Gland Biol. Neoplasia 8:145-58). More recently mammary stem cells have been shown to be activated by Wnt signaling (Liu et al., 2004, *Proc. Nat'l Acad. Sci.* 101:4158). In human breast cancer, β-catenin accumulation implicates activated Wnt signaling in over 50% of carcinomas, and though specific mutations have not been identified, upregulation of Frizzled receptor expression has been observed (Brennan & Brown, 2004, *J. Mammary Gland Neoplasia* 9:119-31; Malovanovic et al., 2004, *Int. J. Oncol.* 25:1337-42).

[0010] FZD10, FZD8, FZD7, FZD4, and FZD5 are five of ten identified human Wnt receptors. Fzd10 is co-expressed with Wnt7b in the lungs, and cell transfection studies have demonstrated that the Fzd10/LRP5 co-receptor activates the canonical Wnt signaling pathway in response to Wnt7b (Wang et al., 2005, *Mol. Cell Biol.* 25:5022-30). FZD10 mRNA is upregulated in numerous cancer cell lines, including cervical, gastric, and glioblastoma cell lines, and in primary cancers including approximately 40% of primary gastric cancers, colon cancers, and synovial sarcomas (Saitoh et al., 2002, *Int. J. Oncol.* 20:117-20; Terasaki et al., 2002, *Int. J. Mol. Med.* 9:107-12; Nagayama et al., 2005, *Oncogene* 1-12). FZD8 is upregulated in several human cancer cell lines, primary gastric cancers, and renal carcinomas (Saitoh et al., 2001, *Int. J. Oncol.* 18:991-96; Kirikoshi et al., 2001, *Int. J. Oncol.* 19:111-5; Janssens et al., 2004, *Tumor Biol.* 25:161-71). FZD7 is expressed throughout the gastrointestinal tract and is up-regulated in one out of six cases of human primary gastric cancer (Kirikoshi et al., 2001, *Int. J. Oncol.* 19:111-5). Expression of the FZD7 ectodomain by a colon cancer cell line induced morphological changes and decreased tumor growth in a xenograft model (Vincan et al., 2005, *Differentiation* 73:142-53). FZD5 plays an essential role in yolk sac and placental angiogenesis (Ishikawa et al., 2001, *Dev.* 128:25-33) and is upregulated in renal carcinomas in association with activation of Wnt/β-catenin signaling (Janssens et al., 2004, *Tumor Biology* 25:161-71). FZD4 is highly expressed in intestinal crypt epithelial cells and is one of several factors that display differential expression in normal versus neoplastic tissue (Gregorieff et al., 2005, *Gastroenterology* 129:626-38). The identification of FZD receptors as markers of cancer stem cells thus makes these proteins ideal targets for cancer therapeutics.

SUMMARY OF THE INVENTION

[0010a] In one aspect, the present invention provides an isolated monoclonal antibody that specifically binds one or more human frizzled receptors selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1); a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2); and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3); and

(b) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:7); a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:8); and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:9); or a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:4); a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:5); and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:6).

[0010b] In another aspect, the present invention provides an isolated monoclonal antibody that specifically binds one or more human frizzled receptors selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, wherein the antibody:

(a) binds at least part of a sequence Q(DE/ED)AGLEVHQF(Y/W)PL (SEQ ID NO:24) within the human frizzled receptor(s); and/or

(b) binds at least part of a sequence (K/Q)(F/Y)GF(Q/A) (SEQ ID NO:69) within the human frizzled receptor(s); and

wherein the antibody comprises:

(i) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1); a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2); and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3); and

(ii) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:7); a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:8); and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:9); or a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:4); a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:5); and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:6).

[0010c] In yet another aspect, the present invention provides an isolated cell producing the antibody as defined herein.

[0010d] In yet another aspect, the present invention provides a pharmaceutical composition comprising the antibody as defined herein and a pharmaceutically acceptable vehicle.

[0010e] In yet another aspect, the present invention provides the use of the antibody as defined herein, in the manufacture of a medicament for the treatment of cancer.

[0010f] In yet another aspect, the present invention provides the antibody as defined herein, for use as a medicament.

[0010g] In yet another aspect, the present invention provides the use of the antibody as defined herein, in the manufacture of a medicament for inhibiting tumor growth.

[0010h] In yet another aspect, the present invention provides an isolated polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs: 10-15.

[0010i] In yet another aspect, the present invention provides an isolated polynucleotide comprising:

(a) a polynucleotide encoding the antibody as defined herein or the polypeptide as defined herein; or

(b) a sequence selected from the group consisting of SEQ ID NOs:17-22, 87-90, 92, and 94-95.

[0010j] In yet another aspect, the present invention provides a vector comprising the polynucleotide as defined herein.

[0011] The present ~~invention~~disclosure provides novel agents that bind to one or more human frizzled receptors (FZDs), including, but not limited to, antibodies or other agents that bind two or more human frizzled receptors, and methods of using the agents. The present

~~invention~~disclosure further provides novel polypeptides, such as antibodies that bind one or more human frizzled receptors, fragments of such antibodies, and other polypeptides related to such antibodies. In certain ~~embodiments~~examples, the agent, antibodies, other polypeptides, or agents that bind a FZD, bind to a region of the FZD referred to herein as the Biological Binding Site (BBS) that the inventors have now for the first time identified as a target for inhibiting Wnt signaling and/or tumor growth. Antibodies and other polypeptides that comprise an antigen-binding site that binds more than one FZD are also provided. Polynucleotides comprising nucleic acid sequences encoding the polypeptides are also provided, as are vectors comprising the polynucleotides. Cells comprising the polypeptides and/or polynucleotides of the ~~invention~~disclosure are further provided. Compositions (e.g., pharmaceutical compositions) comprising the novel FZD-binding agents or antibodies are also provided. In addition, methods of making and using the novel FZD-binding agents or antibodies are also provided, such as methods of using the novel FZD-binding agents or antibodies to inhibit tumor growth and/or treat cancer.

[0012] Also disclosed is~~Thus, in one aspect, the invention provides~~ an agent that specifically binds a human frizzled receptor. In certain ~~embodiments~~examples, the agent inhibits the binding of a ligand (e.g., a Wnt) to the Biological Binding Site (BBS) of the human frizzled receptor. In certain ~~embodiments~~examples, the agent binds to at least part of the Biological Binding Site (BBS) within the human frizzled receptor. In certain ~~embodiments~~examples, the binding of the agent to the BBS results in an inhibition of Wnt signaling and/or tumor growth. In certain ~~embodiments~~examples, the human frizzled receptor is FZD8 and the agent binds to at least a part of (a) a conformational epitope of FZD8 formed by amino acids 72(F), 74-75(PL), 78(I), 92(Y), 121-122(LM), and 129-132(WPDR (SEQ ID NO:70)); (b) a region of FZD8 consisting of the sequence QDEAGLEVHQFWPL (SEQ ID NO:67); and/or (c) a region of FZD8 consisting of the sequence QYGFA (SEQ ID NO:66). In certain ~~embodiments~~examples, the human frizzled receptor is selected from the group consisting of FZD1, FZD2, FZD5, FZD7, or FZD8, and the agent binds to at least part of the sequence Q(DE/ED)AGLEVHQF(Y/W)PL (SEQ ID NO:24) within the human frizzled receptor. For instance, in certain ~~embodiments~~examples, the human frizzled receptor is FZD8 and the agent binds to at least part of the sequence QDEAGLEVHQFWPL (SEQ ID NO:67) within FZD8. In certain ~~embodiments~~examples, the agent binds to at least part of the sequence GLEVHQ (SEQ ID NO:25). In certain ~~embodiments~~examples, the human frizzled receptor is FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD9, or FZD10, and the agent binds to at least part of a region of the human frizzled receptor corresponding to the region of FZD8 consisting of QDEAGLEVHQFWPL (SEQ ID NO:67). In certain ~~embodiments~~examples, the agent

binds to at least part of a sequence (K/Q)(F/Y)GF(Q/A) (SEQ ID NO:69) within FZD1, FZD2, FZD5, FZD7, and/or FZD8. For example, in certain ~~embodiments~~examples, the human frizzled receptor is FZD8 and the agent binds to at least part of a sequence QYGFA (SEQ ID NO:66) within FZD8. In certain alternative ~~embodiments~~examples, the human frizzled receptor is FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD9, or FZD10, and the agent binds to at least part of a region of the human frizzled receptor corresponding to the region of FZD8 consisting of QYGFA (SEQ ID NO:66). In certain ~~embodiments~~examples, the agent specifically binds to two or more, three or more, or four or more human frizzled receptors. In certain ~~embodiments~~examples, the agent specifically binds to human frizzled receptors comprising FZD5 and FZD8.

[0013] Also disclosed is~~In another aspect, the invention provides~~ an agent that competes for specific binding to a human frizzled receptor with an antibody (e.g., in an *in vitro* competitive binding assay), wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:10 and a light chain variable region comprising SEQ ID NO:12 or SEQ ID NO:14. In certain ~~embodiments~~examples, the antibody comprises a heavy chain variable region comprising SEQ ID NO:10 and a light chain variable region comprising SEQ ID NO:14. In certain ~~embodiments~~examples, the agent competes for specific binding to two or more, three or more, or four or more human frizzled receptors. In certain ~~embodiments~~examples, the agent competes for specific binding to FZD1, FZD2, FZD5, FZD7, or FZD8.

[0014] Also disclosed is~~In another aspect, the invention provides~~ an agent that competes for specific binding to a human FZD5 and/or FZD8 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:85 and a light chain variable region comprising SEQ ID NO:86.

[0015] Also disclosed is~~In another aspect, the invention provides~~ an agent that specifically binds to two or more human frizzled receptors. In certain ~~embodiments~~examples, the two or more frizzled receptors comprise: (a) FZD1 and a second frizzled receptor selected from the group consisting of FZD2, FZD5, FZD7, and FZD8; (b) FZD2 and a second frizzled receptor selected from the group consisting of FZD5, FZD7, and FZD8; (c) FZD5 and FZD7; or (d) FZD7 and FZD8. In certain ~~embodiments~~examples, the agent specifically binds three or more (i.e., 3, 4, or 5) human frizzled receptors, wherein the three or more human frizzled receptors comprise FZD1, FZD2, FZD5, FZD7, and/or FZD8. In certain ~~embodiments~~examples, the three or more human receptors comprise FZD5 and FZD8. In certain ~~embodiments~~examples, the three or more human frizzled receptors further comprise FZD3, FZD4, FZD6, FZD9, and/or FZD10.

[0016] Also disclosed is~~In a further aspect, the invention provides~~ a polypeptide that specifically binds a human frizzled receptor, wherein the polypeptide comprises a heavy chain variable region comprising: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and/or (c) a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain ~~embodiments~~examples, the polypeptide specifically binds FZD1, FZD2, FZD5, FZD7, and/or FZD8. In certain ~~embodiments~~examples, the polypeptide specifically binds two or more human frizzled receptors including FZD5 and FZD8. In certain ~~embodiments~~examples, the amino acid substitutions are conservative substitutions.

[0017] Also disclosed is~~In an additional aspect, the invention provides~~ a polypeptide that specifically binds a human frizzled receptor, wherein the polypeptide comprises a light chain variable region comprising: (a) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:4) or SGDNIGSFYVH (SEQ ID NO:7), or a variant of SEQ ID NO:4 or SEQ ID NO:7 comprising 1, 2, 3, or 4 amino acid substitutions; (b) a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:5) or DKSNRPSG (SEQ ID NO:8), or a variant of SEQ ID NO:5 or SEQ ID NO:8 comprising 1, 2, 3, or 4 amino acid substitutions; and/or (c) a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:6) or QSYANTLSL (SEQ ID NO:9), or a variant of SEQ ID NO:6 or SEQ ID NO:9 comprising 1, 2, 3, or 4 amino acid substitutions. In certain ~~embodiments~~examples, the polypeptide specifically binds FZD1, FZD2, FZD5, FZD7, and/or FZD8. In certain ~~embodiments~~examples, the polypeptide specifically binds two or more human frizzled receptors including FZD5 and FZD8. In certain ~~embodiments~~examples, the amino acid substitutions are conservative substitutions.

[0018] Also disclosed is~~In another aspect, the invention provides~~ a polypeptide comprising: (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3); and/or (b) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:4) or SGDNIGSFYVH (SEQ ID NO:7), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:5) or DKSNRPSG (SEQ ID NO:8), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:6) or QSYANTLSL (SEQ ID NO:9). In certain ~~embodiments~~examples, the polypeptide specifically binds a human frizzled receptor. In certain ~~embodiments~~examples, the polypeptide specifically binds two or more (e.g., at least FZD5 and FZD8), three or more, or four or more human frizzled receptors.

[0019] Also disclosed is~~In a further aspect, the invention provides~~ an antibody that specifically binds a human frizzled receptor selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8, wherein the antibody comprises a heavy chain variable region comprising: (a)a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and/or (b) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:4), SGDNIGSFYVH (SEQ ID NO:7), or a variant of either SEQ ID NO:4 or SEQ ID NO:7 comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:5), DKSNRPSG (SEQ ID NO:8), or a variant of either SEQ ID NO:5 or SEQ ID NO:8 comprising 1, 2, 3, or 4 conservative amino acid substitutions; and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:6), QSYANTLSL (SEQ ID NO:9), or a variant of either SEQ ID NO:6 or SEQ ID NO:9 comprising 1, 2, 3, or 4 conservative amino acid substitutions. In certain ~~embodiments~~examples, the antibody comprises (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3); and/or (b) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:4) or SGDNIGSFYVH (SEQ ID NO:7), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:5) or DKSNRPSG (SEQ ID NO:8), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:6) or QSYANTLSL (SEQ ID NO:9). In certain ~~embodiments~~examples, the antibody comprises (a) a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:1), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:2), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:3); and/or (b) a light chain CDR1 comprising SGDNIGSFYVH (SEQ ID NO:7), a light chain CDR2 comprising DKSNRPSG (SEQ ID NO:8), and a light chain CDR3 comprising QSYANTLSL (SEQ ID NO:9).

[0020] Also disclosed is~~In another aspect, the invention provides~~ a polypeptide comprising (a) a polypeptide having at least about 80% sequence identity to SEQ ID NO:10; and/or (b) a polypeptide having at least about 80% sequence identity to SEQ ID NO:12 or SEQ ID NO:14. In certain ~~embodiments~~examples, the ~~invention~~disclosure provides a polypeptide comprising (a) a polypeptide having at least about 80% sequence identity to SEQ ID NO:10; and/or (b) a polypeptide having at least about 80% sequence identity to SEQ ID NO:14. In certain

~~embodiments~~examples, the polypeptide specifically binds a human frizzled receptor. In certain ~~embodiments~~examples, the polypeptide specifically binds two or more, three or more, or four or more human frizzled receptors. In certain ~~embodiments~~examples, the human frizzled receptor(s) are selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8.

[0021] Also disclosed is~~In still another aspect, the invention provides~~ an agent such as an antibody that specifically binds human FZD5 and/or FZD8, wherein the antibody comprises: (a) a heavy chain CDR1 comprising GFTFSSYYIT (SEQ ID NO:77), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising TISYSSSNTYYADSVKG (SEQ ID NO:78), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and a heavy chain CDR3 comprising SIVFDY (SEQ ID NO:79), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising SGDALGNRYVY (SEQ ID NO:80), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising SG (SEQ ID NO:81), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and a light chain CDR3 comprising GSWDTRPYPKY (SEQ ID NO:82), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions. In certain ~~embodiments~~examples, the antibody comprises: (a) a heavy chain CDR1 comprising GFTFSSYYIT (SEQ ID NO:77), a heavy chain CDR2 comprising TISYSSSNTYYADSVKG (SEQ ID NO:78), and a heavy chain CDR3 comprising SIVFDY (SEQ ID NO:79); and/or (b) a light chain CDR1 comprising SGDALGNRYVY (SEQ ID NO:80), a light chain CDR2 comprising SG (SEQ ID NO:81), and a light chain CDR3 comprising GSWDTRPYPKY (SEQ ID NO:82).

[0022] Also disclosed is~~In an additional aspect, the invention provides~~ a polypeptide that specifically binds FZD5 and/or FZD8, wherein said polypeptide comprises: (a) a polypeptide having at least about 80% identity to SEQ ID NO:85; and/or (b) a polypeptide having at least about 80% identity to SEQ ID NO: 86.

[0023] Also disclosed is~~In a further aspect, the invention provides~~ an agent that competes for specific binding to human FZD1, FZD2, FZD5, FZD7, and/or FZD8 with any one of the following IgG antibodies: 18R8, 18R5, 18R4605, and 18R4805.

[0024] Also disclosed is~~In a still further aspect, the invention provides~~ an agent that competes for specific binding to human FZD5 and/or FZD8 with the anti-FZD IgG antibody 44R24.

[0025] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide is an antibody. In certain alternative ~~embodiments~~examples, the agent is not an antibody.

[0026] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide or antibody specifically binds to the extracellular domain (ECD) of the human frizzled receptor or receptors to which it binds. In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide or antibody specifically binds to the Fri domain (Fri) of the human frizzled receptor or receptors to which it binds.

[0027] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described elsewhere herein, an individual antigen-binding site of the antibody or other polypeptide specifically binds (or is capable of binding) more than one human frizzled receptor.

[0028] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide or antibody inhibits binding of a ligand to the human frizzled receptor(s). In certain ~~embodiments~~examples, the ligand is a Wnt.

[0029] In certain ~~embodiments~~examples, of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide or antibody that binds to the FZD(s) is an antagonist of the FZD(s).

[0030] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide or antibody inhibits Wnt signaling. In certain ~~embodiments~~examples, the Wnt signaling that is inhibited is canonical Wnt signaling. In some ~~embodiments~~examples, the Wnt signaling that is inhibited by the FZD-binding agent is non-canonical Wnt signaling. In certain ~~embodiments~~examples, the Wnt signaling is non-canonical Wnt signaling.

[0031] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the FZD-binding agent or polypeptide or antibody inhibits tumor growth.

[0032] The ~~invention~~disclosure further provides the antibodies 18R8, 18R5, 18R4605, 44R24, and 18R4805, as well as fragments thereof.

[0033] The ~~invention~~disclosure further provides compositions, such as pharmaceutical compositions, comprising a FZD-binding agent or antibody.

[0034] Methods of inhibiting Wnt signaling (e.g., canonical Wnt signaling) and/or inhibiting tumor growth in a subject comprising administering a therapeutically effective amount of the FZD-binding agent or polypeptide or antibody are provided.

[0035] Methods of reducing the tumorigenicity of a tumor that comprises cancer stem cells are also provided. In certain ~~embodiments~~examples, the methods comprise administering a therapeutically effective amount of the FZD-binding agent or polypeptide or antibody to a

subject comprising the tumor. In certain ~~embodiments~~examples, the frequency of cancer stem cells in the tumor is reduced by administration of the antibody. In certain ~~embodiments~~examples, administration of the FZD-binding agent results in the differentiation of tumorigenic cells in the tumor to a non-tumorigenic state.

[0036] Also provided are methods of inducing cells in a tumor in a subject to differentiate, said methods comprising administering a therapeutically effective amount of the FZD-binding, agent, polypeptide, or antibody to the subject.

[0037] Methods of treating cancer in a subject, comprising administering a therapeutically effective amount of the FZD-binding agent, polypeptide, or antibody to the subject are further provided.

[0038] In addition, methods of reducing myofibroblast activation in the stroma of a solid tumor, comprising contacting the stroma with an effective amount of the FZD-binding agent, polypeptide, or antibody are also provided.

[0039] In certain ~~embodiments~~examples, the methods comprising administration of the FZD-binding agent, polypeptide, or antibody further comprise administering a second anti-cancer agent (e.g., a chemotherapeutic agent) to the subject. In certain ~~embodiments~~examples, the second agent is gemcitabine, irinotecan, or paclitaxel. In certain ~~embodiments~~examples, the second agent is an angiogenesis inhibitor and/or an inhibitor of Notch signaling.

[0040] Also disclosed is~~In another aspect, the invention provides~~ a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs: 10-15. Polypeptides comprising a sequence selected from the group consisting of SEQ ID NOs: 85-86 are likewise provided. Polynucleotides comprising nucleic acid sequences encoding such polypeptides are also provided.

[0041] Also disclosed is~~In a further aspect, the invention provides~~ a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 17-22. Polynucleotides comprising a sequence selected from the group consisting of SEQ ID NOs: 87-90, 92, and 94-95 are further provided.

[0042] Also disclosed is~~In a still further aspect, the invention provides~~ a polynucleotide that comprises a polynucleotide that hybridizes to a polynucleotide selected from the group consisting of SEQ ID NOs: 17, 19, 21, 87-90, 92, and 94-95, or a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NOs: 10, 12, 14, and 85-86 under conditions of high stringency. In certain ~~embodiments~~examples, the ~~invention~~disclosure comprises a polynucleotide that hybridizes to a polynucleotide consisting of a sequence SEQ ID NOs: 17, 19, or 21, or a polynucleotide encoding SEQ ID NOs: 10, 12, or 14 under conditions of high stringency.

[0043] In certain ~~embodiments~~examples of each of the aforementioned ~~aspects~~disclosures, as well as other ~~aspects~~disclosures described herein, the agent or polypeptide or antibody or polynucleotide is isolated. In certain ~~embodiments~~examples, the agent or polypeptide or antibody or polynucleotide is substantially pure.

[0044] The present ~~invention~~disclosure further provides a Wnt gene signature useful for the identification of tumors and/or patients likely to respond to treatment with a FZD-binding agent (e.g., an antagonist of a human frizzled receptor and/or an inhibitor of Wnt signaling) or other inhibitors of Wnt signaling. Methods of using the Wnt gene signature to select patients for treatment with a FZD-binding agent or other inhibitor of Wnt signaling are also provided. In certain ~~embodiments~~examples, the methods involve the assessment of the level of one or more genes in the Wnt gene signature. Methods of screening drug candidates against tumors identified using the Wnt gene signature are also provided. Arrays, kits, and other compositions useful in the methods are also provided.

[0045] The present ~~invention~~disclosure also provides methods of screening potential drug candidates or other agents. These methods include, but are not limited to, methods comprising comparing the levels of one or more differentiation markers in a first solid tumor that has been exposed to the agent relative to the levels of the one or more differentiation markers in a second solid tumor that has not been exposed to the agent. In certain ~~embodiments~~examples, these methods include comprising (a) exposing a first solid tumor, but not a second solid tumor, to the agent; (b) assessing the levels of one or more differentiation markers in the first and second solid tumors; and (c) comparing the levels of the one or more differentiation markers in the first and second solid tumors.

[0046] 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

[0047] Figure 1. 18R8 binds multiple human frizzled receptors. FACS analysis demonstrated that 18R8 binds to FZD1, FZD2, FZD5, FZD7, and FZD8 sequences on transiently transfected HEK293 cells. FACS plots are shown for the binding of 18R8 to

FZD7, and/or FZD8, QDEAGLEVHQFWPL (SEQ ID NO:67) within FZD8, AGLEVHQF (SEQ ID NO:68) within FZD8, and/or GLEVHQ (SEQ ID NO:25) within FZD8, and/or a sequence corresponding to any of these sequences in a different human frizzled receptor (as defined in Table 2, above) further binds to at least part of QYGFA (SEQ ID NO:66) within FZD8 or YGFA (SEQ ID NO:74) within FZD8, and/or a sequence corresponding to one of these sequences within FZD1-7, FZD9, or FZD10 (as defined in Table 2, above). In certain embodiments, the FZD binding agent binds to at least part of the sequence GLEVHQ (SEQ ID NO:25) within FZD8, as well as to at least part of the sequence YGFA (SEQ ID NO:74) within FZD8. In certain embodiments, the FZD-binding agent binds to at least part of a region of FZD1-7, FZD9, or FZD10 corresponding to the sequence GLEVHQ (SEQ ID NO:25) in FZD8, as well as to at least part of a region of FZD1-7, FZD9, or FZD10 corresponding to the sequence YGFA (SEQ ID NO:74) in FZD8. In certain embodiments, the FZD-binding agent that binds to the indicated sequences also binds to one or more sequences elsewhere within the human frizzled receptor(s) to which it binds. In other words, in certain embodiments, the epitope to which the FZD-binding agent or antibody binds is a region within the FZD extracellular domain that overlaps only partially with the above-indicated sequences. In certain alternative embodiments, the entire epitope to which the FZD-binding agent binds is entirely contained within the above-indicated sequences.

[00161] In certain embodiments, the agent 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.

[00162] The antibodies or other agents of the present invention can be assayed for specific binding by any method known in the art. The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as BIAcore analysis, FACS analysis, immunofluorescence, immunocytochemistry, Western blots, radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitation reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York~~, which is incorporated by reference herein in its entirety~~).

to test the efficacy of an anti-FZD antibody is provided in Example 8, below. 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~~.

[00173] In certain embodiments, antibodies to human frizzled receptors mediate cell death of a cell expressing the FZD protein via antibody-dependent cellular cytotoxicity (ADCC). ADCC involves cell lysis by effector cells that recognize the Fc portion of an antibody. Many lymphocytes, monocytes, tissue macrophages, granulocytes and eosinophiles, for example, have Fc receptors and can mediate cytolysis (Dillman, 1994, J. Clin. Oncol. 12:1497).

[00174] In certain embodiments, antibodies to one or more FZDs trigger cell death of a cell expressing the FZD protein(s) by activating complement-dependent cytotoxicity (CDC). CDC involves binding of serum complement to the Fc portion of an antibody and subsequent activation of the complement protein cascade, resulting in cell membrane damage and eventual cell death. Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf, Textbook of Immunology, 2nd Edition, Williams & Wilkins, p. 218 (1984)). Antibodies of different classes and subclasses differ in this respect, as do antibodies of the same subclass but from different species. Of human antibodies, IgM is the most efficient class of antibodies to bind complement, followed by IgG1, IgG3, and IgG2 whereas IgG4 appears quite deficient in activating the complement cascade (Dillman, 1994, J. Clin. Oncol. 12:1497; Jefferis et al., 1998, Immunol. Rev. 163:59-76). According to the present invention, antibodies of those classes having the desired biological activity are prepared.

[00175] The ability of any particular antibody against one or more FZDs to mediate lysis of the target cell by complement activation and/or ADCC can be assayed. The cells of interest are grown and labeled *in vitro*; the antibody is added to the cell culture in combination with either serum complement or immune cells which can be activated by the antigen antibody complexes. Cytolysis of the target cells is detected, for example, by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the in vitro test can then be used therapeutically in that particular patient.

[00200] 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.

[00201] 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.

[00202] This invention also encompasses bispecific antibodies that specifically recognize a human frizzled receptor. 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 on different molecules such that both, for example, the antibodies can specifically recognize and bind a human frizzled receptor as well as, for example, 1) an effector molecule on a leukocyte such as a T-cell receptor (e.g. CD3) or Fc receptor (e.g. CD64, CD32, or CD16) or 2) a cytotoxic agent as described in detail below. In certain embodiments, the bispecific antibody specifically binds at least one human frizzled receptor, as well as either VEGF, a Notch ligand, such as a delta-like ligand (for example, DLL4) or jagged, or at least one Notch receptor selected from the group consisting of Notch 1, Notch 2, Notch 3, and Notch 4. Bispecific antibodies can be intact antibodies or antibody fragments.

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~~.

[00230] 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).

[00231] 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.

[00232] 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 FZD-binding agent. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

[00233] 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.

[00234] Methods known in the art for purifying antibodies and other proteins 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~~.

[00235] In certain embodiments, the FZD-binding agent is a polypeptide that is not an antibody. A variety of methods for identifying and producing non-antibody polypeptides that bind with high affinity to a protein target are known in the art. See, e.g., Skerra, 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~~. In certain embodiments, phage display technology has been used to identify/produce the FZD-binding polypeptide. In certain embodiments, the polypeptide 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.

[00236] In some embodiments, the agent is a non-protein molecule. In certain embodiments, the agent is a small molecule. Combinatorial chemistry libraries and techniques useful in the identification of non-protein FZD-binding agents are known to those skilled in the art. See, e.g., Kennedy et al., J. Comb. Chem, 10:345-354 (2008), Dolle et al, J.

Comb. Chem., 9:855-902 (2007), and Bhattacharyya, Curr. Med. Chem., 8:1383-404 (2001), ~~each of which is incorporated by reference herein in its entirety~~. In certain further embodiments, the agent is a carbohydrate, a glycosaminoglycan, a glycoprotein, or a proteoglycan.

[00237] In certain embodiments, the agent is a nucleic acid aptamer. Aptamers are polynucleotide molecules that have been selected (e.g., from random or mutagenized pools) on the basis of their ability to bind to another molecule. In some embodiments, the aptamer comprises a DNA polynucleotide. In certain alternative embodiments, the aptamer comprises an RNA polynucleotide. In certain embodiments, the aptamer comprises one or more modified nucleic acid residues. Methods of generating and screening nucleic acid aptamers for binding to proteins are well known in the art. See, e.g., U.S. Patent No. 5,270,163, U.S. Patent No. 5,683,867, U.S. Patent No. 5,763,595, U.S. Patent No. 6,344,321, U.S. Patent No. 7,368,236, U.S. Patent No. 5,582,981, U.S. Patent No. 5,756,291, U.S. Patent No. 5,840,867, U.S. Patent No. 7,312,325, U.S. Patent No. 7,329,742, International Patent Publication No. WO 02/077262, International Patent Publication No. WO 03/070984, U.S. Patent Application Publication No. 2005/0239134, U.S. Patent Application Publication No. 2005/0124565, and U.S. Patent Application Publication No. 2008/0227735, ~~each of which is incorporated by reference herein in its entirety~~.

[00238] The present invention further provides methods of screening agents for efficacy in inhibiting Wnt signaling, for anti-tumor efficacy, and/or efficacy against cancer stem cells. These methods include, but are not limited to, methods comprising comparing the levels of one or more differentiation markers in a first solid tumor that has been exposed to the agent relative to the levels of the one or more differentiation markers in a second solid tumor that has not been exposed to the agent. In certain embodiments, these methods include (a) exposing a first solid tumor, but not a second solid tumor, to the agent; (b) assessing the levels of one or more differentiation markers in the first and second solid tumors; and (c) comparing the levels of the one or more differentiation markers in the first and second solid tumors. In certain embodiments, the agent is an inhibitor of the canonical Wnt signaling pathway, and/or inhibits binding of one or more human Wnt proteins to one or more human frizzled receptors. In certain embodiments, the agent is an antibody that specifically binds to one or more human frizzled receptor. In certain embodiments, increased levels of one or more differentiation markers in the first solid tumor relative to the second solid tumor indicates efficacy against solid tumor stem cells. In certain alternative embodiments, decreased levels of one or more differentiation markers (i.e., negative markers for differentiation) in the first

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 vinka 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. In certain embodiments where the chemotherapeutic agent administered in combination with the FZD-binding agent or polypeptide or antibody comprises an antimitotic agent, the cancer or tumor being treated is breast cancer or a breast tumor.

[00284] In certain embodiments, the treatment involves the combined administration of an antibody (or other agent) of the present invention and radiation therapy. Treatment with the antibody (or agent) can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Any dosing schedules for such radiation therapy can be used as determined by the skilled practitioner.

[00285] In some embodiments, the second anti-cancer agent comprises an antibody. Thus, treatment can involve the combined administration of antibodies (or other agents) of the present invention with other antibodies against additional 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,

series of amino acid variants of FZD8 previously shown to disrupt binding of 18R8 (see Example 5 and Figures 6 and 7). Amino acids 126-127 of FZD8 were found to be required for binding 44R24 as indicated by reduced staining within the co-transfected (GFP positive) cell population. The results of the FACS experiments are shown in Figure 57. These results show that 44R24 binds to an epitope that overlaps with the epitope of 18R8 and comprises shared amino acids 126-127.

EXAMPLE 25

C28 colon tumor growth study with anti-FZD antibodies 18R5, 18R8 and 44R24.

[00426] C28 tumor cells were injected sub-cutaneously in Nod-Scid mice. Tumors were allowed to grow until they had reached an average volume of 126 mm^3. Tumor bearing animals were groups were randomized into four groups (n =10 mice per group) and treated with either Control Ab (black squares), 18R8 (gray triangles), 44R24 (black open circles), or 18R5 (gray circles) (Figure 58). Antibodies were dosed intraperitoneally at 15 mg/kg, twice per week. Tumor volumes are indicated. Treatment with antibodies 44R24 and 18R5 reduced growth relative to the control group, while 18R8 had no effect (Figure 58).

[00427] Following treatment of the animals in the experiment shown in Figure 58, tumors were harvested, fixed in formalin, embedded in paraffin, and cut in 5 micron sections. Tumor sections were analyzed for Cytokeratin 7 expression, a marker of colon cell differentiation, by immunohistochemistry (Vectastain kit, Vector Labs). Cytokeratin 7 expression was seen to be elevated after treatment with 44R24 or 18R5 (Figure 59).

~~**[00428]** All publications, patents, patent applications, internet sites, and accession numbers/database sequences (including both polynucleotide and polypeptide sequences) cited herein 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.~~