232 组成。在一些实施方式中，该第二多肽由 SEQ ID NO：95 的氨基酸 3 至 232 组成。在一些实施方式中，该第二多肽由 SEQ ID NO：95 的氨基酸 6 至 232 组成。在一些实施

方式中，该第一多肽系 SEQ ID NO：28 且该第二多肽系 SEQ ID NO：95。在一些实施方式中，该第一多肽系 SEQ ID NO：28 且该第二多肽系 SEQ ID NO：94。在一些实施方式中，该第一多肽系 SEQ ID NO：28 且该第二多肽系 SEQ ID NO：93。

[0148] 在一些实施方式中，可用于本发明的方法中的可溶性受体包含选自 SEQ ID NO：109 至 121 的氨基酸序列。在某些可供选择的实施方式中，该可溶性受体包含选自 SEQ ID NO：109 至 121 的氨基酸序列，且包含一或多个（例如一、二、三、四、五、六、七、八、九、十个等）保守性取代。在某些实施方式中，可溶性受体包含与选自 SEQ ID NO：109 至 121 的氨基酸序列具有至少约 90％、约 95％或约 98％序列一致性的序列。在某些实施方式中，该变异可溶性受体维持其与一或多种人 Wnt 结合的能力。

[0149] 在某些实施方式中，该可用于本发明的方法中的可溶性受体包含 SEQ ID NO：109 的序列。在某些实施方式中，该可溶性受体包含 SEQ ID NO：115 的序列。在一些实施方式中，该可溶性受体系由 SEQ ID NO：115 所组成的多肽形成的同型二聚体组成。在某些实施方式中，该可溶性受体包含 SEQ ID NO：117 的序列。在一些实施方式中，该可溶性受体系由 SEQ ID NO：117 所组成的多肽形成的同型二聚体组成。

[0150] 在一些实施方式中，该可用于本发明的方法中的可溶性受体（例如 FZD8Fri.Fc）抑制神经内分泌肿瘤或肿瘤细胞的生长。在一些实施方式中，该可溶性受体诱导神经内分泌肿瘤细胞分化。在一些实施方式中，该可溶性受体诱导神经内分泌肿瘤或肿瘤细胞上的分化标志的表达。在某些实施方式中，该可溶性受体减少神经内分泌肿瘤中的癌干细胞的频率。在某些实施方式中，该可溶性受体抑制 Wnt 依赖性神经内分泌肿瘤的生长。在一些实施方式中，包含 SEQ ID NO：115 的可溶性受体抑制神经内分泌肿瘤生长的程度高于包含 SEQ ID NO：109 的可溶性受体。在一些实施方式中，包含 SEQ ID NO：117 的可溶性受体抑制神经内分泌肿瘤生长的程度高于包含 SEQ ID NO：109 的可溶性受体。在一些实施方式中，可溶性受体抑制肿瘤生长的程度高于包含 FZD 结构域成分、Fc 结构域及连接该 FZD 结构域成分与该 Fc 结构域的连接子成分的可溶性受体。在一些实施方式中，该连接子成分系插入肽连接子。

[0151] 在某些实施方式中，该可用于本发明的方法中的可溶性受体（在信号序列切割以前）包含 SEQ ID NO：115 及选自 SEQ ID NO：104 至 108 的信号序列。在一些实施方式中，该可溶性受体（在信号序列切割以前）包含 SEQ ID NO：117 及选自 SEQ ID NO：104 至 108 的信号序列。在一些实施方式中，该可溶性受体包含 SEQ ID NO：105 及 SEQ ID NO：115。在一些实施方式中，该可溶性受体包含 SEQ ID NO：105 及 SEQ ID NO：117。在一些实施方式中，该可溶性受体包含 SEQ ID NO：106 及 SEQ ID NO：115。在一些实施方式中，该可溶性受体包含 SEQ ID NO：106 及 SEQ ID NO：117。在一些实施方式中，该可溶性受体包含 SEQ ID NO：133。

[0152] 在一些实施方式中，该可溶性受体（例如 FZD8Fri.Fc）系实质上经纯化的多肽，其包含选自 SEQ ID NO：109、SEQ ID NO：111、SEQID NO：113、SEQ ID NO：115 及 SEQ ID NO：117 的氨基酸序列。在某些实施方式中，该实质上经纯化的可溶性受体多肽包含至少 80％、至少 90％、至少 95％、至少 97％、至少 98％或至少 99％的多肽具有 ASA 的 N 端序列。在某些实施方式中，该实质上经纯化的可溶性受体多肽系由具有 ASA 的 N 端序列的多肽组成。在一些实施方式中，该新生可溶性受体多肽包含选自 SEQ ID NO：101 至 108 的信号序列。在

一些实施方式中，该新生可溶性受体多肽包含SEQ ID NO：106的信号序列。在一些实施方式中，该新生可溶性受体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。

[0153] 在某些实施方式中，该可溶性FZD受体多肽系OMP-54F28。OMP-54F28系由二条各自由SEQ ID NO：117组成的多肽链形成的同型二聚体。其他有关OMP-54F28的信息可见于美国专利申请案公开号2011/0305695，其系以引用方式整体纳入此处。OMP-54F28在美国专利申请案公开号2011/0305695中通常被称为“54F28”。

[0154] 在某些实施方式中，可用于本发明的方法中的可溶性受体（例如FZD8Fri.Fc）包含免疫球蛋白的Fc区。在某些实施方式中，至少一部分的该Fc区已被删除或以其他方式改变，以提供相较于大约相同免疫原性的包含天然或未经改变的Fc恒定区的可溶性受体所欲的生化或生物特征，诸如增加癌细胞定位、增加肿瘤穿透、减少血清半衰期或增加血清半衰期、减少或不具ADCC活性、减少或不具补体依赖性细胞毒性(CDC)。对Fc区的修饰可能包括添加、删除或取代一或多个结构域中的一或多个氨基酸。其他包含经修饰的Fc区的可溶性受体（例如可溶性FZD受体）系揭示于US2011/0305695，其系以引用方式整体纳入此处。

[0155] 在某些实施方式中，可用于本发明的方法中的可溶性受体（例如FZD8Fri.Fc）以约1μM或更低、约100nM或更低、约40nM或更低、约20nM或更低或约10nM或更低的解离常数(K_D)与至少一种Wnt结合。该可溶性受体的特异性结合可使用该领域已知的任何方法检测。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,eds,1994,Current Protocols in Molecular Biology,Vol.1,John Wiley&Sons,Inc.,New York，其系以引用方式整体纳入此处）。

[0156] 在某些实施方式中，可用于本发明的方法中的可溶性受体（例如FZD8Fri.Fc）系该可溶性受体所结合的至少一种Wnt（即1、2、3、4、5、6、7、8、9或10种Wnt）的拮抗剂。在某些实施方式中，该可溶性受体抑制至少约10％、至少约20％、至少约30％、至少约50％、至少约75％、至少约90％或约100％的该经结合的人Wnt的一或多种活性。用于测定可溶性受体是否抑制Wnt信号传导的活体内及体外测定系该领域所知。适当方法系揭示于US2011/0305695，其系以引用方式整体纳入此处。

[0157] 在某些实施方式中，可用于本发明的方法中的可溶性受体（例如FZD8Fri.Fc）系经水溶性聚合物衍生化。适当的水溶性聚合物包括但不限于聚乙二醇(PEG)、乙二醇/丙二醇的共聚物、羧基甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚-1,3-二草酸酯、聚-1,3,6-三氧杂环已烷(poly1,3,6-trioxane)、乙烯/顺丁烯二酸酐共聚物、聚氨基酸（不论是同元共聚物或随机共聚物）、葡聚糖、聚(n-乙烯基吡咯烷酮)-聚乙二醇、丙二醇同元共聚物、聚环氧丙烷/环氧乙烷共聚物、聚氧乙基化多元醇（例如甘油）、聚乙烯醇及彼等的混合物。在某些实施方式中，该水溶性聚合物系聚乙二醇(PEG)。

[0158] 在某些实施方式中，该可用于本发明的方法中的可溶性受体（例如FZD8Fri.Fc）于小鼠、食蟹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，该可溶性受体以约2mg/kg至约10mg/kg的剂量经尾静脉投予至大鼠时，具有至少约50小时的半衰期。在某些实施方式中，该可溶性受体系包含人FZD受体的Fri结构域（或与一或多种Wnt结合的该Fri结构域的片段或变异体）及人Fc区的可溶性FZD受体，且相较于包含该FZD受体的胞外域及

人 Fc 区的可溶性 FZD 受体具有更长的活体内（例如小鼠或大鼠）半衰期。

5. 抗 -Wnt 抗体

[0159] 本发明的方法的其他态样系抗 Wnt 抗体于治疗神经内分泌肿瘤的用途。在某些实施方式中，该可用于本发明的方法中的抗 Wnt 抗体与一或多种 Wnt 多肽特异性结合。在某些实施方式中，该抗体与二、三、四、五、六、七、八、九、十或多种 Wnt 特异性结合。与该抗体结合的人 Wnt 可能选自 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，与该抗体或其他抗体结合的一或多种（或二或多种、三或多种、四或多种、五或多种等）Wnt 包含 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（或二或多种、三或多种、四或多种、五或多种等）Wnt 包含 Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt8a、Wnt8b、Wnt10a 及 Wnt10b。

[0160] 在某些实施方式中，可用于本发明的方法中的 Wnt 结合抗体的个别抗原结合部位能与一、二、三、四或五（或更多）种人 Wnt 结合。在某些实施方式中，该 Wnt 结合抗体的个别抗原结合部位能与选自 Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a 及 Wnt10b 的一、二、三、四或五种人 Wnt 特异性结合。

[0161] 在某些实施方式中，该可用于本发明的方法中的 Wnt 结合抗体与人 Wnt 的 C 端多半胱氨酸区结合。在某些实施方式中，该抗体与选自 SEQ ID NO：122 至 132 的结构域（位于与该抗体结合的该一或多种 Wnt 蛋白之内）结合。在一些实施方式中，该 Wnt 结合抗体与 SEQ ID NO：122 以内结合。在一些实施方式中，该 Wnt 结合抗体与 Wnt1 的氨基酸 288 至 370 以内结合。

[0162] 在某些实施方式中，该可用于本发明的方法中的 Wnt 结合抗体以约 1μM 或更低、约 100nM 或更低、约 40nM 或更低、约 20nM 或更低或约 10nM 或更低的解离常数（K_D）与一或多种（例如二或多种、三或多种或四或多种）Wnt 结合。举例来说，在某些实施方式中，与超过一种 Wnt 结合的可用于本发明的方法中的 Wnt 结合抗体以约 100nM 或更低、约 20nM 或更低或约 10nM 或更低的 K_D 与该些 Wnt 结合。在某些实施方式中，该 Wnt 结合抗体以约 40nM 或更低的解离常数与下列一或多种（例如 1、2、3、4 或 5 种）Wnt 的各者结合：Wnt1、Wnt2、Wnt2b、Wnt3、Wnt3a、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt10a 及 Wnt10b。

[0163] 在某些实施方式中，该可用于本发明的方法中的抗 Wnt 抗体系 IgG1 抗体或 IgG2 抗体。在某些实施态样中，该抗体系单克隆抗体。在某些实施方式中，该抗体系人抗体或人源化抗体。在某些实施方式中，该抗体系抗体片段。

[0164] 本发明的抗体或其他抗体的特异性结合可使用该领域已知的任何方法检测。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, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley&Sons, Inc., New York，其系以引用方式整体纳入此处）。

[0165] 在某些实施方式中，可用于本发明的方法中的 Wnt 结合抗体系该抗体所结合的至少一种 Wnt（即 1、2、3、4、5、6、7、8、9 或 10 种 Wnt）的拮抗剂。在某些实施方式中，该抗体抑制至少约 10％、至少约 20％、至少约 30％、至少约 50％、至少约 75％、至少约 90％或约 100％的该经结合的人 Wnt 的一或多种活性。

[0166] 在某些实施方式中，该可用于本发明的方法中的 Wnt 结合抗体抑制配体与该至少

一种人 Wnt 的结合。在某些实施方式中，该 Wnt 结合抗体抑制人 Wnt 蛋白与彼的一或多种配体的结合。十九种人 Wnt 蛋白已被识别 :Wnt1、Wnt2、Wnt2B/13、Wnt3、Wnt3a、Wnt4、Wnt5a、Wnt5b、Wnt6、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt9a（前称 Wnt14）、Wnt9b（前称 Wnt15）、Wnt10a、Wnt10b、Wnt11 及 Wnt16。十种人 FZD 受体蛋白已被识别 (FZD1、FZD2、FZD3、FZD4、FZD5、FZD6、FZD7、FZD8、FZD9 及 FZD10)。在某些实施方式中，该 Wnt 结合抗体抑制 FZD4、FZD5 及 / 或 FZD8 与一或多种 Wnt（例如 Wnt3a) 的结合。在某些实施方式中，由该 Wnt 结合抗体所提供的对特定配体与 Wnt 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 与配体诸如 FZD 结合的抗体另抑制 Wnt 信号传导（例如抑制典型 Wnt 信号传导）。

[0167] 在某些实施方式中，该可用于本发明的方法中的 Wnt 结合抗体抑制 Wnt 信号传导。应了解的是，抑制 Wnt 信号传导的 Wnt 结合抗体在某些实施方式中可抑制藉由一或多种 Wnt 的信号传导，但不一定抑制藉由所有 Wnt 的信号传导。在某些选择性实施方式中，藉由所有人 Wnt 的信号传导皆可被抑制。在某些实施方式中，藉由选自 Wnt1、Wnt2、Wnt2b/13、Wnt3、Wnt3a、Wnt4、Wnt5a、Wnt5b、Wnt6、Wnt7a、Wnt7b、Wnt8a、Wnt8b、Wnt9a（前称 Wnt14）、Wnt9b（前称 Wnt15）、Wnt10a、Wnt10b、Wnt11 及 Wnt16 的一或多种 Wnt 的信号传导系经抑制。在某些实施方式中，该经抑制的 Wnt 信号传导系由 Wnt1、Wnt2、Wnt3、Wnt3a、Wnt7a、Wnt7b 及 / 或 Wnt10b 信号传导。在某些实施方式中，该抗体抑制藉由（至少）Wnt1、Wnt3a、Wnt7b 及 Wnt10b 的信号传导。在特定实施方式中，该抗体抑制藉由（至少）Wnt3a 的信号传导。在某些实施方式中，由该 Wnt 结合抗体所提供的对藉由 Wnt 信号传导的抑制系减少该 Wnt 信号传导量至少约 10％、至少约 25％、至少约 50％、至少约 75％、至少约 90％或至少约 95％。在某些实施方式中，该被抑制的 Wnt 信号传导系典型 Wnt 信号传导。

[0168] 用于测定 Wnt 结合抗体是否抑制 Wnt 信号传导的活体内及体外测定系该领域所知。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典型 Wnt 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)。在一或多种 Wnt（例如由转染细胞表达或由 Wnt 条件培养基提供的 Wnt) 存在且有该 Wnt 结合抗体存在时的 Wnt 信号传导量系与无该 Wnt 结合抗体存在时的信号传导量比较。除了 TCF/Luc 报告子测定之外，Wnt 结合抗体（或候选抗体）对典型 Wnt 信号传导的影响可于体外或活体内藉由测量该抗体对 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 / 或纤维黏连蛋白 (Gradl et al.1999,Mol.Cell Biol.,19 :5576-87)。在某些实施方式中，抗体对 Wnt 信号传导的影响亦可能藉由测量该抗体对 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。

[0169] 在某些实施方式中，可用于本发明的方法中的 Wnt 结合抗体具有一或多种下列效应 :抑制神经内分泌肿瘤细胞增生、藉由减少肿瘤中的癌干细胞的频率以减少该神经内分泌肿瘤的肿瘤发生性、抑制神经内分泌肿瘤生长、诱发神经内分泌肿瘤细胞的细胞死亡、使神经内分泌肿瘤发生性细胞分化成非肿瘤发生性状态、预防神经内分泌肿瘤细胞转移或减少存活。

[0170] 在某些实施方式中，可用于本发明的方法中的 Wnt 结合抗体能抑制神经内分泌肿

瘤生长。在某些实施方式中，该 Wnt 结合抗体可抑制活体内的神经内分泌肿瘤生长（例如在异种移植小鼠模型及 / 或于罹患癌的人体内）。

[0171] 在某些实施方式中，可用于本发明的方法中的 Wnt 结合抗体能减少神经内分泌肿瘤的肿瘤发生性。在某些实施方式中，该抗体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神经内分泌肿瘤的肿瘤发生性。在某些实施方式中，神经内分泌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、美国专利申请案公开号 2008/0064049 及美国专利申请案公开号 2008/0178305，彼等各自以引用方式整体纳入此处。

[0172] 在某些实施方式中，该可用于本发明的方法中的 Wnt 结合抗体于小鼠、食蟹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 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 结合抗体系于小鼠、食蟹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 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。

[0173] 在某些实施方式中，可用于本发明的方法中的抗 Wnt 抗体系特异性辨识人 Wnt 的双特异性抗体。双特异性抗体系可特异性辨识及结合至少二种不同表位的抗体。在一实施方式中，该双特异性抗 Wnt 抗体特异性辨识相同人 Wnt 内的不同表位。在另一实施方式中，该双特异性抗 Wnt 抗体特异性辨识不同人 Wnt 内或在不同 Wnt 上的不同表位。

[0174] 另外，在某些可供选择的实施方式中，可用于本发明的方法中的抗 Wnt 抗体不是双特异性抗体。

[0175] 在某些实施方式中，可用于本发明的方法中的抗 Wnt 抗体是单特异性。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相同的一或多种人 Wnt。在某些实施方式中，该单特异性抗体的抗原结合部位能与一、二、三、四或五（或更多）种人 Wnt 结合。

[0176] 可用于本发明的方法中的抗 Wnt 抗体系揭示于国际专利公开号 WO 2011/088127，其系以引用方式整体纳入。

6. 抗体及其制备

[0177] 可用于本发明的方法中的抗体（例如抗 FZD 及抗 Wnt 抗体）可藉由该领域已知的任何适当方法生成。多克隆抗体可利用任何已知的方法制备。多克隆抗体系藉由多次皮下或腹腔内注射该相关抗原（经纯化的肽片段、全长重组蛋白、融合蛋白等）至动物（例如兔、大鼠、小鼠、驴等）以免疫培养，该等相关抗原可任意选择地与钥孔状帽贝血蓝素 (KLH)、血清蛋白等共轭并经无菌盐水稀释且与佐剂（例如完全或不完全弗氏佐剂）组合以形成安定乳剂。然后自经如此免疫的动物的血液、腹水及类似体液收集该多克隆抗体。使收集的血液凝集，倒出血清，离心澄清并测试抗体力价。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，该等方法包括亲和性层析、离子交换层析、胶体电泳、透析等。

[0178] 单克隆抗体可利用杂交瘤方法制备，诸如该些于 Kohler and Milstein(1975) Nature 256 :495 中所描述者。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，以诱发淋巴细胞生成将与免疫抗原特异性结合的抗体。淋巴细胞亦可在体

外免疫。在免疫后，该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。经免疫沉淀、免疫转渍或体外结合试验（例如放射性免疫测定 (RIA)、酶连接免疫吸附测定 (ELISA)）测定后，生成特异性拮抗选定抗原的单克隆抗体的杂交瘤接着可利用标准方法于体外培养复制 (Goding, Monoclonal Antibodies :Principles and Practice, Academic Press, 1986)，或于动物活体内以腹水肿瘤复制。该单克隆抗体接着可如上述制备多克隆抗体的方法自该培养基或腹水液体纯化。

[0179] 另外，单克隆抗体亦可利用美国专利第 4,816,567 号所述的重组 DNA 方法制备。编码单克隆抗体的多核苷酸系自成熟 B 细胞或杂交瘤细胞分离，像是藉由 RT-PCR 使用寡核苷酸引子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常规技术测定。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，该载体经转染至原本不产生免疫球蛋白的宿主细胞诸如大肠杆菌 (E. coli) 细胞、类人猿 COS 细胞、中国仓鼠卵巢 (CHO) 细胞或骨髓瘤细胞后，该宿主细胞生成单克隆抗体。同样地，所欲物种的重组单克隆抗体或彼的片段可如文献所述地自表达该所欲物种的 CDR 的噬菌体展示库分离 (McCafferty et al., 1990, Nature, 348 :552-554、Clackson et al., 1991, Nature, 352 : 624-628 及 Marks et al., 1991, J. Mol. Biol., 222 :581-597)。

[0180] 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，以生成可供选择的抗体。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 1) 可被例如人抗体的该些区域取代以生成嵌合抗体，或 2) 以非免疫球蛋白多肽取代以生成融合抗体。在一些实施方式中，该等恒定区系经截短或移除以生成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。

[0181] 在一些实施方式中，该可用于本发明的方法中的单克隆抗体系人源化抗体。在某些实施方式中，该等抗体系于治疗上使用以减少当投予至人个体时的抗原性及 HAMA（人抗小鼠抗体）反应。人源化抗体可利用该领域已知的多种技术制备。在某些可供选择的实施方式中，该可用于本发明的方法中的抗体系人抗体。

[0182] 人抗体可利用该领域已知的多种技术直接制备。生成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生成，或可自免疫个体分离（见例如 Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) ; Boemer et al., 1991, J. Immunol., 147 (1) :86-95 ; and U. S. Patent 5,750,373)。同样地，该人抗体可选自噬菌体库，其中该噬菌体库表达人抗体，例如于 Vaughan et al., 1996, Nat. Biotech., 14 :309-314, Sheets et al., 1998, Proc. Nat' l. Acad. Sci., 95 : 6157-6162, Hoogenboom and Winter, 1991, J. Mol. Biol., 227 :381, and Marks et al., 1991, J. Mol. Biol., 222 :581 中所述。生成及使用抗体噬菌体库的技术亦描述于美国专利第 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., 2007, J. Mol. Bio., doi :10. 1016/j. jmb. 2007. 12. 018（各文献以引用方式整体纳入此处）。亲和性成熟策略及链改组策略 (Marks et al., 1992, Bio/Technology10 :779-783，以引用方式整体纳入）系该领域所知且可能被应用以生成高亲和性人抗体。

[0183] 人源化抗体亦可于包含人免疫球蛋白基因座的转基因小鼠中制备，如此在动物经免疫时能生成整套人抗体而不生成内源性免疫球蛋白。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。

[0184] 在某些实施方式中，可用于本发明的方法中的抗体系特异性辨识人卷曲受体或人Wnt多肽的双特异性抗体。双特异性抗体系可特异性辨识及结合至少二种不同表位的抗体。不同的表位可位于相同分子的内（例如相同的人卷曲受体或相同的人Wnt多肽）或位于不同分子上。双特异性抗体可为完整抗体或抗体片段。

[0185] 另外，在某些可供选择的实施方式中，可用于本发明的抗体不是双特异性抗体。

[0186] 在某些实施方式中，可用于本发明的抗体是单特异性。举例来说，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相同的人FZD受体或相同的人Wnt多肽。在某些实施方式中，单特异性抗体的抗原结合部位能与一、二、三、四或五（或更多）种人卷曲受体或人Wnt多肽结合。

[0187] 在某些实施方式中，可用于本发明的方法中的抗体是抗体片段。抗体片段相对于全抗体可展现增加的肿瘤穿透性。多种技术已知可用于生成抗体片段。传统上，该些片段系得自完整抗体的蛋白水解消化（例如Morimoto et al.,1993,Journal of Biochemical and Biophysical Methods 24:107-117;Brennan et al,1985,Science,229:81)。在某些实施方式中，抗体片段系经重组产生。Fab、Fv及scFv抗体片段皆可在大肠杆菌或其他宿主细胞中表达及分泌，因此允许大量生成这些片段。该等抗体片段亦可自如上讨论的抗体噬菌体库分离。该抗体片段亦可为例如美国专利第5,641,870号所述的线性抗体，且可为单特异性或双特异性。可用于本发明的方法中的单链抗体可如例如美国专利第4,946,778号所述制备。此外，可改变方法以建构允许快速有效地识别对FZD受体或Wnt多肽具有所欲特异性的单克隆Fab片段的Fab表达库(Huse,et al.,Science 246:1275-1281(1989))。抗体片段可藉由该领域的技术生成，包括但不限于：(a)藉由胃蛋白酶消化抗体分子所生成的F(ab')2片段；(b)藉由减少F(ab')2片段的双硫键所生成的Fab片段；(c)藉由以木瓜酶及还原剂处理抗体分子所生成的Fab片段；及(d)Fv片段。其它用于生成抗体片段的技术将为技术人员所显而易见。

[0188] 另外尤其以抗体片段来说所欲的是，修饰抗体以增加彼的血清半衰期。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。

[0189] 在某些实施方式中，可用于本发明的方法中的抗体是异源共轭抗体。异源共轭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）。考虑到该等抗体可利用已知的合成蛋白质化学方法于体外制备，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氨基硫醇盐及甲基-4-巯基丁亚氨酸酯。

[0190] 该领域已知的是恒定Fc区介导数种效应功能。举例来说，补体的C1成分与抗体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。另外，抗体或可溶性受体可经由Fc区与细胞结

合，藉由抗体上的 Fc 受体部位使 Fc 区与细胞上的 Fc 受体 (FcR) 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（称为抗体依赖性细胞媒介性细胞毒性或 ADCC）、释放发炎介质、胚胎转移及控制免疫球蛋白的生成。

[0191] 在某些实施方式中，可用于本发明的方法中的 Wnt 拮抗剂多肽（抗体及包含可溶性受体的 Fc）提供改变的效应功能，因此影响该投予多肽的生物活性。举例来说，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与 Fc 受体结合，藉此增加肿瘤定位。在其他情况中，修饰恒定区可能影响补体结合，因此减少共轭细胞毒素的血清半衰期及非特异性结合。对恒定区的其他修饰可被用于消除双硫键结或寡糖结构单元以允许促进定位，因为增加抗原特异性或抗体柔韧性。同样地，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。

[0192] 在某些实施方式中，可用于本发明的方法中的包含 Fc 区的 Wnt 拮抗剂多肽（抗体及包含可溶性受体的 Fc）不具有一或多种效应功能。例如在一些实施方式中，该多肽不具抗体依赖性细胞性细胞毒性 (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，该多肽不与 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。

[0193] 本发明亦关于免疫共轭物的用途，该免疫共轭物包含与细胞毒性剂共轭的 Wnt 拮抗剂多肽（例如抗 FZD 及抗 Wnt 抗体）。细胞毒性剂包括化学治疗剂、生长抑制剂、毒素（例如细菌、真菌、植物或动物来源的酶活性毒素或其片段）、放射性同位素（即放射共轭物）等。可用于生成该免疫共轭物的化学治疗剂包括例如甲胺喋呤 (methotrexate)、甲烯土霉素 (adriamycin)、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。可被使用的酶活性毒素及彼等的片段包括白喉毒素 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)。多种放射性核种可用于生成经放射共轭的抗体，包括 ^{212}Bi、^{131}I、^{131}In、^{90}Y 及 ^{186}Re。抗体与细胞毒性剂的共轭物可利用各种双官能性蛋白偶合剂制备，例如 N- 琥珀酰亚氨基 -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）、双迭氮化合物（诸如双（对 - 迭氮苯甲酰基）己二胺）、双重氮衍生物（诸如双 -（对 - 重氮苯甲酰基）- 乙二胺）、二异氰酸酯（诸如 2,6- 二异氰酸甲苯酯）及双活性氟化合物（诸如 1,5- 二氟 -2,4- 二硝苯）。本发明亦可使用抗体与一或多种小分子毒素的共轭物，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。

[0194] 共轭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）。考虑到该等抗体可利用已知的合成蛋白质化学方法于体外制备，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氨基硫醇盐及甲基 -4- 巯基丁亚胺酸酯。

[0195] 无论如何获得可用的量，可用于本发明的方法中的 Wnt 拮抗剂多肽（例如抗体及可溶性受体）可被用于任何一种共轭（即免疫共轭物）或非共轭形式。另外，该多肽可以非共轭或“未经修饰 (naked)”的形式使用。在某些实施方式中，该多肽可以非共轭形式被使用以驾驭个体的天然防御机制，包括补体依赖性细胞毒性 (CDC) 及抗体依赖性细胞毒性 (ADCC)，以消灭恶性细胞。在一些实施方式中，该多肽可利用任何广为周知的螯合剂或直接表示法以与放射性同位素共轭，诸如 ^{90}Y、^{125}I、^{131}I、^{123}I、^{111}In、^{105}Rh、^{153}Sm、^{67}Cu、^{67}Ga、^{166}Ho、^{177}Lu、^{186}Re 及 ^{188}Re。在其他实施方式中，该组合物可包含与药物、前药或生物反应调节剂偶合的 Wnt 拮抗剂多肽，诸如甲胺喋呤 (methotrexate)、甲烯土霉素 (adriamycin) 及淋巴介质诸如干扰素。其他实施方式包含使用与特定生物毒素共轭的 Wnt 拮抗剂多肽，诸如蓖麻毒蛋白 (ricin) 或白喉毒素。又其他实施方式中，该 Wnt 拮抗剂多肽可与其他免疫活性配体（例如抗体或其片段）复合，其中该形成的分子与肿瘤细胞及效应细胞诸如 T 细胞结合。选择使用何种共轭或非共轭 Wnt 拮抗剂多肽将取决于神经内分泌肿瘤的类型及分期、辅佐治疗的使用（例如化学疗法或外部放射）及病患情况。将了解的是该领域的技术人员可根据此处的揭示轻易做出选择。

[0196] 该多肽及类似物可另经修饰以包含正常非该蛋白的部分的额外化学基团。该等衍生化基团可增进该蛋白的溶解性、生物半衰期或吸收。该等基团亦可减少或消除该蛋白及类似物的任何非所欲的不良反应。对该等基团的综述可见 Remington’s Pharmaceutical Sciences,20th ed.,Mack Publishing Co.,Easton,PA(2000)。

[0197] 最适合用于衍生的化学基团包括水溶性聚合物。水溶性聚合物系为所欲，因为其所连接的该蛋白不会沉淀于水性环境，诸如生理环境。在一些实施方式中，该聚合物将为医药上可接受用于制备治疗用的产品或组合物。该领域的技术人员将能根据该聚合物 / 蛋白共轭物是否将作为治疗用途的考虑及若作为治疗用途的所欲剂量、循环时间、对蛋白水解的抗性及其他考虑选择该所欲的聚合物。衍生化的有效性可藉由投予所欲形式（即藉由渗透泵，或藉由注射或输注，或进一步调制成口服、经肺或其他递送途径）的该衍生物并测定其有效性加以确定。适当的水溶性聚合物包括但不限于聚乙二醇 (PEG)、乙二醇 / 丙二醇的共聚物、羧基甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚 -1,3- 二草酸酯、聚 -1,3,6- 三氧杂环己烷、乙烯 / 顺丁烯二酸酐共聚物、聚氨基酸（不论是同元共聚物或随机共聚物）、葡聚糖、聚 (n- 乙烯基吡咯烷酮）- 聚乙二醇、丙二醇同元共聚物、聚环氧丙烷 / 环氧乙烷共聚物、聚氧乙基化多元醇（例如甘油）、聚乙烯醇及彼等的混合物。聚乙二醇丙醛具有制造优点，因为其在水中稳定。

[0198] 可用于本发明的方法中的分离多肽（例如抗体及可溶性受体）可藉由该领域已知的任何适当方法生成。该等方法从直接蛋白质合成方法至建构编码分离多肽序列的 DNA 序列及在适当转化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彼的功能性类似物。见例如 Zoeller et al.,Proc.Nat'l. Acad.Sci.USA81 :5662-5066(1984) 及美国专利第 4,588,585 号。

[0199] 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。该等寡核苷酸可根据该所欲多肽的氨基酸序列设计,并选择该些将生成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分离多核苷酸序列。举例来说,完全氨基酸序列可被用于建构回译基因 (back-translated gene)。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。

[0200] 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分离多肽的多核苷酸序列将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。

[0201] 在某些实施方式中,重组表达载体系用于扩增及表达 Wnt 拮抗剂多肽 (例如抗体或可溶性受体)。重组表达载体为可复制的 DNA 建构体,其具有与适当转录或翻译调节组件可操作性连接的编码感兴趣多肽的合成性或 cDNA 衍生性 DNA 片段,该等适当转录或翻译调节组件系源自哺乳动物、微生物、病毒或昆虫基因。转录单位通常包含下列的组合 :(1) 于基因表达中具有调节作用的基因组件,例如转录启动子或增强子,(2) 经转录成 mRNA 然后翻译成蛋白质的结构或编码序列,及 (3) 适当的转录及翻译启动及终止序列,如下列详述。该等调节组件可包括操作子序列以控制转录。通常由复制起点授予的于宿主内复制的能力及有利转化体辨识的选择基因可被额外纳入。当 DNA 区彼此之间系功能性相关时,彼等系可操作性连接。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。适用于酵母菌表达系统的结构组件包括使宿主细胞得以胞外分泌经翻译的蛋白质的前导序列。或者,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。

[0202] 表达控制序列及表达载体的选择将取决于宿主选择。多样化的表达宿主 / 载体组合可被采用。可用于真核宿主的表达载体包括例如包含源自 SV40、牛乳头状瘤病毒、腺病毒及巨细胞病毒的表达控制序列的载体。可用于细菌宿主的表达载体包括已知的细菌质粒,像是源自大肠杆菌的质粒 (包括 pCR 1、pBR322、pMB9 及彼等的衍生物),广泛宿主范围质粒像是 M13 及丝状单股 DNA 噬菌体。

[0203] 用于表达 Wnt 拮抗剂多肽 (例如抗体或可溶性受体) 的适当宿主细胞包括在适当启动子控制下的原核生物、酵母菌、昆虫或高级真核细胞。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 (bacilli)。高级真核细胞包括如下所述的哺乳动物来源的株化细胞系。不含细胞的翻译系统亦可被采用。用于细菌性、真菌性、酵母菌性及哺乳动物细胞性宿主的适当克隆及表达载体系描述于 Pouwels et al.(Cloning Vectors :ALaboratory Manual,Elsevier,N.Y.,1985),其相关揭示以引用方

式纳入此处。有关蛋白质生成（包括抗体生成）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 WO04009823，这些文献各自以引用方式整体纳入此处。

[0204] 多种哺乳动物或昆虫细胞培养系统亦可被有利地采用以表达重组蛋白。于哺乳动物细胞中表达重组蛋白可被实施，因为这些蛋白通常经过正确折叠、适当修饰且具完全功能性。适当的哺乳动物宿主细胞系实例包括由 Gluzman(Cell23 :175, 1981) 所述的猴肾细胞 COS-7 系，及其他能表达适当载体的细胞系，包括例如 L 细胞、C127、3T3、中国仓鼠卵巢 (CHO)、HeLa 及 BHK 细胞系。哺乳动物表达载体可包含非转录组件（诸如复制起点、与所欲表达的基因相连的适当启动子及增强子，及其他 5’ 或 3’ 侧翼非转录序列）及 5’ 或 3’ 非翻译序列（诸如必要的核糖体结合位点、聚腺苷酸化位点、剪接供点及受点，及转录终止序列）。用于在昆虫细胞中生成异源性蛋白的杆状病毒 (baculovirus) 系统系由 Luckow 及 Summers 回顾 (Bio/Technology6 :47(1988))。

[0205] 由经转化的宿主生成的蛋白质可根据任何适当方法纯化。该等标准方法包括层析（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 (influenza coat sequence) 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。经分离的蛋白亦可利用诸如蛋白水解、核磁共振及 x 光结晶技术进行物理特征分析。

[0206] 举例来说，源自分泌重组蛋白质至培养基的系统的上清液可利用商用蛋白质浓缩过滤器先行浓缩，例如使用阿密康 (Amicon) 或密里博 (Millipore)Pellicon 超过滤单位浓缩。在浓缩步骤之后，该浓缩液可被加至适当纯化基材。或者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。另外，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。最后，使用疏水性 RP-HPLC 介质（例如具有悬垂甲基或其他脂肪基的硅胶）之一或多种逆相高效液相层析 (RP-HPLC) 步骤可被实行，以进一步纯化 Wnt 拮抗剂多肽（例如抗体或可溶性受体）。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。

[0207] 于细菌培养中生产的重组蛋白可被分离，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。高效液相层析 (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。

[0208] 该领域已知的用于纯化 Wnt 拮抗剂多肽（例如抗体或可溶性受体）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 中所述者，各文献以引用方式整体纳入此处。

7. 药物组合物

[0209] Wnt 拮抗剂多肽（例如抗体及可溶性受体）可藉由该领域已知的任何适当方法被调制成医药组合物。在某些实施方式中，该医药组合物包含医药上可接受的载体。该医药组合物于人病患中具有抑制神经内分泌肿瘤生长及治疗神经内分泌肿瘤的用途。

[0210] 在某些实施方式中，制剂系藉由组合经纯化的 Wnt 拮抗剂（例如抗 FZD 抗

体或可溶性FZD受体）与医药上可接受的载体（例如载剂、赋形剂）制备以供储存及使用(Remington,The Science and Practice of Pharmacy 20th Edition Mack Publishing,2000)。适当的医药上可接受的载体包括但不限于非毒性缓冲剂诸如磷酸盐、柠檬酸盐及其他有机酸；盐诸如氯化钠；抗氧化剂包括抗坏血酸及甲硫胺酸；防腐剂（例如十八基二甲基苄基氯化铵、六甲氯胺、氯化苯甲烃铵、氯化苄乙氧铵、酚醇、丁醇、苄醇、烷基对羟苯甲酸酯类诸如对羟苯甲酸甲酯或对羟苯甲酸丙酯、儿茶酚、间苯二酚、环己醇、3-戊醇及间甲酚）；低分子量多肽（例如少于约10个氨基酸残基）；蛋白质诸如血清白蛋白、明胶或免疫球蛋白；亲水性聚合物诸如聚乙烯基吡咯烷酮；氨基酸诸如甘氨酸、谷氨酰胺、天冬酰胺酸、组氨酸、精氨酸或赖氨酸；碳水化合物诸如单醣、双醣、葡萄糖、甘露糖或葡聚糖；螯合剂诸如EDTA；糖类诸如蔗糖、甘露醇、海藻糖或山梨醇；盐形成抗衡离子诸如钠；金属复合物（例如锌蛋白质复合物）；及非离子性界面活性剂诸如TWEEN或聚乙二醇(PEG)。

[0211] 在某些实施方式中，该医药组合物系经冷冻。在某些替代性实施方式中，该医药组合物系经冷冻干燥。

[0212] 本发明的医药组合物可以任何数量的方式经局部或全身投予。投予可为局部（诸如投予至黏膜包括阴道及直肠投予）诸如经皮贴片、软膏、乳液、乳膏、凝胶剂、滴剂、栓剂、喷雾剂、液剂及粉剂；经肺（例如藉由吸入或喷入粉剂或气雾剂，包括藉由喷雾器、气管内、鼻内、表皮及经皮投予）；经口；或非经肠投予包括静脉内、动脉内、皮下、腹膜内或肌肉内注射或输注；或颅内（例如脊椎鞘内或心室内）给药。

[0213] 该治疗制剂可呈单位剂量形式。该等制剂包括片剂、丸剂、胶囊、粉剂、颗粒剂、水或非水性介质的溶液或悬浮液，或用于口服、非经肠或直肠投予或用于藉由吸入投予的栓剂。在固态组合物诸如片剂中，该主要活性成分系与医药载剂混合。常规的制片剂成分包括玉米淀粉、乳糖、蔗糖、山梨醇、滑石、硬脂酸、硬脂酸镁、磷酸二钙或胶及其他稀释剂（例如水）混合，以形成含有本发明的化合物的均质混合物的或其非毒性医药上可接受的盐的固体预调制组合物。该固体预调制组合物接着被分成上述种类的单位剂量形式。该新颖组合物的片剂、丸剂等可经包覆或经其他方式复合以提供提供具有长效优点的剂型。举例来说，片剂或丸剂可包含由外部成份包覆的内部组合物。另外，该两种成份可以肠溶层分开，该肠溶层用来抵抗崩解，以使该内部成份完整通过胃或被延迟释放。多种物质可被用于该肠溶层或包覆层，该等物质包括多种聚合酸及聚合酸与诸如虫胶、鲸蜡醇及醋酸纤维素的物质的混合物。

[0214] Wnt拮抗剂（例如抗FZD抗体或可溶性FZD受体）亦可被包封于微胶囊中。该等微胶囊系藉由例如凝聚技术或藉由界面聚合化制备，例如分别于胶体药物递送系统（例如脂质体、白蛋白微球、微乳化液、纳米微粒及纳米微囊）或于巨乳化液中的羟甲基纤维素或明胶微胶囊及聚（异丁烯酸甲酯）微胶囊，如Remington,The Science and Practice of Pharmacy,20th Ed.Mack Publishing(2000)所述。

[0215] 在某些实施方式中，医药制剂包括与脂质体复合的Wnt拮抗剂（例如抗FZD抗体或可溶性FZD受体）(Epstein,et al.,1985,Proc.Natl.Acad.Sci.USA82:3688;Hwang,et al.,1980,Proc.Natl.Acad.Sci.USA77:4030;及U.S.Patent 4,485,045及4,544,545)。循环时间延长的脂质体系揭露于美国专利第5,013,556号。一些脂质体可利用逆相蒸发以包含磷脂酰胆碱、胆固醇及PEG-衍生性磷脂酰乙醇胺(PEG-PE)的脂质组合物生成。脂质体

被挤压通过定义孔径大小的滤网以产生具有所欲直径的脂质体。

[0216] 此外可制备缓释制剂。缓释制剂的适当实例包括含有该抗体的固相疏水性聚合物的半透性基体，该基体系呈形状对象的形式（例如膜或微胶囊）。缓释基体的实例包括聚酯、水凝胶（诸如聚（2-羟乙基-甲基丙烯酸酯）或聚乙烯醇）、聚交酯（美国专利第3,773,919号）、L-谷氨酸及7乙基-L-谷氨酸盐的共聚物、不可降解的乙烯-乙酸乙烯酯、可降解的乳酸-乙醇酸共聚物诸如LUPRON DEPOT TM（由乳酸-乙醇酸共聚物及醋酸亮丙瑞林(leuprolideacetate)所组成的注射型微球）、蔗糖乙酸异丁酸酯及聚-D-(-)-3-羟丁酸。

实施例

[0217] 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。

实施例1

在第1a期临床研究中神经内分泌肿瘤对OMP-18R5反应

[0218] 在OMP-18R5人抗FZD抗体用于晚期实质肿瘤病患的第一期临床试验中，三名先前接受过多次其他治疗的末期神经内分泌肿瘤病患以周期性低剂量的OMP-18R5作为单一剂治疗。所有三名神经内分泌病患的延长稳定疾病显示，即使以低剂量、单一剂OMP-18R5治疗，可能对神经内分泌肿瘤具有意外程度的疗效，包括具有类癌组织学及胰腺神经内分泌肿瘤的神经内分泌肿瘤。

[0219] 病患3在加入OMP-18R5试验时为59岁女性。她于2004年被诊断为具有神经内分泌肿瘤（类癌瘤）。当时她接受小肠切除手术，并以射频烧蚀治疗肝病灶。在参加OMP-18R5试验之前，她接受trametinib(MEK1/2MAP激酶抑制剂）与GSK2141795Akt抑制剂的组合的全身性治疗，但疾病在治疗1个月后恶化。在OMP-18R5试验中，病患3接受每周0.5mg/kg OMP-18R5的剂量共112天。她的疾病在OMP-18R5治疗期间维持稳定，但在第112天发生骨折后离开试验。有鉴于她接受先前治疗时疾病快速恶化，此病患在接受OMP-18R5治疗时延长疾病控制期间特别显示该抗体可能具有令人意外的临床疗效，即使以低剂量单一剂使用。

[0220] 病患10在加入OMP-18R5试验时为69岁罹患胰腺神经内分泌肿瘤的女性。她在2001年确诊并接受手术治疗包括80％远端胰切除、脾切除及胃后壁楔状切除。在参与OMP-18R5试验之前，她接受下列全身性治疗：(1)regorafenib（部分反应：3年）；(2)抗LOXL2抗体（稳定疾病：5.5个月）；及(3)抗CSFR1抗体（试验后6周进行性疾病）。在2013年1月25日，OMP-18R5试验中的病患10已接受每二周0.5mg/kg OMP-18R5共279天。病患10持续接受每二周0.5mg/kg OMP-18R5共448天。在以OMP-18R5治疗112天之后，计划主持人发现病患10的目标肿瘤肝脏转移减少21％。肿瘤减少系由独立放射评估证实（如表4所示）。见图1A至1C。对照非标靶疾病病灶在相同治疗期间未显示改变。放射检查进一步显示病患10在以OMP-18R5治疗112天之后，肿瘤病灶出现钙化征象（图1C）。观察到的肿瘤病灶钙化可能表示OMP-18R5诱导肿瘤细胞分化及/或肿瘤坏死。后续于第168、224、280、336及392天的计算机断层(CT)扫描显示该病患仍无进行性疾病。病患10在第448天出现进行性疾病征候后停止治疗。此病患接受OMP-18R5治疗时的延长疾病控制期，为该抗体即使以低剂量单一剂每二周使用一次时可能具有令人意外的临床疗效的额

外证据。

表 4. 病患 10: 独立放射学检测 RECIST 1.1

病灶(mm)	**2012 年 3 月 27 日 (基准期)**	**2012 年 6 月 11 日**	**2012 年 8 月 6 日**
1. 肝脏: 右叶 (前-外) (目标)	13.6 x 22.9	17.3 x 23.9	13.3 x 20.5
2. 肝脏: 下腔静脉 (目标)	16.2 x 16.2	15.9 x 15.9	10.8 x 13.1
3. 肝脏: 右顶叶 (目标)	7.9 x 11.6	7.1 x 11.4	4.9 x 8.5
4. 门腔淋巴结 (目标)*	16.6 x 23.9	14.1 x 15.5	9.1 x 14.2
5. 门腔淋巴结 (非目标)	11.1 x 14.5	10.5 x 12.6	9.5 x 14.8
总 计 : 目 标 (mm, %Δ) 总计: 非目标	67.3 Non-PD	65.3 **(-3%)** Non-PD	51.2 **(-24%)** 正常**

* 依照 RECIST 1.1 :LN 最短直径≥ 15mm 是可测量

** 依照 RECIST 1.1 :LN 最短直径< 10mm 被认为是“正常”

Non-PD: 非进行性疾病

[0221] 病患 12 在加入 OMP-18R5 试验时为 77 岁罹患神经内分泌肿瘤（类癌瘤）的女性，于 2006 年确诊。在参加 OMP-18R5 试验之前，她接受下列全身性治疗 :(1)sandostatin（稳定疾病 :20 个月）;(2) 热休克蛋白 90 的抑制剂（稳定疾病 :23 个月）;及 (3)sandostatin 与抗血管生成素 2 抗体的组合（稳定疾病 :4 个月）。在 2013 年 1 月 25 日，OMP-18R5 试验中的病患 12 已接受每三周 1mg/kg OMP-18R5 共 210 天。在 2013 年 10 月 4 日，病患 12 接受每三周 1mg/kg OMP-18R5 治疗共 465 天。此病患于第 56、112、168、224、280、336、392 及 448 天评估时疾病状况稳定。病患在此延长的时间期间维持参与临床试验且无疾病恶化，进一步支持 OMP-18R5 对神经内分泌肿瘤的临床疗效。

[0222] 图 2A 显示每位参加 OMP-18R5 第 1a 期临床试验的病患 (n = 18)，在 2013 年 1 月 25 日时已参与该 OMP-18R5 第 1a 期试验的天数。图 2B 显示每位参加 OMP-18R5 第 1a 期临床试验的病患 (n = 29)，在 2013 年 10 月 4 日时已参与该 OMP-18R5 第 1a 期试验的天数。接受 OMP-18R5 治疗的罹患神经内分泌肿瘤的病患维持参与试验的时间，令人意外地比罹

患其他肿瘤类型（包括结直肠癌、乳癌、黑色素瘤及胰癌）的其他第 1a 期病患更长。

[0223] 同样地，在 2013 年 1 月 25 日，三名神经内分泌肿瘤病患因接受 OMP-18R5 治疗而维持稳定疾病的时间，比他们接受先前治疗到疾病恶化的时间延长约 2 至 7 倍。使用生长调节指数的工具测量该观察到的活性（目前治疗时间除以先前治疗到疾病恶化前时间；GMI ≥ 1.33 被视为优异；Von Hoff；Clinical Cancer Research 4：1079-1086，1998），所有三名神经内分泌肿瘤 (NET) 病患都明显超过此标准（病患 12：1.8；病患 10：6.3；病患 3，退出试验：3.8）。病患 10 的最终 GMI 为 10.7，在 2013 年 10 月 4 日时病患 12 的 GMI 为 1.4。三名神经内分泌肿瘤病患在 2013 年 1 月 25 日时维持参与 OMP-18R5 试验的时间与他们维持先前治疗的时间的比较系显示于图 3A。三名神经内分泌肿瘤病患在 2013 年 10 月 4 日时维持参与 OMP-18R5 试验的时间与他们维持先前治疗的时间的比较系显示于图 3B。

实施例 2

体内采用 Wnt 拮抗剂预防神经内分泌肿瘤生长

[0224] 此实施例描述使用 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）预防异种移植模型中的神经内分泌肿瘤生长。在某些实施方式中，制备已异种移植于小鼠中传代生长的来自病患样本（实质肿瘤活体样本或胸膜渗液）的神经内分泌肿瘤细胞，以再传代于实验动物。在无菌状态下移除神经内分泌肿瘤组织，切成小块，利用无菌刀片完全剁碎，藉由酶消化及机械破坏获得单细胞悬浮液。特别地，将胸膜渗液细胞或该形成的肿瘤块与超纯胶原酶 III 于培养基中混合（每 mL 有 200 至 250 单位的胶原酶），于 37℃下培养 3 至 4 小时，每 15 至 20 分钟以 10mL 吸管抽吸混合一次。经消化的细胞以 45 μM 耐纶网片过滤，以 RPMI/20％ FBS 清洗，再以 HBSS 清洗二次。经解离的神经内分泌肿瘤细胞接着经皮下注射至 NOD/SCID 小鼠的乳腺脂肪垫以诱发肿瘤生长。

[0225] 在某些实施方式中，经解离的神经内分泌肿瘤细胞首先根据细胞表面标志分成肿瘤发生性及非肿瘤发生性细胞，然后才注射至实验动物。特别地，如上所述经解离的神经内分泌肿瘤细胞以包含 2％热失活小牛血清 (HICS) 的 HEPES 缓冲盐水溶液 (HBSS) 清洗二次，重悬为每 100 μl 10^6细胞。加入抗体，使细胞于冰上培养 20 分钟，然后以 HBSS/2％ HICS 清洗二次。抗体包括抗 ESA(Biomeda，Foster City，CA)、抗 CD44、抗 CD24 及细胞系标志抗 CD2、抗 CD3、抗 CD10、抗 CD16、抗 CD18、抗 CD31、抗 CD64 及抗 CD140b（总称为 Lin；PharMingen，San Jose，CA）。抗体与荧光染料直接共轭以阳性或阴性选择表达这些标志的细胞。小鼠细胞藉由筛选 $H2K^{d+}$细胞消除，死亡细胞利用存活染料 7AAD 消除。流式细胞分析系于 FACSVantage(Becton Dickinson，Franklin Lakes，NJ) 上进行。侧向散射及前向散射特性被用于消除细胞团。经分离的 ESA+、CD44+、CD24-/low、Lin- 肿瘤发生性细胞接着经皮下注射至 NOD/SCID 小鼠以诱发肿瘤生长。

[0226] 以实例说明，分析 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）减少神经内分泌肿瘤细胞生长的能力。经解离的神经内分泌肿瘤细胞（每只动物 10，000 个）经皮下注射至 6 至 8 周龄 NOD/SCID 小鼠的侧腹区域。肿瘤细胞注射二天后，每周以 10mg/kg 抗 FZD 抗体或可溶性 FZD 受体腹腔注射 (i.p.) 动物二次。每周监测肿瘤生长直到发现到生长，之后每周测量二次肿瘤生长共 8 周。如此识别相较于 PBS 注射对照组显著减少肿瘤生长的 FZD 结合抗体。

实施例 3

体内使用 Wnt 拮抗剂治疗神经内分泌肿瘤

[0227] 此实施例描述使用 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗异种移植模型中的神经内分泌癌。在某些实施方式中，制备已异种移植于小鼠中传代生长的来自病患样本（实质肿瘤活体样本或胸膜渗液）的神经内分泌肿瘤细胞，以再传代于实验动物。移除神经内分泌肿瘤组织，切成小块，利用无菌刀片完全剁碎，藉由酶消化及机械破坏获得单细胞悬浮液。经解离的神经内分泌肿瘤细胞接着经皮下注射至 NOD/SCID 小鼠的乳腺脂肪垫（乳房肿瘤）或侧腹（非乳房肿瘤）以诱发肿瘤生长。另外，ESA+、CD44+、CD24-/low、Lin- 肿瘤发生性肿瘤细胞系如上述分离及注射。

[0228] 在肿瘤细胞注射之后，监测动物的肿瘤生长情况。一旦神经内分泌肿瘤到达约 150 至 200mm 的平均大小，即开始 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗。每只动物接受 100μg Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）或对照剂腹腔注射每周二至五次共 6 周。在这 6 周期间，每周评估二次肿瘤大小。如此决定 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）相较于对照剂预防进一步神经内分泌肿瘤生长或减少神经内分泌肿瘤大小的能力。

[0229] 在抗体治疗结束时，收集肿瘤进行进一步分析。在一些实施方式中，一部分的神经内分泌肿瘤藉由免疫荧光分析以检测 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）的肿瘤穿透性及肿瘤反应。一部分收集自 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗及对照小鼠的神经内分泌肿瘤系于液态氮中新鲜冷冻，包埋于 O.C.T.，在冷冻切片机上切成 10μm 切片。在一些实施方式中，一部分的各神经内分泌肿瘤系经福尔马林固定及石蜡包埋，于切片机上切成 10μm 切片。切片经后固定，与特异性识别该经注射的 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）的发色标记抗体一起培养，以检测存在于该肿瘤生体样本中的 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）或对照剂。另外，检测不同肿瘤及肿瘤浸润细胞类型的抗体，诸如举例来说检测血管内皮细胞的抗 VE 钙黏素 (CD144) 或抗 PECAM-1(CD31) 抗体、检测血管平滑肌细胞的抗平滑肌 α-肌动蛋白抗体、检测增生细胞的抗 Ki67 抗体、检测死亡细胞的 TUNEL 试验、检测 Wnt 信号传导的抗 β-钙黏素抗体及检测缺口信号传导的抗细胞内结构域 (ICD) 缺口片段抗体，可被用于检测 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗对例如血管生成、肿瘤生长及肿瘤型态的影响。

[0230] 在某些实施方式中，Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗对神经内分泌肿瘤细胞基因表达的影响亦经评估。自经 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗及对照抗体治疗的小鼠收集的一部分神经内分泌肿瘤萃取总 RNA，用于进行定量 RT-PCR。分析 FZD 受体、Wnt 信号传导途径的成分（包括例如 Wnt1 及 β-连环蛋白）及其他先前识别的癌干细胞标志（例如 CD44）的表达量，以看家基因 GAPDH 作为内部对照比较。如此测定以 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗时神经内分泌肿瘤细胞基因表达的改变。

[0231] 此外，评估 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗对神经内分泌肿瘤中的癌干细胞频率的影响。来自经 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗与对照剂治疗的小鼠的神经内分泌肿瘤样本被切成小块，利用无菌刀片完全切碎，藉由酶消化及机械破坏获得单细胞悬浮液。经解离的神经内分泌肿瘤细胞接着以 FACS 分析肿瘤发生性癌干细胞的存在，根据如上详述的 ESA+、CD44+、CD24-/low、Lin- 表面细胞标志表达。

[0232] 接着可评估在 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗后，根据 ESA+、CD44+、CD24-/low、Lin- 表达而分离的细胞的肿瘤发生性。自经 Wnt 拮抗剂（例如 OMP-18R5 或 OMP-54F28）治疗与对照剂治疗的小鼠分离的 ESA+、CD44+、CD24-/low、Lin- 癌干细胞再度皮下注射至 NOD/SCID 小鼠的乳腺脂肪垫。接着根据形成一致神经内分泌肿瘤所需的注射细胞数量测定癌干细胞的肿瘤发生性。

实施例 4

利用抗 FZD 受体的抗体或可溶性 FZD 受体治疗人神经内分泌肿瘤

[0233] 此实施例描述利用拮抗 FZD 受体的抗体以治疗神经内分泌肿瘤的某些方法，以包含癌干细胞及 / 或其中检测到 FZD 受体表达的肿瘤细胞及 / 或具有 Wnt 基因卷标显示对 Wnt 信号传导的抑制有所反应的肿瘤细胞的神经内分泌肿瘤为目标。

[0234] 在一些实施方式中，癌干细胞标志或 FZD 受体的存在或 Wnt 基因标签中的一或多种基因的表达可先从肿瘤活体样本中检测。在无菌条件下自诊断为罹患神经内分泌肿瘤的病患的活体样本取出肿瘤细胞。在一些实施方式中，使该组织活体样本于液态氮中新鲜冷冻，包埋于 O.C.T.，在冷冻切片机上切成 10μm 切片。在一些实施方式中，该组织活体样本系经福尔马林固定及石蜡包埋，于切片机上切成 10μm 切片。

[0235] 切片与抗 FZD 受体的抗体一起培养，以检测 FZD 蛋白表达。另外，分析切片中 Wnt 基因标签中的一或多种基因的存在。

[0236] 亦可测定癌干细胞的存在。组织活体样本被切成小块，利用无菌刀片完全切碎，藉由酶消化及机械破坏细胞以获得单细胞悬浮液。经解离的神经内分泌肿瘤细胞接着与抗 ESA、抗 CD44、抗 CD24、抗 Lin 及抗 FZD 抗体一起培养以检测癌干细胞，ESA+、CD44+、CD24-/low、Lin-、FZD+ 肿瘤干细胞的存在藉由以上详述的流式细胞分析测定。

[0237] 癌病患的神经内分泌肿瘤经诊断为表达 FZD 受体及 / 或 Wnt 基因标签中的一或多种基因，接受抗 FZD 受体抗体或可溶性 FZD 受体的治疗。在某些实施方式中，人源化或人单克隆抗 FZD 受体抗体或可溶性 FZD 受体系经纯化并与适当医药载体调制以供注射。在一些实施方式中，病患以该 FZD 抗体或可溶性 FZD 受体治疗一个月至少一次至少共 10 周。在一些实施方式中，病患以该 FZD 抗体或可溶性 FZD 受体治疗一周至少一次至少约 14 周。每次投予的该抗体或可溶性 FZD 受体应为医药有效剂量。在一些实施方式中，投予约 2 至约 100mg/ml 的抗 FZD 抗体或可溶性 FZD 受体。在一些实施方式中，投予约 5 至约 40mg/ml 的抗 FZD 抗体或可溶性 FZD 受体。该抗体或可溶性 FZD 受体可于标准放射疗法或使用一或多种化学治疗剂的化学疗法进行之前、的同时或之后投予。病患受到监测以决定该治疗是否导致抗肿瘤反应，例如根据肿瘤消退、新肿瘤发生率减少、较低肿瘤抗原表达、减少癌干细胞数量或评估疾病预后的其他方式。

[0238] 所有此处所提及的公开数据、专利、专利申请案、网站及编号 / 数据库序列（包括多核苷酸及多肽序列），以及美国专利申请案 61/717,294(2012 年 10 月 23 日提出），在此系以引用方式整体纳入以符合所有目的，和特别且独立指出个别公开资料、专利、专利申请案、网站或编号 / 数据库序列各自以引用方式被纳入的范围相同。

序列

人 FZD1 全长氨基酸序列 (SEQ ID NO:1 ;画线部分为 ECD)

MAEEEAPKKSRAAGGGASWELCAGALSARLAEEGSGDAGGRRRPPVDPRRLARQLLLLLWLLEAPLLLGVRAQ

AAGQGPGQGPGPGQQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEV
HQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELC
VGQNTSDKGTPTPSLLPEFWTSNPQHGGGGHRGGFPGGAGASERGKFSCPRALKVPSYLNYHFLGEKDCGAPCEPTK
VYGLMYFGPEELRFSRTWIGIWSVLCCASTLFTVLTYLVDMRRFSYPERPIIFLSGCYTAVAVAYIAGFLLEDRVVC
NDKFAEDGARTVAQGTKKEGCTILFMMLYFFSMASSIWWVILSLTWFLAAGMKWGHEAIEANSQYFHLAAWAVPAIK
TITILALGQVDGDVLSGVCFVGLNNVDALRGFVLAPLFVYLFIGTSFLLAGFVSLFRIRTIMKHDGTKTEKLEKLMV
RIGVFSVLYTVPATIVIACYFYEQAFRDQWERSWVAQSCKSYAIPCPHLQAGGGAPPHPPMSPDFTVFMIKYLMTLI
VGITSGFWIWSGKTLNSWRKFYTRLTNSKQGETTV

人 FZD2 全长氨基酸序列 (SEQ ID NO :2 ;画线部分为 ECD)

MRPRSALPRLLLPLLLLPAAGPAQFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEV
HQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQIC
VGQNHSEDGAPALLTTAPPPGLQPGAGGTPGGPGGGGAPPRYATLEHPFHCPRVLKVPSYLSYKFLGERDCAAPCEP
ARPDGSMFFSQEETRFARLWILTWSVLCCASTFFTVTTYLVDMQRFRYPERPIIFLSGCYTMVSVAYIAGFVLQERV
VCNERFSEDGYRTVVQGTKKEGCTILFMMLYFFSMASSIWWVILSLTWFLAAGMKWGHEAIEANSQYFHLAAWAVPA
VKTITILAMGQIDGDLLSGVCFVGLNSLDPLRGFVLAPLFVYLFIGTSFLLAGFVSLFRIRTIMKHDGTKTEKLERL
MVRIGVFSVLYTVPATIVIACYFYEQAFREHWERSWVSQHCKSLAIPCPAHYTPRMSPDFTVYMIKYLMTLIVGITS
GFWIW

人 FZD3 全长氨基酸序列 (SEQ ID NO :3 ;画线部分为 ECD)

MAMTWIVFSLWPLTVFMGHIGGHSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDC
SRDFRPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPYPRLVDLNLAGEPT
EGAPVAVQRDYGFWCPRELKIDPDLGYSFLHVRDCSPPCPNMYFRREELSFARYFIGLISIICLSATLFTFLTFLID
VTRFRYPERPIIFYAVCYMMVSLIFFIGFLLEDRVACNASIPAQYKASTVTQGSHNKACTMLFMILYFFTMAGSVWW
VILTITWFLAAVPKWGSEAIEKKALLFHASAWGIPGTLTIILLAMNKIEGDNISGVCFVGLYDVDALRYFVLAPLCL
YVVVGVSLLLAGIISLNRVRIEIPLEKENQDKLVKFMIRIGVFSILYLVPLLVVIGCYFYEQAYRGIWETTWIQERC
REYHIPCPYQVTQMSRPDLILFLMKYLMALIVGIPSVFWVGSKKTCFEWASFFHGRRKKEIVNESRQVLQEPDFAQS
LLRDPNTPIIRKSRGTSTQGTSTHASSTQLAMVDDQRSKAGSIHSKVSSYHGSLHRSRDGRYTPCSYRGMEERLPHG
SMSRLTDHSRHSSSHRLNEQSRHSSIRDLSNNPMTHITHGTSMNRVIEEDGTSA

人 FZD4 全长氨基酸序列 (SEQ ID NO :4 ;画线部分为 ECD)

MLAMAWRGAGPSVPGAPGGVGLSLGLLLQLLLLLGPARGFGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHE
LQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSK
FPPQNDHNHMCMEGPGDEEVPLPHKTPIQPGEECHSVGTNSDQYIWVKRSLNCVLKCGYDAGLYSRSAKEFTDIWMA
VWASLCFISTAFTVLTFLIDSSRFSYPERPIIFLSMCYNIYSIAYIVRLTVGRERISCDFEEAAEPVLIQEGLKNTG
CAIIFLLMYFFGMASSIWWVILTLTWFLAAGLKWGHEAIEMHSSYFHIAAWAIPAVKTIVILIMRLVDADELTGLCY
VGNQNLDALTGFVVAPLFTYLVIGTLFIAAGLVALFKIRSNLQKDGTKTDKLERLMVKIGVFSVLYTVPATCVIACY
FYEISNWALFRYSADDSNMAVEMLKIFMSLLVGITSGMWIWSAKTLHTWQKCSNRLVNSGKVKREKRGNGWVKPGKG
SETVV

人 FZD5 全长氨基酸序列 (SEQ ID NO :5 ;画线部分为 ECD)

MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPL
VEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDY

NRSEATTAPPRPFPAKPTLPGPPGAPASGGECPAGGPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQPSF
SADERTFATFWIGLWSVLCFISTSTTVATFLIDMERFRYPERPIIFLSACYLCVSLGFLVRLVVGHASVACSREHNH
IHYETTGPALCTIVFLLVYFFGMASSIWWVILSLTWFLAAGMKWGNEAIAGYAQYFHLAAWLIPSVKSITALALSSV
DGDPVAGICYVGNQNLNSLRGFVLGPLVLYLLVGTLFLLAGFVSLFRIRSVIKQGGTKTDKLEKLMIRIGIFTLLYT
VPASIVVACYLYEQHYRESWEAALTCACPGHDTGQPRAKPEYWVLMLKYFMCLVVGITSGVWIWSGKTVESWRRFTS
RCCCRPRRGHKSGGAMAAGDYPEASAALTGRTGPPGPAATYHKQVSLSHV

人 FZD6 全长氨基酸序列 (SEQ ID NO :6 ;画线部分为 ECD)

MEMFTFLLTCIFLPLLRGHSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNI
ETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFDPHTEFLGPQKKT
EQVQRDIGFWCPRHLKTSGGQGYKFLGIDQCAPPCPNMYFKSDELEFAKSFIGTVSIFCLCATLFTFLTFLIDVRRF
RYPERPIIYYSVCYSIVSLMYFIGFLLGDSTACNKADEKLELGDTVVLGSQNKACTVLFMLLYFFTMAGTVWWVILT
ITWFLAAGRKWSCEAIEQKAVWFHAVAWGTPGFLTVMLLAMNKVEGDNISGVCFVGLYDLDASRYFVLLPLCLCVFV
GLSLLLAGIISLNHVRQVIQHDGRNQEKLKKFMIRIGVFSGLYLVPLVTLLGCYVYEQVNRITWEITWVSDHCRQYH
IPCPYQAKAKARPELALFMIKYLMTLIVGISAVFWVGSKKTCTEWAGFFKRNRKRDPISESRRVLQESCEFFLKHNS
KVKHKKKHYKPSSHKLKVISKSMGTSTGATANHGTSAVAITSHDYLGQETLTEIQTSPETSMREVKADGASTPRLRE
QDCGEPASPAASISRLSGEQVDGKGQAGSVSESARSEGRISPKSDITDTGLAQSNNLQVPSSSEPSSLKGSTSLLVH
PVSGVRKEQGGGCHSDT

人 FZD7 全长氨基酸序列 (SEQ ID NO :7 ;画线部分为 ECD)

MRDPGAAAPLSSLGLCALVLALLGALSAGAGAQPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLLGH
TNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCEN
FPVHGAGEICVGQNTSDGSGGPGGGPTAYPTAPYLPDLPFTALPPGASDGRGRPAFPFSCPRQLKVPPYLGYRFLGE
RDCGAPCEPGRANGLMYFKEEERRFARLWVGVWSVLCCASTLFTVLTYLVDMRRFSYPERPIIFLSGCYFMVAVAHV
AGFLLEDRAVCVERFSDDGYRTVAQGTKKEGCTILFMVLYFFGMASSIWWVILSLTWFLAAGMKWGHEAIEANSQYF
HLAAWAVPAVKTITILAMGQVDGDLLSGVCYVGLSSVDALRGFVLAPLFVYLFIGTSFLLAGFVSLFRIRTIMKHDG
TKTEKLEKLMVRIGVFSVLYTVPATIVLACYFYEQAFREHWERTWLLQTCKSYAVPCPPGHFPPMSPDFTVFMIKYL
MTMIVGITTGFWIWSGKTLQSWRRFYHRLSHSSKGETAV

人 FZD8 全长氨基酸序列 (SEQ ID NO :8 ;画线部分为 ECD)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFW
PLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMD
YNRTDLTTAAPSPPRRLPPPPPGEQPPSGSGHGRPPGARPPHRGGGRGGGGGDAAAPPARGGGGGGKARPPGGGAAP
CEPGCQCRAPMVSVSSERHPLYNRVKTGQIANCALPCHNPFFSQDERAFTVFWIGLWSVLCFVSTFATVSTFLIDME
RFKYPERPIIFLSACYLFVSVGYLVRLVAGHEKVACSGGAPGAGGAGGAGGAAAGAGAAGAGAGGPGGRGEYEELGA
VEQHVRYETTGPALCTVVFLLVYFFGMASSIWWVILSLTWFLAAGMKWGNEAIAGYSQYFHLAAWLVPSVKSIAVLA
LSSVDGDPVAGICYVGNQSLDNLRGFVLAPLVIYLFIGTMFLLAGFVSLFRIRSVIKQQDGPTKTHKLEKLMIRLGL
FTVLYTVPAAVVVACLFYEQHNRPRWEATHNCPCLRDLQPDQARRPDYAVFMLKYFMCLVVGITSGVWVWSGKTLES
WRSLCTRCCWASKGAAVGGGAGATAAGGGGGPGGGGGGGPGGGGGPGGGGGSLYSDVSTGLTWRSGTASSVSYPKQM
PLSQV

人 FZD9 全长氨基酸序列 (SEQ ID NO :9 ;画线部分为 ECD)

MAVAPLRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAEL

AEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHA
LCMEAPENATAGPAEPHKGLGMLPVAPRPARPPGDLGPGAGGSGTCENPEKFQYVEKSRSCAPRCGPGVEVFWSRRD
KDFALVWMAVWSALCFFSTAFTVLTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAGALYVI
QEGLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHEAIEAHGSYFHMAAWGLPALKTIVILTLRKVAG
DELTGLCYVASTDAAALTGFVLVPLSGYLVLGSSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIGVFSILYTVP
ATCVIVCYVYERLNMDFWRLRATEQPCAAAAGPGGRRDCSLPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQ
TWQSLCYRKIAAGRARAKACRAPGSYGRGTHCHYKAPTVVLHMTKTDPSLENPTHL

人 FZD10 全长氨基酸序列 (SEQ ID NO:10 ;画线部分为 ECD)

MQRPGPRLWLVLQVMGSCAAISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAP
LVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLCMEA
PNNGSDEPTRGSGLFPPLFRPQRPHSAQEHPLKDGGPGRGGCDNPGKFHHVEKSASCAPLCTPGVDVYWSREDKRFA
VVWLAIWAVLCFFSSAFTVLTFLIDPARFRYPERPIIFLSMCYCVYSVGYLIRLFAGAESIACDRDSGQLYVIQEGL
ESTGCTLVFLVLYYFGMASSLWWVVLTLTWFLAAGKKWGHEAIEANSSYFHLAAWAIPAVKTILILVMRRVAGDELT
GVCYVGSMDVNALTGFVLIPLACYLVIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRIGLFSVLYTVPATCV
IACYFYERLNMDYWKILAAQHKCKMNNQTKTLDCLMAASIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQQVC
SRRLKKKSRRKPASVITSGGIYKKAQHPQKTHHGKYEIPAQSPTCV

含信号序列的人 FZD1ECD(SEQ ID NO:11)

MAEEEAPKKSRAAGGGASWELCAGALSARLAEEGSGDAGGRRRPPVDPRRLARQLLLLLWLLEAPLLLGVR
AQAAGQGPGQGPGPGQQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAG
LEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHG
AGELCVGQNTSDKGTPTPSLLPEFWTSNPQHGGGGHRGGFPGGAGASERGKFSCPRALKVPSYLNYHFLGEKDCG
APCEPTKVYGLMYFGPEELRFSRT

含信号序列的人 FZD2ECD(SEQ ID NO:12)

MRPRSALPRLLLPLLLLPAAGPAQFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGL
EVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAE
QICVGQNHSEDGAPALLTTAPPPGLQPGAGGTPGGPGGGGAPPRYATLEHPFHCPRVLKVPSYLSYKFLGERDCAAP
CEPARPDGSMFFSQEETRFARLWILT

含信号序列的人 FZD3ECD(SEQ ID NO:13)

MAMTWIVFSLWPLTVFMGHIGGHSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNL
DCSRDFRPFLCALYAPICMEYGRVTLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPYPRLVDLNLAG
EPTEGAPVAVQRDYGFWCPRELKIDPDLGYSFLHVRDCSPPCPNMYFRREELSFARY

含信号序列的人 FZD4ECD(SEQ ID NO:14)

MLAMAWRGAGPSVPGAPGGVGLSLGLLLQLLLLLGPARGFGDEEERRCDPIRISMCQNLGYNVTKMPNLVG
HELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLN
CSKFPPQNDHNHMCMEGPGDEEVPLPHKTPIQPGEECHSVGTNSDQYIWVKRSLNCVLKCGYDAGLYSRSAKEFTDI

含信号序列的人 FZD5ECD(SEQ ID NO:15)

MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFW
PLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLC
MDYNRSEATTAPPRPFPAKPTLPGPPGAPASGGECPAGGPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQ

PSFSADERT

含信号序列的人 FZD6ECD(SEQ ID NO:16)

MEMFTFLLTCIFLPLLRGHSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSP NIETFLCKAFVPTCIEQIHVVPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFDPHTEFLGPQ KKTEQVQRDIGFWCPRHLKTSGGQGYKFLGIDQCAPPCPNMYFKSDELEFAKSFIGTVSI

含信号序列的人 FZD7ECD(SEQ ID NO:17)

MRDPGAAAPLSSLGLCALVLALLGALSAGAGAQPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLL GHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLR CENFPVHGAGEICVGQNTSDGSGGPGGGPTAYPTAPYLPDLPFTALPPGASDGRGRPAFPFSCPRQLKVPPYLGYRF LGERDCGAPCEPGRANGLMYFKEEERRFARL

含信号序列的人 FZD8ECD(SEQ ID NO:18)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTL CMDYNRTDLTTAAPSPPRRLPPPPPGEQPPSGSGHGRPPGARPPHRGGGRGGGGGDAAAPPARGGGGGGKARPPGGG AAPCEPGCQCRAPMVSVSSERHPLYNRVKTGQIANCALPCHNPFFSQDERAFT

含信号序列的人 FZD9ECD(SEQ ID NO:19)

MAVAPLRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAA ELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRND PHALCMEAPENATAGPAEPHKGLGMLPVAPRPARPPGDLGPGAGGSGTCENPEKFQYVEKSRSCAPRCGPGVEVFWS RRDKDF

含信号序列的人 FZD10ECD(SEQ ID NO:20)

MQRPGPRLWLVLQVMGSCAAISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEF APLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLC MEAPNNGSDEPTRGSGLFPPLFRPQRPHSAQEHPLKDGGPGRGGCDNPGKFHHVEKSASCAPLCTPGVDVYWSREDK RFA

人 FZD1Fri 结构域氨基酸序列 (SEQ ID NO:21 ;SEQ ID NO:1 的氨基酸 87 至 237)

QQPPPPPQQQQSGQQYNGERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLV KVQCSAELKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELCVGQNTS DKGT

人 FZD2Fri 结构域氨基酸序列 (SEQ ID NO:22 ;SEQ ID NO:2 的氨基酸 24 至 159)

QFHGEKGISIPDHGFCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMY APVCTVLEQAIPPCRSICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQICVGQNHSEDG

人 FZD3Fri 结构域氨基酸序列 (SEQ ID NO:23 ;SEQ ID NO:3 的氨基酸 23 至 143)

HSLFSCEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRV TLPCRRLCQRAYSECSKLMEMFGVPWPEDMECSRFPDCDEPYPRLVDL

人 FZD4Fri 结构域氨基酸序列 (SEQ ID NO:24 ;SEQ ID NO:4 的氨基酸 40 至 170)

FGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEK INIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEV

人 FZD5Fri 结构域氨基酸序列 (SEQ ID NO:25 ;SEQ ID NO:5 的氨基酸 27 至 157)

ASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYH KPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATT

人 FZD6Fri 结构域氨基酸序列 (SEQ ID NO:26 ;SEQ ID NO:6 的氨基酸 19 至 146)

HSLFTCEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVPTCIEQIHV VPPCRKLCEKVYSDCKKLIDTFGIRWPEELECDRLQYCDETVPVTFDPHTEFLG

人 FZD7Fri 结构域氨基酸序列 (SEQ ID NO:27 ;SEQ ID NO:7 的氨基酸 33 至 170)

QPYHGEKGISVPDHGFCQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSM YAPVCTVLDQAIPPCRSLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEICVGQNTSDGSG

人 FZD8Fri 结构域氨基酸序列 (SEQ ID NO:28 ;SEQ ID NO:8 的氨基酸 28 至 158)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTT

人 FZD9Fri 结构域氨基酸序列 (SEQ ID NO:29 ;SEQ ID NO:9 的氨基酸 23 至 159)

LEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSL YAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALCMEAPENA

人 FZD10Fri 结构域氨基酸序列 (SEQ ID NO:30 ;SEQ ID NO:10 的氨基酸 21 至 154)

ISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAP MCTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLCMEAPNNG

18R5 VH CDR1(SEQ ID NO:31)

GFTFSHYTLS

18R5 VH CDR2(SFQ ID NO:32)

VISGDGSYTYYADSVKG

18R5 VH CDR3(SEQ ID NO:33)

NFIKYVFAN

18R5 VL CDR1(SEQ ID NO:34)

SGDNIGSFYVH

18R5 VL CDR2(SEQ ID NO:35)

DKSNRPSG

18R5 VL CDR3(SEQ ID NO:36)

QSYANTLSL

18R5 VH(SEQ ID NO:37)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGDGSYTYYADSVKGRFTISSD NSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSS

18R5 VL(SEQ ID NO:38)

DIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLT ISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG

18R5 重链 (IgG2) 氨基酸序列,画线部分为 VH(SEQ ID NO:39)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVI SGDGSYTYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTC

NVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK

18R5 轻链（λ）氨基酸序列，画线部分为 VL(SEQ ID NO:40)

MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRP
SGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA
TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVA
PTECS

18R8 VL CDR2(SEQ ID NO:42)

EKDNRPSG

18R8 VL CDR3(SEQ ID NO:43)

SSFAGNSLE

18R8 VL(SEQ ID NO:44)

DIELTQPPSVSVAPGQTARISCSGDKLGKKYASWYQQKPGQAPVLVIYEKDNRPSGIPERFSGSNSGNTATL
TISGTQAEDEADYYCSSFAGNSLEVFGGGTKLTVLG18R8 轻链（λ）氨基酸序列，画线部分为 VL(SEQ ID NO:45)

MAWALLLLTLLTQGTGSWADIELTQPPSVSVAPGQTARISCSGDKLGKKYASWYQQKPGQAPVLVIYEKDNRP
SGIPERFSGSNSGNTATLTISGTQAEDEADYYCSSFAGNSLEVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA
TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVA
PTECS

44R24 VH CDR1(SEQ ID NO:46)

GFTFSSYYIT

44R24 VH CDR2(SEQ ID NO:47)

TISYSSSNTYYADSVKG

44R24 VH CDR3(SEQ ID NO:48)

SIVFDY

44R24 VL CDR1(SEQ ID NO:49)

SGDALGNRYVY

44R24 VL CDR2(SEQ ID NO:50)

SG

44R24 VL CDR3(SEQ ID NO:51)

GSWDTRPYPKY

44R24 VH(SEQ ID NO:52)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYITWVRQAPGKGLEWVSTISYSSSNTYYADSVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCARSIVFDYWGQGTLVTVSS

44R24 VL(SEQ ID NO:53)

DIELTQPPSVSVAPGQTARISCSGDALGNRYVYWYQQKPGQAPVLVIPSGIPERFSGSNS
GNTATLTISGTQAEDEADYYCGSWDTRPYPKYVFGGGTKLTVLG

SEQ ID NO:54

CPLYFPLYC

SEQ ID NO:55

CPLVWPLIC

SEQ ID NO:56

CPLAWPLIC

SEQ ID NO:57

CPVKYPLVC

SEQ ID NO:58

CPLRFPLFC

SEQ ID NO:59

CPLAWPLIC

SEQ ID NO:60

CPVAFPLYC

SEQ ID NO:61

CPVNYPLYC

SEQ ID NO:62

CPVKFPLYC

SEQ ID NO:63

CPLTYPLYC

SEQ ID NO:64

CPLRWPLMC

SEQ ID NO:65

CPLQYPLMC

SEQ ID NO:66

CPLSFPLYC

SEQ ID NO:67

CPLNWPLMC

SEQ ID NO:68

CP(L/V)X(Y/F/W)PL(Y/F/I/V/M)C

SEQ ID NO:69

DTLSALIERGLM

SEQ ID NO:70

DVWWLGSTWLKR

SEQ ID NO:71

FGNYLNDVRFLI

SEQ ID NO:72

TNLADIAHWISG

最小 FZD 及 SFRP Fri 结构域序列

h-FZD1 氨基酸 116 至 227(SEQ ID NO :73)

CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAELKFFLCSMYAPVCTVLEQALPPCR SLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAGELC

h-FZD2 氨基酸 39 至 150(SEQ ID NO :74)

CQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLEQAIPPCR SICERARQGCEALMNKFGFQWPERLRCEHFPRHGAEQIC

h-FZD3 氨基酸 28 至 133(SEQ ID NO :75)

CEPITLRMCQDLPYNTTFMPNLLNHYDQQTAALAMEPFHPMVNLDCSRDFRPFLCALYAPICMEYGRVTLPCR RLCQRAYSECSKLMEMFGVPWPEDMECSRFPDC

h-FZD4 氨基酸 48 至 161(SEQ ID NO :76)

CDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPC GGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMC

h-FZD5 氨基酸 33 至 147(SEQ ID NO :77)

CQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPC RSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLC

h-FZD6 氨基酸 24 至 129(SEQ ID NO :78)

CEPITVPRCMKMAYNMTFFPNLMGHYDQSIAAVEMEHFLPLANLECSPNIETFLCKAFVPTCIEQIHVVPPCR KLCEKVYSDCKKLIDTFGIRWPEELECDRLQYC

h-FZD7 氨基酸 49 至 160(SEQ ID NO :79)

CQPISIPLCTDIAYNQTILPNLLGHTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVCTVLDQAIPPCR SLCERARQGCEALMNKFGFQWPERLRCENFPVHGAGEIC

h-FZD8 氨基酸 35 至 148(SEQ ID NO :80)

CQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPC RSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLC

h-FZD9 氨基酸 39 至 152(SEQ ID NO :81)

CQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPMCTDQVSTPIPAC RPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALC

h-FZD10 氨基酸 34 至 147(SEQ ID NO :82)

CQPIEIPMCKDIGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPAC RVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPNYLC

h-SFRP1 氨基酸 57 至 165(SEQ ID NO :83)

CVDIPADLRLCHNVGYKKMVLPNLLEHETMAEVKQQASSWVPLLNKNCHAGTQVFLCSLFAPVCLDRPIYPCR WLCEAVRDSCEPVMQFFGFYWPEMLKCDKFPEGDVC

h-SFRP2 氨基酸 40 至 152(SEQ ID NO :84)

CKPIPANLQLCHGIEYQNMRLPNLLGHETMKEVLEQAGAWIPLVMKQCHPDTKKFLCSLFAPVCLDDLDETIQ PCHSLCVQVKDRCAPVMSAFGFPWPDMLECDRFPQDNDLC

h-SFRP3 氨基酸 35 至 147(SEQ ID NO :85)

CEPVRIPLCKSLPWNMTKMPNHLHHSTQANAILAIEQFEGLLGTHCSPDLLFFLCAMYAPICTIDFQHEPIKP CKSVCERARQGCEPILIKYRHSWPENLACEELPVYDRGVC

h-SFRP4 氨基酸 24 至 136(SEQ ID NO :86)

CEAVRIPMCRHMPWNITRMPNHLHHSTQENAILAIEQYEELVDVNCSAVLRFFFCAMYAPICTLEFLHDPIKPCKSVCQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVC

h-SFRP5 氨基酸 53 至 162(SEQ ID NO :87)

CLDIPADLPLCHTVGYKRMRLPNLLEHESLAEVKQQASSWLPLLAKRCHSDTQVFLCSLFAPVCLDRPIYPCRSLCEAVRAGCAPLMEAYGFPWPEMLHCHKFPLDNDLC

h-ROR1 最小 Fri 结构域 (SEQ ID NO :88)

CQPYRGIACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCHYAFPYCDETSSVPKPRDLCRDECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPESPFAANC

h-ROR2 最小 Fri 结构域 (SEQ ID NO :89)

CQPYRGIACARFIGNRTIYVDSLQMQGEIENRITAAFTMIGTSTHLSDQCSQFAIPSFCHFVFPLCDARSRTPKPRELCRDECEVLESDLCRQEYTIARSNPLILMRLQLPKCEALPMPESPDAANC

人 FZD4Fri 结构域 (划底线为预测信号序列)(SEQ ID NO :90)

MLAMAWRGAGPSVPGAPGGVGLSLGLLLQLLLLLGPARGFGDEEERRCDPIRISMCQNLGYNVTKMPNLVGHELQTDAELQLTTFTPLIQYGCSSQLQFFLCSVYVPMCTEKINIPIGPCGGMCLSVKRRCEPVLKEFGFAWPESLNCSKFPPQNDHNHMCMEGPGDEEV

人 FZD5Fri 结构域 (划底线为预测信号序列)(SEQ ID NO :91)

MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSVCERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATT

人 FZD8Fri 结构域 (划底线为预测信号序列)(SEQ ID NO :92)

MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNKTDLTT

人 IgG1Fc 区 (SEQ ID NO :93)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

人 IgG1Fc 区 (SEQ ID NO :94)

KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

人 IgG1Fc 区 (SEQ ID NO :95)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

连接子 (SEQ ID NO:96)

ESGGGGVT

连接子 (SEQ ID NO:97)

LESGGGGVT

连接子 (SEQ ID NO:98)

GRAQVT

连接子 (SEQ ID NO:99)

WRAQVT

连接子 (SEQ ID NO:100)

ARGRAQVT

信号序列 (SEQ ID NO:101)

MEWGYLLEVTSLLAALALLQRSSGAAA

信号序列 (SEQ ID NO:102)

MEWGYLLEVTSLLAALALLQRSSGALA

信号序列 (SEQ ID NO:103)

MEWGYLLEVTSLLAALALLQRSSGVLA

信号序列 (SEQ ID NO:104)

MEWGYLLEVTSLLAALLLLQRSPIVHA

信号序列 (SEQ ID NO:105)

MEWGYLLEVTSLLAALFLLQRSPIVHA

信号序列 (SEQ ID NO:106)

MEWGYLLEVTSLLAALLLLQRSPFVHA

信号序列 (SEQ ID NO:107)

MEWGYLLEVTSLLAALLLLQRSPIIYA

信号序列 (SEQ ID NO:108)

MEWGYLLEVTSLLAALLLLQRSPIAHA

FZD8-Fc 氨基酸序列 - 变异体 54F03(无预测信号序列;划线部分为在该融合蛋白的 FZD8 序列与 Fc 序列之间的 GRA 连接子序列)(SEQ ID NO:109)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDY KKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGRADKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体

FZD8-Fc 变异体 54F03 氨基酸序列(无预测信号序列;选择性切割)(SEO ID NO:110)

AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTGRADKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT

VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F09 氨基酸序列（无预测信号序列）(SEQ ID NO:111)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPSPPDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPFNNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F09 氨基酸序列（无预测信号序列;选择性切割）(SEQ ID NO:112)

AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPSPPDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F15 氨基酸序列（无预测信号序列）(SEQ ID NO:113)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F15 氨基酸序列（无预测信号序列;选择性切割）(SEQ ID NO:114)

AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTAAPDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F16、54F17、54F18、54F23、54F25、54F27、54F29、54F31 及 54F34 氨基酸序列（无预测信号序列）(SEQ ID NO:115)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F16 氨基酸序列（无预测信号序列;选择性切割）(SEQ ID NO:116)

AAAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHTCPPCP

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

FZD8-Fc 变异体 54F19、54F20、54F24、54F26、54F28、54F30、54F32、54F34 及 54F35 氨基酸序列（无预测信号序列）(SEQ ID NO:117)

ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLE
DYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F19 氨基酸序列（无预测信号序列;选择性切割）(SEQ ID NO:118)

ALAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F20 氨基酸序列（无预测信号序列;选择性切割）(SEQ ID NO:119)

VLAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPI
CLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F34 氨基酸序列（无预测信号序列）(SEQ ID NO:120)

KELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYK
KPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTEPKSSDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FZD8-Fc 变异体 54F33 氨基酸序列（无预测信号序列）(SEQ ID NO:121)

KELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLKFFLCSMYTPICLEDYK
KPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTKSSDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

h-Wnt1 C 端多半胱氨酸区 (aa 288-370)(SEQ ID NO:122)

DLVYFEKSPNFCTYSGRLGTAGTAGRACNSSSPALDGCELLCCGRGHRTRTQRVTERCNCTFHWCCHVSCRNC
THTRVLHECL

h-Wnt2 C 端多半胱氨酸区 (aa 267-360)(SEQ ID NO :123)

DLVYFENSPDYCIRDREAGSLGTAGRVCNLTSRGMDSCEVMCCGRGYDTSHVTRMTKCGCKFHWCCAVRCQDC LEALDVHTCKAPKNADWTTAT

h-Wnt2b C 端多半胱氨酸区 (aa 298-391)(SEQ ID NO :124)

DLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSKGTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAVRCKEC RNTVDVHTCKAPKKAEWLDQT

h-Wnt3 C 端多半胱氨酸区 (aa 273-355)(SEQ ID NO :125)

DLVYYENSPNFCEPNPETGSFGTRDRTCNVTSHGIDGCDLLCCGRGHNTRTEKRKEKCHCIFHWCCYVSCQEC IRIYDVHTCK

h-Wnt3a C 端多半胱氨酸区 (aa 270-352)(SEQ ID NO :126)

DLVYYEASPNFCEPNPETGSFGTRDRTCNVSSHGIDGCDLLCCGRGHNARAERRREKCRCVFHWCCYVSCQEC TRVYDVHTCK

h-Wnt7a C 端多半胱氨酸区 (aa 267-359)(SEQ ID NO :127)

DLVYIEKSPNYCEEDPVTGSVGTQGRACNKTAPQASGCDLMCCGRGYNTHQYARVWQCNCKFHWCCYVKCNTC SERTEMYTCK

h-Wnt7b C 端多半胱氨酸区 (aa 267-349)(SEQ ID NO :128)

DLVYIEKSPNYCEEDAATGSVGTQGRLCNRTSPGADGCDTMCCGRGYNTHQYTKVWQCNCKFHWCCFVKCNTC SERTEVFTCK

h-Wnt8a C 端多半胱氨酸区 (aa 248-355)(SEQ ID NO :129)

ELIFLEESPDYCTCNSSLGIYGTEGRECLQNSHNTSRWERRSCGRLCTECGLQVEERKTEVISSCNCKFQWCC TVKCDQCRHVVSKYYCARSPGSAQSLGRVWFGVYI

h-Wnt8b C 端多半胱氨酸区 (aa 245-351)(SEQ ID NO :130)

ELVHLEDSPDYCLENKTLGLLGTEGRECLRRGRALGRWELRSCRRLCGDCGLAVEERRAETVSSCNCKFHWCC AVRCEQCRRRVTKYFCSRAERPRGGAAHKPGRKP

h-Wnt10a C 端多半胱氨酸区 (aa 335-417)(SEQ ID NO :131)

DLVYFEKSPDFCEREPRLDSAGTVGRLCNKSSAGSDGCGSMCCGRGHNILRQTRSERCHCRFHWCCFVVCEEC RITEWVSVCK

h-Wnt10b C 端多半胱氨酸区 (aa 307-389)(SEQ ID NO :132)

ELVYFEKSPDFCERDPTMGSPGTRGRACNKTSRLLDGCGSLCCGRGHNVLRQTRVERCHCRFHWCCYVLCDEC KVTEWVNVCK

含划底线的预测信号序列的 FZD8-Fc 变异体 54F28(SEQ ID NO :133)

MEWGYLLEVTSLLAALLLLQRSPFVHAASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQFW PLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMD YNRTDLTTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK

[0001]

申请人或代理人档案号 2293095PC02	国际申请号 PCT/US2013/066087

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 36 页，第 17 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 PTA-9540
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
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[0002]

微生物保藏(2)	
A.对说明书第 36 页，第 22 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 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	

微生物保藏(3)	
A.对说明书第 36 页，第 27 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 2009-08-26	保藏号 PTA-10307
C.补充说明（必要时）	更多信息在附加页中 ☐

[0003]

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

微生物保藏(4)	
A.对说明书第_36_页，第_27_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 2009-08-26	保藏号 PTA-10309
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）	

[0004]

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

微生物保藏(5)	
A.对说明书第 36 页，第 27 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 2009-08-26	保藏号 PTA-10311
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	

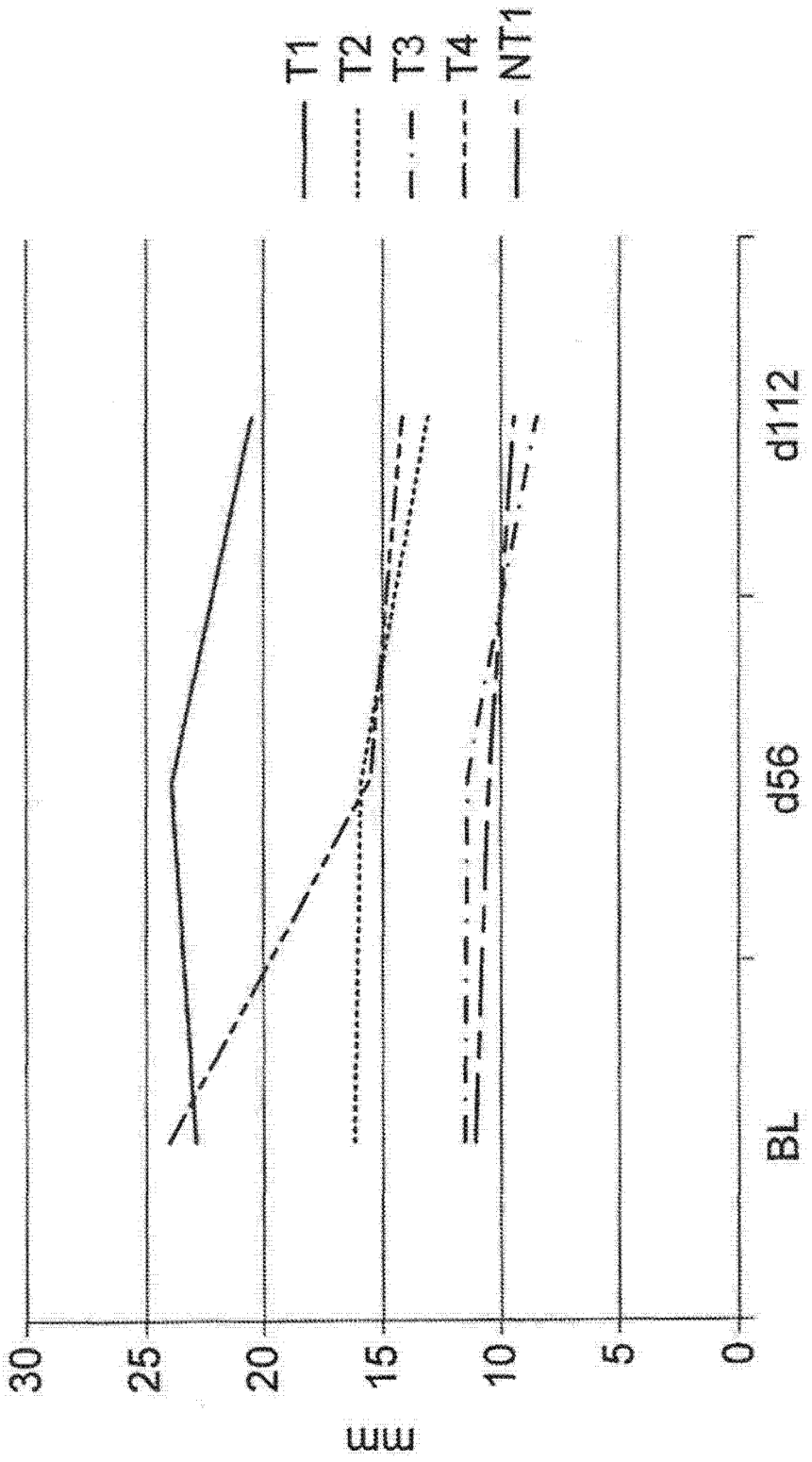
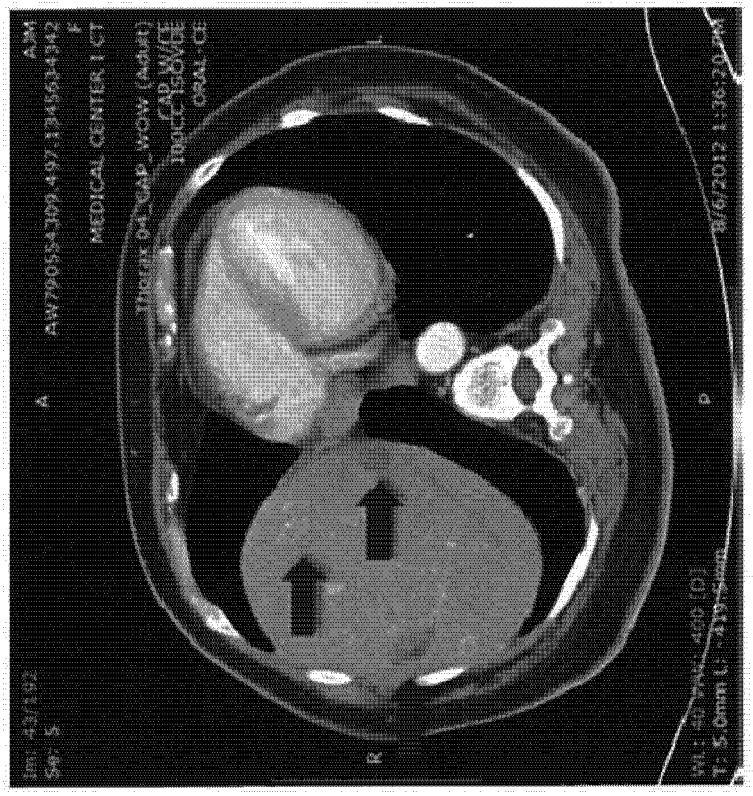
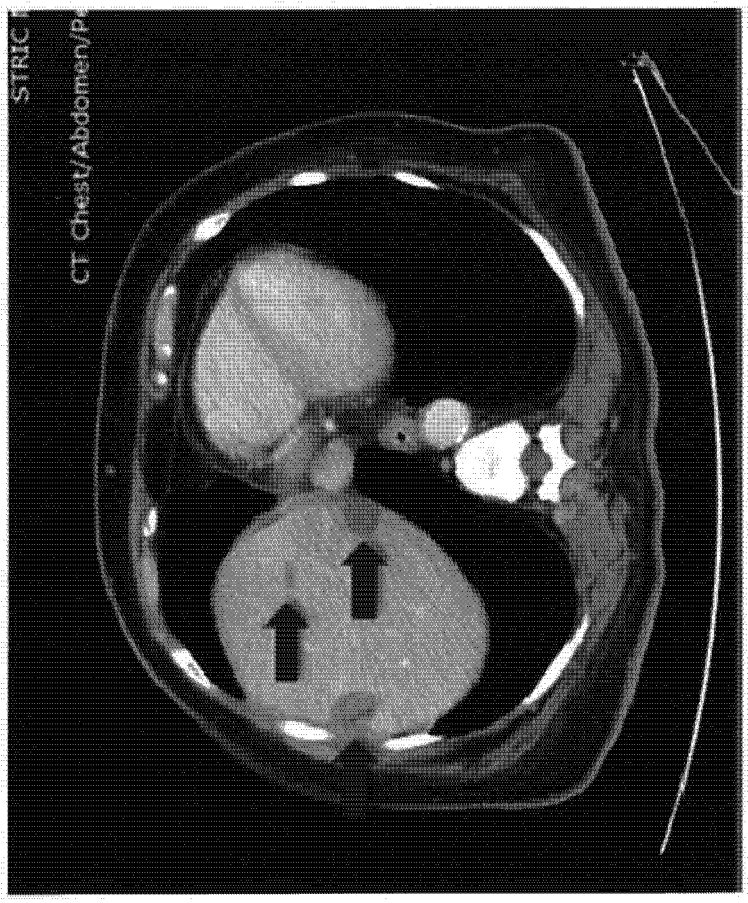


图 1A



第112天



基准期

图 1B

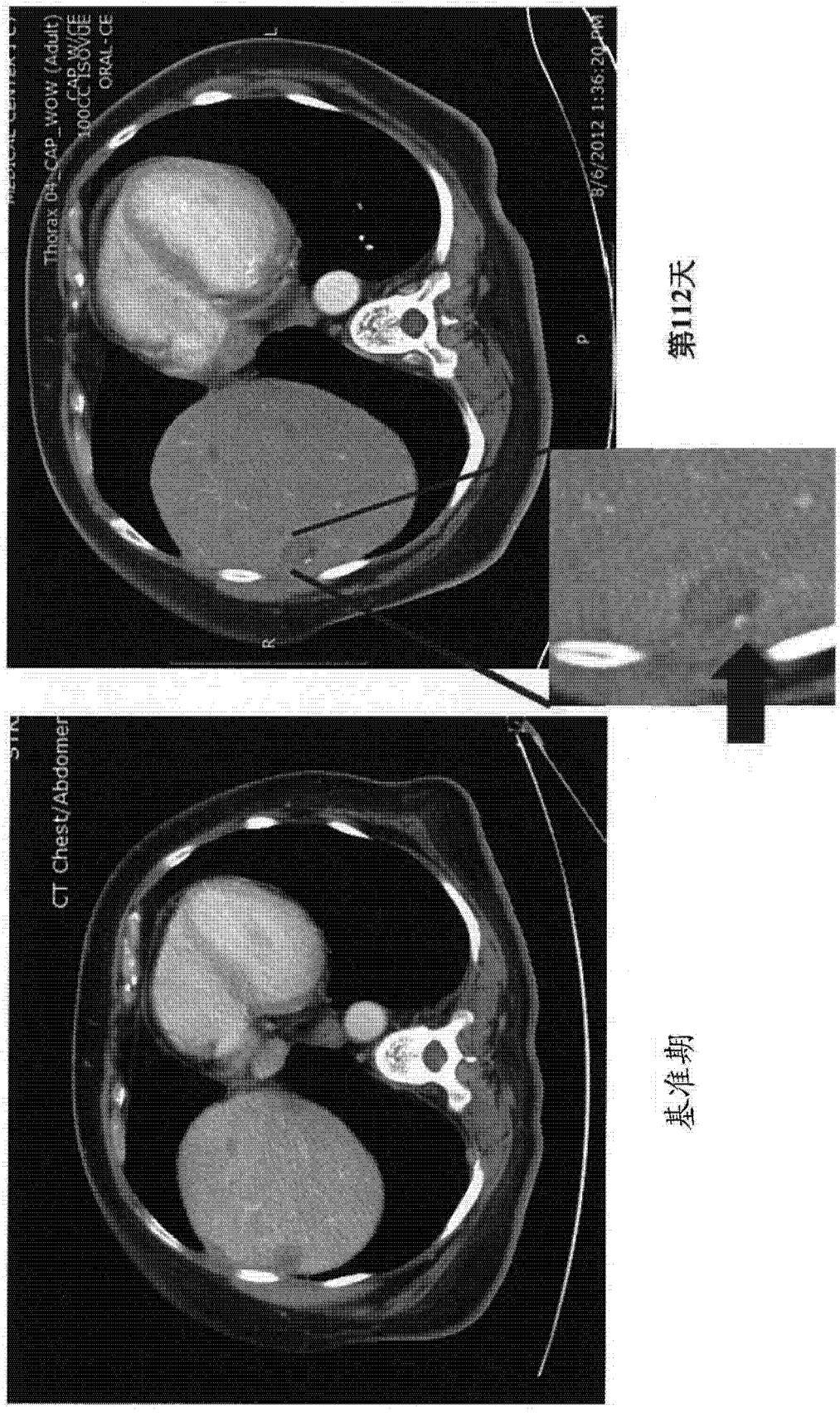

Thorax 0B_CAP_WOW (Adult)
CAP W/CE
100CC ISOVUE
ORAL-CE
8/6/2012 1:36:20 PM
R
L
P
第112天
CT Chest/Abdomen
基准期

图 1C

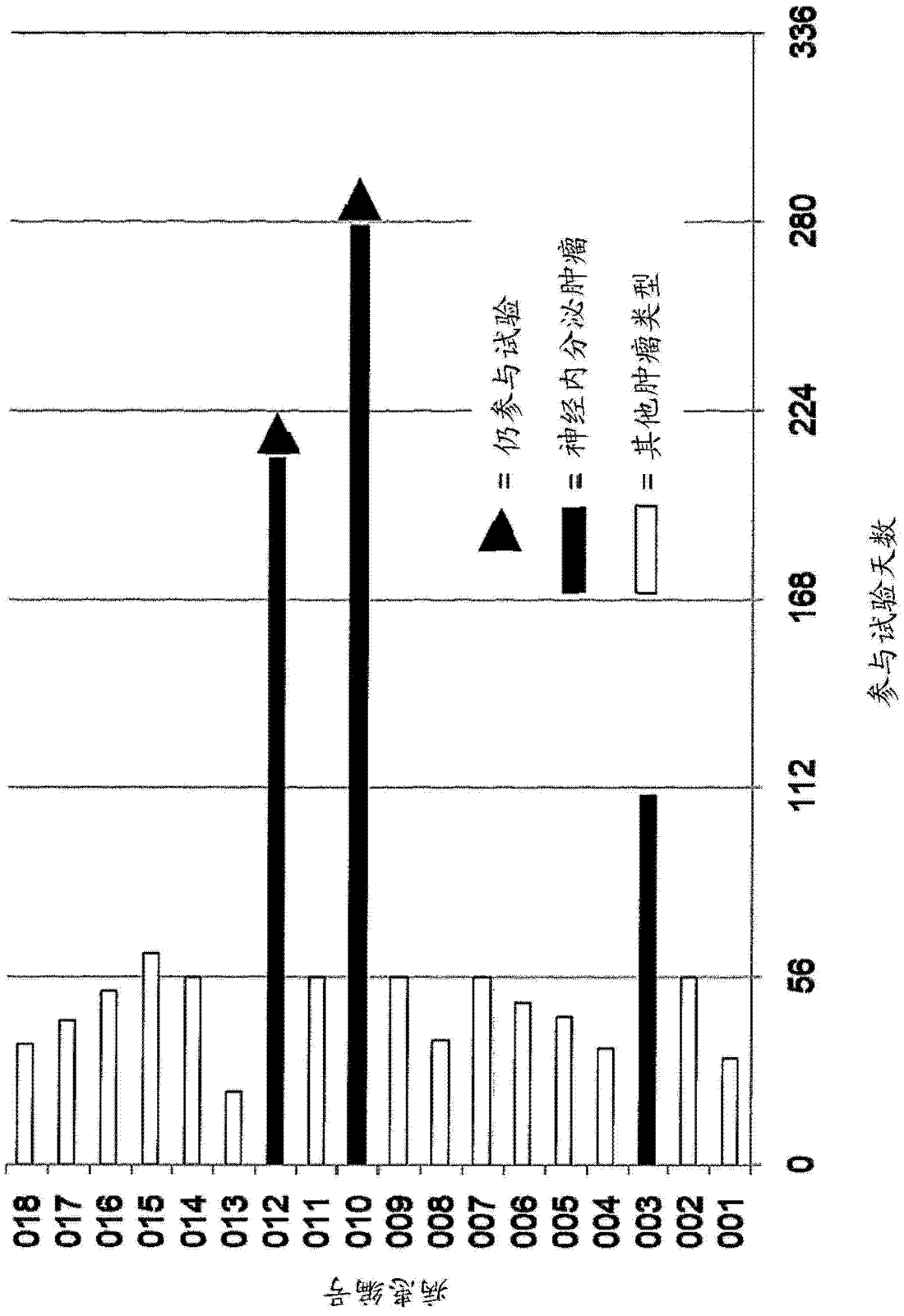

336
280
224
168
112
56
0
参与试验天数
= 仍参与试验
= 神经内分泌肿瘤
= 其他肿瘤类型
018
017
016
015
014
013
012
011
010
009
008
007
006
005
004
003
002
001
病患编号

图 2A

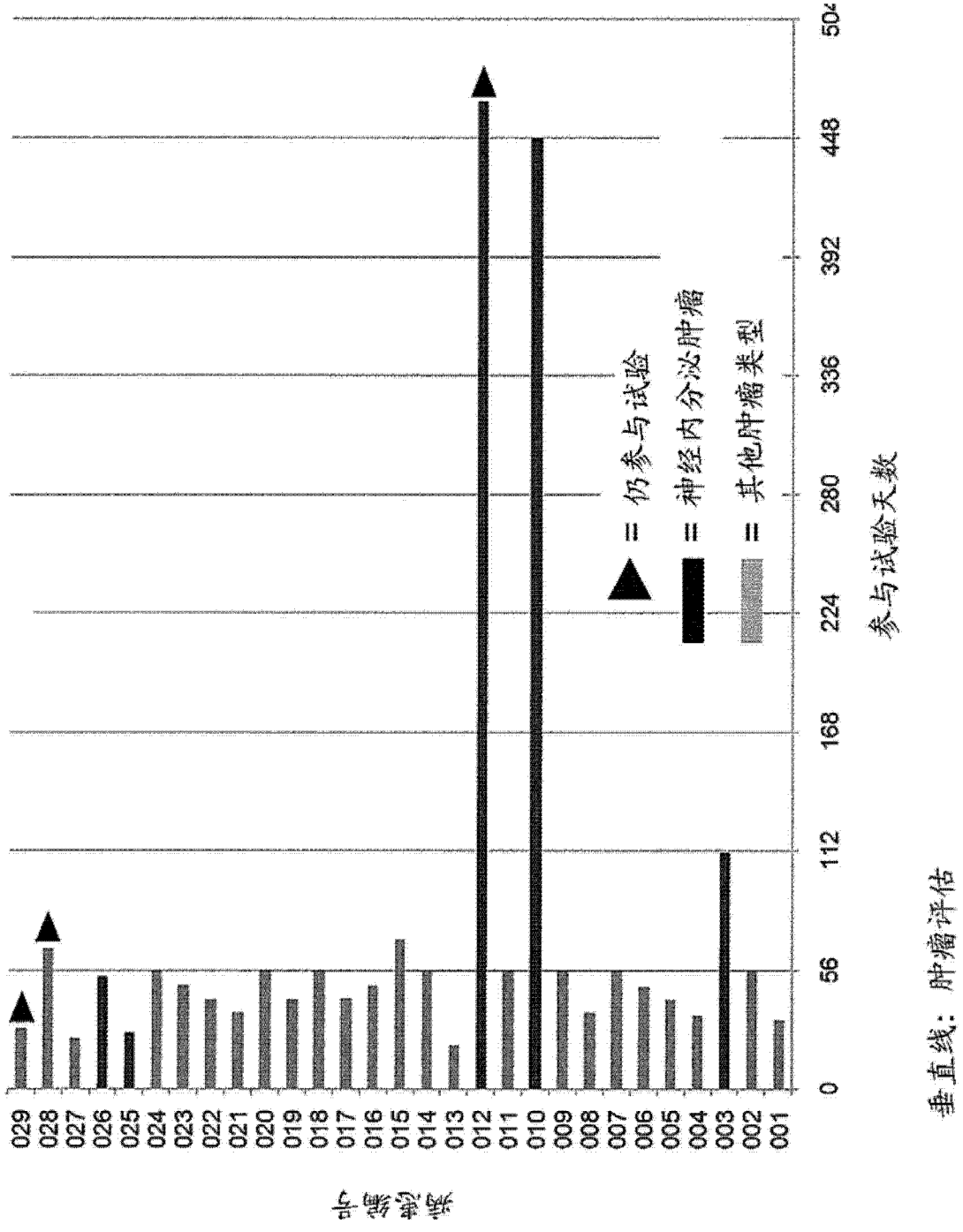
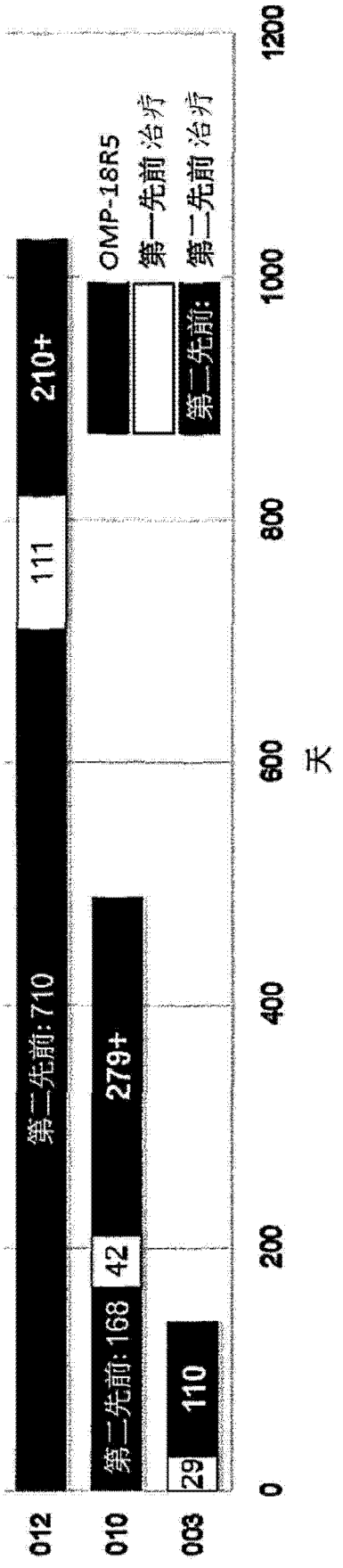

0
56
112
168
224
280
336
392
448
504
参与试验天数
= 仍参与试验
= 神经内分泌肿瘤
= 其他肿瘤类型
垂直线：肿瘤评估
029
028
027
026
025
024
023
022
021
020
019
018
017
016
015
014
013
012
011
010
009
008
007
006
005
004
003
002
001
疾患编号

图 2B



病患编号	类型	年龄	性别	诊断	全身性治疗
003	类癌瘤	59	女	2004: 切除（意图治疗）	1. GSK 1120212 (MEK 抑制剂)+ GSK 2141795 (AKT 抑制剂)* 2. OMP-18R5*
010	胰神经内分泌肿瘤 (PNET)	69	女	2001: 切除（意图治疗） 2006: 肝转移	1. Regorafenib (1093 天) 2. AB0024 (αLOXL2)* 3. AMG820 (αCSFR1)* 4. OMP-18R5*
012	类癌瘤	77	女	2006: 肝转移	1. Sandostatin (569 天) 2. XL888 (HSP90 抑制剂)* 3. Sand + REGN910 (αAng2)* 4. OMP-18R5*

* 这些治疗的参与试验天数显示于上图

图 3A

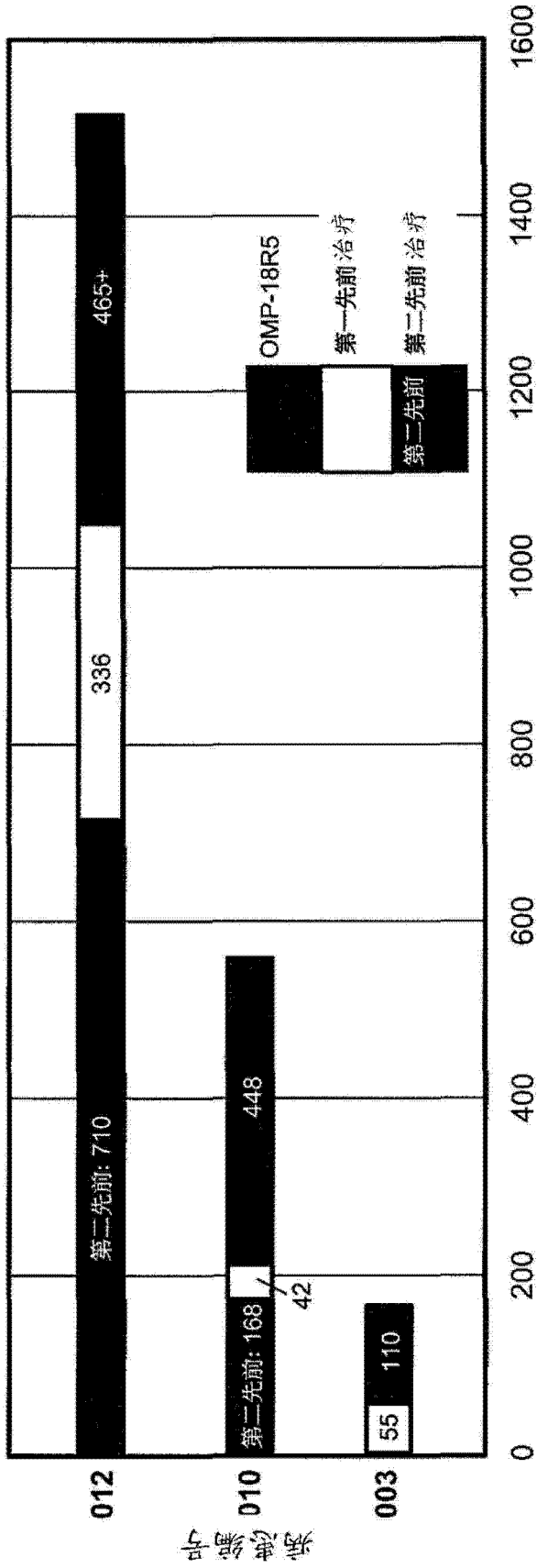

0
200
400
600
800
1000
1200
1400
1600
012
010
003
受试者编号
第二先前: 710
336
465+
第二先前: 168
42
448
55
110
OMP-18R5
第一先前治疗
第二先前治疗
第二先前

图 3B

1. 一种用于治疗神经内分泌癌的 Wnt 拮抗剂，其中所述 Wnt 拮抗剂是 (i) 特异性结合至少一种卷曲 (FZD) 受体的抗体或 (ii) 可溶性 FZD 受体。

2. 一种用于抑制神经内分泌癌的生长的 Wnt 拮抗剂，其中所述 Wnt 拮抗剂是 (i) 特异性结合至少一种卷曲 (FZD) 受体的抗体或 (ii) 可溶性 FZD 受体。

3. 如权利要求 1 或 2 所述的用于所述用途的 Wnt 拮抗剂，其中所述抗体与选自 FZD1、FZD2、FZD5、FZD7 及 FZD8 的一或多种人 FZD 受体特异性结合。

4. 如权利要求 1-3 中任一项所述的用于所述用途的 Wnt 拮抗剂，其中所述抗体包含：

(a) 包含 GFTFSHYTLS(SEQ ID NO:31) 的重链 CDR1，包含 VISGDGSYTYYADSVKG(SEQ ID NO:32) 的重链 CDR2，和包含 NFIKYVFAN(SEQ ID NO:33) 的重链 CDR3，以及包含 SGDNIGSFYVH(SEQ ID NO:34) 的轻链 CDR1，包含 DKSNRPSG(SEQ ID NO:35) 的轻链 CDR2 和包含 QSYANTLSL(SEQ ID NO:36) 的轻链 CDR3；或

(b) 包含 GFTFSHYTLS(SEQ ID NO:31) 的重链 CDR1，包含 VISGDGSYTYYADSVKG(SEQ ID NO:32) 的重链 CDR2，和包含 NFIKYVFAN(SEQ ID NO:33) 的重链 CDR3，以及包含 SGDKLGKKYAS(SEQ ID NO:41) 的轻链 CDR1，包含 EKDNRPSG(SEQ ID NO:42) 的轻链 CDR2 和包含 SSFAGNSLE(SEQ ID NO:43) 的轻链 CDR3。

5. 如权利要求 1-4 中任一项所述的用于所述用途的 Wnt 拮抗剂，其中所述抗体包含：

(a) 包含 SEQ ID NO:37 的氨基酸序列的重链可变区；和 / 或

(b) 包含 SEQ ID NO:38 或 SEQ ID NO:44 的氨基酸序列的轻链可变区。

6. 如权利要求 1-5 中任一项所述的用于所述用途的 Wnt 拮抗剂，其中所述抗体包含：

(a) 包含 SEQ ID NO:39 的氨基酸序列的重链；和 / 或

(b) 包含 SEQ ID NO:40 或 SEQ ID NO:45 的氨基酸序列的轻链。

7. 如权利要求 3-6 中任一项所述的用于所述用途的 Wnt 拮抗剂，其中所述抗体是单克隆抗体、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、和 / 或抗体片段。

8. 如权利要求 1-6 中任一项所述的用于所述用途的 Wnt 拮抗剂，其中所述抗体是 OMP-18R5。

9. 如权利要求 1 或 2 所述的用于所述用途的 Wnt 拮抗剂，其中所述可溶性 FZD 受体包含所述人 FZD8 的 Fri 结构域。

10. 如权利要求 9 所述的用于所述用途的 Wnt 拮抗剂，其中所述可溶性 FZD 受体进一步包含人 Fc 结构域。

11. 如权利要求 9 或 10 所述的用于所述用途的 Wnt 拮抗剂，其中所述 FZD8Fri 结构域包含 SEQ ID NO:28 的氨基酸序列。

12. 如权利要求 10 或 11 所述的用于所述用途的 Wnt 拮抗剂，其中所述人 Fc 结构域包含 SEQ ID NO:95 的氨基酸序列。

13. 如权利要求 1 或 2 所述的用于所述用途的 Wnt 拮抗剂，其中所述可溶性 FZD 受体包含 SEQ ID NO:117 的氨基酸序列。

14. 如权利要求 1 或 2 所述的用于所述用途的 Wnt 拮抗剂，其中所述可溶性 FZD 受体是 OMP-54F28。

15. 如权利要求 1-14 中任一项所述的用于所述用途的 Wnt 拮抗剂，其中所述神经内分

泌癌选自：胰腺神经内分泌癌、类癌瘤、胃肠胰腺神经内分泌瘤、嗜铬细胞瘤、副神经节瘤、甲状腺髓质癌、肺神经内分泌瘤及胸腺神经内分泌瘤。

16. 如权利要求 15 所述的用于所述用途的 Wnt 拮抗剂，其中所述神经内分泌癌是胰腺神经内分泌癌。

17. 如权利要求 15 所述的用于所述用途的 Wnt 拮抗剂，其中所述神经内分泌癌是类癌瘤。

18. 如权利要求 1-17 中任一项所述的用于所述用途的 Wnt 拮抗剂，其进一步包括采用至少一种与所述 Wnt 拮抗剂组合使用的额外的治疗剂。

19. 如权利要求 18 所述的用于所述用途的 Wnt 拮抗剂，其中所述额外的治疗剂是化学治疗剂。

20. 如权利要求 18 或 19 所述的用于所述用途的 Wnt 拮抗剂，其中所述额外的治疗剂是

(a) 白蛋白结合型太平洋紫杉醇 (ABRAXANE)；

(b) 吉西他滨；或

(c) 白蛋白结合型太平洋紫杉醇和吉西他滨。

21. 用于治疗神经内分泌癌的包含 Wnt 拮抗剂的药物组合物，其中所述 Wnt 拮抗剂是 (i) 特异性结合至少一种卷曲 (FZD) 受体的抗体或 (ii) 可溶性 FZD 受体。