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(54) Title: METHODS OF TREATING PANCREATIC CANCER

(57) Abstract: Novel methods of treating pancreatic cancer are provided. In one embodiment, the method comprises determining NOTCH mRNA expression levels in pancreatic cancer cells. In another embodiment, the method further comprises administering to a subject in need thereof a therapeutically effective dose of a NOTCH antagonist.

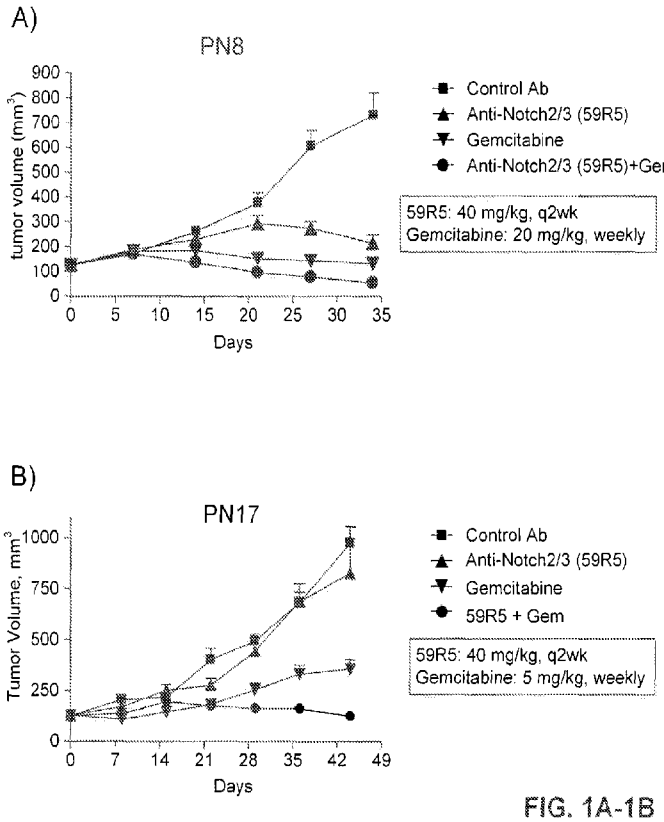

FIG. 1A-1B

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METHODS OF TREATING PANCREATIC CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the priority benefit of U.S. Provisional Application No. 61/794,788, filed March 15, 2013, which is hereby incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[002] The field of this invention generally relates to methods of treating pancreatic cancer. In one embodiment, the method comprises determining NOTCH gene expression levels in pancreatic cancer cells. In another embodiment, the method further comprises administering to a subject in need thereof a therapeutically effective dose of a NOTCH antagonist.

BACKGROUND OF THE INVENTION

[003] The NOTCH signaling pathway is one of several critical regulators of embryonic pattern formation, post-embryonic tissue maintenance, and stem cell biology. Unregulated NOTCH signaling 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 (Brennan and Brown, 2003, *Breast Cancer Res.* 5:69). Thus, carcinogenesis can proceed by usurping homeostatic mechanisms controlling normal development and tissue repair by stem cell populations (Beachy et al., 2004, *Nature* 432:324).

[004] The NOTCH receptor is a single-pass transmembrane receptor containing numerous tandem epidermal growth factor (EGF)-like repeats and three cysteine-rich NOTCH/LIN-12 repeats within a large extracellular domain (Wharton et al., 1985, *Cell* 43:567; Kidd et al., 1986, *Mol. Cell Biol.* 6:3094; reviewed in Artavanis et al., 1999, *Science* 284:770). Four mammalian NOTCH proteins have been identified (NOTCH1, NOTCH2, NOTCH3, and NOTCH4), and mutations in these receptors invariably result in developmental abnormalities and human pathologies including several cancers as described in detail below (Gridley, 1997, *Mol. Cell Neurosci.* 9:103; Joutel & Tournier-Lasserve, 1998, *Semin. Cell Dev. Biol.* 9:619-25).

[005] Aberrant NOTCH signaling has been implicated in a number of human malignancies, for example, T-cell acute lymphoblastic leukemia, breast cancer, cervical cancer, renal cell carcinoma, head and neck squamous cell carcinoma. Aberrant NOTCH signaling has also been implicated in the development of pancreatic cancer. See, e.g., Mazur et al., *Proc. Natl. Acad. Sci. U S A.* 107(30):13438-43 (2010), Wang et al., *Cancer Res.* 69(6):2400-7 (2009), Doucas et al., *J. Surg. Oncol.* 97(1):63-8 (2008), Yao and Qian, *Med. Oncol.* 27(3):1017-22 (2010); and Gungor et al., *Cancer Res.* 71(14):5009-19 (2011).

[006] Pancreatic cancer is the fourth leading cause of cancer deaths with a median survival of 6 months and a dismal 5-year survival rate of 3–5% and this figure has remained relatively unchanged over the past 25 years (Iovanna et al., *Front. Oncol.* 2012; 2: 6). Even for patients diagnosed with local disease, the 5-year survival rate is only 15%. The lethal nature of pancreatic cancer stems from its propensity to rapidly disseminate to the lymphatic system and distant organs. The presence of occult or clinical metastases at the time of diagnosis together with the lack of effective chemotherapies contributes to the high mortality in patients with pancreatic cancer.

[007] Pancreatic cancer is one of the most intrinsically drug-resistant tumors and resistance to chemotherapeutic agents is a major cause of treatment failure in pancreatic cancer. Gemcitabine is the standard chemotherapeutic drug for patients with advanced pancreatic cancer (Burris et al., *Eur. J. Cancer* 1997, 33:S18-22). Recently, a polychemotherapy regimen combining 5-FU, irinotecan, and oxaliplatin (FOLFIRINOX) was shown to nearly double overall survival compared to gemcitabine, at the expense of a manageable but increased toxicity, limiting its use to good performance status patients. In addition, overall survival was less than 12 months (Conroy et al., *N. Engl. J. Med.* 2011, 364:1817-25). Therefore, there is a need for designing new and targeted therapeutic strategies that can overcome the drug-resistance and improve the clinical outcome for patients diagnosed with pancreatic cancer.

SUMMARY OF THE INVENTION

[008] In one aspect, the invention provides methods for selecting a pancreatic cancer patient for treatment with a NOTCH inhibitor comprising: (a) determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and (b) selecting the patient based on the expression level of the one or more biomarkers.

[009] In another aspect, the invention provides methods for determining whether a patient diagnosed with pancreatic cancer is likely to respond to a NOTCH inhibitor-based therapy comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers indicates that the patient is likely to respond to therapy.

[010] In another aspect, the invention provides methods for determining whether a patient diagnosed with pancreatic cancer should be administered a NOTCH inhibitor, comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers is predictive of said patient having a favorable response to treatment with a NOTCH inhibitor.

[011] In another aspect, the invention provides methods to determine whether a patient diagnosed with pancreatic cancer should continue treatment with a NOTCH inhibitor, comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or

more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers indicates that the patient is likely to respond to therapy.

[012] In another aspect, the invention provides methods to determine whether a patient diagnosed with pancreatic cancer should continue treatment with a NOTCH inhibitor, comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers is predictive of said patient having a favorable response to treatment with said NOTCH inhibitor.

[013] In another aspect, the invention provides methods for determining the therapeutic efficacy of a NOTCH inhibitor for treating pancreatic cancer in a patient comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers is indicative of the therapeutic efficacy of said NOTCH inhibitor.

[014] In another aspect, the invention provides methods of treating pancreatic cancer in a patient comprising: (a) determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3; and (b) administering to said patient a therapeutically effective amount of a NOTCH inhibitor.

[015] In another aspect, the invention provides methods for stratifying a pancreatic cancer patient population for treatment with a NOTCH inhibitor comprising: (a) determining the level of expression of one or more biomarkers in tumor cells from said patients, wherein the one or more biomarkers comprise NOTCH3, and (b) stratifying the patient population based on the level of expression of the one or more biomarkers in the tumor cells.

[016] In certain embodiments, the level of NOTCH3 expression is determined to be above a reference level for NOTCH3 expression. In certain embodiments, each of the biomarkers is determined to be expressed at a level above a reference level for the biomarker.

[017] In certain embodiments, the expression level of the one or more biomarkers is determined by determining the level of the biomarker mRNA or the biomarker protein. In certain embodiments, the level of NOTCH3 expression is determined by determining the level of NOTCH3 mRNA in the tumor cells. In certain embodiments, the NOTCH3 mRNA level is determined by quantitative polymerase chain reaction. In certain embodiments, the NOTCH3 mRNA level is determined using: (a) a forward primer having a nucleotide sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:38, and SEQ ID NO:41; (b) a reverse primer having a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:39, and SEQ ID NO:42; and/or (c) a probe comprising an oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:40, and SEQ ID NO:43. In certain embodiments, the NOTCH3 mRNA level is determined using: (a) a forward primer having the sequence of SEQ ID NO:35, a reverse primer having the sequence of SEQ ID NO:36, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:37; (b) a forward primer having the sequence of SEQ ID NO:38, a reverse

primer having the sequence of SEQ ID NO:39, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:40; or (c) a forward primer having the sequence of SEQ ID NO:41, a reverse primer having the sequence of SEQ ID NO:42, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:43. In certain embodiments, the NOTCH3 mRNA level is determined by array hybridization. In certain embodiments, the level of NOTCH3 expression is determined by determining the level of NOTCH3 protein expressed by the tumor cells.

[018] In certain embodiments, the one or more biomarkers consist of NOTCH3. In certain embodiments, the one or more biomarkers further comprise MAML2 and the level of MAML2 expression is determined to be above a reference level for MAML2 expression. In certain embodiments, the one or more biomarkers consist of NOTCH3 and MAML2. In certain embodiments, the level of MAML2 expression is determined by determining the level of MAML2 mRNA in the tumor cells. In certain embodiments, the level of MAML2 expression is determined by determining the level of MAML2 protein expressed by the tumor cells.

[019] In another aspect, the invention provides methods of treating pancreatic cancer in a patient comprising administering to said patient a therapeutically effective amount of a NOTCH inhibitor, wherein at least some of the pancreatic tumor cells from said patient express each of one or more biomarkers at a level above a reference level for that biomarker and/or have been previously determined to express each of one or more biomarkers at a level above a reference level for that biomarker, wherein the one or more biomarkers comprise NOTCH3. In certain embodiments, the level of NOTCH3 expression is determined as the level of NOTCH3 mRNA. In certain embodiments, the level of NOTCH3 expression is determined as the level of NOTCH3 protein. In certain embodiments, the one or more biomarkers consist of NOTCH3. In certain embodiments, the one or more biomarkers further comprise MAML2 and the level of MAML2 expression is above a reference level for MAML2 expression. In certain embodiments, the one or more biomarkers consist of NOTCH3 and MAML2.

[020] In certain embodiments of the methods described herein, the reference level of a biomarker is a predetermined value. In certain embodiments, the reference level of a biomarker is the level of expression of that biomarker in a control sample. In certain embodiments, the reference level for NOTCH3 expression is the 25th percentile, the 30th percentile, the 40th percentile, the 50th percentile, the 60th percentile, the 70th percentile, the 75th percentile, or the 80th percentile for NOTCH3 expression in pancreatic cancers or a subset of pancreatic cancers. In certain embodiments, the reference level for NOTCH3 expression is the 75th percentile for NOTCH3 expression in pancreatic cancers. In certain embodiments, the reference level for NOTCH3 expression is the 50th percentile for NOTCH3 expression in pancreatic cancers. In certain embodiments, the reference level for NOTCH3 expression is the 25th percentile for NOTCH3 expression in pancreatic cancers. In certain embodiments, the reference level for NOTCH3 expression is the 75th percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node

metastatic pancreatic tumors, or chemotherapy-resistant pancreatic cancers. In certain embodiments, the reference level for NOTCH3 expression is the 50th percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node metastatic pancreatic tumors or chemotherapy-resistant pancreatic cancers. In certain embodiments, the reference level for NOTCH3 expression is the 25th percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node metastatic pancreatic tumors or chemotherapy-resistant pancreatic cancers.

[021] In certain embodiments, a method described herein further comprises obtaining a body sample from said patient. In certain embodiments, the level of expression of NOTCH3 is the level in a body sample from the patient. In certain embodiments, the sample is whole blood, plasma, serum, or tissue. In certain embodiments, the sample is a pancreatic tumor sample. In certain embodiments, the sample is from a pancreatic tumor that has metastasized to the liver. In certain embodiments, the sample is formalin-fixed paraffin embedded (FFPE) tissue.

[022] In certain embodiments of the methods described herein, the patient is a human or said patient population is a human population.

[023] In certain embodiments of the methods described herein, the pancreatic cancer is adenocarcinoma. In certain embodiments, the pancreatic cancer is chemotherapy-resistant.

[024] In certain embodiments, a method described herein comprises administering the NOTCH inhibitor to said patient. In certain embodiments, the NOTCH inhibitor is a gamma-secretase inhibitor. In certain embodiments, the NOTCH inhibitor is an anti-NOTCH antibody.

[025] In certain embodiments, the anti-NOTCH antibody specifically binds to human NOTCH2 or human NOTCH3. In certain embodiments, the anti-NOTCH antibody specifically binds to human NOTCH2 and NOTCH3. In certain embodiments, the anti-NOTCH antibody specifically binds to EGF repeat 10 of human NOTCH2. In certain embodiments, the anti-NOTCH antibody specifically binds to EGF repeat 9 of human NOTCH3. In certain embodiments, the anti-NOTCH antibody comprises an antigen-binding site that binds both the EGF repeat 9 of human NOTCH3 and the EGF repeat 10 of NOTCH2.

[026] In certain embodiments, the NOTCH inhibitor is an antagonist of human NOTCH2 and/or NOTCH3. In certain embodiments, the NOTCH inhibitor inhibits binding of a ligand to human NOTCH2 and/or NOTCH3. In certain embodiments, the NOTCH inhibitor inhibits signaling of human NOTCH2 and/or NOTCH3.

[027] In certain embodiments, the anti-NOTCH antibody is encoded by the polynucleotide deposited with ATCC as PTA-9547.

[028] In certain embodiments, the anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9); and (b) a light chain CDR1 comprising

RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8). In certain embodiments, the anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises: (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:5); and (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8).

[029] In certain embodiments, the anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises: (a) a heavy chain variable region having at least about 90% sequence identity to SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:26; and (b) a light chain variable region having at least about 90% sequence identity to SEQ ID NO:29 or SEQ ID NO:27. In certain embodiments, the anti-NOTCH antibody comprises: (a) a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:17; and (b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:29. In certain embodiments, the anti-NOTCH antibody comprises: (a) a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:18; and (b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:29. In certain embodiments, the anti-NOTCH antibody comprises: (a) a heavy chain variable region comprising SEQ ID NO:18; and (b) a light chain variable region comprising SEQ ID NO:29. In certain embodiments, the anti-NOTCH antibody comprises: (a) a heavy chain variable region comprising SEQ ID NO:17; and (b) a light chain variable region comprising SEQ ID NO:29.

[030] In certain embodiments, the anti-NOTCH antibody competes for specific binding to human NOTCH2 and/or NOTCH3 with an antibody selected from the group consisting of: (a) an antibody comprising a heavy chain variable region comprising SEQ ID NO:17 or SEQ ID NO:18, and a light chain variable region comprising SEQ ID NO:29; (b) an antibody comprising a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9), and a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8); and (c) an antibody encoded by the polynucleotide deposited with ATCC as PTA-9547.

[031] In certain embodiments, the anti-NOTCH antibody is a monoclonal antibody. In certain embodiments, the anti-NOTCH antibody is a chimeric antibody, a humanized antibody, a human antibody, or an antibody fragment.

[032] In certain embodiments, a method described herein further comprises administering a second therapeutic agent. In certain embodiments, the second therapeutic agent is a chemotherapeutic agent. In certain embodiments, the second therapeutic agent is a nucleoside analogue or a mitotic inhibitor.

In certain embodiments, the second therapeutic agent is gemcitabine, paclitaxel, albumin-bound paclitaxel, or combinations thereof.

[033] In another aspect, the invention provides a diagnostic composition comprising an isolated polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35-43. In certain embodiments, the diagnostic composition comprises: (a) a polynucleotide having the sequence of SEQ ID NO:35, a polynucleotide having the sequence of SEQ ID NO:36, and a polynucleotide having the sequence of SEQ ID NO:37; (b) a polynucleotide having the sequence of SEQ ID NO:38, a polynucleotide having the sequence of SEQ ID NO:39, and a polynucleotide having the sequence of SEQ ID NO:40; or (c) a polynucleotide having the sequence of SEQ ID NO:41, a polynucleotide having the sequence of SEQ ID NO:42, and a polynucleotide having the sequence of SEQ ID NO:43.

[034] In another aspect, the invention provides methods of detecting NOTCH3 mRNA in a sample, comprising contacting the sample with a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35-43. In certain embodiments, the method comprises contacting the sample with: (a) a forward primer having the sequence of SEQ ID NO:35, a reverse primer having the sequence of SEQ ID NO:36, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:37; (b) a forward primer having the sequence of SEQ ID NO:38, a reverse primer having the sequence of SEQ ID NO:39, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:40; or (c) a forward primer having the sequence of SEQ ID NO:41, a reverse primer having the sequence of SEQ ID NO:42, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:43.

[035] In another aspect, the invention provides kits for detecting NOTCH3 mRNA in a sample, comprising a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35-43. In certain embodiments, the kit comprises: (a) a polynucleotide having the sequence of SEQ ID NO:35, a polynucleotide having the sequence of SEQ ID NO:36, and a polynucleotide having the sequence of SEQ ID NO:37; (b) a polynucleotide having the sequence of SEQ ID NO:38, a polynucleotide having the sequence of SEQ ID NO:39, and a polynucleotide having the sequence of SEQ ID NO:40; or (c) a polynucleotide having the sequence of SEQ ID NO:41, a polynucleotide having the sequence of SEQ ID NO:42, and a polynucleotide having the sequence of SEQ ID NO:43.

[036] In another aspect, the invention provides primers having a sequence selected from the group consisting of: SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, and SEQ ID NO:42.

[037] In another aspect, the invention provides probes comprising an oligonucleotide having a sequence selected from the group consisting of: SEQ ID NO:37, SEQ ID NO:40, and SEQ ID NO:43.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[038] Figure 1. Activity of OMP-59R5 as a single agent, or in combination with a chemotherapeutic agent in (Fig. 1A) PN8 pancreatic tumor cells, (Fig. 1B) PN17 pancreatic tumor cells, (Fig. 1C) PN11 pancreatic tumor cells, (Fig. 1D) UM-PE13 breast tumor cells, (Fig. 1E) UM-T1 breast tumor cells, (Fig. 1F) OMP-Lu40 lung tumor cells, and (Fig. 1G) OMP-Lu53 lung tumor cells.

[039] Figure 2. Correlation of NOTCH3 gene expression and OMP-59R5 tumor inhibition. (Fig. 2A) Extent of pancreatic tumor inhibition by the OMP-59R5 antibody, in combination with gemcitabine, significantly correlates with the levels of NOTCH3 gene expression in the pancreatic tumor cells. (Fig. 2B) Distribution of NOTCH3 gene expression in pancreatic tumors that are responsive (R) and non-responsive (NR) to OMP-59R5 antibody treatment in combination with gemcitabine. NOTCH3 gene expression distribution is shown as a boxplot depicting the sample minimum, lower quartile, median, upper quartile and sample maximum.

[040] Figure 3. NOTCH3 gene expression in pancreatic tumors that are responsive and non-responsive to OMP-59R5 antibody treatment, in combination with gemcitabine, as determined by RNAseq. NOTCH3 gene expression was measured as RPKM (Reads Per Kilobase of transcript per Million mapped reads).

[041] Figure 4. Predicted probability of response to OMP-59R5 antibody treatment, in combination with gemcitabine, in pancreatic tumors based on NOTCH3 gene expression as a predictive indicator.

[042] Figure 5. Predicted probability of response to OMP-59R5 antibody treatment, in combination with gemcitabine, in pancreatic tumors based on NOTCH3 and MAML2 gene expression as a predictive indicator.

[043] Figure 6. NOTCH3 expression in pancreatic tumors. (Fig. 6A) NOTCH3 gene and protein expression in pancreatic tumors. (Fig. 6B) Distribution of NOTCH3 protein expression in pancreatic tumors that are responsive (R) and non-responsive (NR) to OMP-59R5 antibody treatment in combination with gemcitabine. NOTCH3 protein expression distribution is shown as a boxplot depicting the sample minimum, lower quartile, median, upper quartile and sample maximum.

[044] Figure 7. NOTCH3 gene expression in pancreatic cancer metastatic tissues. NOTCH3 gene expression was measured by RT-PCR. NOTCH3 gene expression distribution is shown as a boxplot depicting the sample minimum, lower quartile, median, upper quartile and sample maximum observed within samples of a particular tumor type. Vertical dashed lines represent 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile NOTCH3 expression values observed across all metastatic pancreatic tumor samples.

[045] Figure 8. NOTCH3 gene expression in liver and lymph node pancreatic cancer metastatic tissues, and xenografted tumors. NOTCH3 gene expression was measured by RT-PCR. NOTCH3 gene expression distribution is shown as a boxplot depicting the sample minimum, lower quartile, median, upper quartile and sample maximum observed within samples of a particular tumor type.

Vertical dashed lines represent 10th, 25th, 50th, 75th, and 90th percentile NOTCH3 expression values observed in the lymph node and liver metastatic pancreatic tumor samples.

[046] Figure 9. OMP-59R5 is active in combination with gemcitabine and ABRAXANE™ (protein bound paclitaxel) in pancreatic tumors.

DETAILED DESCRIPTION OF THE INVENTION

[047] The present invention is broadly directed to methods of treating pancreatic cancer using a NOTCH inhibitor. The invention provides methods for stratifying a pancreatic cancer patient population for treatment with a NOTCH inhibitor, methods for selecting a pancreatic patient for treatment with a NOTCH inhibitor, methods for determining whether a patient diagnosed with pancreatic cancer is likely to respond to a NOTCH inhibitor-based therapy, methods for determining whether a patient diagnosed with pancreatic cancer should be administered a NOTCH inhibitor, methods to determine whether a patient diagnosed with pancreatic cancer should continue treatment with a NOTCH inhibitor, and methods for determining the therapeutic efficacy of a NOTCH inhibitor for treating pancreatic cancer in a patient. In some embodiments, the methods comprise determining the level of NOTCH3 gene expression in tumor cells from a patient. In some embodiments, the methods provided herein further comprise determining the level of MAML2 gene expression in tumor cells from a patient. In some embodiments, the methods provided herein comprise administering a NOTCH inhibitor. In some embodiments, the NOTCH inhibitor is an antibody that specifically binds to one or binds to more than one human NOTCH receptor. In some embodiments, the antibody is administered in combination with a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is a nucleoside analogue or a mitotic inhibitor.

1. Definitions

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

[049] "NOTCH" is a membrane-bound transcription factor that regulates many cellular processes, especially in development. In response to ligand binding, its intracellular domain (ICD) is released by two proteases. The released intracellular domain enters the nucleus and interacts with a DNA-bound protein to activate transcription. The extracellular domain of NOTCH and related proteins contains up to 36 EGF-like domains, followed by three notch (DSL) domains. The intracellular domain (ICD) contains six ankyrin repeats and a carboxyl-terminal extension that includes a PEST domain. The NOTCH1 and NOTCH2 ICDs additionally comprise a transactivation domain (TAD). "NOTCH" encompasses all members of the NOTCH receptor family. A description of the NOTCH signaling pathway and conditions affected by it can be found, for example, in WO 98/20142 and WO 00/36089.

[050] There are four members of the NOTCH family in mammals: NOTCH1 (TAN1), NOTCH2, NOTCH3 and NOTCH4/Int-4. Exemplary sequences for the human NOTCH proteins include, but are not limited to: human NOTCH1 is encoded by the mRNA sequence set forth as Genbank Acc. No. NM_017617.3, and has the amino acid sequence set forth as Genbank Acc. No. NP_060087; human NOTCH2 is encoded by the mRNA sequence set forth as Genbank Acc. No. NM_024408, and has the amino acid sequence set forth as Genbank Acc. No. NP_077719; human NOTCH3 is encoded by the mRNA sequence of Genbank Acc. No. NM_000435.2, and has the amino acid sequence of Genbank Acc. No. NP_000426; and human NOTCH4 is encoded by the mRNA sequence of Genbank Acc. No. NM_004557, and has the amino acid sequence of Genbank Acc. No. NP_004548.

[051] A "NOTCH inhibitor," "NOTCH antagonist," "anti-NOTCH therapeutic agent," or "anti-NOTCH agent" as used herein includes any compound that partially or fully blocks, inhibits, or neutralizes a biological activity of the NOTCH pathway. Exemplary NOTCH inhibiting compounds include, but are not limited to gamma-secretase inhibitors such as, III-31-C, N-[N-(3,5-difluorophenacetyl)-L-alanyl]S-phenylglycine t-butyl ester) (DAPT), compound E, D-helical peptide 294, isocoumarins, BOC-Lys(Cbz)Ile-Leu-epoxide, and (Z-LL)2-ketone (see, Kornilova et al., *J. Biol. Chem.* 2003, 278:16479-16473); and those compounds described in WO 01/90084, WO 02/30912, WO 01/70677, WO 03/013506, WO 02/36555, WO 03/093252, WO 03/093264, WO 03/093251, WO 03/093253, WO 2004/039800, WO 2004/039370, WO 2005/030731, WO 2005/014553, WO 2004/089911, WO 02/081435, WO 02/081433, WO 03/018543, WO 2004/031137, WO 2004/031139, WO 2004/031138, WO 2004/101538, WO 2004/101539 and WO 02/47671 and U.S. Patent Application No. 2003/0114496. Specific gamma-secretase inhibitor compounds are also described in U.S. Pat. Nos. 6,984,663 and 7,304,094. Specific antibody NOTCH inhibitors are described herein, as well as in WO 2010/005566, and WO 2010/005567, all of which are herein incorporated by reference. NOTCH inhibitors also include NOTCH ligand antagonists.

[052] "NOTCH inhibitors," "NOTCH antagonists," "anti-NOTCH therapeutic agents," or "anti-NOTCH agents" also encompass antibodies that bind the NOTCH receptor. 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.

[053] 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., *J. Molec. Biol.* 1997, 273:927-948)). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[054] 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.

[055] 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.

[056] 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. Patent 5,225,539.

[057] 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.

[058] The term "chimeric antibodies" refers to antibodies wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammals (e.g. mouse, rat, rabbit, etc.) with the desired specificity, affinity, and capability while the constant regions are homologous to the sequences in antibodies derived from another (usually human) to avoid eliciting an immune response in that species.

[059] 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.

[060] 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 NOTCH receptor in more than one species. Likewise, because of homology between different paralogues (*e.g.*, the different human NOTCH 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 NOTCH 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 NOTCH proteins, such as NOTCH1, NOTCH2, NOTCH3 and/or NOTCH4). 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 NOTCH1, NOTCH2, NOTCH3 and/or NOTCH4). 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 NOTCH receptor, such as human NOTCH2, and further comprises a second, different antigen-binding site that recognizes a different epitope on a second NOTCH receptor, such as human NOTCH3. Generally, but not necessarily, reference to binding means specific binding.

[061] 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 NOTCH-dependent cancers. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia.

[062] "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.

[063] "Metastasis" as used herein refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at the new location. A "metastatic" or "metastasizing" cell is one that loses adhesive contacts with neighboring cells and migrates via the bloodstream or lymph from the primary site of disease to invade neighboring body structures.

[064] 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.

[065] 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.

[066] 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.

[067] 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. A "normal" subject or sample from a "normal" subject as used herein for quantitative and qualitative data refers to a subject who has or would be assessed by a physician as not having pancreatic cancer.

[068] A "control sample" means a separate sample from a control cell. The control cell can be disease free, or can be a pancreatic cancer cell. The control cell can be from the same subject or from another subject. The control cell can be from the same tissue or from a different tissue. The control cell can be from an immortalized cell line.

[069] The term "prognosis" is used herein to refer to the prediction of the likelihood of cancer attributable to death or progression, including recurrence, metastatic spread, and drug resistance, of a neoplastic disease, such as pancreatic cancer. As used herein, the term "predicting" or "prediction" refers to making a finding that a subject has a significantly enhanced or reduced probability of an outcome--favorable prognosis versus an unfavorable prognosis. It can also include the likelihood that a NOTCH inhibitor may be therapeutically effective versus one that is not found to be therapeutic. The term may also be used to refer to the likelihood that a patient will respond either favorably or

unfavorably to a drug or set of drugs, and also the extent of those responses, or that a patient will survive, following surgical removal or the primary tumor and/or chemotherapy for a certain period of time without cancer recurrence. The predictive methods of the present invention can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular patient. Towards this end, the predictive methods of the present invention are valuable tools in predicting if a patient is likely to respond favorably to a NOTCH-based treatment regimen, such as anti-NOTCH antibody treatment, chemotherapy with a given drug or drug combination, e.g. gamma-secretase inhibitor or another NOTCH inhibitor, or whether long-term survival of the patient, following a treatment protocol with a NOTCH inhibitor and/or termination of chemotherapy or other treatment modalities is likely.

[070] 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.

[071] 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.

[072] As used herein, the term "stratifying" refers to sorting subjects into different classes or strata based on the features of a particular disease state or condition. For example, stratifying a population of subjects with pancreatic cancer involves assigning the subjects based on the NOTCH3 gene expression levels in the tumor cells and/or on the basis of the severity of the disease (e.g., pre-malignant, malignant, metastatic etc.).

[073] 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.

[074] 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.

[075] As used herein, the terms "biopsy" or "biopsy tissue" refer to a sample of tissue or fluid that is removed from a subject for the purpose of determining if the sample contains cancerous tissue. In some embodiments, biopsy tissue or fluid is obtained because a subject is suspected of having cancer. The biopsy tissue or fluid is then examined for the presence or absence of cancer.

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

[077] 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.

[078] 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. NOTCH3 evaluation methods

[079] As shown in detail below, the sensitivity of human pancreatic tumors to the anti-NOTCH2/3 antibody OMP-59R5 significantly correlated with increased NOTCH3 expression. Surprisingly, while both NOTCH3 mRNA and protein expression correlated with OMP-59R5 sensitivity in human pancreatic tumors, the correlation was increased between NOTCH3 mRNA expression and treatment sensitivity than between NOTCH3 protein expression and treatment sensitivity. These data strikingly contrast the expression data from human breast, tumor and colon tumors which showed that there was no significant correlation between either NOTCH2 or NOTCH3 expression and tumor sensitivity to OMP-59R5 treatment. Similarly, no correlation between OMP-59R5 sensitivity and NOTCH2 expression was seen in human pancreatic tumors.

[080] The correlation between increased or elevated NOTCH3 expression (e.g., NOTCH3 over-expresssion) and sensitivity to OMP-59R5 treatment in pancreatic cancers (therapeutic efficacy) can be exploited to improve methods of treating pancreatic cancer by selecting pancreatic cancer patients for OMP-59R5 therapy whose tumor cells are characterized by elevated or increased NOTCH3 expression, NOTCH3 overexpression or NOTCH3 expression at or above a predetermined level. The terms "elevated NOTCH3 expression," "increased NOTCH3 expression," and "NOTCH3 overexpression" are, in some instances, used interchangeably herein. Therapeutic efficacy can also be improved by not selecting pancreatic cancer patients for OMP-59R5 therapy whose tumor cells are characterized by normal or reduced NOTCH3 expression, or NOTCH3 expression below a predetermined level. In certain embodiments, the predetermined NOTCH3 expression level can be the level of expression in a control sample, e.g., control cell. In certain embodiments, the predetermined NOTCH3 expression level can be the median level of NOTCH3 expression in pancreatic cancers, or the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 expression in pancreatic cancers.

[081] In certain embodiments, a patient has a pancreatic tumor in which at least some of the tumor cells demonstrate elevated NOTCH3 expression levels. In one embodiment, elevated NOTCH3 expression level is a level at or above the median level of NOTCH3 expression in pancreatic cancers. In another embodiment, elevated NOTCH3 expression level is a level that is at or above the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 gene expression of pancreatic cancers. In certain embodiments, the median level of NOTCH3 expression of pancreatic cancers is the median level of NOTCH3 expression of pancreatic adenocarcinomas, metastatic pancreatic cancers, liver and/or lymph node metastatic pancreatic cancers, chemotherapy-resistant pancreatic cancers, or advanced, refractory or recurrent pancreatic cancers. In certain embodiments, the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 expression in pancreatic cancers is the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic cancers, liver and/or lymph node metastatic

pancreatic cancers, chemotherapy-resistant pancreatic cancers, or advanced, refractory or recurrent pancreatic cancers.

[082] In certain embodiments, elevated NOTCH3 expression level is a level that is at or above a predetermined standard level, or reference level, or control level. The terms "predetermined standard," "reference level," and "control level" are, in some instances, used interchangeably herein. In one embodiment, a predetermined standard demonstrates NOTCH3 expression levels as measured in a control sample, *e.g.*, a sample containing pancreatic cells that does not comprise pancreatic tumor or pancreatic cancer cells. In another embodiment, a predetermined standard demonstrates NOTCH3 expression levels as measured in a sample comprising pancreatic tumor cells, e.g., adenocarcinomas, metastatic tumor cells and liver or lymph node metastatic tumor cells. In a further embodiment, a predetermined standard demonstrates NOTCH3 expression levels as measured in a sample comprising pancreatic cancer cells that do not respond to treatment with a NOTCH inhibitor, e.g., OMP-59R5. In a further embodiment, a predetermined standard demonstrates NOTCH3 expression levels as measured in a sample comprising pancreatic cancer cells that respond to treatment with a NOTCH inhibitor, e.g., OMP-59R5. In another embodiment, a predetermined standard is NOTCH3 expression levels in an isolated cell line. The cell line can be derived from a pancreatic cancer sample. The cell line can also be recombinantly manipulated to express NOTCH3. In certain embodiments, a predetermined standard or reference level for NOTCH3 expression is the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 gene expression in pancreatic cancers, for example, in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node metastatic pancreatic tumors, chemotherapy-resistant pancreatic cancers, or advanced, refractory or recurrent pancreatic cancers.

[083] In certain embodiments, a patient is selected for treatment and/or treated with a NOTCH inhibitor (e.g., OMP-59R5) when at least some of the patient's pancreatic tumor cells express NOTCH3 at an elevated level. In certain embodiments, at least some of the patient's pancreatic tumor cells express NOTCH3 at a level that is at or above a reference level. In certain embodiments, at least some of the patient's pancreatic tumor cells express NOTCH3 at a level that is at or above the median level of NOTCH3 expression in pancreatic cancers. In certain embodiments, at least some of the patient's pancreatic tumor cells express NOTCH3 at a level that is at or above the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 gene expression of pancreatic cancers. In certain embodiments, at least some of the patient's pancreatic tumor cells express NOTCH3 at a level that is at or above the 25^{th} percentile for NOTCH3 gene expression of pancreatic cancers. In certain embodiments, at least some of the patient's pancreatic tumor cells also express MAML2 at a level that is at or above a reference level, or at or above the median level of MAML2 expression in pancreatic cancers. In one embodiment, the patient is selected for treatment and/or treated with OMP-59R5. In another embodiment, the patient is selected for treatment and/or treated with an antibody comprising the six CDRs and/or the variable regions of OMP-59R5.

[084] In certain embodiments, a patient is selected for treatment and/or treated with a NOTCH inhibitor (e.g., OMP-59R5) when at least some of the patient's pancreatic tumor cells comprise a level of NOTCH3 mRNA at or above (1) a reference level, (2) the median level of NOTCH3 mRNA in pancreatic cancers; and/or (3) the 95th, 90th, 80th, 75th, 70th, 50th, 40th, 30th, 25th or 10th percentile for NOTCH3 mRNA level in pancreatic cancers. In a particular embodiment, at least some of the patient's pancreatic tumor cells comprise a level of NOTCH3 mRNA at or above the 25th percentile for NOTCH3 mRNA level in pancreatic cancers, e.g., in liver and/or lymph-node metastatic pancreatic cancers. In certain embodiments, at least some of the patient's pancreatic tumor cells also comprise MAML2 mRNA at or above a reference level, or at or above the median level of MAML2 mRNA in pancreatic cancers. In one embodiment, the patient is selected for treatment and/or treated with OMP-59R5. In another embodiment, the patient is selected for treatment and/or treated with an antibody comprising the six CDRs and/or the variable regions of OMP-59R5.

[085] In certain embodiments, a patient is selected for treatment and/or treated with a NOTCH inhibitor (e.g., OMP-59R5) when at least some of the patient's pancreatic tumor cells comprise a level of NOTCH3 protein at or above (1) a reference level, (2) the median level of NOTCH3 protein in pancreatic cancers; and/or (3) the 95th, 90th, 80th, 75th, 70th, 50th, 40th, 30th, 25th or 10th percentile for NOTCH3 protein level in pancreatic cancers. In a particular embodiment, at least some of the patient's pancreatic tumor cells comprise a level of NOTCH3 protein at or above the 25th percentile for NOTCH3 protein level in pancreatic cancers, e.g., in liver and/or lymph-node metastatic pancreatic cancers. In certain embodiments, at least some of the patient's pancreatic tumor cells also comprise MAML2 protein at or above a reference level, or at or above the median level of MAML2 protein in pancreatic cancers. In one embodiment, the patient is selected for treatment and/or treated with OMP-59R5. In another embodiment, the patient is selected for treatment and/or treated with an antibody comprising the six CDRs and/or the variable regions of OMP-59R5.

[086] Methods for detecting the level of NOTCH3 or the expression of another gene/gene product of interest (e.g., MAML2) comprise any method capable of determining the level of NOTCH3 expression at either the nucleic acid or protein level. Such methods are well known in the art and include, but are not limited to Western blots, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunofluorescence, flow cytometry, immunohistochemistry (IHC), nucleic acid hybridization techniques, nucleic acid reverse transcription methods, nucleic acid amplification methods such as PCR or qRT-PCR, RNase protection, microarrays, serial analysis of gene expression (SAGE), high-throughput mass spectrometry (MS), whole transcriptome shotgun sequencing (WTSS), massively parallel signature sequencing (MPSS), in situ hybridization, and Northern blotting.

[087] The median or percentile expression level of NOTCH3 in pancreatic cancers can be determined at any time relative to measuring NOTCH3 expression in a patient's pancreatic tumor cells. In certain embodiments, the NOTCH3 expression levels are measured contemporaneously. In

another embodiment, the median or percentile expression level of NOTCH3 in pancreatic cancers is determined prior to measurement of the NOTCH3 expression level in a patient's sample.

[088] In one embodiment, NOTCH3 expression is measured in a body sample. The phrase "body sample" as used herein, is intended any sample comprising a cell, a tissue, or a bodily fluid in which the level of NOTCH3 expression can be detected. Examples of such body samples include, but are not limited to, blood, lymph, urine, gynecological fluids, biopsies, amniotic fluid and smears. Body samples can be obtained from a patient by a variety of techniques. Methods for collecting various body samples are well known in the art. In certain embodiments, the body sample is a pancreatic tumor sample. In certain embodiments, the body sample can be a fixed sample, e.g. a formalin fixed, paraffin-embedded (FFPE) sample, or a frozen sample.

[089] In particular embodiments, the level of NOTCH3 expression is detected at the mRNA level. Various methods for determining expression of mRNA include, but are not limited to, quantitative real time PCR (qRT-PCR), microarray analysis, serial analysis of gene expression (SAGE), etc. In certain embodiments, the mRNA level in pancreatic tumor cells is determined using quantitative real time PCR (qRT-PCR) or microarray analysis. Many expression detection methods use isolated RNA. Any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA from body samples (see, e.g., Ausubel, ed., 1999, *Current Protocols in Molecular Biology* (John Wiley & Sons, New York). Additionally, large numbers of tissue samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski (U.S. Pat. No. 4,843,155).

[090] The term "probe" refers to any molecule that is capable of selectively binding to a specifically intended target biomolecule, for example, a nucleotide transcript of NOTCH3. Probes can be synthesized by one of skill in the art using known techniques, or derived from appropriate biological preparations. Probes can be specifically designed to be labeled with a detectable label. Examples of molecules that can be used as probes include, but are not limited to, RNA, DNA, proteins (including peptides), antibodies, and organic molecules.

[091] NOTCH3 mRNA from pancreatic tumor cells can be detected in hybridization or amplification assays that include, but are not limited to, mRNA sequencing methods, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to an mRNA or genomic DNA encoding NOTCH3. Hybridization of an mRNA with the probe indicates that the gene in question is being expressed.

[092] In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel

to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array (Santa Clara, Calif.). Known mRNA detection methods can be readily adapted for use in determining NOTCH3 mRNA in pancreatic tumor cells.

[093] An alternative method for determining the level of NOTCH3 mRNA in a sample involves the process of nucleic acid amplification, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany, 1991, *Proc. Natl. Acad. Sci. USA*, 88:189 193), self sustained sequence replication (Guatelli, 1990, *Proc. Natl. Acad. Sci. USA*, 87:1874 1878), transcriptional amplification system (Kwoh, 1989, *Proc. Natl. Acad. Sci. USA*, 86:1173 1177), Q-Beta Replicase (Lizardi, 1988, *Bio/Technology*, 6:1197), rolling circle replication (Lizardi, U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of NOTCH3 mRNA is assessed by quantitative fluorogenic RT-PCR (i.e., the TaqMan® System). Such methods typically use pairs of oligonucleotide primers that flank introns within the NOTCH3 gene. Methods for designing oligonucleotide primers specific for a known sequence are known in the art.

[094] In one embodiment, the present invention provides primer sets that are suitable for determining the level of NOTCH3 mRNA in a sample using quantitative RT-PCR. In one embodiment, the primer set comprises three isolated polynucleotides comprising the sequence of SEQ ID NO:35, 36, and 37. In one embodiment, the primer set comprises three isolated polynucleotides comprising the sequence of SEQ ID NO:38, 39, and 40. In one embodiment, the primer set comprises three isolated polynucleotides comprising the sequence of SEQ ID NO:41, 42, and 43. In a further aspect, the present invention provides a method for detecting the presence of NOTCH3 mRNA in a sample comprising contacting the sample with at least one isolated oligonucleotide comprising the sequence of SEQ ID NO:35-43. The primer sets provided herein can be used for quantitating NOTCH3 mRNA levels in a sample following standard qRT-PCR procedures.

[095] In one embodiment of the invention, microarrays are used to determine NOTCH3 mRNA levels in biological samples. Microarrays are particularly well suited for this purpose because of their reproducibility. DNA microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes or a large number of oligonucleotide probes directed to different parts of a molecule of interest. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by for example, laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. See, U.S. Pat. Nos. 6,040,138, 5,800,992 and 6,020,135, 6,033,860, and 6,344,316,

which are incorporated herein by reference. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNAs in a sample.

[096] Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261, incorporated herein by reference in its entirety. Although a planar array surface is preferred, the array can be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays can be peptides or nucleic acids on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate, see U.S. Pat. Nos. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992, each of which is hereby incorporated in its entirety. Arrays can be packaged in such a manner as to allow for diagnostics or other manipulation of an all-inclusive device. See, for example, U.S. Pat. Nos. 5,856,174 and 5,922,591 herein incorporated by reference.

[097] Methods for detecting the level of NOTCH3 protein in tumor cells can comprise any method that detects the presence of NOTCH3 protein in a biological sample. Such methods are well known in the art and include, but are not limited to, Western blots, slot blots, ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunocytochemistry, immunohistochemistry (IHC), and mass spectroscopy. Such immunoassay methods can be performed manually or in an automated fashion. Antibodies that bind any region of NOTCH3 are useful in the detection methods described herein. In one embodiment, the level of NOTCH3 protein in a tumor sample is determined using IHC.

[098] Techniques for detecting antibody binding are well known in the art. Antibody binding to NOTCH3 protein can be detected through the use of chemical reagents that generate a detectable signal that corresponds to the level of antibody binding and, accordingly, to the level of NOTCH3 protein. In one embodiment, antibody binding is detected through the use of a secondary antibody that is conjugated to a labeled polymer. Examples of labeled polymers include but are not limited to polymer-enzyme conjugates. The enzymes in these complexes are typically used to catalyze the deposition of a chromogen at the antigen-antibody binding site, thereby resulting in cell staining that corresponds to expression level of the mutation of interest. Enzymes of particular interest include horseradish peroxidase (HRP) and alkaline phosphatase (AP). Commercial antibody detection systems, such as, for example the Dako Envision+ system (Dako North America, Inc., Carpinteria, Calif.) and Mach 3 system (Biocare Medical, Walnut Creek, Calif.), can be used to practice the present invention.

[099] Detection of antibody binding can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein,

fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H.

[0100] In one embodiment, the level of NOTCH3 protein is determined using an agent that specifically binds to NOTCH3. Any molecular entity that displays specific binding to NOTCH3 can be employed to determine the level of NOTCH3 protein in a sample. Specific binding agents include, but are not limited to, antibodies, antibody mimetics, and polynucleotides (e.g., aptamers). One of skill understands that the degree of specificity required is determined by the particular assay used to detect NOTCH3 protein. For example, an agent that specifically binds to both full length NOTCH3 and NOTCH3 ICD can be used in a method that involves the separation of polypeptides based on their size, e.g. Western blot.

[0101] In one embodiment, the level of NOTCH3 protein is determined using an antibody specific for NOTCH3. In another embodiment, the antibody is a monoclonal antibody. NOTCH3 specific antibodies can be generated according to any method known to one of skill in the art. See, e.g., Tagami et al., 2008 *Mol. Cell. Biol.*, 28(1):165-176. NOTCH3 specific antibodies are also available from commercial sources. See, e.g., R&D Systems, Anti-Human NOTCH3 Polyclonal Antibody, Catalog # BAF1559. The anti-NOTCH3 antibody can be monoclonal antibody, polyclonal antibody, humanized antibody, human antibody, chimeric antibody or an antigen binding fragment thereof. In a further embodiment, the antibody specifically binds to NOTCH3 in a fixed and embedded tissue sample. The tissue sample can be a formalin fixed tissue sample. The tissue sample can be a paraffin embedded tissue sample.

3. NOTCH inhibitors

[0102] Another aspect of the methods of the invention is the use of a NOTCH inhibitor (*e.g.*, anti-NOTCH antibody) for treating pancreatic cancer patients whose NOTCH3 expression levels have been determined. In certain embodiments, the NOTCH inhibitor is an anti-NOTCH antibody. In certain embodiments, the anti-NOTCH antibody specifically binds to an EGF10 domain (or an equivalent of an EGF10 domain) of one or more human NOTCH receptors. In certain embodiments, the anti-NOTCH antibody specifically binds to EGF10 of human NOTCH2 and/or EGF9 of human NOTCH3. EGF9 is the EGF within human NOTCH3 that is equivalent to EGF10 in the other human NOTCH receptors NOTCH1, NOTCH2, and NOTCH4. In some embodiments, the anti-NOTCH antibody specifically binds to EGF10 of NOTCH2. In some embodiments, the anti-NOTCH antibody specifically binds to EGF10 of NOTCH2 and to EGF9 of NOTCH3. In some embodiments, the anti-NOTCH antibody specifically binds to EGF9 of NOTCH3. In other embodiments, the anti-NOTCH antibody binds to at least part of the sequence HKGAL (SEQ ID NO:1) within NOTCH2 EGF10. In some embodiments, the anti-NOTCH antibody binds to at least part of the sequence HEDAI (SEQ ID

NO:2) within NOTCH3 EGF9. Exemplary antibodies that bind NOTCH2 and NOTCH3 are described in U.S. Pat. No. 8,226,943, which is incorporated herein by reference in its entirety.

[0103] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention inhibits binding of a ligand to human NOTCH2 and/or NOTCH3. In some embodiments, the anti-NOTCH antibody inhibits binding of a ligand to human NOTCH2. In some embodiments, the anti-NOTCH antibody inhibits binding of a ligand to NOTCH2 and NOTCH3. In other embodiments, the anti-NOTCH antibody inhibits binding of a ligand to NOTCH3. In certain embodiments, the ligand is DLL4, JAG1 or JAG2. In other embodiments, the anti-NOTCH antibody inhibits signaling of human NOTCH2 and/or NOTCH3. In some embodiments, the anti-NOTCH antibody inhibits signaling of human NOTCH2. In some embodiments, the anti-NOTCH antibody inhibits signaling of NOTCH2 and NOTCH3. In other embodiments, the anti-NOTCH antibody inhibits signaling of NOTCH3. In some embodiments NOTCH2 and/or NOTCH3 signaling is induced by DLL4, JAG1 or JAG2.

[0104] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8). In some embodiments, the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions.

[0105] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:5); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8). In certain embodiments, the antibody specifically binds NOTCH2. In some embodiments, the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID

NO:3), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:5), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions.

[0106] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises (a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and/or a heavy chain CDR3 comprising (G/S)(I/S)F(F/Y)(A/P)(I/T/S/N) (SEQ ID NO:10); and/or (b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and/or a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8). In some embodiments, the antibody comprises a heavy chain CDR3 comprising SIFYPT (SEQ ID NO:11). In some embodiments, the antibody comprises a heavy chain CDR3 comprising SSSFFAS (SEQ ID NO:12). In other embodiments, the antibody comprises a heavy chain CDR3 comprising SSFYAS (SEQ ID NO:13). In certain embodiments, the antibody comprises a heavy chain CDR3 comprising SSFFAT (SEQ ID NO:14). In some embodiments, the antibody comprises a heavy chain CDR3 comprising SIFYPS (SEQ ID NO:15). In yet other embodiments, the antibody comprises a heavy chain CDR3 comprising SSFFAN (SEQ ID NO:16).

[0107] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention comprises: (a) a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:26 (with or without signal sequence); and/or (b) a light chain variable region having at least about 80% sequence identity to SEQ ID NO:29, SEQ ID NO:27 or SEQ ID NO:28 (with or without signal sequence). In certain embodiments, the anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3. In some embodiments, the anti-NOTCH antibody specifically binds to human NOTCH2. In some embodiments, the anti-NOTCH antibody binds to NOTCH2 and NOTCH3. In other embodiments, the anti-NOTCH antibody binds to NOTCH3. In certain embodiments, the anti-NOTCH antibody comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:18 or SEQ ID NO:17. In certain embodiments, the anti-NOTCH antibody comprises a light chain variable region having at

least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:29.

[0108] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention comprises: (a) a heavy chain having at least about 80% sequence identity to SEQ ID NO:30, SEQ ID NO:31, or SEQ ID NO:32 (with or without signal sequence); and/or (b) a light chain having at least about 80% sequence identity to SEQ ID NO:33, or SEQ ID NO:34 (with or without signal sequence). In certain embodiments, the anti-NOTCH antibody comprises a heavy chain having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:19, and a light chain having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:28. In certain embodiments, the anti-NOTCH antibody comprises a heavy chain having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:30, and a light chain having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:28.

[0109] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention comprises: (a) a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:17; and/or (b) a light chain variable region having at least about 80% sequence identity to SEQ ID NO:29. In certain embodiments, the anti-NOTCH antibody comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:17, and a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or about 100% sequence identity to SEQ ID NO:29.

[0110] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention comprises, consists, or consists essentially of a 59R1 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NOs:31 and 33 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on October 15, 2008, and assigned designation number PTA-9547.

[0111] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention comprises, consists or consists essentially of a 59R5 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NO:30 and SEQ ID NO:33 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the ATCC on July 6, 2009, and assigned designation number PTA-10170. In certain embodiments, the anti-NOTCH antibody useful in the methods of the invention comprises the heavy chains and light chains of the 59R5 IgG2 antibody (with or without the leader sequence). In certain embodiments, the anti-NOTCH antibody that is useful in the methods of the invention is the 59R5 IgG2 antibody. The 59R5 IgG2 antibody is also referred to herein as OMP-59R5. Additional information regarding the OMP-59R5 antibody can be

found, for example, in U.S. Patent No. 8,226,943, which is incorporated by reference herein in its entirety. In U.S. Patent No. 8,226,943, the OMP-59R5 antibody is generally referred to as "59R5" or the "59R5 IgG2 antibody."

[0112] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention competes for specific binding to human NOTCH2 and/or NOTCH3 with an antibody comprising a heavy chain variable region comprising SEQ ID NO:18 and a light chain variable region comprising SEQ ID NO:29. In certain embodiments, the antibody competes for specific binding with a 59R1 IgG2 antibody comprising the heavy chain and light chain of SEQ ID NOs:31 and 33 (with or without signal sequence), respectively, or as encoded by the DNA deposited with the ATCC on October 15, 2008, and assigned designation number PTA-9547. In some embodiments, the antibody competes for binding to human NOTCH2. In some embodiments, the antibody competes for binding to human NOTCH2 and NOTCH3. In other embodiments, the antibody competes for binding to human NOTCH3.

[0113] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention competes for specific binding to human NOTCH2 and/or NOTCH3 with an antibody comprising a heavy chain variable region comprising SEQ ID NO:17 and a light chain variable region comprising SEQ ID NO:29. In some embodiments, the antibody competes for specific binding with a 59R5 antibody comprising the heavy chain and light chain of SEQ ID NOs:30 and 33, respectively, or as encoded by the DNA deposited with the ATCC on July 6, 2009, and assigned designation number PTA-10170. In some embodiments, the antibody competes for binding to human NOTCH2. In some embodiments, the antibody competes for binding to human NOTCH2 and NOTCH3. In other embodiments, the antibody competes for binding to human NOTCH3.

[0114] In certain embodiments, an anti-NOTCH antibody that is 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.

[0115] In certain embodiments, an anti-NOTCH antibody that is 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 59R1 or 59R5 antibody.

[0116] Further examples of anti-NOTCH antibodies useful in the methods of the invention are disclosed in U.S. Patent 8,226,943, which is incorporated by reference herein in its entirety.

[0117] In certain embodiments, an anti-NOTCH antibody that is useful in the methods of the invention is a bispecific antibody that specifically recognizes a human NOTCH receptor. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. In one embodiment, the bispecific anti-NOTCH antibody specifically recognizes different epitopes within the same human NOTCH receptor. In another embodiment, the bispecific anti-

NOTCH antibody specifically recognizes different epitopes within a human NOTCH receptor or on different human NOTCH receptors.

[0118] Alternatively, in certain alternative embodiments, an anti-NOTCH antibody that is useful in the methods of the invention is not a bispecific antibody.

[0119] In certain embodiments, an anti-NOTCH antibody that is useful in 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 NOTCH receptors. In certain embodiments, an antigen-binding site of the monospecific anti-NOTCH antibody is capable of binding (or binds) one, two, three, or four human NOTCH receptors.

[0120] Another aspect of the methods of the invention is the use of a NOTCH inhibitor (*e.g.*, anti-NOTCH antibody) in the treatment of pancreatic cancer. In certain embodiments, the NOTCH inhibitors are inhibitors for gamma-secretase. Because gamma-secretase inhibitors are also able to prevent NOTCH receptor activation, several forms of gamma-secretase inhibitors have been tested for antitumor effects. First, an original gamma-secretase inhibitor, IL-X (cbz-IL-CHO), was shown to have NOTCH1-dependent antineoplastic activity in Ras-transformed fibroblasts. A tripeptide gamma-secretase inhibitor (z-Leu-leu-Nle-CHO) was reported to suppress tumor growth in cell lines and/or xenografts in mice from melanoma and Kaposi sarcoma (Curry CL et al., *Oncogene* 24:6333–44(2005)). Treatment with dipeptide gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) also resulted in a marked reduction in medulloblastoma growth and induced G0-G1 cell cycle arrest and apoptosis in a T-ALL animal model (O'Neil J. et al., *Blood* 107:781–5 (2006)). Another gamma-secretase inhibitor, dibenzazepine, has been shown to inhibit epithelial cell proliferation and induce goblet cell differentiation in intestinal adenomas in *Apc*−/− (min) mice (van Es JH, et al., *Nature* 435:959–63 (2005)). More recently, functional inactivation of NOTCH3 either by tripeptide gamma-secretase inhibitor or NOTCH3-specific small interfering RNA results in suppression of cell proliferation and induction of apoptosis in the tumor cell lines that overexpressed NOTCH3 but not in those with minimal amounts of NOTCH3 expression (Park JT et al., *Cancer Res.* 66: 6312-8 (2006)). Furthermore, a phase I clinical trial for a NOTCH inhibitor, MK0752 (developed by Merck, Whitehouse Station, NJ), has been launched for relapsed or refractory T-ALL patients and advanced breast cancers.

4. Methods of treatment

[0121] As described above, NOTCH inhibitors (e.g., OMP-59R5) can be used to treat pancreatic cancer in a patient whose tumor cells have been determined to possess increased levels of NOTCH3 expression (e.g., NOTCH3 mRNA expression), e.g., levels at or above the median level for NOTCH3 expression in pancreatic cancers, levels at or above the 95th, 90th, 80th, 75th, 70th, 50th, 40th, 30th, 25th or 10th percentile for NOTCH3 expression of pancreatic cancers, or levels at or above the level of NOTCH3 expression of a control sample. In certain embodiments, the tumor cells have also been

determined to possess increased levels of MAML2 expression (e.g., MAML2 mRNA expression), for example, levels at or above the median level for MAML2 expression in pancreatic cancers, or levels at or above the level of MAML2 expression of a control sample. In certain embodiments, the NOTCH inhibitors (e.g., OMP-59R5) are useful in inhibiting tumor growth, inducing differentiation, and/or reducing tumor volume. In addition, the invention provides a method of reducing the tumorigenicity of a pancreatic tumor in a subject, comprising administering a therapeutically effective amount of a NOTCH inhibitor (e.g., OMP-59R5) to a patient whose tumor cells have been determined to express increased levels of NOTCH3 as described herein. In certain embodiments, the tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the NOTCH inhibitor (e.g., OMP-59R5).

[0122] In one embodiment, NOTCH inhibitors (e.g., OMP-59R5) can be used to treat a pancreatic cancer whose tumor cells are characterized by having a level of NOTCH3 expression at or above the level of NOTCH3 expression in a control sample or cell. In one embodiment, NOTCH inhibitors (e.g., OMP-59R5) can be used to treat a pancreatic cancer whose tumor cells are characterized by having a level of NOTCH3 gene expression at or above the median level of NOTCH3 expression of pancreatic cancers. In certain embodiments, the pancreatic cancer treated comprises tumor cells characterized by having a level of NOTCH3 expression at or above the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 expression in pancreatic cancers. In certain embodiments, the median level of NOTCH3 expression of pancreatic cancers is the median level of NOTCH3 expression of pancreatic adenocarcinomas, metastatic pancreatic cancers, or liver and/or lymph node metastatic pancreatic cancers. In certain embodiments, the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 expression in pancreatic cancers is the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic cancers, or liver and/or lymph node metastatic pancreatic cancers. In certain embodiments, NOTCH3 expression level is determined using qRT-PCR. In certain embodiments, NOTCH3 expression level is determined using the probes described herein, for example, using a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:35-43.

[0123] In one embodiment, NOTCH inhibitors (e.g., OMP-59R5) can be used to treat a pancreatic cancer that comprises tumor cells at least some of which demonstrate a level of MAML2 expression at or above the level of MAML2 expression in a control cell. In one embodiment, NOTCH inhibitors (e.g., OMP-59R5) can be used to treat a pancreatic cancer that comprises tumor cells at least some of which demonstrate a level of MAML2 expression at or above the median level of MAML2 expression of pancreatic cancers. In certain embodiments, the pancreatic cancer treated comprises tumor cells at least some of which demonstrate a level of MAML2 expression at or above the 95^{th}, 90^{th}, 80^{th}, 75^{th}, 70^{th}, 50^{th}, 40^{th}, 30^{th}, 25^{th} or 10^{th} percentile for MAML2 expression in pancreatic cancers. In certain embodiments, the median level of MAML2 expression of pancreatic cancers is the median level of

MAML2 expression of pancreatic adenocarcinomas, metastatic pancreatic cancers, or liver and/or lymph node metastatic pancreatic cancers. In certain embodiments, the 95th, 90th, 80th, 75th, 70th, 50th, 40th, 30th, 25th or 10th percentile for MAML2 expression in pancreatic cancers is the 95th, 90th, 80th, 75th, 70th, 50th, 40th, 30th, 25th or 10th percentile for MAML2 expression in pancreatic adenocarcinomas, metastatic pancreatic cancers, or liver and/or lymph node metastatic pancreatic cancers. In certain embodiments, MAML2 expression level is determined using qRT-PCR.

[0124] In certain embodiments, the pancreatic cancer that is treated with a NOTCH inhibitor (e.g., OMP-59R5) is an exocrine tumor of the pancreas. In certain embodiments, the pancreatic cancer treated is acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, or solid and pseudopapillary tumor. In certain embodiments, the pancreatic cancer treated is adenocarcinoma. In certain embodiments, the pancreatic cancer treated is a neuroendocrine tumor. In certain embodiments, the pancreatic neuroendocrine tumor is a gastrinoma, glucagonoma, insulinoma, nonfunctional islet cell tumor, VIPoma, or somatostatinoma. In certain embodiments, the pancreatic cancer treated is not a neuroendocrine tumor.

[0125] In certain embodiments, the pancreatic cancer that is treated with a NOTCH inhibitor (e.g., OMP-59R5) is resectable tumor, locally advanced cancer, or metastatic pancreatic cancer. In certain embodiments, the pancreatic cancer is a grade 1, 2, 3 or 4 cancer as determined according to the AJCC TNM system.

[0126] In one embodiment, the NOTCH inhibitors (e.g., OMP-59R5) are particularly useful in treating pancreatic cancer patients that have already undergone some form of treatment. In another embodiment, the NOTCH inhibitors (e.g., OMP-59R5) are used to treat a pancreatic cancer patient that previously failed with a cancer therapy. Failed cancer therapies can include, but are not limited to, chemotherapy, adjuvant therapy, neoadjuvant therapy, and combinations thereof. In one embodiment, the NOTCH inhibitors (e.g., OMP-59R5) are used to treat chemotherapy resistant tumors. In another embodiment, the NOTCH inhibitors (e.g., OMP-59R5) are used to treat chemotherapy resistant pancreatic cancer.

[0127] In one embodiment, the treatment method involves first testing a biological sample containing pancreatic cancer cells from a patient to determine whether they express the NOTCH3 gene at or above a predetermined standard, e.g., at or above the median level for NOTCH3 expression in pancreatic cancer. Patients whose samples demonstrate elevated level of NOTCH3 expression would then be treated using a NOTCH inhibitor (e.g., OMP-59R5) that interferes with NOTCH receptor activity. The dosage administered will depend upon the particular condition being treated, the route of administration and clinical considerations that are well known in the art. Dosages can be gradually increased until a beneficial effect, e.g., a slowing of tumor growth, is detected. The NOTCH inhibitors (e.g., OMP-59R5) can then be provided in either single or multiple dosage regimens and can be given either alone or in conjunction with other therapeutic agents.

[0128] Treatment of pancreatic cancers with increased NOTCH3 expression is compatible with any route of administration and dosage form. Depending upon the particular condition being treated, certain dosage forms will tend to be more convenient or effective than others. For example, NOTCH inhibitors can be administered parenterally, topically, orally, perorally, internally, intranasally, rectally, vaginally, lingually and transdermally. Specific dosage forms include tablets, pills, capsules, powders, aerosols, suppositories, skin patches, parenterals and oral liquids including suspensions, solutions and emulsions. Sustained release dosage forms can also be used. All dosage forms can be prepared using methods that are standard in the art (see, e.g., *Remington's Pharmaceutical Sciences*, 16th ed., Easton, Pa. (1980)).

[0129] In certain embodiments, the administration of a NOTCH inhibitor (e.g., OMP-59R5) can be by intravenous injection or intravenously. In some embodiments, the administration is by intravenous infusion. In some embodiments, the administration of the NOTCH inhibitor (e.g., OMP-59R5) can be by a non-intravenous route.

[0130] The appropriate dosage of a NOTCH inhibitor (e.g., OMP-59R5) therapeutic agent depends on the severity and course of the disease, the responsiveness of the disease, whether the antibody or NOTCH inhibitor 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 antibody or other NOTCH inhibitor 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 disease state 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 antibody or other NOTCH inhibitor. The administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. In general, dosage of an anti-NOTCH antibody (e.g., OMP-59R5) is from 0.01 μg to 100 mg per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the antibody or agent in bodily fluids or tissues.

[0131] As is known by those of skill in the art, doses used will vary depending on the clinical goals to be achieved. In some embodiments, each dose of the anti-NOTCH antibody (e.g., OMP-59R5) is about 0.25mg/kg to about 15mg/kg. In some embodiments, each dose is about 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg. In certain embodiments, each dose is about 0.5mg/kg. In certain embodiments, each dose is about 1mg/kg. In certain embodiments, each dose is about 2.5mg/kg. In certain embodiments, each dose is about 5mg/kg. In certain embodiments, each dose is about 7.5mg/kg. In certain embodiments, each dose is about 10mg/kg. In certain embodiments, each dose is about 12.5 mg/kg. In certain embodiments, each dose is about 15mg/kg.

[0132] In certain embodiments, the NOTCH inhibitor (e.g., OMP-59R5) used in the methods described herein is administered to the patient using an intermittent dosing regimen, which may in

some instances reduce side effects and/or toxicities associated with administration of the NOTCH inhibitor (e.g., OMP-59R5). As used herein, "intermittent dosing" refers to a dosing regimen using a dosing interval of more than once a week, e.g., dosing once every 2 weeks, once every 3 weeks, once every 4 weeks, etc. In some embodiments, a method for treating pancreatic cancer in a human patient comprises administering to the patient an effective dose of a NOTCH inhibitor (e.g., OMP-59R5) according to an intermittent dosing regimen. In some embodiments, a method for treating pancreatic cancer in a human patient comprises administering to the patient an effective dose of a NOTCH inhibitor (e.g., OMP-59R5) according to an intermittent dosing regimen, and increasing the therapeutic index of the NOTCH inhibitor (e.g., OMP-59R5). In some embodiments, the intermittent dosing regimen comprises administering an initial dose of a NOTCH inhibitor (e.g., OMP-59R5) to the patient, and administering subsequent doses of the NOTCH inhibitor (e.g., OMP-59R5) about once every 2 weeks. In some embodiments, the intermittent dosing regimen comprises administering an initial dose of a NOTCH inhibitor (e.g., OMP-59R5) to the patient, and administering subsequent doses of the NOTCH inhibitor (e.g., OMP-59R5) about once every 3 weeks. In some embodiments, the intermittent dosing regimen comprises administering an initial dose of a NOTCH inhibitor (e.g., OMP-59R5) to the patient, and administering subsequent doses of the NOTCH inhibitor (e.g., OMP-59R5) about once every 4 weeks.

[0133] In some alternative embodiments, the anti-NOTCH antibody used in the methods is OMP-59R5, or an antibody comprising the six CDRs and/or the variable regions of OMP-59R5, and the antibody is administered to subjects intravenously at a dosage of about 2.5 mg/kg to about 7.5 mg/kg (e.g., about 2.5 mg/kg, about 5 mg/kg, or about 7.5 mg/kg) approximately every two to three weeks.

[0134] In certain embodiments, in addition to administering a NOTCH inhibitor (e.g., OMP-59R5), the method or treatment further comprises administering at least one additional therapeutic agent or therapy. An additional therapeutic agent or therapy can be administered prior to, concurrently with, and/or subsequently to, administration of the anti-NOTCH therapeutic agent. In some embodiments, the at least one additional therapeutic agent or therapy comprises 1, 2, 3, or more additional therapeutic agents or therapies.

[0135] Combination therapy with at least two therapeutic agents often uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action may result in additive or synergetic effects. Combination therapy may allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects. Combination therapy may decrease the likelihood that resistant cancer cells will develop.

[0136] It will be appreciated that the combination of a NOTCH inhibitor (e.g., OMP-59R5) and an additional therapeutic agent or therapy can be administered in any order or concurrently. In some embodiments, the NOTCH inhibitor (e.g., OMP-59R5) will be administered to patients that have previously undergone treatment with a second therapeutic agent or therapy. In certain other embodiments, the NOTCH inhibitor (e.g., OMP-59R5) and a second therapeutic agent or therapy will

be administered substantially simultaneously or concurrently. For example, a subject can be given the NOTCH inhibitor (e.g., OMP-59R5) agent while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In certain embodiments, the NOTCH inhibitor (e.g., OMP-59R5) will be administered within 1 year of the treatment with a second therapeutic agent. In certain alternative embodiments, the NOTCH inhibitor (e.g., OMP-59R5) will be administered within 10, 8, 6, 4, or 2 months of any treatment with a second therapeutic agent. In certain other embodiments, the NOTCH inhibitor (e.g., OMP-59R5) will be administered within 4, 3, 2, or 1 weeks of any treatment with a second therapeutic agent. In some embodiments, the NOTCH inhibitor (e.g., OMP-59R5) will be administered within 5, 4, 3, 2, or 1 days of any treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments can be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[0137] As is known by those of skill in the art, doses used will vary depending on the clinical goals to be achieved. In some embodiments, each dose of an anti-NOTCH antibody (e.g., OMP-59R5) is about 0.25 mg/kg to about 15 mg/kg. In some embodiments, each dose is about 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg. In certain embodiments, each dose is about 0.5 mg/kg. In certain embodiments, each dose is about 1 mg/kg. In certain embodiments, each dose is about 2.5 mg/kg. In certain embodiments, each dose is about 5 mg/kg. In certain embodiments, each dose is about 7.5 mg/kg. In certain embodiments, each dose is about 10 mg/kg. In certain embodiments, each dose is about 12.5 mg/kg. In certain embodiments, each dose is about 15 mg/kg. .

[0138] In certain embodiments, a method treating pancreatic cancer described herein comprises the administration of a NOTCH inhibitor (e.g., OMP-59R5) in combination with one or more chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the combined administration of a NOTCH inhibitor (e.g., OMP-59R5) and a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. In certain embodiments, a method described herein comprises administering to a pancreatic cancer patient a therapeutically effective amount of the OMP-59R5 antibody in combination with gemcitabine and ABRAXANE™ (protein bound paclitaxel). Treatment with a NOTCH inhibitor (e.g., OMP-59R5) can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Editor M. C. Perry, Williams & Wilkins, Baltimore, MD (1992).

[0139] Chemotherapeutic agents useful in the instant invention include, but are not limited to, alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel and docetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide; ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine; 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 anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0140] 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, and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these.

[0141] In certain embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain embodiments, a method described herein comprises administering to a pancreatic cancer patient a therapeutically effective amount of the OMP-59R5 antibody in combination with an anti-metabolite. In certain embodiments, the anti-metabolite is a nucleoside analogue. In certain embodiments, a method described herein comprises administering to a pancreatic cancer patient a therapeutically effective amount of the OMP-59R5 antibody in combination with gemcitabine.

[0142] In certain embodiments, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some embodiments, the agent is a taxane. In certain embodiments, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain 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 certain embodiments, a method described herein comprises administering to a pancreatic cancer patient a therapeutically effective amount of the OMP-59R5 antibody in combination with an antimitotic agent. In certain embodiments, the anti-metabolite is a taxane. In certain embodiments, a method described herein comprises administering to a pancreatic cancer patient a therapeutically effective amount of the OMP-59R5 antibody in combination with ABRAXANE™ (protein bound paclitaxel).

[0143] In certain embodiments, the treatment involves the combined administration of an NOTCH inhibitor (e.g., OMP-59R5) and radiation therapy. Treatment with the NOTCH inhibitor (e.g., OMP-59R5) can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Dosing schedules for such radiation therapy can be determined by the skilled medical practitioner. In some embodiments, the NOTCH inhibitor (e.g., OMP-59R5) is administered after radiation treatment. In some embodiments, the NOTCH inhibitor (e.g., OMP-59R5) is administered with radiation therapy.

[0144] In some embodiments, a second therapeutic agent comprises an antibody. Thus, treatment can involve the combined administration of an anti-NOTCH antibody (e.g., OMP-59R5) or other NOTCH inhibitor with other antibodies against additional tumor-associated antigens including, but not limited to, antibodies that bind to EGFR, ErbB2, DLL4, or NF-κB. Exemplary anti-DLL4 antibodies are described, for example, in U.S. Patent No. 7,750,124. Additional anti-DLL4 antibodies are described in, e.g., International Patent Pub. Nos. WO 2008/091222 and WO 2008/0793326, and U.S. Patent Application Pub. Nos. 2008/0014196; 2008/0175847; 2008/0181899; and 2008/0107648. 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.

[0145] Furthermore, treatment with the NOTCH inhibitor (e.g., OMP-59R5) can include combination treatment with one or more cytokines (e.g., lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of tumors, cancer cells or any other therapy deemed necessary by a treating physician.

5. Antibodies and production thereof

[0146] Additional 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.

[0147] 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.

[0148] 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).

[0149] 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.

[0150] 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.

[0151] 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 can be employed to generate high affinity human antibodies.

[0152] 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.

[0153] In certain embodiments, the antibody useful in the methods of the invention is a bispecific antibody that specifically recognizes a human NOTCH 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 NOTCH receptor) or on different molecules. Bispecific antibodies can be intact antibodies or antibody fragments.

[0154] Alternatively, in certain alternative embodiments, antibodies useful for the invention are not bispecific antibodies.

[0155] 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 NOTCH receptor. In certain embodiments, an antigen-binding site of a monospecific antibody is capable of binding (or binds) one, two, three, or four human NOTCH receptors.

[0156] 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 NOTCH receptor. Antibody fragments can 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.

[0157] 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).

[0158] 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.

[0159] 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 opsonisation 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.

[0160] In certain embodiments, the NOTCH 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 can easily be made using well known biochemical or molecular engineering techniques well within the purview of the skilled artisan.

[0161] In certain embodiments, a NOTCH 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.

[0162] The invention also pertains to the use of immunoconjugates comprising a NOTCH antagonist polypeptide (*e.g.*, anti-NOTCH 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, adriamicin, 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.

[0163] 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.

[0164] Regardless of how useful quantities are obtained, the NOTCH 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 NOTCH 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 NOTCH antagonist polypeptides conjugated to specific biotoxins such as ricin or diptheria toxin. In yet other embodiments the NOTCH 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 NOTCH 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.

[0165] 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).

[0166] 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.

[0167] 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.

[0168] 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.

[0169] 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.

[0170] In certain embodiments, recombinant expression vectors are used to amplify and express NOTCH 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.

[0171] 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, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from Escherichia coli, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

[0172] Suitable host cells for expression of a NOTCH 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.

[0173] 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).

[0174] 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.

[0175] 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 NOTCH 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.

[0176] 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.

[0177] Methods known in the art for purifying a NOTCH 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.

6. Pharmaceutical compositions

[0178] The NOTCH antagonist polypeptides (*e.g.*, anti-NOTCH antibodies) 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.

[0179] In certain embodiments, formulations are prepared for storage and use by combining a purified NOTCH antagonist (*e.g.*, an anti-NOTCH antibody) 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).

[0180] In certain embodiments, the pharmaceutical composition is frozen. In certain alternative embodiments, the pharmaceutical composition is lyophilized.

[0181] 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.

[0182] 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.

[0183] The NOTCH antagonists (*e.g.*, anti-NOTCH antibodies) 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).

[0184] In certain embodiments, pharmaceutical formulations include the NOTCH antagonists (*e.g.*, anti-NOTCH antibodies) 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.

[0185] 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.

7. Kits

[0186] Kits for practicing the methods of the invention are further provided. By "kit" is intended any manufacture (e.g., a package or a container) comprising at least one reagent, e.g., a nucleic acid probe, etc. for specifically detecting the level of NOTCH3 gene expression in a sample, e.g., cell, cell line, tumor, or tissue. The kit can be promoted, distributed, or sold as a unit for performing the methods of the present invention. Additionally, the kits can contain a package insert describing the kit and including instructional material for its use.

[0187] In one embodiment, kits for practicing the methods of the invention are provided. Such kits are compatible with both manual and automated screening. For qRT-PCR assays, the kits comprise at least the probes disclosed herein for the detection of NOTCH3 gene expression. The kits can further comprise reagents for RNA extraction, reverse transcription, and/or PCR amplifications. In certain embodiments, a kit according to the present invention comprises at least one oligonucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:35-43.

[0188] Positive and/or negative controls can be included in the kits to validate the activity and correct usage of reagents employed in accordance with the invention. Controls can include samples, such as RNA preparations, formalin fixed tissues, etc., known to be either positive or negative for the presence of NOTCH3 mRNA. The design and use of controls is standard and well within the routine capabilities of those in the art.

[0189] It will be further appreciated that any or all steps in the methods of the invention could be implemented by personnel or, alternatively, performed in an automated fashion. Thus, the steps of body sample preparation, sample freezing or fixing, RNA extraction, and/or detection of NOTCH3 transcript level can be automated.

EXAMPLES

[0190] 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

In vivo prevention of tumor growth using the OMP-59R5 anti-NOTCH2/3 receptor antibody as a single agent and in combination with a chemotherapeutic agent.

[0191] 20,000 OMP-PN8 tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 22 days until they had reached an average volume of 125 mm^3. Tumor bearing mice were randomized into 4 groups and treated with control antibody, OMP-59R5 (anti-NOTCH2/3), gemcitabine, or the combination of OMP-59R5 and gemcitabine. Antibodies were dosed every other week at 40 mg/kg. Gemcitabine was dosed at 20 mg/kg weekly. Tumor volumes were measured on

the indicated days post-treatment. OMP-59R5 strongly inhibited OMP-PN8 tumor growth as a single agent or in combination with gemcitabine (Figure 1A).

[0192] The ability of anti-NOTCH2/3 OMP-59R5 antibody to inhibit the *in vivo* growth of OMP-PN17 pancreatic tumor was determined using substantially identical methods. As shown in Figure 1B, OMP-59R5 strongly inhibited OMP-PN17 tumor growth as a single agent or in combination with gemcitabine.

[0193] 50,000 OMP-PN11 tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 21 days until they had reached an average volume of 120 mm^3. Tumor bearing mice were randomized into 4 groups and treated with control antibody, OMP-59R5 (anti-NOTCH2/3), gemcitabine, or the combination of OMP-59R5 and gemcitabine. Antibodies were dosed every other week at 40 mg/kg. Gemcitabine was dosed at 20 mg/kg weekly. Tumor volumes were measured on the indicated days post-treatment. As shown in Figure 1C, OMP-59R5 had no effect on OMP-PN11 tumor growth either as a single agent or in combination with gemcitabine.

[0194] 20,000 UM-PE13 breast (NOTCH3 high expressing) tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 37 days until they had reached an average volume of 140 mm^3. Tumor bearing mice were randomized into 4 groups and treated with control antibody, OMP-59R5, taxol, or the combination of OMP-59R5 and taxol. Antibodies were dosed weekly at 20 mg/kg. Taxol was dosed at 10 mg/kg weekly. Tumor volumes were measured on the indicated days post-treatment. As shown in Figure 1D, OMP-59R5 strongly inhibited UM-PE13 tumor growth as a single agent or in combination with taxol.

[0195] 20,000 UM-T1 breast (NOTCH3 high expressing) tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 28 days until they had reached an average volume of 120 mm^3. Tumor bearing mice were randomized into 4 groups and treated with either control antibody, OMP-59R5 anti-NOTCH2/3 antibody, taxol, or the combination of OMP-59R5 and taxol. Antibodies were dosed weekly at 20 mg/kg. Taxol was dosed at 10 mg/kg weekly. Tumor volumes were measured on the indicated days post-treatment. As shown in Figure 1E, OMP-59R5 had no effect on UM-T1 tumor growth as a single agent or in combination with taxol.

[0196] 50,000 OMP-Lu40 lung (NOTCH3 low expressing) tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 33 days until they had reached an average volume of 140 mm^3. Tumor bearing mice were randomized into 4 groups and treated with either control antibody, OMP-59R5 anti-NOTCH2/3 antibody, taxol, or the combination of OMP-59R5 and taxol. Antibodies were dosed weekly at 20 mg/kg. Taxol was dosed at 10 mg/kg weekly. Tumor volumes were measured on the indicated days post-treatment. As shown in Figure 1F, OMP-59R5 strongly inhibited OMP-Lu40 tumor growth in combination with taxol.

[0197] 50,000 OMP-Lu53 lung (NOTCH3 high expressing) tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 33 days until they had reached an average volume of 120 mm^3. Tumor bearing mice were randomized into 4 groups and treated with control antibody, OMP-

59R5 anti-NOTCH2/3 antibody, taxol, or the combination of OMP-59R5 and taxol. Antibodies were dosed every other week at 40 mg/kg. Taxol was dosed at 10 mg/kg weekly. Tumor volumes were measured on the indicated days post-treatment. As shown in Figure 1G, OMP-59R5 had no effect on OMP-Lu53 tumor growth in combination with taxol.

Example 2

Tumor growth inhibition by OMP-59R5 in combination with gemcitabine significantly correlates with the levels of NOTCH3 gene expression in pancreatic tumors, but not in breast or lung tumors

[0198] NOTCH2 and NOTCH3 gene expression levels were determined in pancreatic, breast and lung tumors assayed in the *in vivo* xenograft assay described in Example 1 using standard microarray technology. Expression data was obtained using Affymetrix® U133 plus 2 arrays according to the manufacturer's instructions. The results are shown in Tables 1-3 below. The Tables also include data on the responsiveness of the particular tumor to treatment with OMP-59R5 anti-NOTCH2/3 antibody in combination with a chemotherapeutic agent in the *in vivo* xenograft assay described in Example 1. The analyses of NOTCH2 and 3 gene expression levels shown in the Tables were based on a cut-off value of 500. However, the overall conclusion from the analyses remained the same when the cut-off value was varied between 300 and 1000. No correlation between NOTCH3 expression and *in vivo* treatment efficacy was observed in the breast tumor and lung tumor samples: only 5 out of 14 breast or lung tumors with high NOTCH3 gene expression were responsive. Further, no correlation between NOTCH2 expression and *in vivo* efficacy was observed in breast, lung, or pancreatic tumor samples. Surprisingly, in pancreatic tumors there was a very strong correlation between high levels of NOTCH3 gene expression and the *in vivo* efficacy of OMP-59R5/gemcitabine treatment: 9 out of the 10 pancreatic tumors with high NOTCH3 gene expression were responsive *in vivo* to treatment with OMP-59R5 and gemcitabine.

Table 1. NOTCH2 and NOTCH3 gene expression levels in pancreatic tumors.

Tumor	**Efficacy (OMP-59R5 + gemcitabine)**	**N3 expression**	**N2 expression**
PN4	+	High (1802)	High (4637)
PN7	-	Low (274)	High (2140)
PN8	+	High (2484)	High (6909)
PN11	-	Low (141)	High (4576)
PN13	-	Low (23)	High (6848)
PN16	+	High (3318)	High (3812)
PN17	+	High (6106)	High (5904)
PN21	+	High (2776)	High (6203)
PN23	-	High (2978)	High (5166)
PN25	+	High (6600)	High (4383)

Table 2. NOTCH2 and NOTCH3 gene expression levels in breast tumors.

Tumor	Efficacy (OMP-59R5 + taxol)	N3 expression	N2 expression
PE13	+	High (5616)	High (6283)
T1	-	High (11708)	High (7551)
B37	+	High (10217)	High (3231)
B40	-	High (11615)	High (10999)

Table 3. NOTCH2 and NOTCH3 gene expression levels in lung tumors. NSCLC – non-small cell lung cancer; SCLC – small cell lung cancer.

	Tumor	Efficacy (OMP-59R5 + taxol)	N3 expression	N2 expression
NSCLC	Lu15	-	Low (440)	High (1995)
NSCLC	Lu24	-	High (5430)	High (3105)
NSCLC	Lu25	-	High (9768)	High (3225)
NSCLC	Lu53	-	High (12294)	High (7828)
SCLC	Lu40	+	Low (423)	High (1040)
SCLC	Lu61	+	High 11732	High (1500)
SCLC	Lu65	+	Low (269)	High (514)
SCLC	Lu66	+	Low (9)	Low (12)
SCLC	Lu67	-	High (682)	High (2214)
SCLC	Lu68	+	High (838)	High (3519)

[0199] The surprising correlation between high levels of NOTCH3 gene expression and the *in vivo* efficacy of OMP-59R5/gemcitabine combination treatment in pancreatic tumors was further analyzed. NOTCH3 gene expression levels were determined in the PN11, PN13, PN23, PN04, PN08, PN16, PN17, PN21, and PN25 pancreatic tumor cells using standard multiplex transcript sequencing (e.g., RNASeq). RNASeq was performed using the Illumina® HiSeq™ 2000 Sequencing System according to the manufacturer's instructions. Figure 2A shows that increased NOTCH3 gene expression significantly correlated (0.823; $p<0.021$) with *in vivo* tumor inhibition by OMP-59R5/gemcitabine combination treatment in human pancreatic xenograft models. Figure 3 further shows that NOTCH3 gene expression detected in responsive pancreatic tumors was significantly higher than the expression level detected in non-responsive pancreatic tumors.

[0200] Figure 2B shows the distribution of NOTCH3 gene expression detected in human pancreatic tumors which were responsive to treatment with OMP-59R5 anti-NOTCH2/3 antibody in combination with gemcitabine (R=responders: pval <0.05 compared to gemcitabine treatment alone) and for those xenografts which were found to be non-responsive to treatment with OMP-59R5 anti-NOTCH2/3 antibody in combination with gemcitabine (NR=non-responders: pval >0.05 compared to gemcitabine treatment alone). The distribution of NOTCH3 gene expression levels in non-responsive pancreatic tumors showed a clear separation from the distribution of NOTCH3 gene expression levels in responsive pancreatic tumors.

[0201] Logistic regression, a standard statistical model was used to predict the *in vivo* responsiveness of particular pancreatic cancers to treatment with OMP-59R5 in combination with a chemotherapeutic agent, e.g., gemcitabine, based on the NOTCH3 gene expression level detected in the pancreatic cancer by RNASeq. Alan Agresti: *An Introduction to Categorical Data Analysis*, John Wiley and Sons, Inc. (1996). Results of the analysis are shown in Figure 4. The positive predictive value (PPV), negative predictive value (NPV), sensitivity (SENS) and specificity (SPEC) of the NOTCH3 gene expression data set was 83%, 75%, 83%, and 75%, respectively.

[0202] The accuracy of the prediction of *in vivo* responsiveness of pancreatic cancers to treatment with OMP-59R5 in combination with gemcitabine was further improved by including in the statistical analysis MAML2 gene expression data from the pancreatic cancers. The results obtained by applying logistic regression to the NOTCH3 and MAML2 gene expression data set are shown in Figure 5. The positive predictive value (PPV), negative predictive value (NPV), sensitivity (SENS) and specificity (SPEC) of the NOTCH3 and MAML2 gene expression data set was 100%. The experiment was cross-validated using gene expression data obtained by standard RNASeq methods.

Example 3

NOTCH3 protein expression in pancreatic tumor samples

[0203] NOTCH3 Western blot analysis was performed to determine the expression of NOTCH3 protein in human pancreatic tumors (Figure 6A). The anti-NOTCH3 antibody (Cell signaling #5276) used in this analysis detected both full length NOTCH3 (FL: ~250kDa), and the transmembrane and intracellular regions of NOTCH3 (TM=~98kDa).

[0204] Figure 6B shows the distribution of NOTCH3 protein expression in human pancreatic tumors which were responsive to treatment with OMP-59R5 in combination with gemcitabine (R=responders: pval <0.05 compared to gemcitabine treatment alone) and for those xenografts which were found to be non-responsive to treatment with OMP-59R5 in combination with gemcitabine (NR=non-responders: pval >0.05 compared to Gemcitabine treatment alone) in the xenograft assay described in Example 1. The separation in the distribution of NOTCH3 protein expression between responders and non-responders was less pronounced than the separation in the distribution of NOTCH3 gene expression. Logistic regression was applied to the NOTCH3 protein expression data in pancreatic cancers to predict the sensitivity of particular pancreatic cancers to treatment with OMP-59R5 in combination with gemcitabine. The NOTCH3 protein expression data generated similar performance in predicting the response to OMP-59R5 plus gemcitabine treatment to the performance of the NOTCH3 gene expression data discussed above.

Example 4

NOTCH3 gene expression in metastatic pancreatic tumor samples measured by qRT-PCR

[0205] NOTCH3 gene expression was determined in metastatic pancreatic tumor samples using standard quantitative qRT-PCR. The assay probes were designed using the NOTCH3 RefSeq mRNA sequence NM_000435.2. NOTCH3_A7 detects one of the two potential transcripts while NOTCH3_A1 detects both transcripts predicted by the Ensembl database. The probes and qRT-PCR assay were verified using human fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) human tissue samples.

Table 3. Nucleotide sequence of probes used in NOTCH3 qRT-PCR assays.

NOTCH3_A1	Forward	AGGCAGAGTGGCGACCTC (SEQ ID NO:35)
	Reverse	CGTCCACGTTCACTTCACAATTC (SEQ ID NO:36)
	Probe	AACCCAGGAAGACAGGCACAGTCGT (SEQ ID NO:37)
NOTCH3_A9	Forward	CTGGGTTTGAGGGTCAGAAT (SEQ ID NO:38)
	Reverse	GGGCACTGGCAGTTATAGGT (SEQ ID NO:39)
	Probe	TGACGCCATCCACGCATGTC (SEQ ID NO:40)
NOTCH3_A7	Forward	TGCAGGATAGCAAGGAGGAGAC (SEQ ID NO:41)
	Reverse	GCAGCTTGGCAGCCTCATAG (SEQ ID NO:42)
	Probe	CTCGCGGGCGGCCAGGAATAGGG (SEQ ID NO:43)

[0206] Approximately 100 formalin-fixed paraffin embedded (FFPE) metastatic tumor tissues from first-line pancreatic cancer patients were sourced to determine the levels and distribution of NOTCH3 expression in this cohort (Figure 7). NOTCH3 gene expression was determined with the NOTCH3_A7 primer/probe set using a standard quantitative RT-PCR protocol. ANOVA statistical analysis was performed to determine if the levels of NOTCH3 correlated with factors including sample age, sex, patient age etc. NOTCH3 levels were not found to be correlated with any of these factors except for site of metastasis with liver showing significance and a wider NOTCH3 gene expression distribution. Figure 7 displays the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile for NOTCH3 gene expression across all metastatic tumor samples examined.

[0207] NOTCH3 gene expression levels from the sourced human liver and lymph node metastatic pancreatic cancer tissues and the primary human pancreatic tumors used in the xenograft assays were normalized in order to compare the data. The mean of data was subtracted and divided by the standard deviation in each data set. The grey (Light) dots represent the human pancreatic tumors that were non-responsive to treatment with OMP-59R5 in combination with gemcitabine in the xenograft assay described in Example 1, and the black (Dark) dots represent the human pancreatic tumors that were responsive in the xenograft assay (Figure 8). The responsive tumors showed higher levels of NOTCH3 gene expression than the non-responsive ones, indicating that NOTCH3 gene expression can be used to predict *in vivo* responsiveness of pancreatic tumors to treatment with, for example, OMP-59R5 in combination with a chemotherapeutic agent. Figure 8 also displays the 10^{th}, 25^{th}, 50^{th},

75th, and 90th percentile for NOTCH3 gene expression in the human liver and lymph node metastatic pancreatic cancer tissues examined.

Example 6

The OMP-59R5 anti-NOTCH2/3 antibody in combination with gemcitabine and ABRAXANE™ inhibits *in vitro* growth of pancreatic tumors

[0208] 20,000 OMP-PN8 (NOTCH3 high expressing) tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow 26 days until they had reached an average volume of 110 mm^3. Tumor bearing mice were randomized into 3 groups (n = 9 mice per group) and treated with control antibody, gemcitabine plus ABRAXANE™ (albumin bound paclitaxel), or the combination of OMP-59R5 anti-NOTCH2/3 antibody and gemcitabine plus ABRAXANE™. OMP-59R5 was dosed every other week at 40 mg/kg. Gemcitabine was dosed at 10 mg/kg weekly and ABRAXANE™ at 30 mg/kg weekly. Tumor volumes were measured on the indicated days post-treatment. OMP-59R5 strongly inhibited OMP-PN8 tumor growth in combination with gemcitabine plus ABRAXANE™, and was more active than gemcitabine plus ABRAXANE™ alone (Figure 9). The top and bottom graphs show data obtained from the same experiment on different scales. The bottom graph shows data obtained from the active treatment groups only, but not data obtained from control treated animals. The results indicate that NOTCH3 expression levels can be used to predict *in vivo* responsiveness of pancreatic tumors to treatment with OMP-59R5 antibody in combination with various chemotherapeutic agents.

[0209] 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.

SEQUENCES

SEQ ID NO:1
HKGAL

SEQ ID NO:1
HEDAI

SEQ ID NO:3: 59R1Heavy chain CDR1
SSSGMS

SEQ ID NO:4: 59R1Heavy chain CDR2
VIASSGSNTYYADSVKG

SEQ ID NO:5: 59R1Heavy chain CDR3
GIFFAI

SEQ ID NO:6: 59R1 Light chain CDR1
RASQSVRSNYLA

SEQ ID NO:7: 59R1 Light chain CDR2
GASSRAT

SEQ ID NO:8: 59R1 Light chain CDR3
QQYSNFPI

SEQ ID NO:9: 59R5 Heavy chain CDR3
SIFYTT

SEQ ID NO:10 (heavy chain CDR3 consensus sequence):
(G/S)(I/S)F(F/Y)(A/P)(I/T/S/N)

SEQ ID NO:11 (alternative heavy chain CDR3)
SIFYPT

SEQ ID NO:12 (alternative heavy chain CDR3)
SSFFAS

SEQ ID NO:13 (alternative heavy chain CDR3)
SSFYAS

SEQ ID NO:14 (alternative heavy chain CDR3)
SSFFAT

SEQ ID NO:15 (alternative heavy chain CDR3)
SIFYPS

SEQ ID NO:16 (alternative heavy chain CDR3)
SSFFAN

SEQ ID NO:17: 59R5 Heavy chain variable region
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYTTWGQGTLVTVSSAST

SEQ ID NO:18: 59R1 Heavy chain VH of 59R1 IgG antibody
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

SEQ ID NO:19: 59R1 heavy chain VH plus mammalian signal sequence (underlined)
MKHLWFFLLLVAAPRWVLSQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAP
GKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIF
FAIWGQGTLVTVSSA

SEQ ID NO:20: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYPTWGQGTLVTVSSA

SEQ ID NO:21: Variant 59R1 Heavy chain variable region

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFFASWGQGTLVTVSSA

SEQ ID NO:22: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFYASWGQGTLVTVSSA

SEQ ID NO:23: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFFATWGQGTLVTVSSA

SEQ ID NO:24: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYPSWGQGTLVTVSSA

SEQ ID NO:25: Variant 59R1 Heavy chain variable region
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFFANWGQGTLVTVSSA

SEQ ID NO:26: 59R1 Heavy chain VH of 59RGV antibody (germlined variant of 59R1)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

SEQ ID NO:27: 59R1 Light chain VL of 59RGV antibody (germlined variant of 59R1)
EIVLTQSPATLSLSPGERATLSCRRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGIPARFSGSG
SGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

SEQ ID NO:28: 59R1 light chain VL plus mammalian signal sequence (underlined)
MVLQTQVFISLLLWISGAYGDIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQK
PGQAPRLLIYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFG
QGTKVEIKR

SEQ ID NO:29: 59R1 Light chain VL of 59R1 IgG antibody
DIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGVPARFSGSGS
GTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

SEQ ID NO:30: 59R5 Heavy chain
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYTTWGQGTLVTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK

SEQ ID NO:31: Predicted protein sequence of anti-NOTCH2/3 59R1 IgG2 heavy chain, plus signal sequence. The signal sequence is underlined.
MKHLWFFLLLVAAPRWVLSQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVS
VIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSAS
TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKG
LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:32: Predicted protein sequence of the heavy chain of anti-NOTCH2/3 59RGV (germlined variant of 59R1), plus signal sequence. The signal sequence is underlined.
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVS
VIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSAS
TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKG
LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:33: Predicted protein sequence of anti-NOTCH2/3 59R1 light chain, plus signal sequence. The signal sequence is underlined.
MVLQTQVFISLLLWISGAYGDIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLL
IYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:34: Predicted protein sequence of the light chain of anti-NOTCH2/3 59RGV antibody (germlined variant of 59R1), plus signal sequence. The signal sequence is underlined.
MVLQTQVFISLLLWISGAYGEIVLTQSPATLSLSPGERATLSCRRASQSVRSNYLAWYQQKPGQAPRL
LIYGASSRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:35
AGGCAGAGTGGCGACCTC

SEQ ID NO:36
CGTCCACGTTCACTTCACAATTC

SEQ ID NO:37
AACCCAGGAAGACAGGCACAGTCGT

SEQ ID NO:38
CTGGGTTTGAGGGTCAGAAT

SEQ ID NO:39
GGGCACTGGCAGTTATAGGT

SEQ ID NO:40
TGACGCCATCCACGCATGTC

SEQ ID NO:41
TGCAGGATAGCAAGGAGGAGAC

SEQ ID NO:42
GCAGCTTGGCAGCCTCATAG

SEQ ID NO:43
CTCGCGGGCGGCCAGGAATAGGG

What we claim is:

1. A method for selecting a pancreatic cancer patient for treatment with a NOTCH inhibitor comprising: (a) determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and (b) selecting the patient based on the expression level of the one or more biomarkers.

2. A method for determining whether a patient diagnosed with pancreatic cancer is likely to respond to a NOTCH inhibitor-based therapy comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers indicates that the patient is likely to respond to therapy.

3. A method for determining whether a patient diagnosed with pancreatic cancer should be administered a NOTCH inhibitor, comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers is predictive of said patient having a favorable response to treatment with a NOTCH inhibitor.

4. A method to determine whether a patient diagnosed with pancreatic cancer should continue treatment with a NOTCH inhibitor, comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers indicates that the patient is likely to respond to therapy.

5. A method to determine whether a patient diagnosed with pancreatic cancer should continue treatment with a NOTCH inhibitor, comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers is predictive of said patient having a favorable response to treatment with said NOTCH inhibitor.

6. A method for determining the therapeutic efficacy of a NOTCH inhibitor for treating pancreatic cancer in a patient comprising determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers is indicative of the therapeutic efficacy of said NOTCH inhibitor.

7. A method of treating pancreatic cancer in a patient comprising:

(a) determining the level of expression of one or more biomarkers in tumor cells from said patient, wherein the one or more biomarkers comprise NOTCH3; and

(b) administering to said patient a therapeutically effective amount of a NOTCH inhibitor.

8. A method for stratifying a pancreatic cancer patient population for treatment with a NOTCH inhibitor comprising:

(a) determining the level of expression of one or more biomarkers in tumor cells from said patients, wherein the one or more biomarkers comprise NOTCH3, and

(b) stratifying the patient population based on the level of expression of the one or more biomarkers in the tumor cells.

9. The method of any one of claims 1-8, wherein the level of NOTCH3 expression is determined to be above a reference level for NOTCH3 expression.

10. The method of any one of claims 1-9, wherein each of the biomarkers is determined to be expressed at a level above a reference level for the biomarker.

11. The method of any one of claims 1-10, wherein the expression level of the one or more biomarkers is determined by determining the level of the biomarker mRNA or the biomarker protein.

12. The method of any one of claims 1-11, wherein the level of NOTCH3 expression is determined by determining the level of NOTCH3 mRNA in the tumor cells.

13. The method of claim 12, wherein the NOTCH3 mRNA level is determined by quantitative polymerase chain reaction.

14. The method of claim 13, wherein the NOTCH3 mRNA level is determined using: (a) a forward primer having a nucleotide sequence selected from the group consisting of SEQ ID NO: 35, SEQ ID NO: 38, and SEQ ID NO: 41; (b) a reverse primer having a nucleotide sequence selected from the group consisting of SEQ ID NO: 36, SEQ ID NO: 39, and SEQ ID NO: 42; and/or (c) a probe comprising an oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 40, and SEQ ID NO: 43.

15. The method of claim 14, wherein the NOTCH3 mRNA level is determined using: (a) a forward primer having the sequence of SEQ ID NO: 35, a reverse primer having the sequence of SEQ ID NO: 36, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO: 37;

(b) a forward primer having the sequence of SEQ ID NO: 38, a reverse primer having the sequence of SEQ ID NO: 39, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO: 40; or

(c) a forward primer having the sequence of SEQ ID NO: 41, a reverse primer having the sequence of SEQ ID NO: 42, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO: 43.

16. The method of claim 12, wherein the NOTCH3 mRNA level is determined by array hybridization.

17. The method of any one of claims 1-11, wherein the level of NOTCH3 expression is determined by determining the level of NOTCH3 protein expressed by the tumor cells.

18. The method of any one of claims 1-17, wherein the one or more biomarkers consist of NOTCH3.

19. The method of any one of the claims 1-17, wherein the one or more biomarkers further comprise MAML2 and the level of MAML2 expression is determined to be above a reference level for MAML2 expression.

20. The method of claim 19 wherein the one or more biomarkers consist of NOTCH3 and MAML2.

21. The method of claim 19 or 20, wherein the level of MAML2 expression is determined by determining the level of MAML2 mRNA in the tumor cells.

22. The method of claim 19 or 20, wherein the level of MAML2 expression is determined by determining the level of MAML2 protein expressed by the tumor cells.

23. A method of treating pancreatic cancer in a patient comprising administering to said patient a therapeutically effective amount of a NOTCH inhibitor, wherein at least some of the pancreatic tumor cells from said patient express each of one or more biomarkers at a level above a reference level for that biomarker and/or have been previously determined to express each of one or

more biomarkers at a level above a reference level for that biomarker, wherein the one or more biomarkers comprise NOTCH3.

24. The method of claim 23, wherein the level of NOTCH3 expression is determined as the level of NOTCH3 mRNA.

25. The method of claim 23, wherein the level of NOTCH3 expression is determined as the level of NOTCH3 protein.

26. The method of any one of claims 23-25, wherein the one or more biomarkers consist of NOTCH3.

27. The method of any one of the claims 23-25, wherein the one or more biomarkers further comprise MAML2 and the level of MAML2 expression is above a reference level for MAML2 expression.

28. The method of claim 27 wherein the one or more biomarkers consist of NOTCH3 and MAML2.

29. The method of any one of claims 1-28, wherein the reference level of a biomarker is a predetermined value.

30. The method of any one of claims 1-29, wherein the reference level of a biomarker is the level of expression of that biomarker in a control sample.

31. The method of any one of the claims 1-29, wherein the reference level for NOTCH3 expression is the 25^{th} percentile, the 30^{th} percentile, the 40^{th} percentile, the 50^{th} percentile, the 60^{th} percentile, the 70^{th} percentile, the 75^{th} percentile, or the 80^{th} percentile for NOTCH3 expression in pancreatic cancers or a subset of pancreatic cancers.

32. The method of any one of the claims 1-29, wherein the reference level for NOTCH3 expression is the 75^{th} percentile for NOTCH3 expression in pancreatic cancers.

33. The method of any one of the claims 1-29, wherein the reference level for NOTCH3 expression is the 50^{th} percentile for NOTCH3 expression in pancreatic cancers.

34. The method of any one of the claims 1-29, wherein the reference level for NOTCH3 expression is the 25th percentile for NOTCH3 expression in pancreatic cancers.

35. The method of any one of claims 1-29, wherein the reference level for NOTCH3 expression is the 75th percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node metastatic pancreatic tumors, or chemotherapy-resistant pancreatic cancers.

36. The method of any one of claims 1-29, wherein the reference level for NOTCH3 expression is the 50th percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node metastatic pancreatic tumors or chemotherapy-resistant pancreatic cancers.

37. The method of any one of claims 1-29, wherein the reference level for NOTCH3 expression is the 25th percentile for NOTCH3 expression in pancreatic adenocarcinomas, metastatic pancreatic tumors, liver and/or lymph node metastatic pancreatic tumors or chemotherapy-resistant pancreatic cancers.

38. The method of any of claims 1-22, or 29-37, further comprising obtaining a body sample from said patient.

39. The method of any of claims 1-38, wherein the level of expression of NOTCH3 is the level in a body sample from the patient.

40. The method of claim 38 or 39, wherein said sample is whole blood, plasma, serum, or tissue.

41. The method of claim 38, 39, or 40, wherein said sample is a pancreatic tumor sample.

42. The method of claim 41, wherein the sample is from a pancreatic tumor that has metastasized to the liver.

43. The method of any one of claims 38-42, wherein the sample is formalin-fixed paraffin embedded (FFPE) tissue.

44. The method of any of claims 1-43, wherein said patient is a human or said patient population is a human population.

45. The method of any of claims 1-44, wherein said pancreatic cancer is adenocarcinoma.

46. The methods of any one of claims 1-45, wherein the pancreatic cancer is chemotherapy-resistant.

47. The method of any of claims 1-6, 8-22, or 29-46, further comprising administering the NOTCH inhibitor to said patient.

48. The method of any of claims 1-47, wherein said NOTCH inhibitor is a gamma-secretase inhibitor.

49. The method of any of claims 1-47, wherein said NOTCH inhibitor is an anti-NOTCH antibody.

50. The method of claim 49, wherein said anti-NOTCH antibody is a monoclonal antibody.

51. The method of claim 49 or 50, wherein said anti-NOTCH antibody specifically binds to human NOTCH2 or human NOTCH3.

52. The method of claim 51, wherein said anti-NOTCH antibody specifically binds to human NOTCH2 and NOTCH3.

53. The method of claim 49 or 50, wherein said anti-NOTCH antibody specifically binds to EGF repeat 10 of human NOTCH2.

54. The method of claim 49 or 50, wherein said anti-NOTCH antibody specifically binds to EGF repeat 9 of human NOTCH3.

55. The method of any claim 52, wherein said anti-NOTCH antibody comprises an antigen-binding site that binds both the EGF repeat 9 of human NOTCH3 and the EGF repeat 10 of NOTCH2.

56. The method of any one of claims 1-55, wherein said NOTCH inhibitor is an antagonist of human NOTCH2 and/or NOTCH3.

57. The method of any one of claims 1-56, wherein said NOTCH inhibitor inhibits binding of a ligand to human NOTCH2 and/or NOTCH3.

58 The method of any one of claims 1-57, wherein said NOTCH inhibitor inhibits signaling of human NOTCH2 and/or NOTCH3.

59. The method of claim 52, wherein said anti-NOTCH antibody is encoded by the polynucleotide deposited with ATCC as PTA-9547.

60. The method of claim 49 or 50, wherein said anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9); and

(b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8).

61. The method of claim 49 or 50, wherein said anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising GIFFAI (SEQ ID NO:5); and

(b) a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8).

62. The method of claim 49 or 50, wherein said anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises:

(a) a heavy chain variable region having at least about 90% sequence identity to SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:26; and

(b) a light chain variable region having at least about 90% sequence identity to SEQ ID NO:29 or SEQ ID NO:27.

63. The method of claim 49 or 50, wherein said anti-NOTCH antibody comprises:

(a) a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:17; and

(b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:29.

64. The method of claim 49 or 50, wherein said anti-NOTCH antibody comprises:

(a) a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:18; and

(b) a light chain variable region having at least about 95% sequence identity to SEQ ID NO:29.

65. The method of claim 49 or 50, wherein said anti-NOTCH antibody comprises:

(a) a heavy chain variable region comprising SEQ ID NO:18; and

(b) a light chain variable region comprising SEQ ID NO:29.

66. The method of claim 49 or 50, wherein said anti-NOTCH antibody comprises:

(a) a heavy chain variable region comprising SEQ ID NO:17; and

(b) a light chain variable region comprising SEQ ID NO:29.

67. The method of claim 49 or 50, wherein said anti-NOTCH antibody competes for specific binding to human NOTCH2 and/or NOTCH3 with an antibody selected from the group consisting of:

(a) an antibody comprising a heavy chain variable region comprising SEQ ID NO:17 or SEQ ID NO:18, and a light chain variable region comprising SEQ ID NO:29;

(b) an antibody comprising a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9), and a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8); and

(c) an antibody encoded by the polynucleotide deposited with ATCC as PTA-9547.

68. The method of any one of claims 49-67, wherein said anti-NOTCH antibody is a chimeric antibody, a humanized antibody, a human antibody, or an antibody fragment.

69. The method of any one of claims 7, 23-28, or 47-68, further comprising administering a second therapeutic agent.

70. The method of claim 69, wherein the second therapeutic agent is a chemotherapeutic agent.

71. The method of claim 70, wherein the second therapeutic agent is a nucleoside analogue or a mitotic inhibitor.

72. The method of claim 69, wherein the second therapeutic agent is gemcitabine, paclitaxel, albumin-bound paclitaxel, or combinations thereof.

73. A diagnostic composition comprising an isolated polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 35-43.

74. The diagnostic composition of claim 73, which comprises:

(a) a polynucleotide having the sequence of SEQ ID NO: 35, a polynucleotide having the sequence of SEQ ID NO: 36, and a polynucleotide having the sequence of SEQ ID NO: 37;

(b) a polynucleotide having the sequence of SEQ ID NO: 38, a polynucleotide having the sequence of SEQ ID NO: 39, and a polynucleotide having the sequence of SEQ ID NO: 40; or

(c) a polynucleotide having the sequence of SEQ ID NO: 41, a polynucleotide having the sequence of SEQ ID NO: 42, and a polynucleotide having the sequence of SEQ ID NO: 43.

75. A method of detecting NOTCH3 mRNA in a sample, comprising contacting the sample with a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 35-43.

76. The method of claim 75, which comprises contacting the sample with:

(a) a forward primer having the sequence of SEQ ID NO: 35, a reverse primer having the sequence of SEQ ID NO: 36, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO: 37;

(b) a forward primer having the sequence of SEQ ID NO: 38, a reverse primer having the sequence of SEQ ID NO: 39, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO: 40; or

(c) a forward primer having the sequence of SEQ ID NO: 41, a reverse primer having the sequence of SEQ ID NO: 42, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO: 43.

77. A kit for detecting NOTCH3 mRNA in a sample, comprising a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 35-43.

78. The kit of claim 77, which comprises:

(a) a polynucleotide having the sequence of SEQ ID NO: 35, a polynucleotide having the sequence of SEQ ID NO: 36, and a polynucleotide having the sequence of SEQ ID NO: 37;

(b) a polynucleotide having the sequence of SEQ ID NO: 38, a polynucleotide having the sequence of SEQ ID NO: 39, and a polynucleotide having the sequence of SEQ ID NO: 40; or

(c) a polynucleotide having the sequence of SEQ ID NO: 41, a polynucleotide having the sequence of SEQ ID NO: 42, and a polynucleotide having the sequence of SEQ ID NO: 43.

79. A primer having a sequence selected from the group consisting of: SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 41, and SEQ ID NO: 42.

80. A probe comprising an oligonucleotide having a sequence selected from the group consisting of: SEQ ID NO: 37, SEQ ID NO: 40, and SEQ ID NO: 43.

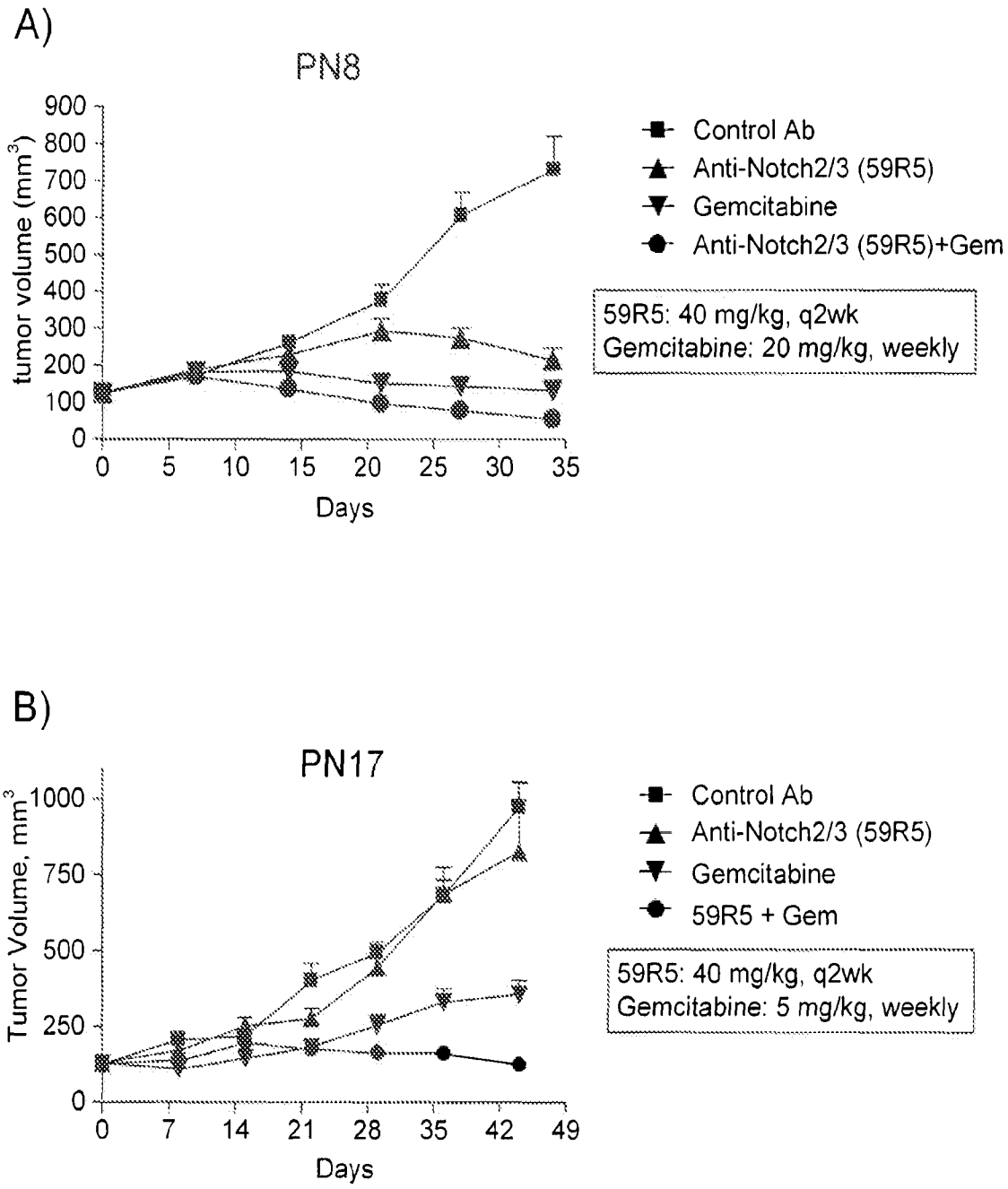

A)
PN8
tumor volume (mm3)
900
800
700
600
500
400
300
200
100
0
0 5 10 15 20 25 30 35
Days
Control Ab
Anti-Notch2/3 (59R5)
Gemcitabine
Anti-Notch2/3 (59R5)+Gem
59R5: 40 mg/kg, q2wk
Gemcitabine: 20 mg/kg, weekly
B)
PN17
Tumor Volume, mm3
1000
750
500
250
0
0 7 14 21 28 35 42 49
Days
Control Ab
Anti-Notch2/3 (59R5)
Gemcitabine
59R5 + Gem
59R5: 40 mg/kg, q2wk
Gemcitabine: 5 mg/kg, weekly

FIG. 1A-1B

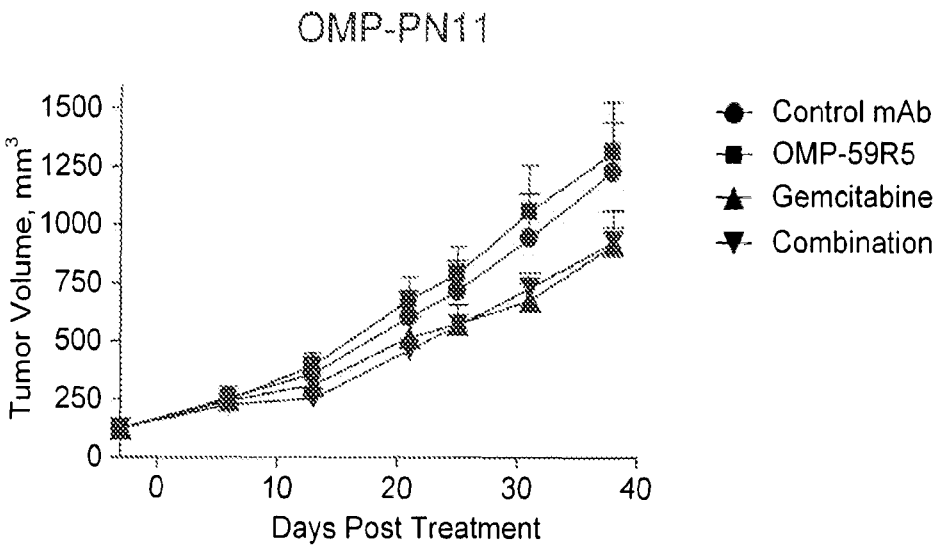
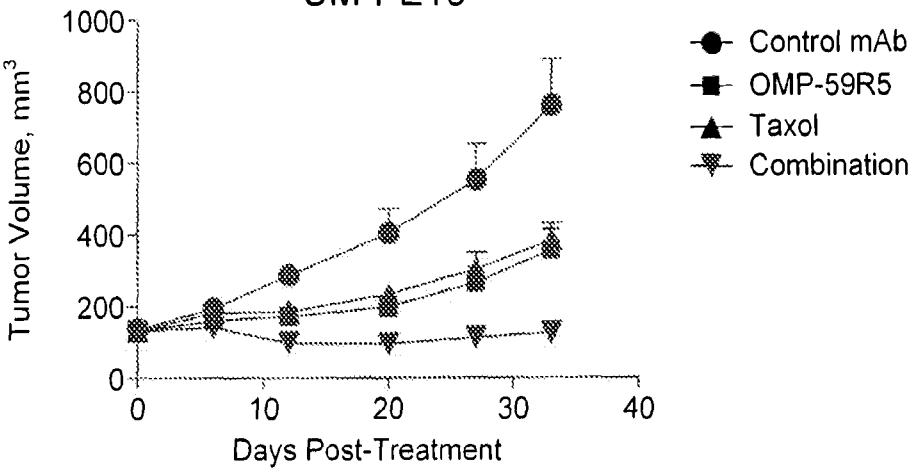

OMP-PN11
Tumor Volume, mm3
1500
1250
1000
750
500
250
0
0
10
20
30
40
Days Post Treatment
Control mAb
OMP-59R5
Gemcitabine
Combination

FIG. 1C

D)


UM-PE13
Tumor Volume, mm3
1000
800
600
400
200
0
0
10
20
30
40
Days Post-Treatment
Control mAb
OMP-59R5
Taxol
Combination

E)

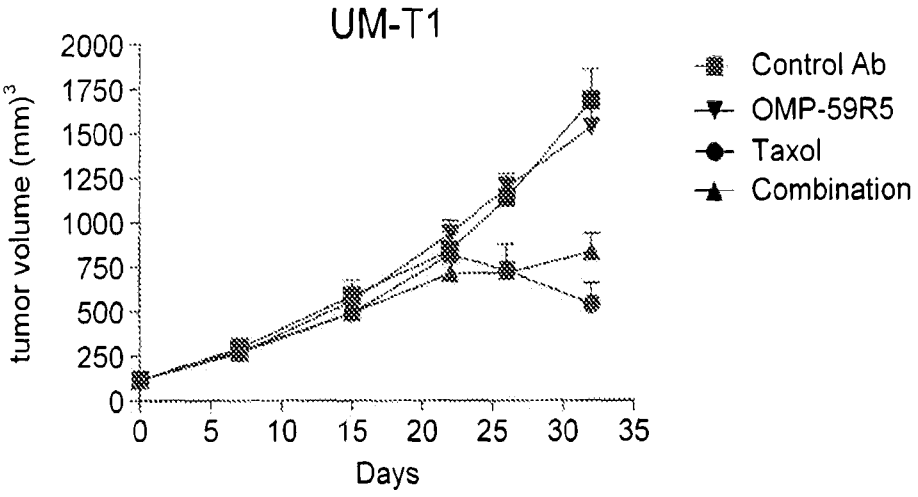
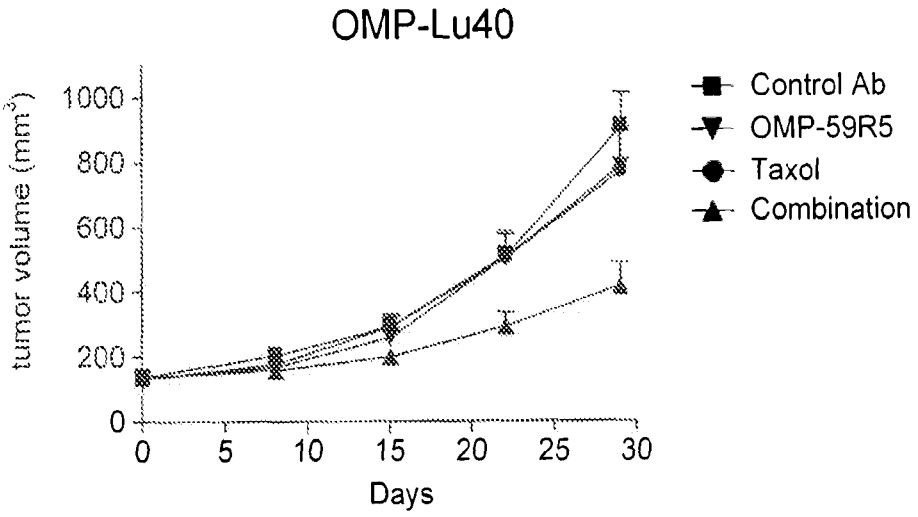

UM-T1
tumor volume (mm)3
2000
1750
1500
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35
Days
Control Ab
OMP-59R5
Taxol
Combination

FIG. 1D-1E

F)


OMP-Lu40
tumor volume (mm3)
1000
800
600
400
200
0
0
5
10
15
20
25
30
Days
Control Ab
OMP-59R5
Taxol
Combination

G)

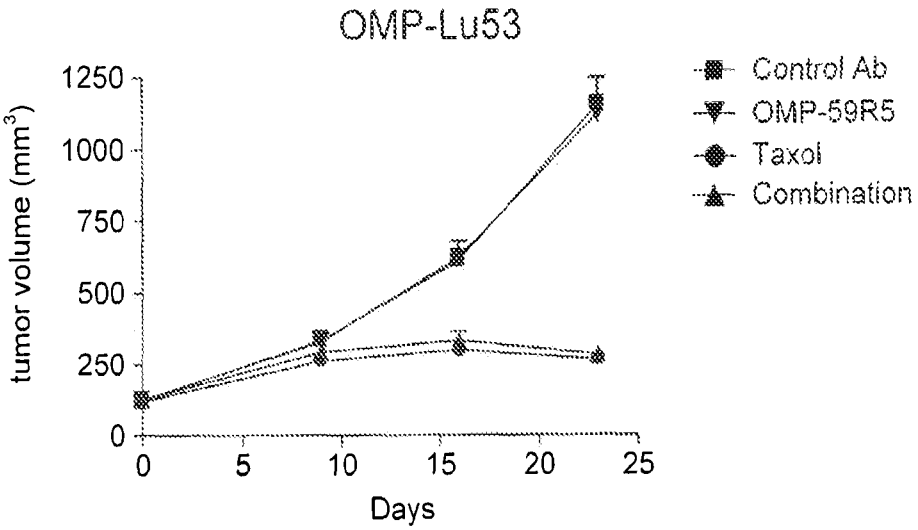

OMP-Lu53
tumor volume (mm3)
1250
1000
750
500
250
0
0
5
10
15
20
25
Days
Control Ab
OMP-59R5
Taxol
Combination

FIG. 1F-1G

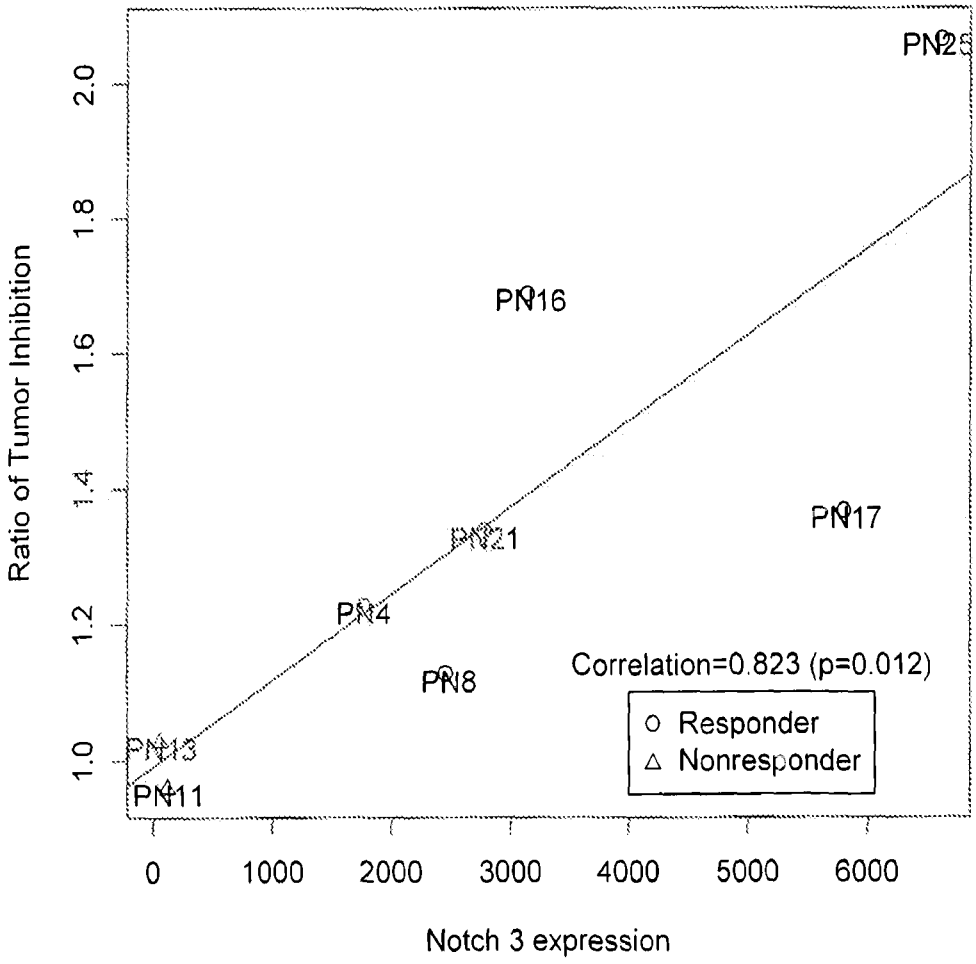

Ratio of Tumor Inhibition
1.0
1.2
1.4
1.6
1.8
2.0
0
1000
2000
3000
4000
5000
6000
Notch 3 expression
PN16
PN17
PN4
PN8
PN11
Correlation=0.823 (p=0.012)
o Responder
△ Nonresponder

FIG. 2A

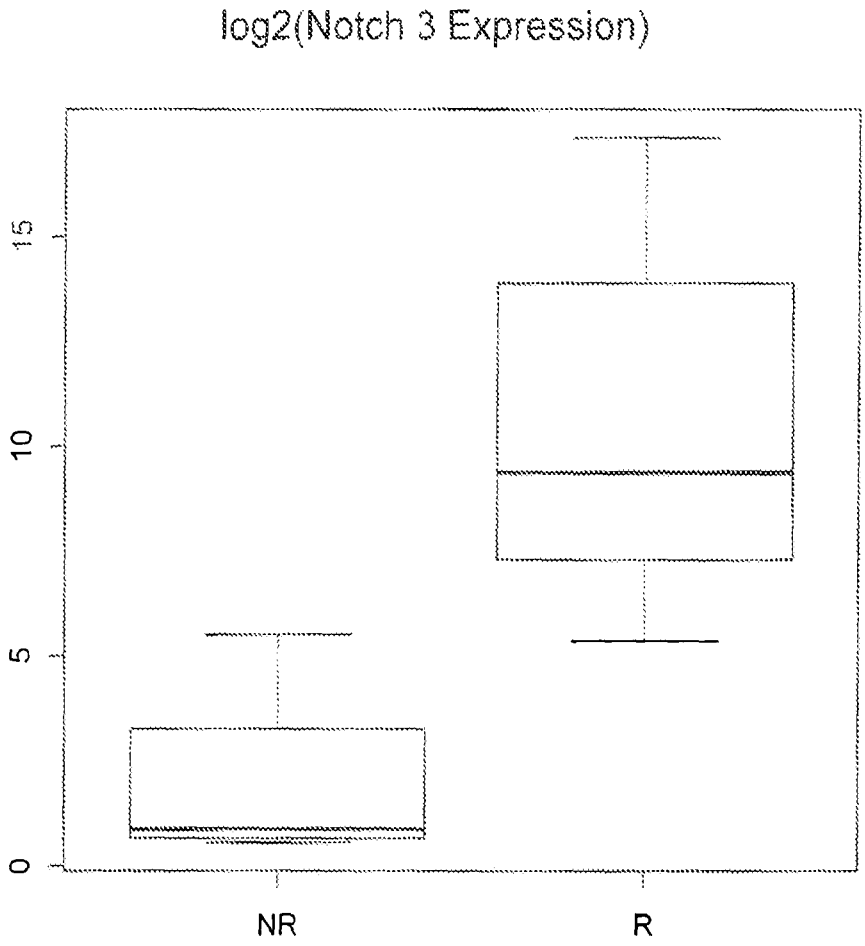
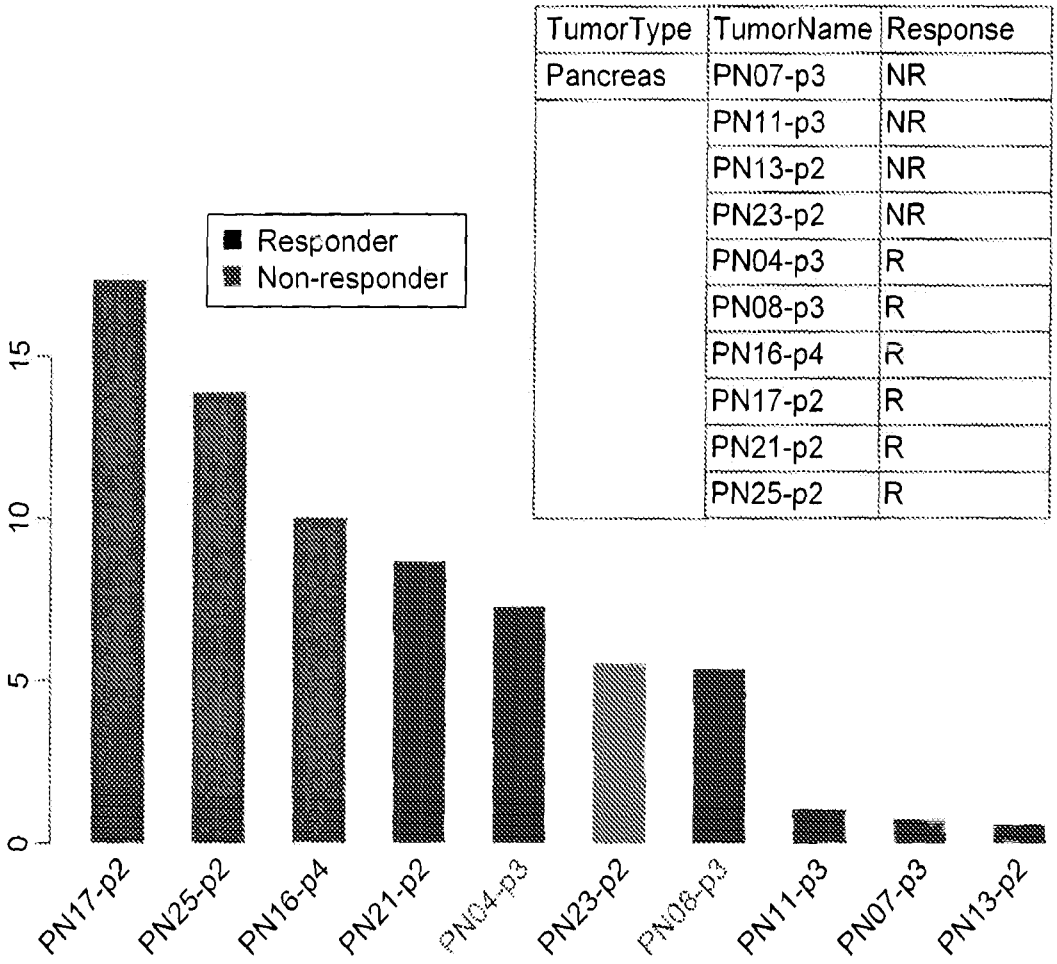


FIG. 2B

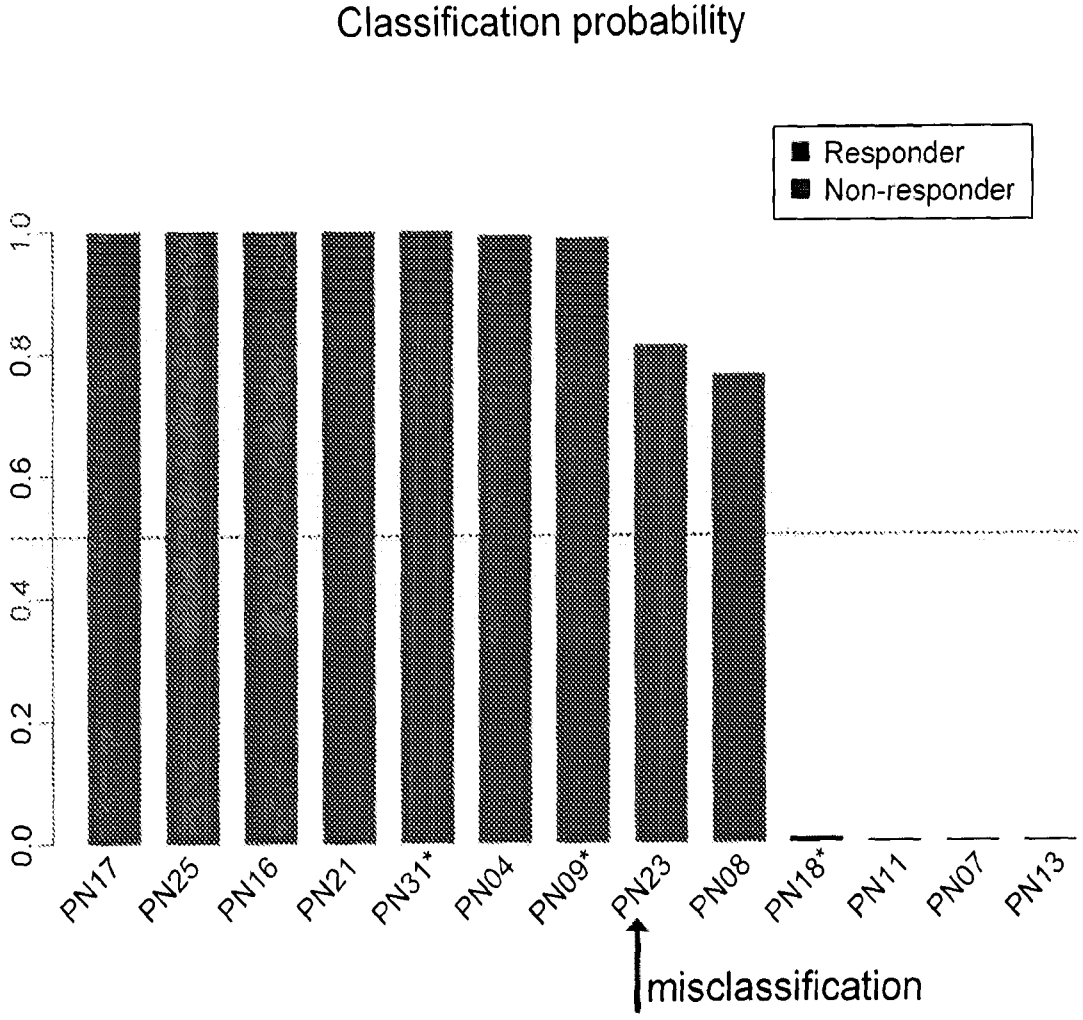
NOTCH3 RPKM counts



TumorType	TumorName	Response
Pancreas	PN07-p3	NR
	PN11-p3	NR
	PN13-p2	NR
	PN23-p2	NR
	PN04-p3	R
	PN08-p3	R
	PN16-p4	R
	PN17-p2	R
	PN21-p2	R
	PN25-p2	R

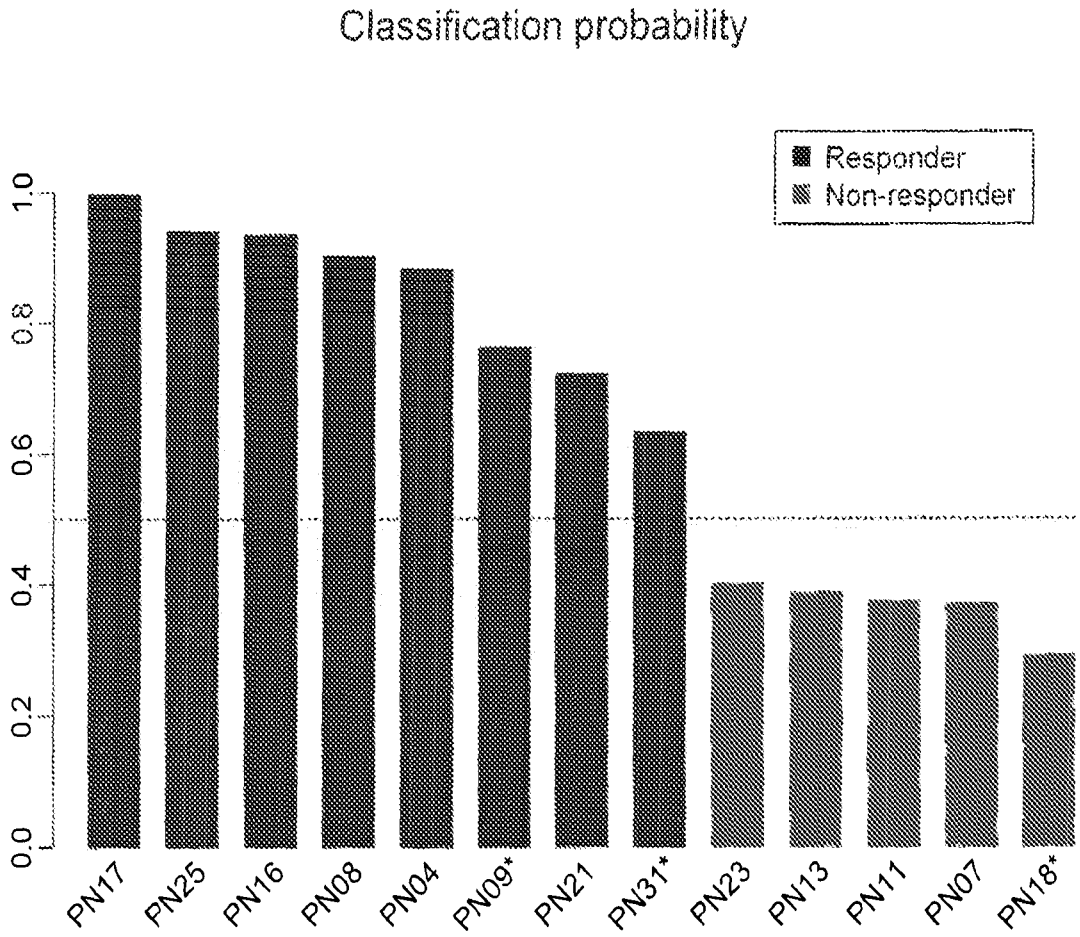
Notch3 is differently expressed between R and NR: p-value = 0.0086

FIG. 3



Classifier: logistic regression.
Cross-validated PPV=83%, NPV=75%, SENS=83%, SPEC=75%
Predicted responder: PN31, PN09
Predicted non-responder: PN18

FIG. 4



Predicted responder: PN09, PN31
Predicted non-responder: PN18
Cross-validated PPV=NPV=SENS=SPEC=100% in RNA-seq

FIG. 5

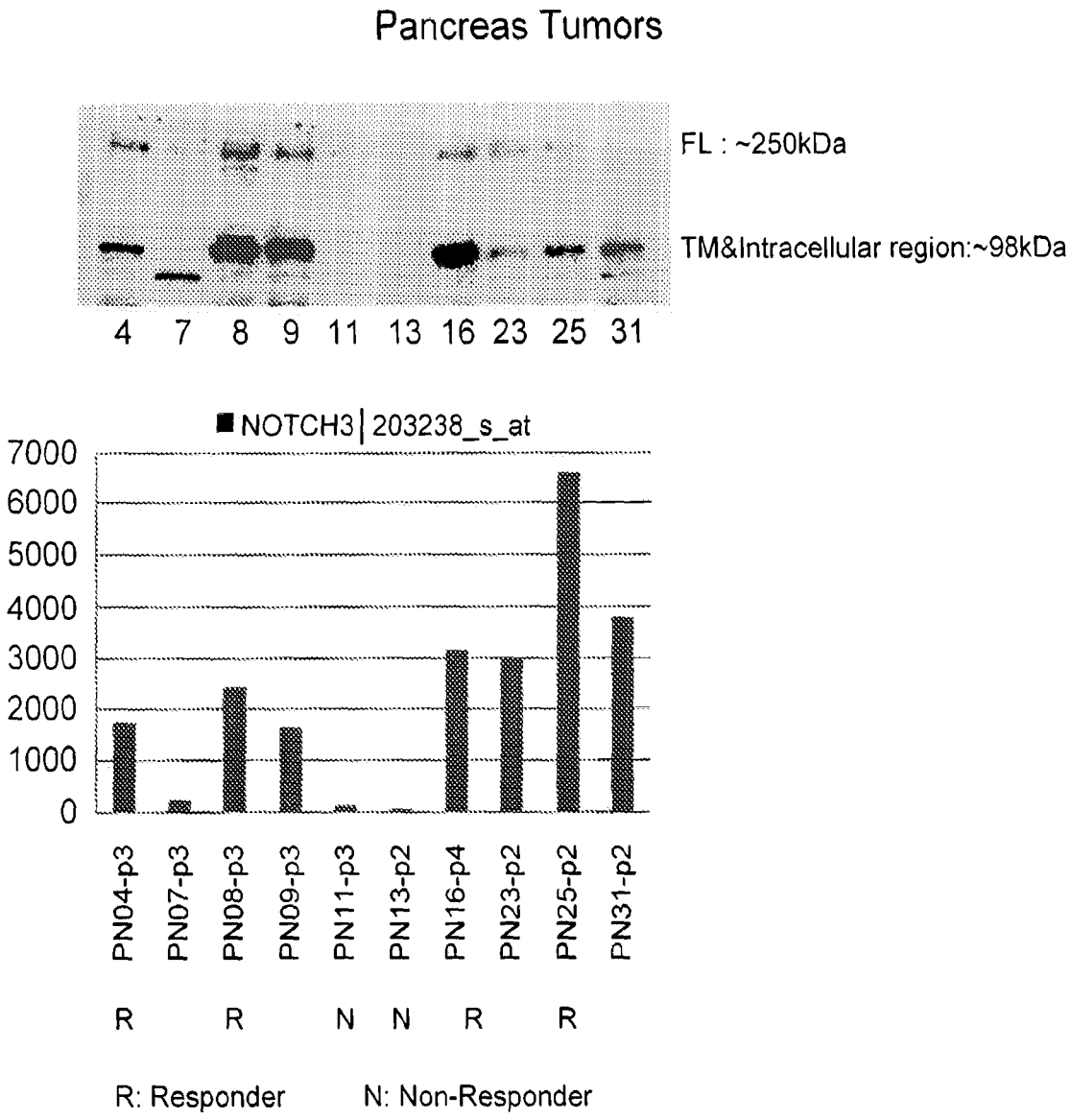

Pancreas Tumors
FL : ~250kDa
TM&Intracellular region:~98kDa
4 7 8 9 11 13 16 23 25 31
NOTCH3 | 203238_s_at
7000
6000
5000
4000
3000
2000
1000
0
PN04-p3
PN07-p3
PN08-p3
PN09-p3
PN11-p3
PN13-p2
PN16-p4
PN23-p2
PN25-p2
PN31-p2
R R N N R R
R: Responder N: Non-Responder

FIG. 6A

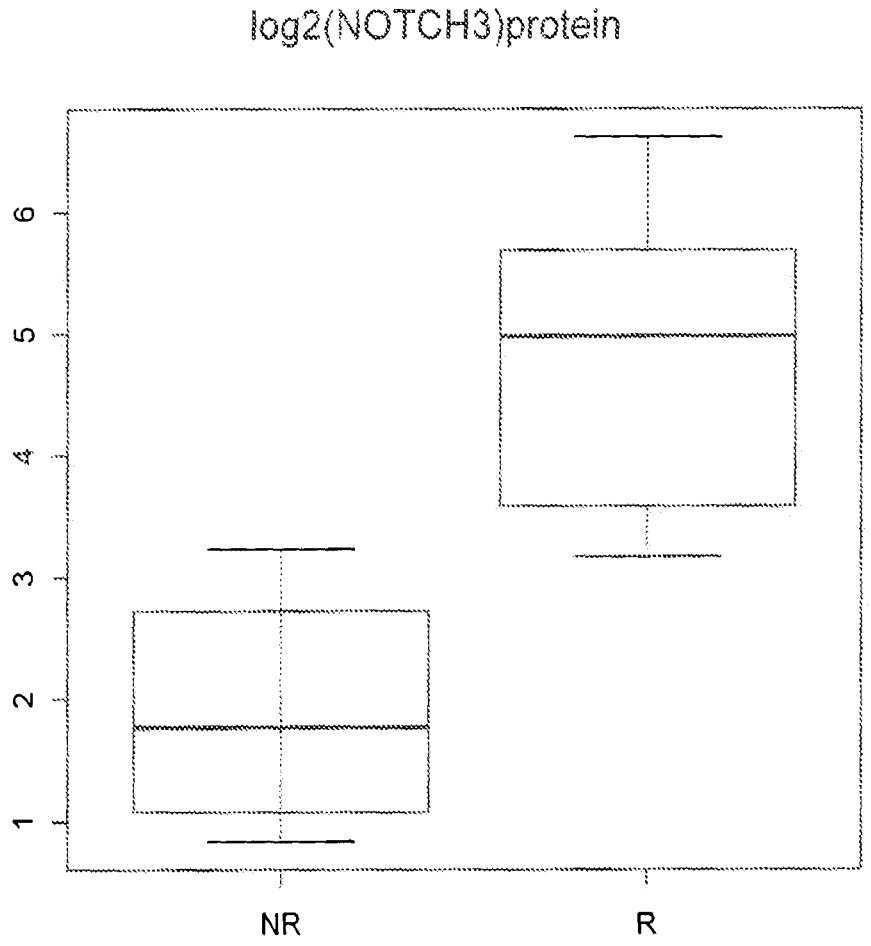


FIG. 6B

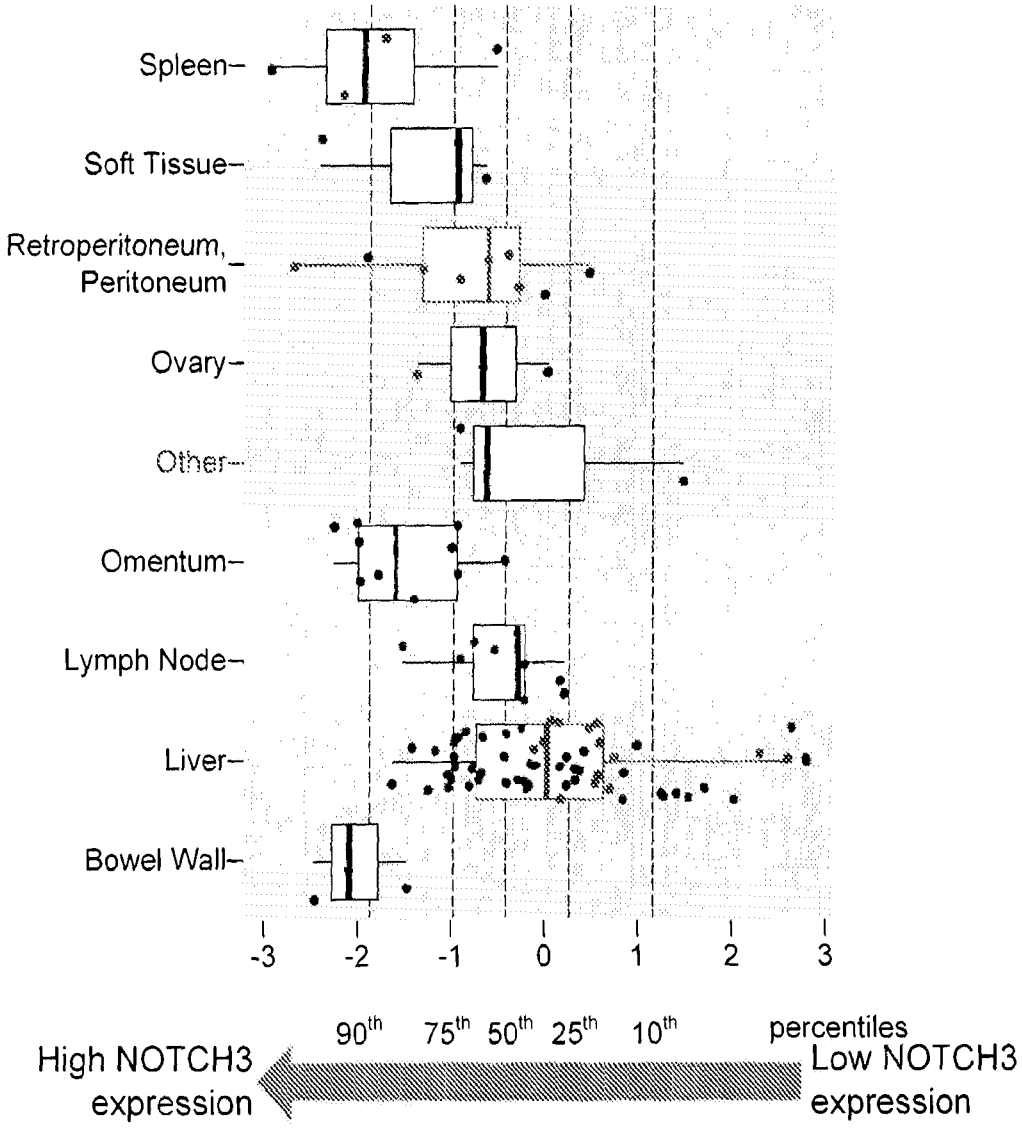

Spleen
Soft Tissue
Retroperitoneum, Peritoneum
Ovary
Other
Omentum
Lymph Node
Liver
Bowel Wall
-3
-2
-1
0
1
2
3
90th 75th 50th 25th 10th
percentiles
High NOTCH3 expression
Low NOTCH3 expression

FIG. 7

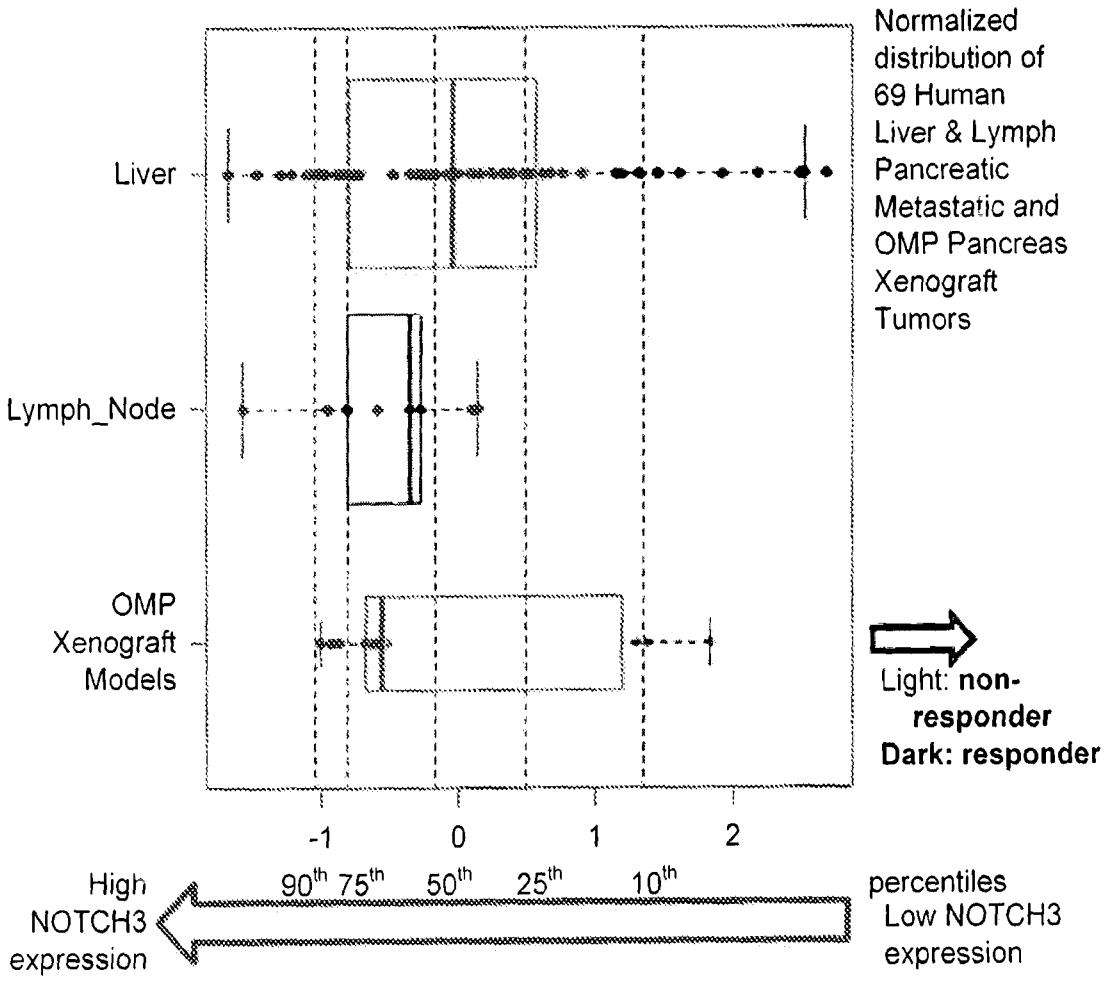

Normalized distribution of 69 Human Liver & Lymph Pancreatic Metastatic and OMP Pancreas Xenograft Tumors
Liver
Lymph_Node
OMP Xenograft Models
Light: non-responder
Dark: responder
-1
0
1
2
High NOTCH3 expression
90th 75th 50th 25th 10th
percentiles
Low NOTCH3 expression

FIG. 8

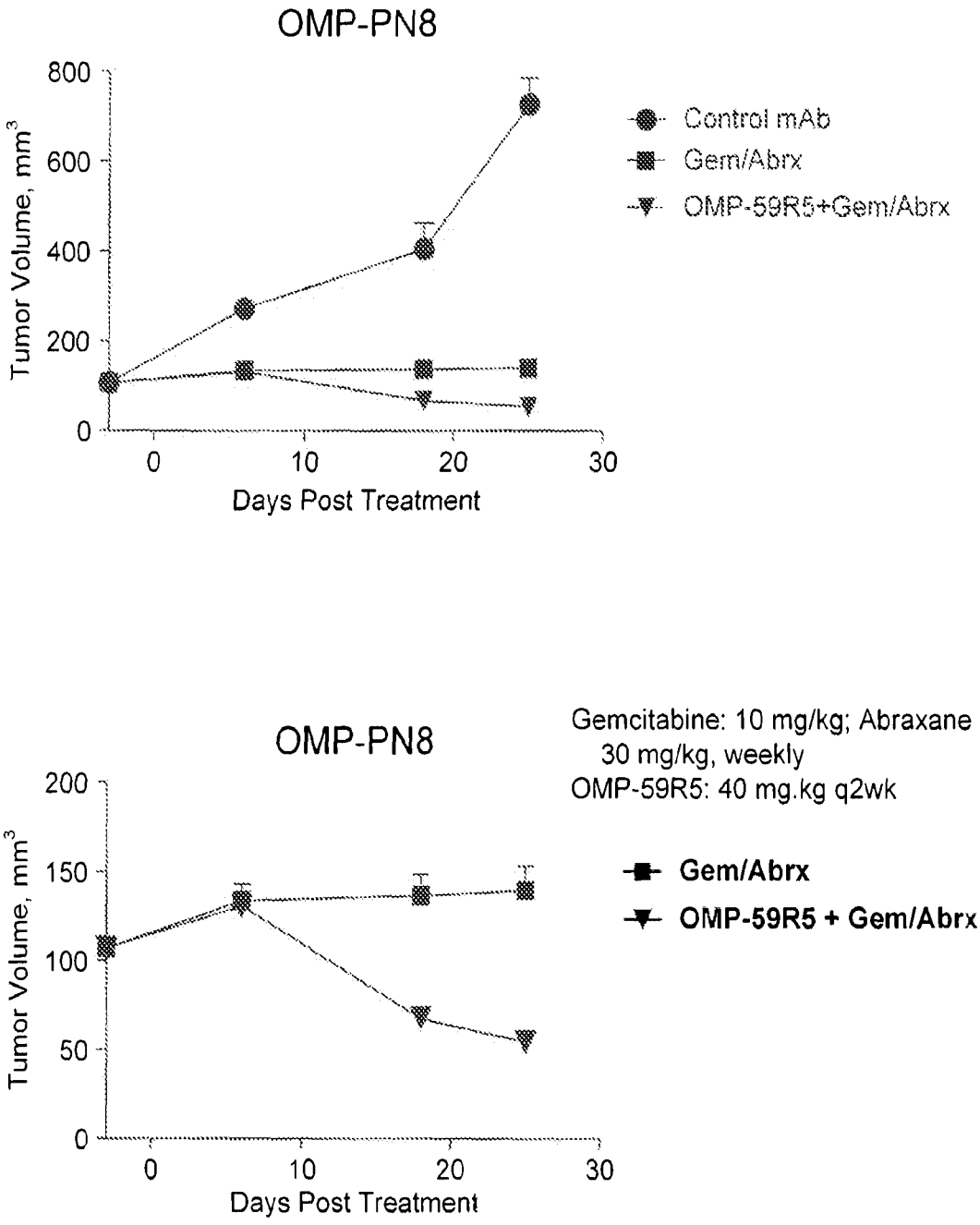

OMP-PN8
Tumor Volume, mm3
800
600
400
200
0
0
10
20
30
Days Post Treatment
Control mAb
Gem/Abrx
OMP-59R5+Gem/Abrx
OMP-PN8
Tumor Volume, mm3
200
150
100
50
0
0
10
20
30
Days Post Treatment
Gemcitabine: 10 mg/kg; Abraxane 30 mg/kg, weekly
OMP-59R5: 40 mg.kg q2wk
Gem/Abrx
OMP-59R5 + Gem/Abrx

FIG. 9

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International Bureau

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G01N 33/53 (2006.01) *A61K 35/00* (2006.01)

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13 March 2014 (13.03.2014)

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(72) Inventors: **HOEY, Timothy, Charles**; 200 Darrell Road, Hillsborough, CA 94010 (US). **ZHANG, Chun**; 570 Ashton Avenue, Palo Alto, CA 94306 (US). **KAPOUN, Ann, M.**; 686 Ehrhorn Avenue, Mountain View, CA 94041 (US).

(74) Agents: **CALVO, Paul, A.** et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, N.W., Washington, DC 20005 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,

[Continued on next page]

(54) Title: METHODS OF TREATING PANCREATIC CANCER

(57) Abstract: Novel methods of treating pancreatic cancer are provided. In one embodiment, the method comprises determining NOTCH mRNA expression levels in pancreatic cancer cells. In another embodiment, the method further comprises administering to a subject in need thereof a therapeutically effective dose of a NOTCH antagonist.

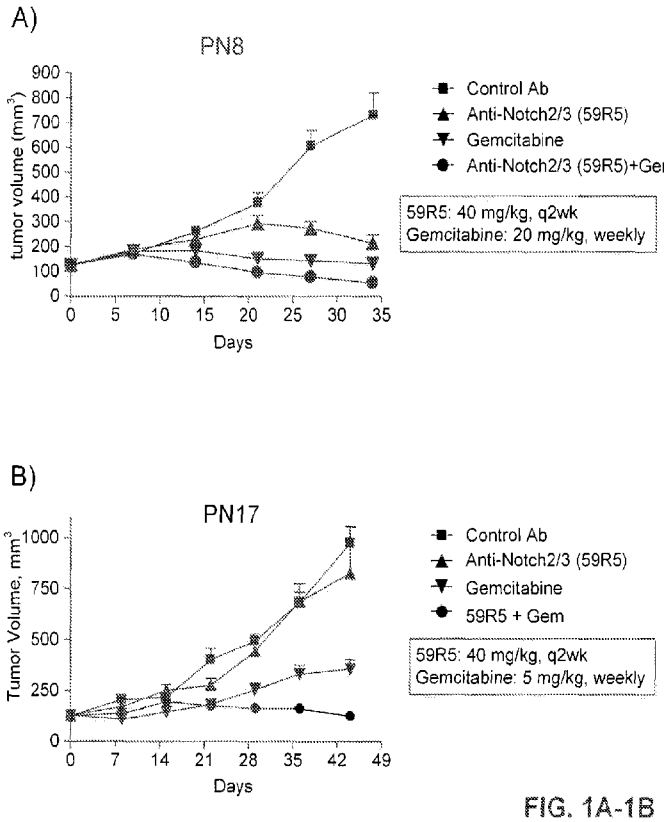

FIG. 1A-1B

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UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

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

— *with amended claims (Art. 19(1))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

(88) Date of publication of the international search report: 27 November 2014

Date of publication of the amended claims: 29 January 2015

AMENDED CLAIMS
received by the International Bureau on 02 December 2014(02.12.2014)

What we claim is:

1. A method for selecting a pancreatic cancer patient for treatment with a NOTCH inhibitor comprising:
   (a) determining the level of expression of one or more biomarkers in tumor cells from the patient, wherein the one or more biomarkers comprise NOTCH3, and
   (b) selecting the patient based on the expression level of the one or more biomarkers.

2. A method for determining whether a patient diagnosed with pancreatic cancer is likely to respond to a NOTCH inhibitor-based therapy or should continue treatment with a NOTCH inhibitor, the method comprising determining the level of expression of one or more biomarkers in tumor cells from the patient, wherein the one or more biomarkers comprise NOTCH3, and the level of expression of the one or more biomarkers indicates that the patient is likely to respond to therapy.

3. A method of treating pancreatic cancer in a patient comprising:
   (a) determining the level of expression of one or more biomarkers in tumor cells from the patient, wherein the one or more biomarkers comprise NOTCH3; and
   (b) administering to the patient a therapeutically effective amount of a NOTCH inhibitor.

4. The method of any one of claims 1-3, wherein each of the biomarkers is determined to be expressed at a level above a reference level for the biomarker.

5. The method of any one of claims 1-4, wherein the expression level of the one or more biomarkers is determined by determining the level of the biomarker mRNA or the biomarker protein.

6. The method of claim 5, wherein the biomarker mRNA level is determined by quantitative polymerase chain reaction or by array hybridization.

7. The method of claim 6, wherein the biomarker is NOTCH3 and the mRNA level is determined using:
   (a) a forward primer having a nucleotide sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:38, and SEQ ID NO:41;
   (b) a reverse primer having a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:39, and SEQ ID NO:42; and/or

AMENDED SHEET (ARTICLE 19)

(c) a probe comprising an oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:40, and SEQ ID NO:43.

8. The method of claim 7, wherein the NOTCH3 mRNA level is determined using:

(a) a forward primer having the sequence of SEQ ID NO:35, a reverse primer having the sequence of SEQ ID NO:36, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:37;

(b) a forward primer having the sequence of SEQ ID NO:38, a reverse primer having the sequence of SEQ ID NO:39, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:40; or

(c) a forward primer having the sequence of SEQ ID NO:41, a reverse primer having the sequence of SEQ ID NO:42, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:43.

9. The method of any one of claims 1-8, wherein the one or more biomarkers further comprise MAML2 and the level of MAML2 expression is determined to be above a reference level for MAML2 expression.

10. The method of any one of claims 1-9, wherein the reference level of a biomarker is a predetermined value or is the level of expression of that biomarker in a control sample.

11. The method of any one of claims 1-10, wherein the reference level for NOTCH3 expression is the 25th percentile, the 30th percentile, the 40th percentile, the 50th percentile, the 60th percentile, the 70th percentile, the 75th percentile, or the 80th percentile for NOTCH3 expression in pancreatic cancers or a subset of pancreatic cancers.

12. The method of any one of claims 1-11, comprising obtaining a sample from the patient.

13. The method of claim 12, wherein the sample is whole blood, plasma, serum, or tissue.

14. The method of claim 12 or claim 13, wherein the sample is a pancreatic tumor sample.

15. The method of any one of claims 12-14, wherein the sample is formalin-fixed paraffin embedded (FFPE) tissue.

16. The method of any one of claims 1, 2, or 4-15, further comprising administering the NOTCH inhibitor to the patient.

17. The method of any one of claims 1-16, wherein the NOTCH inhibitor is a gamma-secretase inhibitor or an anti-NOTCH antibody.

18. The method of claim 17, wherein the anti-NOTCH antibody specifically binds to human NOTCH2 and/or human NOTCH3.

19. The method of claim 18, wherein the anti-NOTCH antibody is encoded by the polynucleotide deposited with ATCC as PTA-9547.

20. The method of claim 17, wherein the anti-NOTCH antibody specifically binds human NOTCH2 and/or NOTCH3, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9) or GIFFAI (SEQ ID NO:5); and a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8); or

(b) a heavy chain variable region having at least about 90% sequence identity to SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:26; and a light chain variable region having at least about 90% sequence identity to SEQ ID NO:29 or SEQ ID NO:27.

21. The method of claim 17, wherein the anti-NOTCH antibody competes for specific binding to human NOTCH2 and/or NOTCH3 with an antibody selected from the group consisting of:

(a) an antibody comprising a heavy chain variable region comprising SEQ ID NO:17 or SEQ ID NO:18, and a light chain variable region comprising SEQ ID NO:29;

(b) an antibody comprising a heavy chain CDR1 comprising SSSGMS (SEQ ID NO:3), a heavy chain CDR2 comprising VIASSGSNTYYADSVKG (SEQ ID NO:4), and a heavy chain CDR3 comprising SIFYTT (SEQ ID NO:9), and a light chain CDR1 comprising RASQSVRSNYLA (SEQ ID NO:6), a light chain CDR2 comprising GASSRAT (SEQ ID NO:7), and a light chain CDR3 comprising QQYSNFPI (SEQ ID NO:8); and

(c) an antibody encoded by the polynucleotide deposited with ATCC as PTA-9547.

22. The method of any one of claims 17-21, wherein the anti-NOTCH antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, or an antibody fragment.

23. The method of any one of claims 3 or 16-22, further comprising administering a second therapeutic agent; optionally where the second therapeutic agent is a chemotherapeutic agent, a nucleoside analogue, or a mitotic inhibitor.

24. A diagnostic composition comprising an isolated polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35-43.

25. The diagnostic composition of claim 24, which comprises:
(a) a polynucleotide having the sequence of SEQ ID NO:35, a polynucleotide having the sequence of SEQ ID NO:36, and a polynucleotide having the sequence of SEQ ID NO:37;
(b) a polynucleotide having the sequence of SEQ ID NO:38, a polynucleotide having the sequence of SEQ ID NO:39, and a polynucleotide having the sequence of SEQ ID NO:40; or
(c) a polynucleotide having the sequence of SEQ ID NO:41, a polynucleotide having the sequence of SEQ ID NO:42, and a polynucleotide having the sequence of SEQ ID NO:43.

26. A method of detecting NOTCH3 mRNA in a sample, comprising contacting the sample with a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35-43.

27. The method of claim 26, which comprises contacting the sample with:
(a) a forward primer having the sequence of SEQ ID NO:35, a reverse primer having the sequence of SEQ ID NO:36, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:37;
(b) a forward primer having the sequence of SEQ ID NO:38, a reverse primer having the sequence of SEQ ID NO:39, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:40; or
(c) a forward primer having the sequence of SEQ ID NO:41, a reverse primer having the sequence of SEQ ID NO:42, and a probe comprising an oligonucleotide having the sequence of SEQ ID NO:43.

28. A kit for detecting NOTCH3 mRNA in a sample, comprising a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35-43.

29. The kit of claim 28, which comprises:

(a) a polynucleotide having the sequence of SEQ ID NO:35, a polynucleotide having the sequence of SEQ ID NO:36, and a polynucleotide having the sequence of SEQ ID NO:37;

(b) a polynucleotide having the sequence of SEQ ID NO:38, a polynucleotide having the sequence of SEQ ID NO:39, and a polynucleotide having the sequence of SEQ ID NO:40; or

(c) a polynucleotide having the sequence of SEQ ID NO:41, a polynucleotide having the sequence of SEQ ID NO:42, and a polynucleotide having the sequence of SEQ ID NO:43.

30. A primer having a sequence selected from the group consisting of: SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, and SEQ ID NO:42.

31. A probe comprising an oligonucleotide having a sequence selected from the group consisting of: SEQ ID NO:37, SEQ ID NO:40, and SEQ ID NO:43.

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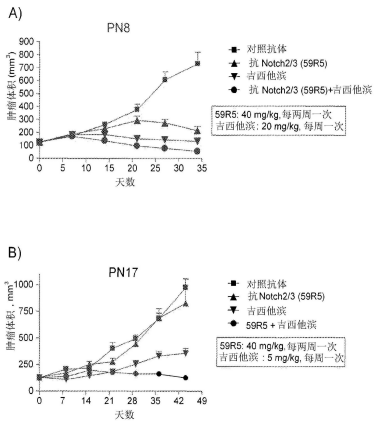
(54)发明名称

治疗胰腺癌的方法

(57)摘要

提供了治疗胰腺癌的新方法。在一个实施方式中,所述方法包括确定胰腺癌细胞中的NOTCH mRNA表达水平。在另一个实施方式中,所述方法还包括向有需要的受试对象施用治疗有效量的NOTCH拮抗剂。



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1. 一种为了用NOTCH抑制剂进行治疗而选择胰腺癌患者的方法，所述方法包括：(a) 确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，和 (b) 基于所述一种或多种生物标志物的表达水平选择患者。

2. 一种确定被诊断为患有胰腺癌的患者是否可能对基于NOTCH抑制剂的疗法产生响应的方法，所述方法包括：确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，并且所述一种或多种生物标志物的表达水平表明患者可能对疗法产生响应。

3. 一种确定是否应当向被诊断为患有胰腺癌的患者施用NOTCH抑制剂的方法，所述方法包括：确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，并且所述一种或多种生物标志物的表达水平预示所述患者对NOTCH抑制剂治疗具有有利的响应。

4. 一种确定被诊断为患有胰腺癌的患者是否应当继续用NOTCH抑制剂进行治疗的方法，所述方法包括：确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，并且所述一种或多种生物标志物的表达水平表明患者可能对疗法产生响应。

5. 一种确定被诊断为患有胰腺癌的患者是否应当继续用NOTCH抑制剂进行治疗的方法，所述方法包括：确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，并且所述一种或多种生物标志物的表达水平预示所述患者对所述NOTCH抑制剂治疗具有有利的响应。

6. 一种确定NOTCH抑制剂在治疗患者的胰腺癌中的治疗功效的方法，所述方法包括：确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，并且所述一种或多种生物标志物的表达水平表明所述NOTCH抑制剂的治疗功效。

7. 一种治疗患者的胰腺癌的方法，所述方法包括：

(a) 确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3；和

(b) 向所述患者施用治疗有效量的NOTCH抑制剂。

8. 一种为了用NOTCH抑制剂进行治疗而将胰腺癌患者群体分阶的方法，所述方法包括：

(a) 确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平，其中，所述一种或多种生物标志物包含NOTCH3，和

(b) 基于所述肿瘤细胞中的所述一种或多种生物标志物的表达水平将所述患者群体分阶。

9. 如权利要求1～8中任一项所述的方法，其中，NOTCH3的表达水平经确定高于NOTCH3表达的参照水平。

10. 如权利要求1～9中任一项所述的方法，其中，每种生物标志物经确定都以高于该生物标志物的参照水平的水平表达。

11. 如权利要求1～10中任一项所述的方法，其中，一种或多种生物标志物的表达水平通过确定生物标志物mRNA或生物标志物蛋白的水平来确定。

12. 如权利要求 1 ～ 11 中任一项所述的方法，其中，NOTCH3 的表达水平通过确定肿瘤细胞中的 NOTCH3 mRNA 水平来确定。

13. 如权利要求 12 所述的方法，其中，所述 NOTCH3 mRNA 水平通过定量聚合酶链式反应来确定。

14. 如权利要求 13 所述的方法，其中，所述 NOTCH3 mRNA 水平使用以下 (a)、(b) 和 / 或 (c) 来确定 ：(a) 核苷酸序列选自由 SEQ ID NO:35、SEQ ID NO:38 和 SEQ ID NO:41 组成的组的正向引物 ；(b) 核苷酸序列选自由 SEQ ID NO:36、SEQ ID NO:39 和 SEQ ID NO:42 组成的组的反向引物 ；和 / 或 (c) 包含寡核苷酸的探针，所述寡核苷酸的核苷酸序列选自由 SEQ ID NO:37、SEQ ID NO:40 和 SEQ ID NO:43 组成的组。

15. 如权利要求 14 所述的方法，其中，所述 NOTCH3 mRNA 水平使用以下 (a)、(b) 或 (c) 来确定 ：

(a) 序列为 SEQ ID NO:35 的正向引物，序列为 SEQ ID NO:36 的反向引物，和包含序列为 SEQ ID NO:37 的寡核苷酸的探针 ；

(b) 序列为 SEQ ID NO:38 的正向引物，序列为 SEQ ID NO:39 的反向引物，和包含序列为 SEQ ID NO:40 的寡核苷酸的探针 ；或

(c) 序列为 SEQ ID NO:41 的正向引物，序列为 SEQ ID NO:42 的反向引物，和包含序列为 SEQ ID NO:43 的寡核苷酸的探针。

16. 如权利要求 12 所述的方法，其中，所述 NOTCH3 mRNA 水平通过阵列杂交来确定。

17. 如权利要求 1 ～ 11 中任一项所述的方法，其中，NOTCH3 的表达水平通过确定肿瘤细胞所表达的 NOTCH3 蛋白水平来确定。

18. 如权利要求 1 ～ 17 中任一项所述的方法，其中，所述一种或多种生物标志物由 NOTCH3 构成。

19. 如权利要求 1 ～ 17 中任一项所述的方法，其中，所述一种或多种生物标志物还包括 MAML2，并且 MAML2 的表达水平经确定高于 MAML2 表达的参照水平。

20. 如权利要求 19 所述的方法，其中，所述一种或多种生物标志物由 NOTCH3 和 MAML2 构成。

21. 如权利要求 19 或 20 所述的方法，其中，所述 MAML2 的表达水平通过确定肿瘤细胞中的 MAML2 mRNA 水平来确定。

22. 如权利要求 19 或 20 所述的方法，其中，所述 MAML2 的表达水平通过确定肿瘤细胞所表达的 MAML2 蛋白水平来确定。

23. 一种治疗患者的胰腺癌的方法，所述方法包括 ：向所述患者施用治疗有效量的 NOTCH 抑制剂，其中，来自所述患者的至少一些胰腺肿瘤细胞表达一种或多种生物标志物中的每一种的水平高于所述生物标志物的参照水平，和 / 或已被预先确定为表达一种或多种生物标志物中的每一种的水平高于所述生物标志物的参照水平 ；其中，所述一种或多种生物标志物包含 NOTCH3。

24. 如权利要求 23 所述的方法，其中，NOTCH3 的表达水平被确定为 NOTCH3 mRNA 水平。

25. 如权利要求 23 所述的方法，其中，NOTCH3 的表达水平被确定为 NOTCH3 蛋白水平。

26. 如权利要求 23 ～ 25 中任一项所述的方法，其中，所述一种或多种生物标志物由 NOTCH3 构成。

27. 如权利要求 23 ～ 25 中任一项所述的方法，其中，所述一种或多种生物标志物还包括 MAML2，并且 MAML2 的表达水平高于 MAML2 表达的参照水平。

28. 如权利要求 27 所述的方法，其中，所述一种或多种生物标志物由 NOTCH3 和 MAML2 构成。

29. 如权利要求 1 ～ 28 中任一项所述的方法，其中，生物标志物的参照水平是预定值。

30. 如权利要求 1 ～ 29 中任一项所述的方法，其中，生物标志物的参照水平是该生物标志物在对照样品中的表达水平。

31. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺癌或胰腺癌亚类中的 NOTCH3 表达的第 25、第 30、第 40、第 50、第 60、第 70、第 75 或第 80 百分位数。

32. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺癌中的 NOTCH3 表达的第 75 百分位数。

33. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺癌中的 NOTCH3 表达的第 50 百分位数。

34. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺癌中的 NOTCH3 表达的第 25 百分位数。

35. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤或抗化疗胰腺癌中的 NOTCH3 表达的第 75 百分位数。

36. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤或抗化疗胰腺癌中的 NOTCH3 表达的第 50 百分位数。

37. 如权利要求 1 ～ 29 中任一项所述的方法，其中，NOTCH3 表达的参照水平是胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤或抗化疗胰腺癌中的 NOTCH3 表达的第 25 百分位数。

38. 如权利要求 1 ～ 22 或 29 ～ 37 中任一项所述的方法，所述方法还包括从所述患者获得身体样品。

39. 如权利要求 1 ～ 38 中任一项所述的方法，其中，NOTCH3 的表达水平是来自患者的身体样品中的水平。

40. 如权利要求 38 或 39 所述的方法，其中，所述样品是全血、血浆、血清或组织。

41. 如权利要求 38、39 或 40 所述的方法，其中，所述样品是胰腺肿瘤样品。

42. 如权利要求 41 所述的方法，其中，所述样品是来自已转移至肝脏的胰腺肿瘤的样品。

43. 如权利要求 38 ～ 42 中任一项所述的方法，其中，所述样品是福尔马林固定的石蜡包埋 (FFPE) 的组织。

44. 如权利要求 1 ～ 43 中任一项所述的方法，其中，所述患者是人，或所述患者群体是人群体。

45. 如权利要求 1 ～ 44 中任一项所述的方法，其中，所述胰腺癌是腺癌。

46. 如权利要求 1 ～ 45 中任一项所述的方法，其中，所述胰腺癌是抗化疗的。

47. 如权利要求 1 ～ 6、8 ～ 22 或 29 ～ 46 中任一项所述的方法，所述方法还包括向所述患者施用 NOTCH 抑制剂。

48. 如权利要求 1 ～ 47 中任一项所述的方法，其中，所述 NOTCH 抑制剂是 γ 分泌酶抑制剂。

49. 如权利要求 1 ～ 47 中任一项所述的方法，其中，所述 NOTCH 抑制剂是抗 NOTCH 抗体。

50. 如权利要求 49 所述的方法，其中，所述抗 NOTCH 抗体是单克隆抗体。

51. 如权利要求 49 或 50 所述的方法，其中，所述抗 NOTCH 抗体特异性地结合人 NOTCH2 或人 NOTCH3。

52. 如权利要求 51 所述的方法，其中，所述抗 NOTCH 抗体特异性地结合人 NOTCH2 和人 NOTCH3。

53. 如权利要求 49 或 50 所述的方法，其中，所述抗 NOTCH 抗体特异性地结合人 NOTCH2 的 EGF 重复 10。

54. 如权利要求 49 或 50 所述的方法，其中，所述抗 NOTCH 抗体特异性地结合人 NOTCH3 的 EGF 重复 9。

55. 如权利要求 52 所述的方法，其中，所述抗 NOTCH 抗体包含与人 NOTCH3 的 EGF 重复 9 和人 NOTCH2 的 EGF 重复 10 都结合的抗原结合位点。

56. 如权利要求 1 ～ 55 中任一项所述的方法，其中，所述 NOTCH 抑制剂是人 NOTCH2 和 / 或人 NOTCH3 的拮抗剂。

57. 如权利要求 1 ～ 56 中任一项所述的方法，其中，所述 NOTCH 抑制剂抑制配体与人 NOTCH2 和 / 或人 NOTCH3 的结合。

58. 如权利要求 1 ～ 57 中任一项所述的方法，其中，所述 NOTCH 抑制剂抑制人 NOTCH2 和 / 或人 NOTCH3 的信号传导。

59. 如权利要求 52 所述的方法，其中，所述抗 NOTCH 抗体由以 PTA-9547 保藏在 ATCC 的多核苷酸所编码。

60. 如权利要求 49 或 50 所述的方法，其中，所述抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或人 NOTCH3，其中，所述抗体包含 ：

(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和包含 SIFYTT(SEQ ID NO:9) 的重链 CDR3 ；和

(b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。

61. 如权利要求 49 或 50 所述的方法，其中，所述抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或人 NOTCH3，其中，所述抗体包含 ：

(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和包含 GIFFAI(SEQ ID NO:5) 的重链 CDR3 ；和

(b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。

62. 如权利要求 49 或 50 所述的方法，抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或人 NOTCH3，其中，所述抗体包含 ：

(a) 与 SEQ ID NO:17、SEQ ID NO:18 或 SEQ ID NO:26 具有至少约 90％序列同一性的重链可变区 ;和

(b) 与 SEQ ID NO:29 或 SEQ ID NO:27 具有至少约 90％序列同一性的轻链可变区。

63. 如权利要求 49 或 50 所述的方法,其中,所述抗 NOTCH 抗体包含 :

(a) 与 SEQ ID NO:17 具有至少约 95％序列同一性的重链可变区 ;和

(b) 与 SEQ ID NO:29 具有至少约 95％序列同一性的轻链可变区。

64. 如权利要求 49 或 50 所述的方法,其中,所述抗 NOTCH 抗体包含 :

(a) 与 SEQ ID NO:18 具有至少约 95％序列同一性的重链可变区 ;和

(b) 与 SEQ ID NO:29 具有至少约 95％序列同一性的轻链可变区。

65. 如权利要求 49 或 50 所述的方法,其中,所述抗 NOTCH 抗体包含 :

(a) 包含 SEQ ID NO:18 的重链可变区 ;和

(b) 包含 SEQ ID NO:29 的轻链可变区。

66. 如权利要求 49 或 50 所述的方法,其中,所述抗 NOTCH 抗体包含 :

(a) 包含 SEQ ID NO:17 的重链可变区 ;和

(b) 包含 SEQ ID NO:29 的轻链可变区。

67. 如权利要求 49 或 50 所述的方法,其中,所述抗 NOTCH 抗体与选自由以下抗体组成的组的抗体竞争对人 NOTCH2 和 / 或人 NOTCH3 的特异性结合 :

(a) 包含含有 SEQ ID NO:17 或 SEQ ID NO:18 的重链可变区和含有 SEQ ID NO:29 的轻链可变区的抗体 ;

(b) 包含含有 SSSGMS(SEQ ID NO:3) 的重链 CDR1、含有 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2 和含有 SIFYTT(SEQ ID NO:9) 的重链 CDR3 以及含有 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1、含有 GASSRAT(SEQ ID NO:7) 的轻链 CDR2 和含有 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3 的抗体 ;和

(c) 由以 PTA-9547 保藏在 ATCC 的多核苷酸所编码的抗体。

68. 如权利要求 49 ～ 67 中任一项所述的方法,其中,所述抗 NOTCH 抗体是嵌合抗体、人源化抗体、人抗体或抗体片段。

69. 如权利要求 7、23 ～ 28 或 47 ～ 68 中任一项所述的方法,所述方法还包括施用第二治疗剂。

70. 如权利要求 69 所述的方法,其中,所述第二治疗剂是化疗剂。

71. 如权利要求 70 所述的方法,其中,所述第二治疗剂是核苷类似物或有丝分裂抑制剂。

72. 如权利要求 69 所述的方法,其中,所述第二治疗剂是吉西他滨、紫杉醇、白蛋白结合的紫杉醇或其组合。

73. 一种包含分离的多核苷酸的诊断用组合物,所述多核苷酸包含选自由 SEQ ID NO:35 ～ 43 组成的组的序列。

74. 如权利要求 73 所述的诊断用组合物,所述诊断用组合物包含 :

(a) 序列为 SEQ ID NO:35 的多核苷酸,序列为 SEQ ID NO:36 的多核苷酸,和序列为 SEQ ID NO:37 的多核苷酸 ;

(b) 序列为 SEQ ID NO:38 的多核苷酸,序列为 SEQ ID NO:39 的多核苷酸,和序列为 SEQ

ID NO:40 的多核苷酸 ;或

(c) 序列为SEQ ID NO:41 的多核苷酸,序列为SEQ ID NO:42 的多核苷酸,和序列为SEQ ID NO:43 的多核苷酸。

75. 一种检测样品中的 NOTCH3 mRNA 的方法,所述方法包括使所述样品接触包含选自由 SEQ ID NO:35 ～ 43 组成的组的序列的多核苷酸。

76. 如权利要求 75 所述的方法,所述方法包括使所述样品接触 :

(a) 序列为 SEQ ID NO:35 的正向引物,序列为 SEQ ID NO:36 的反向引物,和包含序列为 SEQ ID NO:37 的寡核苷酸的探针 ;

(b) 序列为 SEQ ID NO:38 的正向引物,序列为 SEQ ID NO:39 的反向引物,和包含序列为 SEQ ID NO:40 的寡核苷酸的探针 ;或

(c) 序列为 SEQ ID NO:41 的正向引物,序列为 SEQ ID NO:42 的反向引物,和包含序列为 SEQ ID NO:43 的寡核苷酸的探针。

77. 一种用于检测样品中的 NOTCH3 mRNA 的试剂盒,所述试剂盒包含 :包含选自由 SEQ ID NO:35 ～ 43 组成的组的序列的多核苷酸。

78. 如权利要求 77 所述的试剂盒,所述试剂盒包含 :

(a) 序列为SEQ ID NO:35 的多核苷酸,序列为SEQ ID NO:36 的多核苷酸,和序列为SEQ ID NO:37 的多核苷酸 ;

(b) 序列为SEQ ID NO:38 的多核苷酸,序列为SEQ ID NO:39 的多核苷酸,和序列为SEQ ID NO:40 的多核苷酸 ;或

(c) 序列为SEQ ID NO:41 的多核苷酸,序列为SEQ ID NO:42 的多核苷酸,和序列为SEQ ID NO:43 的多核苷酸。

79. 一种引物,其序列选自由 SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:39、SEQ ID NO:41 和 SEQ ID NO:42 组成的组。

80. 一种包含寡核苷酸的探针,所述寡核苷酸的序列选自由 SEQ ID NO:37、SEQ ID NO:40 和 SEQ ID NO:43 组成的组。

治疗胰腺癌的方法

[0001] 对相关申请的交叉引用

[0002] 本申请要求于2013年3月15日递交的美国临时申请第61/794,788号的优先权，将该临时申请通过援引完整地并入本文中。

技术领域

[0003] 本发明的领域总体涉及治疗胰腺癌的方法。在一个实施方式中，所述方法包括确定胰腺癌细胞中的NOTCH基因表达水平。在另一个实施方式中，所述方法还包括向有需要的受试对象施用治疗有效剂量的NOTCH拮抗剂。

背景技术

[0004] NOTCH信号传导途径是胚胎模式形成、胚后期组织维持和干细胞生物学的若干种关键调控因子之一。失调的NOTCH信号传导与多种人类癌症相关，在这些癌症中其能够改变肿瘤细胞的发育命运以将这些细胞保持在未分化的增殖状态(Brennan and Brown,2003,Breast Cancer Res.5:69)。因此，致癌作用可以通过篡夺控制正常发育和凭借干细胞群的组织修复的体内稳态机制而进行(Beachy等,2004,Nature 432:324)。

[0005] Notch受体是单次跨膜受体，在大胞外结构域中含有许多串联的表皮生长因子(EGF)样重复和三个富含半胱氨酸的Notch/LIN-12重复(Wharton等,1985,Cell 43:567；Kidd等,1986,Mol.Cell.Biol.6:3094;Artavanis等的综述,1999,Science 284:770)。已经鉴定出4种哺乳动物Notch蛋白(Notch1、Notch2、Notch3和Notch4)，这些受体中的突变总是导致发育异常和人类病变，所述发育异常和人类病变包括以下详细说明的几种癌(Gridley,1997,Mol.Cell Neurosci.9:103;Joutel&Tournier-Lasserve,1998,Semin.Cell Dev.Biol.9:619-25)。

[0006] 异常Notch信号传导涉及许多人类恶性肿瘤，例如，T细胞急性淋巴母细胞白血病、乳腺癌、宫颈癌、肾细胞癌、头颈鳞状上皮细胞癌。异常Notch信号传导还与胰腺癌的发展有关。参见例如Mazur等,Proc.Natl.Acad.Sci.U S A.107(30):13438-43(2010),Wang等,Cancer Res.69(6):2400-7(2009),Doucas等,J.Surg.Oncol.97(1):63-8(2008),Yao和Qian,Med.Oncol.27(3):1017-22(2010)；和Gungor等,Cancer Res.71(14):5009-19(2011)。

[0007] 胰腺癌是引起癌症死亡的第四大原因，存活期中位数为6个月，5年存活率不幸地为3％～5％，而且这一数字在过去25年一直保持相对不变(Iovanna等,Front.Oncol.2012;2:6)。即使对于被诊断为患有局部病的患者，五年存活率也仅有15％。胰腺癌的致死特性源于其快速扩散至淋巴系统和远端器官的倾向。在诊断时隐匿性转移或临床转移的存在和有效化疗的缺乏共同促成了胰腺癌患者的高死亡率。

[0008] 胰腺癌是固有抗药性最高的肿瘤之一，对化疗剂的抗性是胰腺癌治疗失败的主要原因。吉西他滨是晚期胰腺癌患者的标准化疗剂(Burris等,Eur.J.Cancer 1997,33:S18-22)。最近显示，与吉西他滨相比，联用5-FU、依立替康和奥沙利铂的多重

化疗方案(FOLFIRINOX)几乎使总体存活率翻倍,代价是可控但增加的毒性,这将其应用限制在了良好表现状态的患者。此外,总体存活时间小于12个月(Conroy等,N.Engl.J.Med.2011,364:1817-25)。因此,需要设计新的靶向治疗策略,其能够克服抗药性并改善被诊断为患有胰腺癌的患者的临床结果。

发明内容

[0009] 在一方面,本发明提供为了用NOTCH抑制剂进行治疗而选择胰腺癌患者的方法,所述方法包括:(a)确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,和(b)基于所述一种或多种生物标志物的表达水平选择患者。

[0010] 在另一方面,本发明提供确定被诊断为患有胰腺癌的患者是否可能对基于NOTCH抑制剂的疗法产生响应的方法,所述方法包括:确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,并且所述一种或多种生物标志物的表达水平表明患者可能对疗法产生响应。

[0011] 在另一方面,本发明提供确定是否应当向被诊断为患有胰腺癌的患者施用NOTCH抑制剂的方法,所述方法包括:确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,并且所述一种或多种生物标志物的表达水平预示所述患者对NOTCH抑制剂治疗具有有利的响应。

[0012] 在另一方面,本发明提供确定被诊断为患有胰腺癌的患者是否应当继续用NOTCH抑制剂进行治疗的方法,所述方法包括:确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,并且所述一种或多种生物标志物的表达水平表明患者可能对疗法产生响应。

[0013] 在另一方面,本发明提供确定被诊断为患有胰腺癌的患者是否应当继续用NOTCH抑制剂进行治疗的方法,所述方法包括:确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,并且所述一种或多种生物标志物的表达水平预示所述患者对所述NOTCH抑制剂治疗具有有利的响应。

[0014] 在另一方面,本发明提供确定NOTCH抑制剂在治疗患者的胰腺癌中的治疗功效的方法,所述方法包括:确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,并且所述一种或多种生物标志物的表达水平表明所述NOTCH抑制剂的治疗功效。

[0015] 在另一方面,本发明提供治疗患者的胰腺癌的方法,所述方法包括:(a)确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3;和(b)向所述患者施用治疗有效量的NOTCH抑制剂。

[0016] 在另一方面,本发明提供为了用NOTCH抑制剂进行治疗而将胰腺癌患者群体分阶(stratify)的方法,所述方法包括:(a)确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,和(b)基于所述肿瘤细胞中的所述一种或多种生物标志物的表达水平将所述患者群体分阶。

[0017] 在某些实施方式中,NOTCH3的表达水平经确定高于NOTCH3的参照表达水平。在某些实施方式中,每种生物标志物经确定都以高于该生物标志物的参照水平的水平表达。

[0018] 在某些实施方式中，一种或多种生物标志物的表达水平通过确定生物标志物 mRNA 或生物标志物蛋白的水平来确定。在某些实施方式中，NOTCH3 的表达水平通过确定肿瘤细胞中的 NOTCH3mRNA 水平来确定。在某些实施方式中，NOTCH3mRNA 水平通过定量聚合酶链式反应来确定。在某些实施方式中，NOTCH3mRNA 水平使用以下 (a)、(b) 和 / 或 (c) 来确定 ：(a) 核苷酸序列选自由 SEQ ID NO:35、SEQ ID NO:38 和 SEQ ID NO:41 组成的组的正向引物 ；(b) 核苷酸序列选自由 SEQ ID NO:36、SEQ ID NO:39 和 SEQ ID NO:42 组成的组的反向引物 ；和 / 或 (c) 包含寡核苷酸的探针，所述寡核苷酸的核苷酸序列选自由 SEQ ID NO:37、SEQ ID NO:40 和 SEQ ID NO:43 组成的组。在某些实施方式中，NOTCH3mRNA 水平使用以下 (a)、(b) 或 (c) 来确定 ：(a) 序列为 SEQ ID NO:35 的正向引物，序列为 SEQ ID NO:36 的反向引物，和包含序列为 SEQ ID NO:37 的寡核苷酸的探针 ；(b) 序列为 SEQ ID NO:38 的正向引物，序列为 SEQ ID NO:39 的反向引物，和包含序列为 SEQ ID NO:40 的寡核苷酸的探针 ；或 (c) 序列为 SEQ ID NO:41 的正向引物，序列为 SEQ ID NO:42 的反向引物，和包含序列为 SEQ ID NO:43 的寡核苷酸的探针。在某些实施方式中，NOTCH3mRNA 水平通过阵列杂交来确定。在某些实施方式中，NOTCH3 的表达水平通过确定肿瘤细胞所表达的 NOTCH3 蛋白水平来确定。

[0019] 在某些实施方式中，一种或多种生物标志物由 NOTCH3 构成。在某些实施方式中，一种或多种生物标志物还包括 MAML2，并且 MAML2 的表达水平经确定高于 MAML2 的参照表达水平。在某些实施方式中，一种或多种生物标志物由 NOTCH3 和 MAML2 构成。在某些实施方式中，MAML2 的表达水平通过确定肿瘤细胞中的 MAML2mRNA 水平来确定。在某些实施方式中，MAML2 的表达水平通过确定肿瘤细胞所表达的 MAML2 蛋白水平来确定。

[0020] 在另一方面，本发明提供治疗患者的胰腺癌的方法，所述方法包括 ：向所述患者施用治疗有效量的 NOTCH 抑制剂，其中，来自所述患者的至少一些胰腺肿瘤细胞 ：表达一种或多种生物标志物中的每一种且表达水平高于所述生物标志物的参照水平，且 / 或已被预先确定为表达一种或多种生物标志物中的每一个且表达水平高于所述生物标志物的参照水平 ；其中，所述一种或多种生物标志物包含 NOTCH3。在某些实施方式中，NOTCH3 的表达水平以 NOTCH3mRNA 水平来确定。在某些实施方式中，NOTCH3 的表达水平以 NOTCH3 蛋白水平来确定。在某些实施方式中，一种或多种生物标志物由 NOTCH3 构成。在某些实施方式中，一种或多种生物标志物还包括 MAML2，并且 MAML2 的表达水平高于 MAML2 的参照表达水平。在某些实施方式中，一种或多种生物标志物由 NOTCH3 和 MAML2 构成。

[0021] 在本文所述的方法的某些实施方式中，生物标志物的参照水平是预定值。在某些实施方式中，生物标志物的参照水平是该生物标志物在对照样品中的表达水平。在某些实施方式中，NOTCH3 的参照表达水平是胰腺癌或胰腺癌亚类中的 NOTCH3 表达的第 25、30、40、50、60、70、75 或 80 百分位数 (percentile)。在某些实施方式中，NOTCH3 的参照表达水平是胰腺癌中的 NOTCH3 表达的第 75 百分位数。在某些实施方式中，NOTCH3 的参照表达水平是胰腺癌中的 NOTCH3 表达的第 50 百分位数。在某些实施方式中，NOTCH3 的参照表达水平是胰腺癌中的 NOTCH3 表达的第 25 百分位数。在某些实施方式中，NOTCH3 的参照表达水平是胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤或抗化疗胰腺癌中的 NOTCH3 表达的第 75 百分位数。在某些实施方式中，NOTCH3 的参照表达水平是胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤或抗化疗胰腺癌中的 NOTCH3 表达的第 50 百分位数。

在某些实施方式中，NOTCH3 的参照表达水平是胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤或抗化疗胰腺癌中的 NOTCH3 表达的第 25 百分位数。

[0022] 在某些实施方式中，本文描述的方法还包括从所述患者获得身体样品。在某些实施方式中，NOTCH3 的表达水平是来自患者的身体样品中的水平。在某些实施方式中，样品是全血、血浆、血清或组织。在某些实施方式中，样品是胰腺肿瘤样品。在某些实施方式中，样品是来自已转移至肝脏的胰腺肿瘤的样品。在某些实施方式中，样品是福尔马林固定的石蜡包埋 (FFPE) 的组织。

[0023] 在本文所述的方法的某些实施方式中，患者是人，或所述患者群体是人群体。

[0024] 在本文所述的方法的某些实施方式中，胰腺癌是腺癌。在某些实施方式中，胰腺癌是抗化疗的。

[0025] 在某些实施方式中，本文描述的方法包括向所述患者施用 NOTCH 抑制剂。在某些实施方式中，NOTCH 抑制剂是 γ 分泌酶抑制剂。在某些实施方式中，NOTCH 抑制剂是抗 NOTCH 抗体。

[0026] 在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 或人 NOTCH3。在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 和 NOTCH3。在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 的 EGF 重复 10。在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH3 的 EGF 重复 9。在某些实施方式中，抗 NOTCH 抗体包含与人 NOTCH3 的 EGF 重复 9 和人 NOTCH2 的 EGF 重复 10 都结合的抗原结合位点。

[0027] 在某些实施方式中，NOTCH 抑制剂是人 NOTCH2 和 / 或 NOTCH3 的拮抗剂。在某些实施方式中，NOTCH 抑制剂抑制配体与人 NOTCH2 和 / 或 NOTCH3 的结合。在某些实施方式中，NOTCH 抑制剂抑制人 NOTCH2 和 / 或 NOTCH3 的信号传导。

[0028] 在某些实施方式中，抗 NOTCH 抗体由以 PTA-9547 保藏在 ATCC 的多核苷酸所编码。

[0029] 在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或 NOTCH3，其中，所述抗体包含 :(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和包含 SIFYTT(SEQ ID NO:9) 的重链 CDR3 ；和 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或 NOTCH3，其中，所述抗体包含 :(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和包含 GIFFAI(SEQ ID NO:5) 的重链 CDR3 ；和 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。

[0030] 在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或 NOTCH3，其中，所述抗体包含 :(a) 与 SEQ ID NO:17、SEQ ID NO:18 或 SEQ ID NO:26 具有至少约 90％序列同一性的重链可变区 ；和 (b) 与 SEQ ID NO:29 或 SEQ ID NO:27 具有至少约 90％序列同一性的轻链可变区。在某些实施方式中，抗 NOTCH 抗体包含 :(a) 与 SEQ ID NO:17 具有至少约 95％序列同一性的重链可变区 ；和 (b) 与 SEQ ID NO:29 具有至少约 95％序列同一性的轻链可变区。在某些实施方式中，抗 NOTCH 抗体包含 :(a) 与 SEQ ID NO:18 具有至少约 95％序列同一性的重链可变区 ；和 (b) 与 SEQ ID NO:29 具有至少约 95％序列同一性的轻链可变区。在某些实施方式中，抗 NOTCH 抗体包含 :(a) 包含 SEQ ID NO:18 的重链可变区 ；

和 (b) 包含 SEQ ID NO:29 的轻链可变区。在某些实施方式中，抗 NOTCH 抗体包含 :(a) 包含 SEQ ID NO:17 的重链可变区 ;和 (b) 包含 SEQ ID NO:29 的轻链可变区。

[0031] 在某些实施方式中，抗 NOTCH 抗体与选自由以下抗体组成的组的抗体竞争对人 NOTCH2 和 / 或 NOTCH3 的特异性结合 :(a) 包含含有 SEQ ID NO:17 或 SEQ ID NO:18 的重链可变区和含有 SEQ ID NO:29 的轻链可变区的抗体 ;(b) 包含含有 SSSGMS(SEQ ID NO:3) 的重链 CDR1、含有 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2 和含有 SIFYTT(SEQ ID NO:9) 的重链 CDR3 以及含有 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1、含有 GASSRAT(SEQ ID NO:7) 的轻链 CDR2 和含有 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3 的抗体 ;和 (c) 由以 PTA-9547 保藏在 ATCC 的多核苷酸编码的抗体。

[0032] 在某些实施方式中，抗 NOTCH 抗体是单克隆抗体。在某些实施方式中，抗 NOTCH 抗体是嵌合抗体、人源化抗体、人抗体或抗体片段。

[0033] 在某些实施方式中，本文描述的方法还包括施用第二治疗剂。在某些实施方式中，第二治疗剂是化疗剂。在某些实施方式中，第二治疗剂是核苷类似物或有丝分裂抑制剂。在某些实施方式中，第二治疗剂是吉西他滨、紫杉醇、白蛋白结合的紫杉醇或其组合。

[0034] 在另一方面，本发明提供包含分离的多核苷酸的诊断用组合物，所述多核苷酸包含选自由 SEQ ID NO:35 ～ 43 组成的组的序列。在某些实施方式中，所述诊断用组合物包含 :(a) 序列为 SEQ ID NO:35 的多核苷酸，序列为 SEQ ID NO:36 的多核苷酸，和序列为 SEQ ID NO:37 的多核苷酸 ;(b) 序列为 SEQ ID NO:38 的多核苷酸，序列为 SEQ ID NO:39 的多核苷酸，和序列为 SEQ ID NO:40 的多核苷酸 ;或 (c) 序列为 SEQ ID NO:41 的多核苷酸，序列为 SEQ ID NO:42 的多核苷酸，和序列为 SEQ ID NO:43 的多核苷酸。

[0035] 在另一方面，本发明提供检测样品中的 NOTCH3mRNA 的方法，所述方法包括使所述样品接触包含选自由 SEQ ID NO:35 ～ 43 组成的组的序列的多核苷酸。在某些实施方式中，所述方法包括使样品接触 :(a) 序列为 SEQ ID NO:35 的正向引物，序列为 SEQ ID NO:36 的反向引物，和包含序列为 SEQ ID NO:37 的寡核苷酸的探针 ;(b) 序列为 SEQ ID NO:38 的正向引物，序列为 SEQ ID NO:39 的反向引物，和包含序列为 SEQ ID NO:40 的寡核苷酸的探针 ;或 (c) 序列为 SEQ ID NO:41 的正向引物，序列为 SEQ ID NO:42 的反向引物，和包含序列为 SEQ ID NO:43 的寡核苷酸的探针。

[0036] 在另一方面，本发明提供用于检测样品中的 NOTCH3mRNA 的试剂盒，所述试剂盒包含 :包含选自由 SEQ ID NO:35 ～ 43 组成的组的序列的多核苷酸。在某些实施方式中，所述试剂盒包含 :(a) 序列为 SEQ ID NO:35 的多核苷酸，序列为 SEQ ID NO:36 的多核苷酸，和序列为 SEQ ID NO:37 的多核苷酸 ;(b) 序列为 SEQ ID NO:38 的多核苷酸，序列为 SEQ ID NO:39 的多核苷酸，和序列为 SEQ ID NO:40 的多核苷酸 ;或 (c) 序列为 SEQ ID NO:41 的多核苷酸，序列为 SEQ ID NO:42 的多核苷酸，和序列为 SEQ ID NO:43 的多核苷酸。

[0037] 在另一方面，本发明提供引物，其序列选自由 SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:39、SEQ ID NO:41 和 SEQ ID NO:42 组成的组。

[0038] 在另一方面，本发明提供包含寡核苷酸的探针，所述寡核苷酸的序列选自由 SEQ ID NO:37、SEQ ID NO:40 和 SEQ ID NO:43 组成的组。

附图说明

[0039] 图 1. 作为单一试剂或与化疗剂联用的 OMP-59R5 在 PN8 胰腺肿瘤细胞（图 1A)、PN17 胰腺肿瘤细胞（图 1B)、PN11 胰腺肿瘤细胞（图 1C)、UM-PE13 乳腺肿瘤细胞（图 1D)、UM-T1 乳腺肿瘤细胞（图 1E)、OMP-Lu40 肺肿瘤细胞（图 1F）和 OMP-Lu53 肺肿瘤细胞（图 1G）中的活性。

[0040] 图 2.NOTCH3 基因表达与 OMP-59R5 肿瘤抑制作用的相关性。（图 2A）与吉西他滨联用的 OMP-59R5 抗体对胰腺肿瘤的抑制程度与胰腺肿瘤细胞中的 NOTCH3 基因表达水平显著相关。（图 2B）对 OMP-59R5 抗体与吉西他滨的联合治疗有响应 (R) 和无响应 (NR) 的胰腺肿瘤中的 NOTCH3 基因表达分布。NOTCH3 基因表达分布以箱形图示出，其描绘了样品的最小值、下四分位数、中位数、上四分位数和样品最大值。

[0041] 图 3. 对 OMP-59R5 抗体与吉西他滨的联合治疗有响应和无响应的胰腺肿瘤中的 NOTCH3 基因表达，通过 RNAseq 确定。NOTCH3 基因表达以 RPKM（每 1 百万个匹配的读出序列中每 1 千碱基的转录本上的读出序列数）测量。

[0042] 图 4. 胰腺肿瘤中对 OMP-59R5 抗体与吉西他滨的联合治疗的预计响应概率，基于 NOTCH3 基因表达作为预测指标。

[0043] 图 5. 胰腺肿瘤中对 OMP-59R5 抗体与吉西他滨的联合治疗的预计响应概率，基于 NOTCH3 和 MAML2 基因表达作为预测指标。

[0044] 图 6. 胰腺肿瘤中的 NOTCH3 表达。（图 6A）胰腺肿瘤中的 NOTCH3 基因和蛋白表达。（图 6B）对 OMP-59R5 抗体与吉西他滨的联合治疗有响应 (R) 和无响应 (NR) 的胰腺肿瘤中的 NOTCH3 蛋白表达分布。NOTCH3 蛋白表达分布以箱形图示出，其描绘了样品的最小值、下四分位数、中位数、上四分位数和样品最大值。

[0045] 图 7. 胰腺癌转移组织中的 NOTCH3 基因表达。NOTCH3 基因表达通过 RT-PCR 来测量。NOTCH3 基因表达分布以箱形图示出，其描绘了样品的最小值、下四分位数、中位数、上四分位数和样品最大值，用特定肿瘤类型的样品进行观察。垂直的虚线表示在所有转移胰腺肿瘤样品上观察到的 NOTCH3 表达值的第 10、第 25、第 50、第 75 和第 90 百分位数。

[0046] 图 8. 肝和淋巴结胰腺癌转移组织以及异种移植肿瘤中的 NOTCH3 基因表达。NOTCH3 基因表达通过 RT-PCR 来测量。NOTCH3 基因表达分布以箱形图示出，其描绘了样品的最小值、下四分位数、中位数、上四分位数和样品最大值，用特定肿瘤类型的样品进行观察。垂直的虚线表示在淋巴结和肝转移胰腺肿瘤样品中观察到的 NOTCH3 表达值的第 10、第 25、第 50、第 75 和第 90 百分位数。

[0047] 图 9. 在胰腺肿瘤中，OMP-59R5 在与吉西他滨和 ABRAXANE™（结合蛋白的紫杉醇）联用时具有活性。

具体实施方式

[0048] 本发明总体涉及使用 NOTCH 抑制剂治疗胰腺癌的方法。本发明提供了为了用 NOTCH 抑制剂进行治疗而将胰腺癌患者群体分阶的方法，为了用 NOTCH 抑制剂进行治疗而选择胰腺癌患者的方法，确定被诊断为患有胰腺癌的患者是否可能对基于 NOTCH 抑制剂的疗法产生响应的方法，确定是否应当向被诊断为患有胰腺癌的患者施用 NOTCH 抑制剂的方法，确定被诊断为患有胰腺癌的患者是否应当继续用 NOTCH 抑制剂进行治疗的方法，和确定 NOTCH 抑制剂在治疗患者的胰腺癌中的治疗功效的方法。在一些实施方式中，所述方法

包括确定来自患者的肿瘤细胞中的NOTCH3基因表达水平。在一些实施方式中，本文提供的方法还包括确定来自患者的肿瘤细胞中的MAML2基因表达水平。在一些实施方式中，本文提供的方法包括施用NOTCH抑制剂。在一些实施方式中，NOTCH抑制剂是与一种或多种人NOTCH受体特异性结合的抗体。在一些实施方式中，抗体与化疗剂联合施用。在一些实施方式中，化疗剂是核苷类似物或有丝分裂抑制剂。

[0049] 1. 定义

[0050] 为了便于理解本发明，下文定义了多种术语和短语。

[0051] "NOTCH"是膜结合的转录因子，其调控许多细胞进程，尤其是在发育方面。在响应于配体结合时，其细胞内结构域(ICD)通过两种蛋白酶释放。所释放的细胞内结构域进入细胞核并与DNA结合的蛋白相互作用，从而激活转录。NOTCH及相关蛋的细胞外结构域白包含最多36个EGF样结构域，其后是三个notch(DSL)结构域。细胞内结构域(ICD)包含6个锚蛋白重复和羧基末端延伸(其包含PEST结构域)。NOTCH1和NOTCH2的ICD额外包含反式激活结构域(TAD)。"NOTCH"涵盖了NOTCH受体家族的所有成员。对NOTCH信号传导途径及受该途径影响的病况的描述可见于例如WO 98/20142和WO 00/36089。

[0052] 哺乳动物中的NOTCH家族有四个成员：NOTCH1(TAN1)、NOTCH2、NOTCH3和NOTCH4/Int-4。人NOTCH蛋白的示例性序列包括但不限于：由Genbank登录号NM_017617.3描述的mRNA序列编码的人NOTCH1，其氨基酸序列如Genbank登录号NP_060087所述；由Genbank登录号NM_024408描述的mRNA序列编码的人NOTCH2，其氨基酸序列如Genbank登录号NP_077719所述；由Genbank登录号NM_000435.2描述的mRNA序列编码的人NOTCH3，其氨基酸序列如Genbank登录号NP_000426所述；和由Genbank登录号NM_004557描述的mRNA序列编码的人NOTCH4，其氨基酸序列如Genbank登录号NP_004548所述。

[0053] 本文所用的"NOTCH抑制剂"、"NOTCH拮抗剂"、"抗NOTCH治疗剂"或"抗NOTCH剂"包括部分或全部阻断、抑制或中和Notch途径的生物活性的任何化合物。示例性的NOTCH抑制化合物包括但不限于：γ分泌酶抑制剂，例如III-31-C、N-[N-(3,5-二氟苯乙酰基)-L-丙氨酰基]S-苯基甘氨酸叔丁酯)(DAPT)、化合物E、D-螺旋肽294、异香豆素、BOC-Lys(Cbz)Ile-Leu-环氧化物和(Z-LL)2-酮(参见Kornilova等，J.Biol.Chem.2003,278:16479-16473)；和以下文献中所述的化合物：WO 01/90084、WO 02/30912、WO 01/70677、WO 03/013506、WO 02/36555、WO 03/093252、WO 03/093264、WO 03/093251、WO 03/093253、WO 2004/039800、WO 2004/039370、WO 2005/030731、WO 2005/014553、WO 2004/089911、WO 02/081435、WO 02/081433、WO 03/018543、WO 2004/031137、WO 2004/031139、WO 2004/031138、WO 2004/101538、WO 2004/101539和WO 02/47671以及美国专利临时申请第2003/0114496号。具体的γ分泌酶抑制剂化合物还在美国专利6,984,663和7,304,094中有所描述。具体的抗体NOTCH抑制剂在本文以及WO 2010/005566和WO 2010/005567(将其通过引用全部并入本文)中有所描述。NOTCH抑制剂还包括NOTCH配体拮抗剂。

[0054] "NOTCH抑制剂"、"NOTCH拮抗剂"、"抗NOTCH治疗剂"或"抗NOTCH剂"还涵盖与NOTCH受体结合的抗体。使用术语"抗体"指通过至少一个位于免疫球蛋白分子的可变区内的抗原识别位点来识别和特异性结合诸如蛋白、多肽、肽、碳水化合物、多核苷酸、脂质或上述的组合等靶标的免疫球蛋白分子。本文所用的术语"抗体"包括：完整的多克隆抗体、

完整的单克隆抗体、抗体片段（例如 Fab、Fab'、$F(ab')_2$和 Fv 片段）、单链 Fv(scFv) 突变形式、从至少两个完整抗体产生的诸如双特异性抗体等多特异性抗体、嵌合抗体、人源化抗体、人抗体、包含抗体的抗原决定部分的融合蛋白，和任何其它包含抗原识别位点的经修饰的免疫球蛋白分子，只要所述抗体显示出所需的生物活性即可。抗体可以是五个主要类别免疫球蛋白中的任一种 :IgA、IgD、IgE、IgG 和 IgM，或其亚类（同型）（例如，IgG1、IgG2、IgG3、IgG4、IgA1 和 IgA2），基于其重链恒定结构域的同一性，分别称为 α、δ、ε、γ 和 μ。不同类别的免疫球蛋白具有不同的众所周知的亚基结构和三维构象。抗体可以是裸抗体或与诸如毒素、放射性同位素等其它分子偶联。

[0055] 抗体的“可变区”是指抗体轻链的可变区和 / 或抗体重链的可变区。重链和轻链的可变区各自由通过三个也称为高变区的互补决定区 (CDR) 连接的四个框架区 (FR) 组成。每条链中的 CDR 通过 FR 彼此靠近地保持在一起，并且与来自另一条链的 CDR 一起促成了抗体的抗原结合位点的形成。存在至少两种确定 CDR 的技术 :(1) 基于跨物种序列变异性的方法（即，Kabat 等，Sequences of Proteins of Immunological Interest，（第 5 版，1991，National Institutes of Health，Bethesda Md.)) ;和 (2) 基于抗原 - 抗体复合物的晶体学研究的方法 (Al-lazikani 等，1997，J.Molec.Biol.273 :927-948)。另外，本领域有时使用这两种方法的组合来确定 CDR。

[0056] 术语“抗体片段”表示完整抗体的一部分，和表示完整抗体的抗原性决定可变区。抗体片段的实例包括但不限于 Fab、Fab'、$F(ab')_2$和 Fv 片段、线性抗体、单链抗体和由抗体片段形成的多特异性抗体。

[0057] “单克隆抗体”是指涉及高度特异性识别和结合单抗原决定簇（或表位）的同源性抗体群。这与通常包括针对不同抗原决定簇的不同抗体的多克隆抗体相反。术语“单克隆抗体”包括完整的全长单克隆抗体以及抗体片段（例如，Fab、Fab'、$F(ab')_2$、Fv)、单链 Fv(scFv) 突变形式、包含抗体部分的融合蛋白和任何其它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”是指以包括但不限于通过杂交瘤、噬菌体筛选、重组表达和转基因动物在内的多种方式制得的此类抗体。

[0058] 术语“人源化抗体”是指非人类（例如鼠类）抗体的形式，其为含有最小限度的非人类（例如鼠类）序列的特异性免疫球蛋白链、嵌合免疫球蛋白或其片段。通常而言，人源化抗体是其中来自互补决定区 (CDR) 的残基被来自非人类物种（例如小鼠、大鼠、兔、仓鼠）的具有所需特异性、亲和力和结合力的 CDR 的残基置换的人类免疫球蛋白 (Jones 等，1986，Nature，321:522-525 ;Riechmann 等，1988，Nature，332:323-327 ;Verhoeyen 等，1988，Science，239:1534-1536)。在某些情况下，人类免疫球蛋白的 Fv 框架区 (FR) 残基被来自非人类物种的抗体中的具有所需特异性、亲和力和结合力的相应残基置换。通过对 Fv 框架区中的和 / 或在已置换的非人残基中的其他残基进行替换，可以进一步修饰人源化抗体，从而精炼和优化抗体的特异性、亲和力和 / 或结合力。通常，人源化抗体基本上会包含至少一个且通常两个或三个可变区域中的全部，在该可变区中，CDR 区中的全部或基本上全部都对应于非人免疫球蛋白，而 FR 区中的全部或基本上全部都属于人免疫球蛋白共有序列。人源化抗体还可以包含免疫球蛋白恒定区或恒定区域 (Fc) 的至少一部分，通常为人免疫球蛋白的相应部分。用于产生人源化抗体的方法的实例描述于美国专利第 5,225,539 号。

[0059] 术语“人抗体”是指由人类产生的抗体或通过使用本领域已知的任何技术制备的具有与由人类产生的抗体相对应的氨基酸序列的抗体。人抗体的该定义包括完整或全长抗体、其片段、和/或包含至少一个人类重链和/或轻链多肽的抗体，例如，包含鼠类轻链和人类重链多肽的抗体。

[0060] 术语“嵌合抗体”是指其中免疫球蛋白分子的氨基酸序列源自两种以上物种的抗体。通常，轻链和重链的可变区都对应于源自一种哺乳动物物种（例如小鼠、大鼠、兔等）的具有所需特异性、亲和性和/或结合力的抗体可变区，而恒定区与源自另一物种（通常是人类）的抗体中的序列具有同源性，从而避免引发该物种中的免疫应答。

[0061] 术语“表位”或“抗原决定簇”在本文中可互换使用，指的是特定抗体所能够识别并特异性地结合的抗原的部分。当抗原是多肽时，表位可以由连续氨基酸构成，也可以由借助蛋白的三级结构折叠而并置的不连续氨基酸构成。由连续氨基酸构成的表位通常在蛋白变性时得以保留，然而通过三级结构折叠形成的表位通常在蛋白变性时丢失。表位通常包含独特空间构象中的至少 3 个且更常见为至少 5 个或 8 ～ 10 个氨基酸。

[0062] 多肽或其他试剂（例如抗体或可溶性受体）与蛋白“特异性地结合”的意思是，与替代性物质（包括不相关蛋白）相比，该多肽或其他试剂与该蛋白的反应或联结更频繁、更快速、持续时间更长、亲和力更高或具有上述效果的某种组合。在某些实施方式中，“特异性地结合”意思是，例如，试剂（例如抗体或可溶性受体）与蛋白结合的 K_D 为约 0.1mM 以下，但更常见为小于约 1μM。在某些实施方式中，“特异性地结合”是指，试剂（例如抗体或可溶性受体）与蛋白结合的 K_D 有些时候为至少约 0.1μM 或更小，至少约 0.01μM 或更小，另外一些时候为至少约 1nM 或更小。由于不同物种中同源蛋白之间的序列同一性，特异性结合可以包括在超过一个物种中识别特定蛋白（例如 Notch 受体）的试剂（例如抗体或可溶性受体）。类似地，由于其序列的特定区中的不同旁系同源物（例如不同的人 Notch 蛋白）之间的同源性，特异性结合可以包括识别超过一种旁系同源物（例如多于一种人 NOTCH 蛋白）的试剂（例如抗体或可溶性受体）。应该理解，在特定实施方式中，与第一靶标特异性结合的试剂（例如抗体或可溶性受体）可以与第二靶标特异性结合或不特异性结合。这样，“特异性结合”并非必须（但可以包括）排他性结合，即，与单一靶标结合。因此，在某些实施方式中，试剂（例如抗体或可溶性受体）可以与超过一个靶标（例如，多种不同的人 NOTCH 蛋白，例如 NOTCH1、NOTCH2、NOTCH3 和/或 NOTCH4）特异性结合。在某些实施方式中，抗体上的相同抗原结合位点可以结合抗体的多种靶标。例如，在某些情况下，抗体可以包含两个相同的抗原结合位点，其中每一个都特异性结合两种以上人类卷曲受体（例如，人 NOTCH1、NOTCH2、NOTCH3 和/或 NOTCH4）。在某些替代性实施方式中，抗体可以是双特异性的，并且包含至少两种具有不同的特异性的抗原结合位点。借助于非限制性实例，双特异性抗体可以包含一个识别位于一种 NOTCH 受体（例如人 NOTCH2）上的表位的抗原结合位点，和还包含识别位于第二 NOTCH 受体（例如人 NOTCH3）上的不同表位的第二抗原结合位点。通常而言，但不是必然地，“结合”是指“特异性结合”。

[0063] 术语“癌症”或“癌的”指代或描述哺乳动物的生理病况，其中的细胞群的特征为失调的细胞生长。术语“癌症”应理解为涵盖 NOTCH 依赖性癌症。癌症的实例包括但不限于癌、淋巴瘤、母细胞瘤、肉瘤和白血病。

[0064] “肿瘤”和“赘生物”是指任何由过度的细胞生长或增殖导致的组织团块，其可以是

良性的（非癌的）或恶性的（癌的），包括癌前病变。

[0065] 本文所用的“转移”是指癌从起始位置扩散或转移到身体的其它区域的过程，并且在新位置发展出类似的癌病变。“转移”或“转移性”细胞是失去与相邻细胞的粘合接触并且经由血液或淋巴液从疾病的原发位置迁移从而入侵邻近的身体结构的细胞。

[0066] 术语“癌干细胞”、“肿瘤干细胞”或“实体瘤干细胞”在本文中可互换使用，并且指来自具有以下性质的实体瘤的一群细胞：(1) 具有广泛的增殖能力；2) 能够进行不对称细胞分裂从而产生一种以上的增殖或发育潜能减少的分化子代；和 (3) 能够进行对称分裂以用于自我更新或自我维持。当连续移植到免疫受损的小鼠时，与不能形成肿瘤的大多数肿瘤细胞相比，“癌干细胞”、“肿瘤干细胞”或“实体瘤干细胞”的这些性质使这些癌干细胞具有形成可触知的肿瘤的能力。癌干细胞以无序方式进行自我更新而不是分化，从而形成具有异常细胞类型的肿瘤，当发生突变时所述异常细胞类型可以随时间而改变。

[0067] 术语“癌细胞”、“肿瘤细胞”和语法等效概念是指源自肿瘤或癌前病变的全部细胞群体，包括构成肿瘤细胞群体块的非致瘤性细胞和致瘤性干细胞（癌干细胞）。当仅指缺乏更新和分化能力的那些肿瘤细胞时，本文所用的术语“肿瘤细胞”将用术语“非致瘤性”来修饰，以使其与癌干细胞区分开。

[0068] 术语“致瘤性”是指实体瘤干细胞的功能特征，包括自我更新（产生额外的致瘤性癌干细胞）的性质和增殖以产生所有其它肿瘤细胞（产生分化的、并因此是非致瘤性的肿瘤细胞）的性质，这允许实体瘤干细胞形成肿瘤。与非致瘤性肿瘤细胞（其在连续移植后不能形成肿瘤）相比，这些自我更新并增殖产生所有其他肿瘤细胞的性质使癌干细胞在连续移植到免疫受损的小鼠中后能够形成可触知的肿瘤。已观察到，在从实体瘤获得肿瘤细胞后首次移植到免疫受损小鼠中时非致瘤性肿瘤细胞可以形成肿瘤，但这些非致瘤性肿瘤细胞在连续移植后并不产生肿瘤。

[0069] 术语“受试者”是指任何动物（例如，哺乳动物），包括但不限于人、非人灵长类、啮齿类等，其将是特定治疗的接受者。通常，述及人受试者时，术语“受试者”和“患者”在本文中可以互换使用。在本文中用于获得定量或定性数据的“正常”受试对象或来自“正常”受试对象的样品是已被或将被医师评估为不具有胰腺癌的受试对象。

[0070] “对照样品”是指来自对照细胞的单独样品。对照细胞可以是无疾病的，或可以是胰腺癌细胞。对照细胞可以来自同一受试对象或另一受试对象。对照细胞可以来自同一组织或另一组织。对照细胞可以来自永生化的细胞系。

[0071] 本文使用术语“预后”来指对癌症可导致的死亡或进展的可能性的预测，包括肿瘤疾病（例如胰腺癌）的复发、转移性扩展和药物抗性。本文所用的术语“预测”是指作出受试对象的后果具有显著提高或下降的可能性（有利的预后或不利的预后）的决定。其还可以包括 NOTCH 抑制剂可以是治疗有效的或未发现其具有治疗性的可能性。该术语还用于指以下情况的可能性：患者对药物或药物组产生有利或不利的响应以及这些响应的程度；或患者在手术除去原发性肿瘤和 / 或化学治疗后会存活一定时间且癌症不会复发。本发明的预测性方法可在临床上用于通过为任何具体患者选择最恰当的治疗模式而作出治疗决定。因此，在预测患者是否可能有利地响应于基于 NOTCH 的治疗方案（例如抗 NOTCH 抗体治疗、使用给定药物或药物组合（例如 γ 分泌酶抑制剂或其他 NOTCH 抑制剂）的化学治疗）时，或在预测用 NOTCH 抑制剂进行治疗方案后和 / 或终止化学治疗或其他治疗模式后患者是否

可能长期存活时，本发明的预测性方法是有价值的工具。

[0072] 术语“治疗有效量”是指试剂（例如抗体、可溶性受体、多肽、多核苷酸、有机小分子或其他药剂）的对“治疗”受试对象或哺乳动物的疾病或病症有效的量。对于癌症的情况，治疗有效量的试剂可以：减少癌细胞数量，减小肿瘤尺寸，抑制或终止癌细胞向周边器官的浸润（包括例如癌扩散至软组织和骨中），抑制或终止肿瘤转移，抑制或终止肿瘤生长，在一定程度上减轻一种或多种癌症相关症状、降低发病率和死亡率，提高生活质量，降低肿瘤的致瘤性、成瘤频率或成瘤能力，减少癌干细胞在肿瘤中的数量或比例，使致瘤性细胞分化为非致瘤性细胞，或这些效果的组合。只要试剂会阻止生长和/或杀死已存在的癌细胞，就可以称之为抑制细胞生长的和/或细胞毒性的。

[0073] 本文所用的术语“抑制肿瘤生长”是指能够抑制肿瘤细胞生长的任何机理。在某些实施方式中，通过使肿瘤细胞的增殖变慢来抑制肿瘤细胞生长。在某些实施方式中，通过使肿瘤细胞的增殖停止来抑制肿瘤细胞生长。在某些实施方式中，通过杀死肿瘤细胞来抑制肿瘤细胞生长。在某些实施方式中，通过诱导肿瘤细胞凋亡来抑制肿瘤细胞生长。在某些实施方式中，通过诱导肿瘤细胞分化来抑制肿瘤细胞生长。在某些实施方式中，通过剥夺肿瘤细胞的营养来抑制肿瘤细胞生长。在某些实施方式中，通过防止肿瘤细胞迁移来抑制肿瘤细胞生长。在某些实施方式中，通过防止肿瘤细胞侵袭来抑制肿瘤细胞生长。

[0074] 本文所用的术语“分阶(stratifying)”是指根据特定的疾病状态或状况的特征将受试对象分成不同的类别或阶层。例如，对患有胰腺癌的受试对象群体进行分阶包括基于肿瘤细胞中的NOTCH3基因表达水平和/或基于疾病的严重性（例如恶化前、恶化、转移等）来划分受试对象。

[0075] 术语“治疗”或“处理”或“要治疗”或“缓解”或“要缓解”是指1)治愈、减慢、减轻诊断的病理性病况或疾病的症状和/或使诊断的病理性病况或疾病的进展停止的治疗措施；和2)防止和/或减缓靶向的病理性病况或疾病的发展的预防性或防备性措施。因此，需要治疗的受试对象包括已经患有疾病的受试对象；有倾向患疾病的受试对象和要预防疾病的受试对象。在某些实施方式中，如果患者显示出以下情况的一种或多种，则根据本发明的方法成功地“治疗”了受试对象：癌细胞数量减少或完全不存在；肿瘤尺寸减少；癌细胞向外围器官的浸润（包括例如癌扩散到软组织和骨）受到抑制或不存在；肿瘤转移受到抑制或不存在；肿瘤生长受到抑制或不存在；一种或多种与特定癌相关的症状减轻；发病率和致死率降低；生活质量改善；肿瘤的致瘤性、成瘤频率或成瘤能力降低；癌干细胞在肿瘤中的数量或比例减少；致瘤性细胞分化为非致瘤性状态；或这些效果的组合。

[0076] 术语“多肽”、“肽”和“蛋白”在本文中可互换使用，指任何长度的氨基酸聚合物。该聚合物可以是直链的或支化的，其可以包含经修饰的氨基酸，并且可以被非氨基酸插入。该术语还涵盖已天然修饰或人工修饰的氨基酸聚合物；例如，二硫键形成、糖基化、脂化、酰化、磷酸化或任何其他操纵或修饰，例如与标记组分偶联。该定义还包括例如包含一个或多个氨基酸类似物（包括例如非天然氨基酸等）以及本领域中已知的其他修饰的多肽。应理解的是，由于本发明的多肽基于抗体，因此在某些实施方式中，所述多肽可以作为单链或相互联结的链存在。

[0077] 本文所用的术语“活检”或“活检组织”是指从受试对象取出的组织或流体的样品，用于确定所述样品是否包含癌组织。在一些实施方式中，获取活检组织或流体是因为受试

对象疑似患有癌症。随后检查该活检组织或流体以确定癌症是否存在。

[0078] 除非上下文另有明确规定，本文和权利要求中所用的单数形式“一个”、“一种”和“所述”包含复数形式。

[0079] 应当理解的是，每当在本文用措辞“包含”来描述实施方式时，还提供以“由……组成”和/或“基本上由……组成”描述的其它类似实施方式。

[0080] 本文在短语中使用的术语“和/或”（例如“A和/或B”）意在包括：A和B二者；A或B；A；和B。类似地，在短语中使用的术语“和/或”（例如“A、B和/或C”）意在涵盖以下实施方式中的每一种：A、B和C；A、B或C；A或C；A或B；B或C；A和C；A和B；B和C；A（单独）；B（单独）；和C（单独）。

[0081] 2.NOTCH3评估方法

[0082] 如下文详细示出的，人胰腺肿瘤对抗NOTCH2/3抗体OMP-59R5的敏感性与增加的NOTCH3表达显著相关。令人惊奇的是，尽管NOTCH3mRNA和蛋白表达均与人胰腺肿瘤中的OMP-59R5敏感性相关，但是，相比于NOTCH3蛋白表达与治疗敏感性之间的相关性，NOTCH3mRNA表达与治疗敏感性之间的相关性增加。这些数据与来自人乳腺肿瘤和结肠肿瘤的表达数据惊人地相反，后者显示，在NOTCH2或NOTCH3表达与肿瘤对OMP-59R5治疗的敏感性之间没有显著相关性。类似地，在人胰腺肿瘤中，未观察到OMP-59R5敏感性与NOTCH2表达之间存在相关性。

[0083] 通过选择其肿瘤细胞以升高或增加的NOTCH3表达、NOTCH3过表达或预定水平以上的NOTCH3表达为特征的胰腺癌患者进行OMP-59R5疗法，可以利用增加或升高的NOTCH3表达（例如NOTCH3过表达）与胰腺癌中对OMP-59R5治疗的敏感性（治疗功效）之间的关联来改进治疗胰腺癌的方法。在一些情况下，术语“升高的NOTCH3表达”、“增加的NOTCH3表达”和“NOTCH3过表达”在本文中可以互换使用。还可以通过以下方法来提高治疗功效：不选择其肿瘤细胞以正常或减少的NOTCH3表达或低于预定水平的NOTCH3表达为特征的胰腺癌患者进行OMP-59R5疗法。在某些实施方式中，预定的NOTCH3表达水平可以是对照样品（例如对照细胞）中的表达水平。在某些实施方式中，预定的NOTCH3表达水平可以是胰腺癌中的NOTCH3的中位数表达水平，或者是胰腺癌中的NOTCH3表达水平的第95、90、80、75、70、50、40、30、25或10百分位数。

[0084] 在某些实施方式中，在患者的胰腺肿瘤中，至少一些肿瘤细胞表现出升高的NOTCH3表达水平。在一个实施方式中，升高的NOTCH3表达水平是等于或高于胰腺癌中的NOTCH3中位数表达水平的水平。在另一实施方式中，升高的NOTCH3表达水平是等于或高于胰腺癌中的NOTCH3基因表达水平的第95、90、80、75、70、50、40、30、25或10百分位数的水平。在某些实施方式中，胰腺癌中的NOTCH3中位数表达水平是胰腺腺癌、转移胰腺癌、肝脏和/或淋巴结转移胰腺癌、抗化疗胰腺癌或晚期、难治性或复发性胰腺癌中的NOTCH3中位数表达水平。在某些实施方式中，胰腺癌中的NOTCH3表达水平的第95、90、80、75、70、50、40、30、25或10百分位数是胰腺腺癌、转移胰腺癌、肝脏和/或淋巴结转移胰腺癌、抗化疗胰腺癌或晚期、难治性或复发性胰腺癌中的NOTCH3表达水平的第95、90、80、75、70、50、40、30、25或10百分位数。

[0085] 在某些实施方式中，升高的NOTCH3表达水平是等于或高于预定标准水平或参照水平或对照水平的水平。在一些情况下，术语“预定标准”、“参照水平”和“对照水平”在本文

中可以互换使用。在一个实施方式中，预定标准表示在对照样品（例如，包含胰腺细胞的样品，且该样品不包含胰腺肿瘤或胰腺癌细胞）中测得的 NOTCH3 表达水平。在另一实施方式中，预定标准表示在包含胰腺肿瘤细胞（例如，腺癌、转移性肿瘤细胞和肝脏和 / 或淋巴结转移肿瘤细胞）的样品中测得的 NOTCH3 表达水平。在又一实施方式中，预定标准表示在包含胰腺肿瘤细胞的样品中测得的 NOTCH3 表达水平，其中，所述胰腺肿瘤细胞对 NOTCH 抑制剂（例如 OMP-59R5）治疗无响应。在又一实施方式中，预定标准表示在包含胰腺肿瘤细胞的样品中测得的 NOTCH3 表达水平，其中，所述胰腺肿瘤细胞对 NOTCH 抑制剂（例如 OMP-59R5）治疗有响应。在另一实施方式中，预定标准是分离的细胞系中的 NOTCH3 表达水平。所述细胞系可源自胰腺癌样品。所述细胞系还可以经重组操作而表达 NOTCH3。在某些实施方式中，NOTCH3 表达的预定标准或参照水平是胰腺癌中（例如，胰腺腺癌、转移胰腺肿瘤、肝脏和 / 或淋巴结转移胰腺肿瘤、抗化疗胰腺癌或晚期、难治性或复发性胰腺癌中）的 NOTCH3 基因表达水平的第 95、90、80、75、70、50、40、30、25 或 10 百分位数。

[0086] 在某些实施方式中，当患者的至少一些胰腺肿瘤细胞以升高的水平表达 NOTCH3 时，选择该患者以进行 NOTCH 抑制剂（例如 OMP-59R5）治疗或对该患者进行 NOTCH 抑制剂（例如 OMP-59R5）治疗。在某些实施方式中，患者的至少一些胰腺肿瘤细胞以等于或高于参照水平的水平表达 NOTCH3。在某些实施方式中，患者的至少一些胰腺肿瘤细胞以等于或高于胰腺癌中的 NOTCH3 中位数表达水平的水平表达 NOTCH3。在某些实施方式中，患者的至少一些胰腺肿瘤细胞以等于或高于胰腺癌中的 NOTCH3 基因表达的第 95、90、80、75、70、50、40、30、25 或 10 百分位数的水平表达 NOTCH3。在某些实施方式中，患者的至少一些胰腺肿瘤细胞以等于或高于胰腺癌中的 NOTCH3 基因表达的第 25 百分位数的水平表达 NOTCH3。在某些实施方式中，患者的至少一些胰腺肿瘤细胞还以等于或高于参照水平或等于或高于胰腺癌中的 MAML2 中位数表达水平的水平表达 MAML2。在一个实施方式中，选择患者以进行 OMP-59R5 治疗或对患者进行 OMP-59R5 治疗。在另一实施方式中，选择患者以进行抗体治疗或对患者进行抗体治疗，所述抗体包含 OMP-59R5 的 6 个 CDR 和 / 或可变区。

[0087] 在某些实施方式中，当患者的至少一些胰腺肿瘤细胞所包含的 NOTCH3mRNA 水平等于或高于 (1) 参照水平、(2) 胰腺癌中的 NOTCH3mRNA 中位数水平、和 / 或 (3) 胰腺癌中的 NOTCH3mRNA 水平的第 95、90、80、75、70、50、40、30、25 或 10 百分位数时，选择该患者以进行 NOTCH 抑制剂（例如 OMP-59R5）治疗或对该患者进行 NOTCH 抑制剂（例如 OMP-59R5）治疗。在特定实施方式中，患者的至少一些胰腺肿瘤细胞包含等于或高于胰腺癌中（例如，肝和 / 或淋巴结转移胰腺癌中）的 NOTCH3mRNA 水平的第 25 百分位数的 NOTCH3mRNA 水平。在某些实施方式中，患者的至少一些胰腺肿瘤细胞还包含等于或高于参照水平或等于或高于胰腺癌中的 MAML2mRNA 中位数水平的 MAML2mRNA。在一个实施方式中，选择患者以进行 OMP-59R5 治疗或对患者进行 OMP-59R5 治疗。在另一实施方式中，选择患者以进行抗体治疗或对患者进行抗体治疗，所述抗体包含 OMP-59R5 的 6 个 CDR 和 / 或可变区。

[0088] 在某些实施方式中，当患者的至少一些胰腺肿瘤细胞所包含的 NOTCH3 蛋白水平等于或高于 (1) 参照水平、(2) 胰腺癌中的 NOTCH3 蛋白中位数水平、和 / 或 (3) 胰腺癌中的 NOTCH3 蛋白水平的第 95、90、80、75、70、50、40、30、25 或 10 百分位数时，选择该患者以进行 NOTCH 抑制剂（例如 OMP-59R5）治疗或对该患者进行 NOTCH 抑制剂（例如 OMP-59R5）治疗。在特定实施方式中，患者的至少一些胰腺肿瘤细胞包含等于或高于胰腺癌中（例如，肝

脏和/或淋巴结转移胰腺癌中）的NOTCH3蛋白水平的第25百分位数的NOTCH3蛋白水平。在某些实施方式中，患者的至少一些胰腺肿瘤细胞还包含等于或高于参照水平或等于或高于胰腺癌中的MAML2蛋白中位数水平的MAML2蛋白。在一个实施方式中，选择患者以进行OMP-59R5治疗或对患者进行OMP-59R5治疗。在另一实施方式中，选择患者以进行抗体治疗或对患者进行抗体治疗，所述抗体包含OMP-59R5的6个CDR和/或可变区。

[0089] 检测NOTCH3水平或其他所关注的基因/基因产物（例如MAML2）的表达水平的方法包括能够在核酸或蛋白水平上确定NOTCH3表达水平的任何方法。此类方法在本领域是熟知的，并且包括但不限于蛋白质印迹、酶联免疫吸附测定(ELISA)、免疫沉淀、免疫荧光、流式细胞术、免疫组化(IHC)、核酸杂交技术、核酸逆转录方法、核酸扩增方法（例如PCR或qRT-PCR）、RNA酶保护、微阵列、基因表达系列分析(SAGE)、高通量质谱(MS)、全转录组鸟枪法测序(WTSS)、大规模平行签名测序(MPSS)、原位杂交和RNA印迹。

[0090] 胰腺癌中的NOTCH3的中位数或百分位数表达水平可以在任何时候相对于测量患者的胰腺肿瘤细胞中的NOTCH3表达来确定。在某些实施方式中，NOTCH3的多个表达水平同时测量。在另一实施方式中，胰腺癌中的NOTCH3的中位数或百分位数表达水平在测量患者的样品中的NOTCH3表达水平之前确定。

[0091] 在一个实施方式中，在身体样品中测量NOTCH3表达。此处所用的短语“身体样品”是指可以检出其中的NOTCH3表达水平的任何样品，包括细胞、组织或体液。此类身体样品的实例包括但不限于血液、淋巴液、尿、妇科液(gynecological fluid)、活检、羊水和涂片。身体样品可以通过多种技术从患者获得。收集各种身体样品的方法是本领域公知的。在某些实施方式中，身体样品是胰腺肿瘤样品。在某些实施方式中，身体样品可以是固定的样品，例如，福尔马林固定的石蜡包埋(FFPE)样品，或冷冻样品。

[0092] 在特定实施方式中，NOTCH3的表达水平在mRNA水平上检测。确定mRNA表达的各种方法包括但不限于定量实时PCR(qRT-PCR)、微阵列分析、基因表达系列分析(SAGE)等。在某些实施方式中，胰腺肿瘤细胞中的mRNA水平使用定量实时PCR(qRT-PCR)或微阵列分析来确定。许多表达检测方法使用分离的RNA。可以使用任何不针对mRNA的分离进行选择的RNA分离技术来从身体样品中纯化RNA(参见例如Ausubel编，1999,Current Protocols in Molecular Biology(John Wiley&Sons,New York))。另外，可以使用本领域技术人员公知的技术容易地处理大量组织样品，例如Chomczynski的一步RNA分离法（美国专利第4,843,155号）。

[0093] 术语“探针”是指能够选择性地与特别指定的靶标生物分子（例如，NOTCH3的核苷酸转录本）结合的任何分子。探针可由本领域技术人员使用已知的技术合成，或源自合适的生物学制品。可以将探针特别设计成带有可检测标记。可用作探针的分子的实例包括但不限于RNA、DNA、蛋白质（包括肽）、抗体和有机分子。

[0094] 来自胰腺肿瘤细胞的NOTCH3mRNA可以在杂交或扩增测定中检测，所述测定包括但不限于mRNA测序法、DNA或RNA印迹分析、聚合酶链式反应分析和探针阵列。用于检测mRNA水平的一个方法包括使分离的mRNA接触能够与被检测基因所编码的mRNA杂交的核酸分子（探针）。所述核酸探针可以为例如全长cDNA或其一部分，例如长度为至少7、15、30、50、100、250或500个核苷酸的寡核苷酸，并且足以在严格条件下与编码NOTCH3的mRNA或基因组DNA特异性地杂交。mRNA与探针的杂交表明所关注的基因正在表达。

[0095] 在一个实施方式中，将 mRNA 固定在固相表面上并与探针接触，例如，通过使分离的 mRNA 在琼脂糖凝胶上电泳，并将该 mRNA 从凝胶转移到例如硝酸纤维素等膜上。在替代性实施方式中，将探针固定在固相表面上并使 mRNA 与探针接触，例如与 Affymetrix 基因芯片阵列 (Santa Clara, Calif.) 中的探针接触。可以容易地对已知的 mRNA 检测法进行修改以用于检测胰腺肿瘤细胞中的 NOTCH3mRNA。

[0096] 用于检测样品中的 NOTCH3mRNA 水平的替代性方法包括核酸扩增过程，例如通过 RT-PCR(Mullis 于 1987 年在美国专利第 4,683,202 号中提出的实验实施方式)、连接酶链式反应 (Barany, 1991, Proc. Natl. Acad. Sci. USA, 88:189193)、自维持序列复制 (Guatelli, 1990, Proc. Natl. Acad. Sci. USA, 87:18741878)、转录扩增系统 (Kwoh, 1989, Proc. Natl. Acad. Sci. USA, 86:11731177)、Q-β 复制酶 (Lizardi, 1988, Bio/Technology, 6:1197)、滚动环复制 (Lizardi, 美国专利 5,854,033) 或任何其他核酸扩增方法来进行的扩增过程；然后使用本领域技术人员公知的技术检测所扩增的分子。如果核酸分子以非常低的量存在，则这些检测方案对于检测这些核酸分子而言特别有用。在本发明的特定方面，通过定量发荧光 RT-PCR(即 TaqMan® System) 来评估 NOTCH3mRNA 水平。此类方法通常使用寡核苷酸引物对，该引物对位于 NOTCH3 基因内的内含子两侧。设计对已知序列具有特异性的寡核苷酸引物的方法是本领域已知的。

[0097] 在一个实施方式中，本发明提供适合于用定量 RT-PCR 确定样品中的 NOTCH3mRNA 水平的引物组。在一个实施方式中，引物组包含三种分离的多核苷酸，包括序列 SEQ ID NO:35、36 和 37。在一个实施方式中，引物组包含三种分离的多核苷酸，包括序列 SEQ ID NO:38、39 和 40。在一个实施方式中，引物组包含三种分离的多核苷酸，包括序列 SEQ ID NO:41、42 和 43。在另一方面，本发明提供检测样品中的 NOTCH3mRNA 的存在方法，所述方法包括使所述样品接触包含 SEQ ID NO:35 ～ 43 中的序列的至少一种分离的多核苷酸。本文提供的引物组可用于按照标准 qRT-PCR 程序对样品中的 NOTCH3mRNA 水平进行定量。

[0098] 在本发明的一个实施方式中，使用微阵列来确定生物样品中的 NOTCH3mRNA 水平。由于其可再现性，微阵列特别适合于该目的。DNA 微阵列提供一种同时测量大量基因或针对所关注的分子的不同部位的大量寡核苷酸探针的表达水平的方法。每个阵列都由连接至固相载体的呈可再现模式的捕获探针组成。使经标记的 RNA 或 DNA 在阵列上与互补探针杂交，然后通过例如激光扫描来检测。确定阵列上各探针的杂交强度，并将其转化成代表相对基因表达水平的定量值。参见美国专利第 6,040,138、5,800,992 和 6,020,135、6,033,860 以及 6,344,316，通过援引将它们并入本文。高密度寡核苷酸阵列对于确定样品中的大量 RNA 的基因表达谱而言特别有用。

[0099] 使用机械合成方法合成这些阵列的技术描述于例如美国专利第 5,384,261 号中，通过援引将其全文并入本文中。虽然优选平面阵列表面，但可以在几乎任何形状的表面或甚至多重表面上制造阵列。阵列可以是位于珠、凝胶、聚合物表面、纤维 (例如光纤)、玻璃或任何其他合适的基底上的肽或核酸，参见美国专利第 5,770,358、5,789,162、5,708,153、6,040,193 和 5,800,992 号，在此通过援引将其每一篇都全部并入本文。可以以考虑诊断学或全包式 (all-inclusive) 装置的其他操作的方式来组装阵列。参见例如美国专利第 5,856,174 和 5,922,591 号，通过援引将其并入本文。

[0100] 检测肿瘤细胞中 NOTCH3 蛋白水平的方法可以包括检测生物样品中 NOTCH3 蛋白

的存在的任何方法。此类方法是本领域公知的，并且包括但不限于蛋白质印迹、狭缝印迹、ELISA、免疫沉淀、免疫荧光、流式细胞术、免疫细胞化学、免疫组化（IHC）和质谱。此类免疫测定方法可以手动进行或以自动化方式进行。结合 NOTCH3 的任何区域的抗体可用于本文所述的检测方法中。在一个实施方式中，使用 IHC 确定肿瘤样品中的 NOTCH3 蛋白水平。

[0101] 用于检测抗体结合的技术是本领域公知的。与 NOTCH3 蛋白结合的抗体可以使用化学试剂来检测，所述化学试剂产生可检测信号，该信号对应于抗体结合水平，并因而对应于 NOTCH3 蛋白的水平。在一个实施方式中，使用与带标记的聚合物偶联的二抗来检测抗体结合。带标记的聚合物的实例包括但不限于聚合物 - 酶偶联物。这些复合物中的酶通常用于催化抗原 - 抗体结合部位处的色素原沉积，由此产生与所关注的突变的表达水平对应的细胞染色。特别关注的酶包括辣根过氧化物酶（HRP）和碱性磷酸酶（AP）。可以使用市售的抗体检测系统来实施本发明的方法，例如 Dako Envision+ 系统（Dako North America,Inc.,Carpinteria,Calif.）和 Mach 3 系统（Biocare Medical,Walnut Creek,Calif.）。

[0102] 抗体结合检测可以通过将抗体与可检测物质偶联来促进。可检测物质的实例包括各种酶、辅基、荧光材料、发光材料、生物发光材料和放射性材料。合适的酶的实例包括辣根过氧化物酶、碱性磷酸酶、β - 半乳糖苷酶或乙酰胆碱酯酶；合适的辅基复合物的实例包括抗生蛋白链菌素 / 生物素以及抗生物素蛋白 / 生物素；合适的荧光材料的实例包括伞形酮、荧光素、异硫氰酸荧光素、罗丹明、二氯三嗪基胺荧光素、丹酰氯或藻红蛋白；发光材料的实例包括鲁米诺；生物发光材料的实例包括萤光素酶、萤光素和水母发光蛋白；合适的放射性材料的实例包括 ^{125}I、^{131}I、^{35}S 或 ^{3}H。

[0103] 在一个实施方式中，NOTCH3 蛋白的水平使用特异性结合 NOTCH3 的试剂来确定。任何展示出对 NOTCH3 的特异性结合的分子实体都可以用来确定样品中的 NOTCH3 蛋白的水平。特异性结合剂包括但不限于抗体、抗体模拟物和多核苷酸（例如适体）。本领域技术人员理解，通过用于检测 NOTCH3 蛋白的特定测定来确定所需的特异性程度。例如，在涉及基于多肽尺寸来分离多肽的方法（例如蛋白质印迹）中，可以使用既与全长 NOTCH3 又与 NOTCH3ICD 特异性结合的试剂。

[0104] 在一个实施方式中，NOTCH3 蛋白的水平使用对 NOTCH3 有特异性的抗体来确定。在另一实施方式中，该抗体是单克隆抗体。NOTCH3 特异性抗体可以根据本领域技术人员已知的任何方法产生。参见例如 Tagami 等，2008Mol.Cell.Biol.28(1):165-176。NOTCH3 特异性抗体还可以获自商购来源。参见例如 R&D Systems, 抗人 NOTCH3 多克隆抗体，目录号 BAF1559。抗 NOTCH3 抗体可以是单克隆抗体、多克隆抗体、人源化抗体、人抗体、嵌合抗体或其抗原结合片段。在又一实施方式中，该抗体与固定且包埋的组织样品中的 NOTCH3 特异性结合。该组织样品可以是福尔马林固定的组织样品。该组织样品可以是石蜡包埋的组织样品。

[0105] 3.NOTCH 抑制剂

[0106] 本发明的方法的另一方面是 NOTCH 抑制剂（例如抗 NOTCH 抗体）在治疗 NOTCH3 表达水平已得到确定的胰腺癌患者中的应用。在某些实施方式中，NOTCH 抑制剂是抗 NOTCH 抗体。在某些实施方式中，抗 NOTCH 抗体特异性地结合一种或多种人 NOTCH 受体的 EGF10 结构域（或 EGF10 结构域的等效物）。在某些实施方式中，抗 NOTCH 抗体特异性地结合人

NOTCH2 的 EGF10 和 / 或人 NOTCH3 的 EGF9。EGF9 是人 NOTCH3 内的 EGF，其等同于其他人 NOTCH 受体 NOTCH1、NOTCH2 和 NOTCH4 中的 EGF10。在一些实施方式中，抗 NOTCH 抗体特异性地结合 NOTCH2 的 EGF10。在一些实施方式中，抗 NOTCH 抗体特异性地结合 NOTCH2 的 EGF10 和 NOTCH3 的 EGF9。在一些实施方式中，抗 NOTCH 抗体特异性地结合 NOTCH3 的 EGF9。在其他实施方式中，抗 NOTCH 抗体结合 NOTCH2EGF10 内的序列 HKGAL(SEQ ID NO:1) 的至少一部分。在一些实施方式中，抗 NOTCH 抗体结合 NOTCH3EGF9 内的序列 HEDAI(SEQ ID NO:2) 的至少一部分。结合 NOTCH2 和 NOTCH3 的示例性抗体描述于美国专利 8,226,943 中，通过援引将其整体并入本文中。

[0107] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体抑制配体与人 NOTCH2 和 / 或 NOTCH3 的结合。在一些实施方式中，抗 NOTCH 抗体抑制配体与人 NOTCH2 的结合。在一些实施方式中，抗 NOTCH 抗体抑制配体与 NOTCH2 和 NOTCH3 的结合。在其他实施方式中，抗 NOTCH 抗体抑制配体与 NOTCH3 的结合。在某些实施方式中，配体是 DLL4、JAG1 或 JAG2。在其他实施方式中，抗 NOTCH 抗体抑制人 NOTCH2 和 / 或 NOTCH3 的信号传导。在一些实施方式中，抗 NOTCH 抗体抑制人 NOTCH2 的信号传导。在一些实施方式中，抗 NOTCH 抗体抑制 NOTCH2 和 NOTCH3 的信号传导。在其他实施方式中，抗 NOTCH 抗体抑制 NOTCH3 的信号传导。在一些实施方式中，NOTCH2 和 / 或 NOTCH3 的信号传导由 DLL4、JAG1 或 JAG2 诱导。

[0108] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或 NOTCH3，其中，所述抗体包含 :(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和 / 或包含 SIFYTT(SEQ ID NO:9) 的重链 CDR3 ;和 / 或 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和 / 或包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。在一些实施方式中，抗体包含 :(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，和 / 或包含 SIFYTT(SEQ ID NO:9) 的重链 CDR3 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体 ;和 / 或 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，和 / 或包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体。

[0109] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体特异性地结合人 NOTCH2 和 / 或 NOTCH3，其中，所述抗体包含 :(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和 / 或包含 GIFFAI(SEQ ID NO:5) 的重链 CDR3 ;和 / 或 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和 / 或包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。在某些实施方式中，抗体特异性地结合 NOTCH2。在一些实施方式中，抗体包含 :(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，和 / 或包含 GIFFAI(SEQ ID NO:5) 的重链 CDR3 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体 ;和 / 或 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2 或其包含 1、2、3 或 4 个保守性氨

基酸替换的变体，和/或包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3 或其包含 1、2、3 或 4 个保守性氨基酸替换的变体。

[0110] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体特异性地结合人 NOTCH2 和/或 NOTCH3，其中，所述抗体包含：(a) 包含 SSSGMS(SEQ ID NO:3) 的重链 CDR1，包含 VIASSGSNTYYADSVKG(SEQ ID NO:4) 的重链 CDR2，和/或包含 (G/S)(I/S)F(F/Y)(A/P)(I/T/S/N)(SEQ ID NO:10) 的重链 CDR3；和/或 (b) 包含 RASQSVRSNYLA(SEQ ID NO:6) 的轻链 CDR1，包含 GASSRAT(SEQ ID NO:7) 的轻链 CDR2，和/或包含 QQYSNFPI(SEQ ID NO:8) 的轻链 CDR3。在一些实施方式中，抗体包含含有 SIFYPT(SEQ ID NO:11) 的重链 CDR3。在一些实施方式中，抗体包含含有 SSSFFAS(SEQ ID NO:12) 的重链 CDR3。在其他实施方式中，抗体包含含有 SSFYAS(SEQ ID NO:13) 的重链 CDR3。在某些实施方式中，抗体包含含有 SSFFAT(SEQ ID NO:14) 的重链 CDR3。在一些实施方式中，抗体包含含有 SIFYPS(SEQ ID NO:15) 的重链 CDR3。在其他实施方式中，抗体包含含有 SSFFAN(SEQ ID NO:16) 的重链 CDR3。

[0111] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体包含：(a) 与 SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25 或 SEQ ID NO:26 具有至少约 80％序列同一性的重链可变区（具有或不具有信号序列）；和/或 (b) 与 SEQ ID NO:29、SEQ ID NO:27 或 SEQ ID NO:28 具有至少约 80％序列同一性的轻链可变区（具有或不具有信号序列）。在某些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2 和/或 NOTCH3。在一些实施方式中，抗 NOTCH 抗体特异性地结合人 NOTCH2。在一些实施方式中，抗 NOTCH 抗体结合 NOTCH2 和 NOTCH3。在其他实施方式中，抗 NOTCH 抗体结合 NOTCH3。在某些实施方式中，抗 NOTCH 抗体包含与 SEQ ID NO:18 或 SEQ ID NO:17 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的重链可变区。在某些实施方式中，抗 NOTCH 抗体包含与 SEQ ID NO:29 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的轻链可变区。

[0112] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体包含：(a) 与 SEQ ID NO:30、SEQ ID NO:31 或 SEQ ID NO:32 具有至少约 80％序列同一性的重链（具有或不具有信号序列）；和/或 (b) 与 SEQ ID NO:33 或 SEQ ID NO:34 具有至少约 80％序列同一性的轻链（具有或不具有信号序列）。在某些实施方式中，抗 NOTCH 抗体包含：与 SEQ ID NO:19 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的重链，和与 SEQ ID NO:28 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的轻链。在某些实施方式中，抗 NOTCH 抗体包含：与 SEQ ID NO:30 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的重链，和与 SEQ ID NO:28 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的轻链。

[0113] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体包含：(a) 与 SEQ ID NO:17 具有至少约 80％序列同一性的重链可变区；和 (b) 与 SEQ ID NO:29 具有至少约 80％序列同一性的轻链可变区。在某些实施方式中，抗 NOTCH 抗体包含：与 SEQ ID NO:17 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的重链可变区，和与 SEQ ID NO:29 具有至少约 85％、至少约 90％、至少约 95％、至少约 98％或约 100％序列同一性的轻链可变区。

[0114] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体包含59R1 IgG2抗体、由59R1 IgG2抗体构成或基本由59R1 IgG2抗体构成，所述59R1 IgG2抗体包含分别由SEQ ID NO:31和33表示的重链和轻链（具有或不具有信号序列），或者由依照布达佩斯条约的条款于2008年10月15日保藏在美国典型培养物保藏中心(ATCC)（美国弗吉尼亚州马纳萨斯市大学路10801）并被指定保藏号PTA-9547的DNA编码。

[0115] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体包含59R5IgG2抗体、由59R5IgG2抗体构成或基本由59R5IgG2抗体构成，所述59R5IgG2抗体包含分别由SEQ ID NO:30和33表示的重链和轻链（具有或不具有信号序列），或者由于2009年7月6日保藏在ATCC并被指定保藏号PTA-10170的DNA编码。在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体包含59R5IgG2抗体的重链和轻链（具有或不具有信号序列）。在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体是59R5IgG2抗体。59R5IgG2抗体在本文中还称为OMP-59R5。关于OMP-59R5抗体的其他信息可见于例如美国专利8,226,943中，通过援引将其整体并入本文中。在美国专利8,226,943中，OMP-59R5抗体通常称为“59R5”或“59R5IgG2抗体”。

[0116] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体与包含含有SEQ ID NO:18的重链可变区和含有SEQ ID NO:29的轻链可变区的抗体竞争与人NOTCH2和/或NOTCH3的特异性结合。在某些实施方式中，抗体与59R1 IgG2抗体在特异性结合中竞争，所述59R1 IgG2抗体包含分别由SEQ ID NO:31和33表示的重链和轻链（具有或不具有信号序列），或者由于2008年10月15日保藏在ATCC并被指定保藏号PTA-9547的DNA编码。在一些实施方式中，抗体竞争与人NOTCH2的结合。在一些实施方式中，抗体竞争与人NOTCH2和NOTCH3的结合。在其他实施方式中，抗体竞争与人NOTCH3的结合。

[0117] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体与包含含有SEQ ID NO:17的重链可变区和含有SEQ ID NO:29的轻链可变区的抗体竞争与人NOTCH2和/或NOTCH3的特异性结合。在一些实施方式中，抗体与59R5IgG2抗体在特异性结合中竞争，所述59R5IgG2抗体包含分别由SEQ ID NO:30和33表示的重链和轻链，或者由于2009年7月6日保藏在ATCC并被指定保藏号PTA-10170的DNA编码。在一些实施方式中，抗体竞争与人NOTCH2的结合。在一些实施方式中，抗体竞争与人NOTCH2和NOTCH3的结合。在其他实施方式中，抗体竞争与人NOTCH3的结合。

[0118] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体是IgG1抗体或IgG2抗体。在某些实施方式中，抗体是单克隆抗体。在某些实施方式中，抗体是人抗体或人源化抗体。在某些实施方式中，抗体是抗体片段。

[0119] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体所结合的表位与59R1或59R5抗体的表位相同或有重叠。

[0120] 可用于本发明的方法中的抗NOTCH抗体的其他实例在美国专利8,226,943中公开，通过援引将其整体并入本文中。

[0121] 在某些实施方式中，可用于本发明的方法中的抗NOTCH抗体是特异性地识别人NOTCH受体的双特异性抗体。双特异性抗体是能够特异性地识别并结合至少两种不同的表位的抗体。在一个实施方式中，双特异性抗NOTCH抗体特异性地识别同一人NOTCH受体内的不同表位。在另一个实施方式中，双特异性抗NOTCH抗体特异性地识别人NOTCH受体内

的不同表位或不同的人 NOTCH 受体上的不同表位。

[0122] 或者，在某些替代性实施方式中，可用于本发明的方法中的抗 NOTCH 抗体不是双特异性抗体。

[0123] 在某些实施方式中，可用于本发明的方法中的抗 NOTCH 抗体是单特异性抗体。例如，在某些实施方式中，抗体所包含的一个或多个抗原结合位点结合或能够结合相同的一种或多种人 NOTCH 受体。在某些实施方式中，单特异性抗 NOTCH 抗体的抗原结合位点结合或能够结合一种、两种、三种或四种人 NOTCH 受体。

[0124] 本发明的方法的另一方面是 NOTCH 抑制剂（例如抗 NOTCH 抗体）在治疗胰腺癌中的应用。在某些实施方式中，NOTCH 抑制剂是 γ 分泌酶的抑制剂。由于 γ- 分泌酶抑制剂也能够防止 NOTCH 受体激活，因此已测试了数种形式的 γ- 分泌酶抑制剂的抗肿瘤效应。首先，最初的 γ- 分泌酶抑制剂 IL-X(cbz-IL-CHO) 在 Ras 转化的成纤维细胞中显示出具有 NOTCH1 依赖性抗成瘤活性。据报道，在来自小鼠的黑素瘤和卡波西肉瘤的细胞系和 / 或异种移植物中，三肽 γ- 分泌酶抑制剂 (z-Leu-leu-Nle-CHO) 抑制肿瘤生长 (Curry CL 等，Oncogene 24:6333-44(2005))。用二肽 γ- 分泌酶抑制剂 N-[N-(3,5- 二氟苯乙酰基)-L- 丙氨酰基]S- 苯基甘氨酸叔丁基酯 (DAPT) 进行的治疗也在 T-ALL 动物模型中导致髓母细胞瘤生长的显著减少并诱导 G0-G1 细胞周期阻滞和凋亡 (O'Neil J. 等，Blood 107:781-5(2006))。另一种 γ- 分泌酶抑制剂二苯并氮䓬已经显示出在 Apc-/-(min) 小鼠的肠道腺瘤中抑制上皮细胞增殖并诱导杯形细胞分化 (van Es JH, 等，Nature 435:959-63(2005))。近来，由三肽 γ- 分泌酶抑制剂或 NOTCH3 特异性小干扰 RNA 造成的 NOTCH3 功能性失活在过表达 NOTCH3 的肿瘤细胞系中导致细胞增殖受抑制并诱导凋亡，但在具有最小量的 NOTCH3 表达的细胞系中却非如此 (Park JT 等，Cancer Res.,66:6312-8(2006))。此外，针对复发性或难治性 T-ALL 患者和晚期乳腺癌的 NOTCH 抑制剂 MK0752(由 Merck,Whitehouse Station,NJ 开发) 的 I 期临床试验已经启动。

[0125] 4. 治疗方法

[0126] 如上文所述，NOTCH 抑制剂 (例如 OMP-59R5) 可用于治疗其肿瘤细胞已确定具有增加的 NOTCH3 表达 (例如 NOTCH3mRNA 表达) 水平的患者的胰腺癌，所述增加的水平为例如：等于或高于胰腺癌中的 NOTCH3 的中位数表达水平的水平，等于或高于胰腺癌中的 NOTCH3 表达的第 95、90、80、75、70、50、40、30、25 或 10 百分位数的水平，或等于或高于对照样品的 NOTCH3 表达水平的水平。在某些实施方式中，还已确定肿瘤细胞具有增加的 MAML2 表达 (例如，MAML2mRNA 表达) 水平，例如，等于或高于胰腺癌中的 MAML2 的中位数表达水平的水平，或等于或高于对照样品的 MAML2 表达水平的水平。在某些实施方式中，NOTCH 抑制剂 (例如 OMP-59R5) 可用于抑制肿瘤生长、诱导分化和 / 或减小肿瘤体积。此外，本发明提供一种降低受试对象中的胰腺肿瘤的致瘤性的方法，所述方法包括向已确定其肿瘤细胞表达本文所述的增加水平的 NOTCH3 的患者施用治疗有效量的 NOTCH 抑制剂 (例如 OMP-59R5)。在某些实施方式中，肿瘤包含癌干细胞。在某些实施方式中，通过施用 NOTCH 抑制剂 (例如 OMP-59R5) 来降低癌干细胞在肿瘤中的比例。

[0127] 在一个实施方式中，NOTCH 抑制剂 (例如 OMP-59R5) 可用于治疗胰腺癌，且该胰腺癌的肿瘤细胞的特征在于其 NOTCH3 表达水平等于或高于对照样品或细胞中的 NOTCH3 表达水平。在一个实施方式中，NOTCH 抑制剂 (例如 OMP-59R5) 可用于治疗胰腺癌，且该胰腺癌

的肿瘤细胞的特征在于其NOTCH3基因表达水平等于或高于胰腺癌的NOTCH3中位数表达水平。在某些实施方式中，所治疗的胰腺癌的肿瘤细胞的特征在于其NOTCH3表达水平等于或高于胰腺癌中的NOTCH3表达的第95、90、80、75、70、50、40、30、25或10百分位数。在某些实施方式中，胰腺癌中的NOTCH3中位数表达水平是胰腺腺癌、转移胰腺癌、或者肝脏和/或淋巴结转移胰腺癌中的NOTCH3中位数表达水平。在某些实施方式中，胰腺癌中的NOTCH3表达的第95、90、80、75、70、50、40、30、25或10百分位数是胰腺腺癌、转移胰腺癌、或者肝脏和/或淋巴结转移胰腺癌中的NOTCH3表达的第95、90、80、75、70、50、40、30、25或10百分位数。在某些实施方式中，NOTCH3表达水平使用qRT-PCR来确定。在某些实施方式中，NOTCH3表达水平使用本文所述的探针来确定，例如，包含选自由SEQ ID NO:35～43组成的组的核苷酸序列的多核苷酸。

[0128] 在一个实施方式中，NOTCH抑制剂（例如OMP-59R5）可用于治疗胰腺癌，且该胰腺癌的至少一些肿瘤细胞表现出等于或高于对照细胞中的MAML2表达水平的MAML2表达水平。在一个实施方式中，NOTCH抑制剂（例如OMP-59R5）可用于治疗胰腺癌，且该胰腺癌的至少一些肿瘤细胞表现出等于或高于胰腺癌中的MAML2中位数表达水平的MAML2表达水平。在某些实施方式中，所治疗的胰腺癌的至少一些肿瘤细胞表现出等于或高于胰腺癌中的MAML2表达的第95、90、80、75、70、50、40、30、25或10百分位数的MAML2表达水平。在某些实施方式中，胰腺癌中的MAML2中位数表达水平是胰腺腺癌、转移胰腺癌、或者肝脏和/或淋巴结转移胰腺癌中的MAML2中位数表达水平。在某些实施方式中，胰腺癌中的MAML2表达的第95、90、80、75、70、50、40、30、25或10百分位数是胰腺腺癌、转移胰腺癌、或者肝脏和/或淋巴结转移胰腺癌中的MAML2表达的第95、90、80、75、70、50、40、30、25或10百分位数。在某些实施方式中，MAML2表达水平使用qRT-PCR来确定。

[0129] 在某些实施方式中，用NOTCH抑制剂（例如OMP-59R5）治疗的胰腺癌是外分泌胰腺肿瘤。在某些实施方式中，所治疗的胰腺癌是腺泡细胞癌、腺癌、腺鳞状癌、巨细胞瘤、导管内乳头粘液腺瘤(IPMN)、粘液囊腺癌、胰腺母细胞瘤、浆液囊腺癌或实体假乳头瘤。在某些实施方式中，所治疗的胰腺癌是腺癌。在某些实施方式中，所治疗的胰腺癌是神经内分泌肿瘤。在某些实施方式中，胰腺神经内分泌肿瘤是胃泌素瘤、胰高血糖素瘤、胰岛素瘤、无功能胰岛细胞瘤、舒血管肠肽瘤(VIPoma)或生长抑素瘤。在某些实施方式中，所治疗的胰腺癌不是神经内分泌肿瘤。

[0130] 在某些实施方式中，用NOTCH抑制剂（例如OMP-59R5）治疗的胰腺癌是可切除的肿瘤、局部晚期的癌或转移性胰腺癌。在某些实施方式中，根据AJCC TNM系统所确定，胰腺癌为第1、2、3或4级癌。

[0131] 在一个实施方式中，NOTCH抑制剂（例如OMP-59R5）特别可用于治疗已经经历一些形式的治疗的胰腺癌患者。在另一实施方式中，NOTCH抑制剂（例如OMP-59R5）用于治疗之前用癌症疗法治疗失败的胰腺癌患者。失败的癌症疗法可包括但不限于化疗、辅助疗法、新辅助疗法和它们的组合。在一个实施方式中，NOTCH抑制剂（例如OMP-59R5）用于治疗具有化疗抗性的肿瘤。在另一实施方式中，NOTCH抑制剂（例如OMP-59R5）用于治疗具有化疗抗性的胰腺癌。

[0132] 在一个实施方式中，治疗方法包括首先测试含有来自患者的胰腺癌细胞的生物样品，从而确定这些细胞是否表达等于或高于预定标准（例如等于或高于胰腺癌中NOTCH3的

中位数表达水平）的NOTCH3基因。然后，对于其样品中展现出升高的NOTCH3表达水平的患者，使用干扰NOTCH受体活性的NOTCH抑制剂（例如OMP-59R5）来进行治疗。施用剂量将取决于所治疗的具体病况、施用途径和本领域公知的临床考量因素。剂量可以逐渐增加直到检测到有益效果，例如肿瘤生长减缓。NOTCH抑制剂（例如OMP-59R5）然后可以以单剂量方案或多剂量方案提供，并且可以单独给药，或与其他治疗剂联合给药。

[0133] 对具有增加的NOTCH3表达的胰腺癌的治疗与任何施用途径和剂型都相容。根据所治疗的具体病况，某些剂型倾向于比其他剂型更方便或更有效。例如，NOTCH抑制剂可以胃肠外、局部、口服、经口、内部、鼻内、直肠、阴道、舌部和透皮施用。具体的剂型包括片剂、丸剂、胶囊、粉末、气溶胶、栓剂、皮肤贴片、胃肠外和口服液体（包括悬浮液、溶液和乳液）。也可以使用持释剂型。所有剂型都可以使用本领域的标准方法来制备（参见例如Remington's Pharmaceutical Sciences, 第16版，Easton,Pa.(1980)）。

[0134] 在某些实施方式中，NOTCH抑制剂（例如OMP-59R5）的施用可以是通过静脉内注射来施用或静脉内施用。在一些实施方式中，施用为静脉内输注。在某些实施方式中，NOTCH抑制剂（例如OMP-59R5）的施用可以通过非静脉内途径。

[0135] NOTCH抑制剂（例如OMP-59R5）治疗剂的合适剂量取决于疾病的严重性和进程、疾病的响应性、施用抗体或NOTCH抑制剂是为了治疗目的还是预防目的、之前的治疗、患者临床史等等，所有这些都由治疗医师来裁度。抗体或其他NOTCH抑制剂的施用可以是一次，或者可以在一系列治疗上进行，所述一系列治疗持续数天至数月，或直至实现治愈或疾病状态的减少（例如，肿瘤尺寸的减小）为止。从对药物在患者体内的药物积累的测量中可以计算出最佳定量给药计划，并且该计划会根据单个抗体或其他NOTCH抑制剂的相对效力而有所不同。施用医师能够容易地确定最佳剂量、定量给药方法和重复率。通常，抗NOTCH抗体（例如OMP-59R5）剂量为0.01μg～100mg/千克体重，并且可每天、每周、每月或每年给药一次或多次。治疗医师能够根据抗体或试剂在体液或组织中的测得的停留时间和浓度来估算定量给药的重复率。

[0136] 如本领域技术人员已知的，所用的剂量将视所要实现的临床目标而变化。在一些实施方式中，抗NOTCH抗体（例如OMP-59R5）的每剂量为约0.25mg/kg～约15mg/kg。在一些实施方式中，每个剂量为约0.25mg/kg、0.5mg/kg、1mg/kg、2mg/kg、3mg/kg、4mg/kg、5mg/kg、6mg/kg、7mg/kg、8mg/kg、9mg/kg、10mg/kg、11mg/kg、12mg/kg、13mg/kg、14mg/kg、15mg/kg、16mg/kg、17mg/kg、18mg/kg、19mg/kg或20mg/kg。在某些实施方式中，每个剂量为约0.5mg/kg。在某些实施方式中，每个剂量为约1mg/kg。在某些实施方式中，每个剂量为约2.5mg/kg。在某些实施方式中，每个剂量为约5mg/kg。在某些实施方式中，每个剂量为约7.5mg/kg。在某些实施方式中，每个剂量为约10mg/kg。在某些实施方式中，每个剂量为约12.5mg/kg。在某些实施方式中，每个剂量为约15mg/kg。

[0137] 在某些实施方式中，使用间歇式定量给药方案向患者施用本文描述的方法中所用的NOTCH抑制剂（例如OMP-59R5），该方案在某些情况下可以减少与施用所述NOTCH抑制剂（例如OMP-59R5）相关的副作用和/或毒性。本文所用的“间歇式定量给药”是指使用超过每周1次的定量给药间隔的定量给药方案，例如每2周定量给药一次、每3周1次、每4周1次等等。在一些实施方式中，治疗人患者的胰腺癌的方法包括按照间歇式定量给药方案向患者施用有效剂量的NOTCH抑制剂（例如OMP-59R5）。在一些实施方式中，治疗人患

者的胰腺癌的方法包括按照间歇式定量给药方案向患者施用有效剂量的NOTCH抑制剂(例如OMP-59R5),并且增加所述NOTCH抑制剂(例如OMP-59R5)的治疗指数。在一些实施方式中,间歇式定量给药方案包括向患者施用起始剂量的NOTCH抑制剂(例如OMP-59R5),并且约每2周1次施用后续剂量的NOTCH抑制剂(例如OMP-59R5)。在一些实施方式中,间歇式定量给药方案包括向患者施用起始剂量的NOTCH抑制剂(例如OMP-59R5),并且约每3周1次施用后续剂量的NOTCH抑制剂(例如OMP-59R5)。在一些实施方式中,间歇式定量给药方案包括向患者施用起始剂量的NOTCH抑制剂(例如OMP-59R5),并且约每4周1次施用后续剂量的NOTCH抑制剂(例如OMP-59R5)。

[0138] 在一些替代性实施方式中,用于所述方法中的抗NOTCH抗体是OMP-59R5,或包含OMP-59R5的6个CDR和/或可变区的抗体,并且约每2～3周向受试对象静脉内施用约2.5mg/kg～约7.5mg/kg(例如约2.5mg/kg、约5mg/kg或约7.5mg/kg)的剂量的所述抗体。

[0139] 在某些实施方式中,除了施用NOTCH抑制剂(例如OMP-59R5)之外,所述方法或治疗还包括施用至少一种额外的治疗剂或疗法。额外的治疗剂或疗法可以在施用抗NOTCH治疗剂之前、同时和/或之后施用。在一些实施方式中,至少一种额外的治疗剂或疗法包括1种、2种、3种或更多种额外的治疗剂或疗法。

[0140] 使用至少两种治疗剂的联合疗法常常使用通过不同的作用机制起作用的试剂,但这不是必须的。施用具有不同作用机制的试剂的联合疗法可以产生加成或协同效应。联合疗法可以允许使用与单一疗法相比更低剂量的每种试剂,由此减少毒副作用。联合疗法可以降低发展出抗性癌细胞的可能性。

[0141] 应该意识到的是,NOTCH抑制剂(例如OMP-59R5)与额外的治疗剂或疗法的组合可以以任意顺序施用或同时施用。在一些实施方式中,将向之前已经历第二治疗剂或疗法治疗的患者施用NOTCH抑制剂(例如OMP-59R5)。在某些实施方式中,NOTCH抑制剂(例如OMP-59R5)和第二治疗剂或疗法会基本上同时或同步施用。例如,可以向正在经历第二治疗剂(例如化疗)治疗进程的受试对象施用NOTCH抑制剂(例如OMP-59R5)。在某些实施方式中,在用第二治疗剂治疗1年内施用NOTCH抑制剂(例如OMP-59R5)。在某些替代实施方式中,在用第二治疗剂进行任何治疗10、8、6、4或2个月内施用NOTCH抑制剂(例如OMP-59R5)。在某些其他实施方式中,在用第二治疗剂进行任何治疗4周、3周、2周或1周内施用NOTCH抑制剂(例如OMP-59R5)。在一些实施方式中,在用第二治疗剂进行任何治疗5天、4天、3天、2天或1天内施用NOTCH抑制剂(例如OMP-59R5)。还应该意识到的是,两种(或更多种)试剂或治疗可以在大约数小时或数分钟内(即基本上同时)施用于受试对象。

[0142] 如本领域技术人员已知的,所用的剂量将视所要实现的临床目标而变化。在一些实施方式中,抗NOTCH抗体(例如OMP-59R5)的每个剂量为约0.25mg/kg～约15mg/kg。在一些实施方式中,每个剂量为约0.25mg/kg、0.5mg/kg、1mg/kg、2mg/kg、3mg/kg、4mg/kg、5mg/kg、6mg/kg、7mg/kg、8mg/kg、9mg/kg、10mg/kg、11mg/kg、12mg/kg、13mg/kg、14mg/kg、15mg/kg、16mg/kg、17mg/kg、18mg/kg、19mg/kg或20mg/kg。在某些实施方式中,每个剂量为约0.5mg/kg。在某些实施方式中,每个剂量为约1mg/kg。在某些实施方式中,每个剂量为约2.5mg/kg。在某些实施方式中,每个剂量为约5mg/kg。在某些实施方式中,每个剂量为约7.5mg/kg。在某些实施方式中,每个剂量为约10mg/kg。在某些实施方式中,每个剂量为

约 12.5mg/kg。在某些实施方式中，每个剂量为约 15mg/kg。

[0143] 在某些实施方式中，本文所述的治疗胰腺癌的方法包括联合施用 NOTCH 抑制剂（例如 OMP-59R5）与一种或多种化疗剂。因此，在一些实施方式中，所述方法或治疗包括联合施用 NOTCH 抑制剂（例如 OMP-59R5）和化疗剂或多种不同的化疗剂的混合物。在某些实施方式中，本文所述的方法包括向胰腺癌患者联合施用治疗有效量的 OMP-59R5 抗体和吉西他滨与 ABRAXANE™（蛋白结合的紫杉醇）。用 NOTCH 抑制剂（例如 OMP-59R5）进行的治疗可以出现在施用化疗剂之前、同时或之后。联合施用可以包括：以单一药物制剂方式或使用独立的多个制剂的共施用，或以任何顺序但通常在使所有活性试剂能够同时发挥其生物学活性的时期内进行的连续施用。可以使用制造商的操作说明所述的或技术熟练的从业者凭经验确定的此类化疗剂的制备和定量给药计划。此类化疗剂的制备和定量给药计划还在 Chemotherapy Service M.C.Perry 编，Williams&Wilkins,Baltimore,Md.(1992) 中有所描述。

[0144] 可用于本发明的化疗剂包括但不限于：烷基化剂，例如噻替派和环磷酰胺；烷基磺酸盐，例如白消安、英丙舒凡和哌泊舒凡；氮丙啶，例如苯佐替派、卡波醌、美妥替哌和乌瑞替哌；乙烯亚胺类和甲基蜜胺类 (methylamelamines)，包括六甲蜜胺、三乙烯蜜胺、三乙烯磷酰胺、三乙烯硫代磷酰胺和三羟甲基蜜胺 (trimethylolomelamine)；氮芥类，例如苯丁酸氮芥、萘氮芥、氯代磷酰胺 (cholophosphamide)、雌氮芥、异环磷酰胺、氮芥、盐酸氧化氮芥、美法仑、新氮芥、苯芥胆甾醇、泼尼氮芥、曲磷胺、尿嘧啶氮芥；亚硝基脲类，例如卡莫司汀、氯脲菌素、福莫司汀、洛莫司汀、尼莫司汀、雷莫司汀；抗生素，例如阿克拉霉素、放线菌素、安曲霉素、重氮丝氨酸、博莱霉素、放线菌素 C、加利车霉素、卡拉比星 (carabicin)、洋红霉素、嗜癌霉素、色霉素、放线菌素 D、柔红霉素、地托比星、6- 重氮基 -5- 氧代 -L- 正亮氨酸、多柔比星、表柔比星、依索比星、伊达比星、麻西罗霉素、丝裂霉素、霉酚酸、诺加霉素、橄榄霉素、培洛霉素、泊非霉素、嘌呤霉素、三铁阿霉素、罗多比星、链黑霉素、链脲菌素、杀结核菌素、乌苯美司、净司他丁、佐柔比星；抗代谢药，例如甲氨蝶呤和 5- 氟尿嘧啶 (5-FU)；叶酸类似物，例如二甲叶酸、甲氨蝶呤、蝶罗呤、三甲曲沙；嘌呤类似物，例如氟达拉滨、6- 巯基嘌呤、硫咪嘌呤、硫鸟嘌呤；嘧啶类似物例如安西他滨、阿扎胞苷、6- 阿扎尿苷、卡莫氟、阿糖胞苷、双脱氧尿苷、去氧氟尿苷、依诺他滨、氟尿苷、5-FU；雄激素类，例如卡鲁睾酮、丙酸屈他雄酮、环硫雄醇、美雄烷、睾内酯；抗肾上腺药 (anti-adrenals)，例如氨鲁米特、米托坦、曲洛司坦；叶酸补充剂，例如亚叶酸；醋葡醛内酯；醛磷酰胺糖苷；氨基酮戊酸；安吖啶；贝达布昔 (bestrabucil)；比生群；依达曲沙；地磷酰胺 (defofamine)；秋水仙胺；地吖醌；依氟鸟氨酸 (elfornithine)；依利醋铵；依托格鲁；硝酸镓；羟基脲；香菇多糖；氯尼达明；米托胍腙；米托蒽醌；莫哌达醇；硝氨丙吖啶；喷司他丁；蛋氨氮芥；吡柔比星；鬼臼酸；2- 乙基肼；丙卡巴肼；PSK；丙亚胺；西佐非兰；锗螺胺；替奴佐酸；三亚胺醌；2,2′,2″- 三氯三乙胺；乌拉坦；长春地辛；达卡巴嗪；甘露醇氮芥；二溴甘露醇；二溴卫矛醇；哌泊溴烷；加赛妥辛 (gacytosine)；阿糖胞苷 (Ara-C)；紫杉烷类，例如紫杉醇和多西他赛；苯丁酸氮芥；吉西他滨；6- 硫鸟嘌呤；巯嘌呤；铂类似物，例如顺铂和卡铂；长春碱；铂；依托泊苷；异环磷酰胺；丝裂霉素 C；米托蒽醌；长春新碱；长春瑞滨；诺维本；诺消灵；替尼泊苷；道诺霉素；氨喋呤；希罗达；伊班膦酸盐；CPT-11；拓扑异构酶抑制剂 RFS 2000；二氟甲基鸟氨酸；视黄酸；埃斯波霉素；卡培他滨；以及上述任一种的药学上可接受

的盐、酸或衍生物。化疗剂还包括用于调节或抑制激素对肿瘤的作用的抗激素剂，例如抗雌激素剂，包括例如他莫昔芬、雷洛昔芬、芳香酶抑制性 4(5)- 咪唑、4- 羟基他莫昔芬、曲沃昔芬、易维特 (keoxifene)、LY117018、奥那司酮和托瑞米芬 (Fareston)；以及抗雄激素剂，例如氟他胺、尼鲁米特、比卡鲁胺、亮丙瑞林和戈舍瑞林；以及上述任一种的药学上可接受的盐、酸或衍生物。

[0145] 在一些实施方式中，所述化疗剂是拓扑异构酶抑制剂。拓扑异构酶抑制剂是干扰拓扑异构酶 (例如拓扑异构酶 I 或 II) 的作用的化疗剂。拓扑异构酶抑制剂包括但不限于盐酸多柔比星、柠檬酸柔红霉素、盐酸米托蒽醌、放射菌素 D、依托泊苷、盐酸托泊替康、替尼泊苷和伊立替康，以及这些中任一种的药学上可接受的盐、酸或衍生物。

[0146] 在一些实施方式中，所述化疗剂是抗代谢药。抗代谢药是结构与正常生化反应所需的代谢物类似的化学物质，但是其区别足以干扰细胞的一种或多种正常功能，例如细胞分裂。抗代谢药包括但不限于：吉西他滨、氟尿嘧啶、卡培他滨、甲氨蝶呤钠、雷替曲塞 (ralitrexed)、培美曲塞、喃氟啶、胞嘧啶阿拉伯糖苷、硫鸟嘌呤、5- 氮胞苷、6- 巯基嘌呤、硫唑嘌呤、6- 硫鸟嘌呤、喷司他丁、磷酸氟达拉滨和克拉屈滨，以及这些中任何一种的药学上可接受的盐、酸或衍生物。在某些实施方式中，本文所述的方法包括向胰腺癌患者联合施用治疗有效量的 OMP-59R5 抗体和抗代谢药。在某些实施方式中，抗代谢药是核苷类似物。在某些实施方式中，本文所述的方法包括向胰腺癌患者联合施用治疗有效量的 OMP-59R5 抗体和吉西他滨。

[0147] 在一些实施方式中，所述化疗剂是抗有丝分裂剂，包括但不限于结合微管蛋白的试剂。在一些实施方式中，所述试剂是紫杉烷。在一些实施方式中，所述试剂是紫杉醇或多西他赛，或紫杉醇或多西他赛的药学上可接受的盐、酸或衍生物。在一些替代实施方式中，抗有丝分裂剂包括长春花生物碱，例如长春新碱、长春碱、长春瑞滨或长春地辛，或其药学上可接受的盐、酸或衍生物。在某些实施方式中，本文所述的方法包括向胰腺癌患者联合施用治疗有效量的 OMP-59R5 抗体和抗有丝分裂剂。在某些实施方式中，抗有丝分裂剂是紫杉烷。在某些实施方式中，本文所述的方法包括向胰腺癌患者联合施用治疗有效量的 OMP-59R5 抗体和 ABRAXANE™(蛋白结合的紫杉醇)。

[0148] 在某些实施方式中，治疗包括联合施用 NOTCH 抑制剂 (例如 OMP-59R5) 和放射疗法。用 NOTCH 抑制剂 (例如 OMP-59R5) 进行的治疗可以在施用放射疗法之前、同时或之后发生。此类放射疗法的剂量方案可由熟练的医学从业者来确定。在一些实施方式中，在放射治疗之后施用 NOTCH 抑制剂 (例如 OMP-59R5)。在一些实施方式中，与放射疗法一起施用 NOTCH 抑制剂 (例如 OMP-59R5)。

[0149] 在一些实施方式中，第二治疗剂包括抗体。因此，治疗可以包括联合施用抗 NOTCH 抗体 (例如 OMP-59R5) 或其他 NOTCH 抑制剂与针对另外的肿瘤相关抗原的其他抗体 (包括包括但不限于结合 EGFR、ErbB2、DLL4 或 NF- κ B 的抗体)。示例性的抗 DLL4 抗体描述于例如美国专利第 7,750,124 号中。另外的抗 DLL4 抗体描述于例如国际专利公开 WO2008/091222 和 WO2008/0793326 以及美国专利申请公开第 2008/0014196、2008/0175847、2008/0181899 和 2008/0107648 中。联合施用可以包括：以单一药物制剂方式或使用独立的多个制剂的共施用，或以任何顺序但通常在使所有活性试剂能够同时发挥其生物学活性的时期内进行的连续使用。

[0150] 此外，用 NOTCH 抑制剂（例如 OMP-59R5）进行的治疗可以包括与一种或多种细胞因子（例如，淋巴因子、白细胞介素、肿瘤坏死因子和 / 或生长因子）的联合治疗，或可以伴随有手术摘除肿瘤、癌细胞或治疗医师认为必需的其他任何疗法。

[0151] 5. 抗体及其制备

[0152] 可以用本领域已知的任何合适的方法产生可用于本发明的方法中的其他抗体。多克隆抗体可以用任何已知方法制备。通过多次皮下注射或腹膜内注射相关抗原（纯化的肽片段、全长重组蛋白、融合蛋白等）来使动物（例如兔、大鼠、小鼠、驴等）免疫，从而产生多克隆抗体，其中，所述抗原可选地偶联至钥孔血蓝蛋白 (KLM)、血清白蛋白等，稀释在无菌盐水中，并与佐剂（例如完全或不完全弗氏佐剂）组合以形成稳定的乳液。随后从由此免疫化的动物的血液、腹水等中回收多克隆抗体。使所收集的血液凝结，随后倾析出血清，离心至澄清，并测定抗体滴度。多克隆抗体可以按照本领域中的标准方法（包括亲和色谱、离子交换色谱、凝胶电泳和透析等）从血清或腹水中纯化。

[0153] 单克隆抗体可以使用杂交瘤方法制备，例如 Kohler 和 Milstein(1975) Nature256:495 中描述的杂交瘤方法。使用杂交瘤法按上文所述使小鼠、仓鼠或其他适合的宿主动物免疫，以引发淋巴细胞产生特异性结合免疫抗原的抗体。淋巴细胞也可以在体外免疫化。免疫化之后，分离淋巴细胞，并使用例如聚乙二醇使其与适合的骨髓瘤细胞系融合，从而形成能够随后从未融合的淋巴细胞和骨髓瘤细胞中筛选出的杂交瘤细胞。产生特异性地针对选定抗原的单克隆抗体（这通过免疫沉淀、免疫印迹或体外结合测定（例如放射免疫测定 (RIA)；酶联免疫吸附测定 (ELISA)）来确定）的杂交瘤可以随后用标准方法在体外培养物中扩大培养 (Goding,Monoclonal Antibodies:Principles and Practice,Academic Press,1986) 或作为动物中的腹水肿瘤在体内扩大培养。随后，如上文针对多克隆抗体所述的，可以从培养基或腹水液体中纯化出单克隆抗体。

[0154] 作为另一选择，单克隆抗体也可以使用如美国专利第 4,816,567 号中描述的重组 DNA 方法制造。通过例如使用特异性扩增编码抗体重链和轻链的寡核苷酸引物的 RT-PCR，将编码单克隆抗体的多核苷酸从成熟 B 细胞或杂交瘤细胞中分离出，并使用常规程序确定其序列。随后将编码重链和轻链的分离的多核苷酸克隆至适合的表达载体中，当将该表达载体转染到宿主细胞（例如大肠杆菌细胞、猿 COS 细胞、中华仓鼠卵巢 (CHO) 细胞或不另外产生免疫球蛋白的骨髓瘤细胞）中时，所述宿主细胞产生单克隆抗体。另外，所需物种的重组单克隆抗体或其片段可以按已描述的方法从表达所需物种的 CDR 的噬菌体展示文库中分离出 (McCafferty 等，Nature,348:552-554(1990)；Clackson 等，Nature,352:624-628(1991)；Marks 等，J.Mol.Biol.,222:581-597(1991))。

[0155] 可以使用重组 DNA 技术以多种不同的方式进一步修饰编码单克隆抗体的多核苷酸，以产生替代性抗体。在一些实施方式中，例如小鼠单克隆抗体的轻链和重链的恒定区可以 :1) 被替换成例如人抗体的对应区域以产生嵌合抗体，或 2) 被替换成非免疫球蛋白多肽以产生融合抗体。在一些实施方式中，恒定区被截短或除去，以产生单克隆抗体的所需抗体片段。可以使用定点诱变或高密度诱变来优化单克隆抗体的特异性和亲和力等。

[0156] 在一些实施方式中，可用于本发明的方法中的单克隆抗体是人源化抗体。在某些实施方式中，在施用给人受试对象时，治疗上使用此类抗体来减少抗原性和 HAMA（人抗小鼠抗体）响应。可以使用本领域中已知的各种技术来制造人源化抗体。在某些实施方式中，

可用于本发明的方法中的抗体是人抗体。

[0157] 可以使用本领域中已知的各种技术来直接制造人抗体。可以制造体外免疫化的或从产生针对靶抗原的抗体的免疫化个体分离出的永生化人B淋巴细胞(参见例如Cole等,Monoclonal Antibodies and Cancer Therapy,Alan R.Liss,第77页(1985);Boemer等,1991,J.Immunol.,147(1):86-95;和美国专利第5,750,373号)。另外,人抗体可以从噬菌体文库中选择,其中该噬菌体文库表达人抗体,如例如以下文献中所述:Vaughan等,1996,Nat.Biotech.,14:309-314;Sheets等,1998,Proc.Nat'l.Acad.Sci.,95:6157-6162;Hoogenboom和Winter,1991,J.Mol.Biol.,227:381;Marks等,1991,J.Mol.Biol.,222:581。用于产生和使用抗体噬菌体文库的技术还描述于美国专利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和7,264,963以及Rothe等,2007,J.Mol.Bio.,doi:10.1016/j.jmb.2007.12.018(将其每个都通过援引整体并入本文)。亲和力成熟策略和链改组策略(Marks等,1992,Bio/Technology 10:779-783;将其通过援引完整并入本文)在本领域是已知的,可以用于产生高亲和力的人抗体。

[0158] 还可以在含有人免疫球蛋白基因座的转基因小鼠中制造人源化抗体,所述小鼠在免疫化时能够在不产生内源免疫球蛋白的情况下产生全部人抗体。在美国专利第5,545,807、5,545,806、5,569,825、5,625,126、5,633,425和5,661,016号中描述了该方法。

[0159] 在某些实施方式中,可用于本发明的方法中的抗体是特异性地识别人NOTCH受体的双特异性抗体。双特异性抗体是能够特异性地识别并结合至少两种不同的表位的抗体。所述不同的表位可位于同一分子(例如,同一人NOTCH受体)内或位于不同的分子上。双特异性抗体可以是完整抗体或抗体片段。

[0160] 或者,在某些替代性实施方式中,可用于本发明中的抗体不是双特异性抗体。

[0161] 在某些实施方式中,可用于本发明中的抗体是单特异性的。例如,在某些实施方式中,抗体所包含的一个或多个抗原结合位点结合或能够结合相同的人NOTCH受体。在某些实施方式中,单特异性抗体的抗原结合位点结合或能够结合一种、两种、三种或四种人NOTCH受体。

[0162] 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。抗体片段能够表现出相对于完整抗体而言增加的肿瘤穿透性。用来产生抗体片段的各种技术是已知的。传统上,通过对完整抗体进行蛋白水解消化来得到这些片段(例如Morimoto等,1993,Journal of Biochemical and Biophysical Methods 24:107-117;Brennan等,1985,Science,229:81)。在某些实施方式中,通过重组手段来制造抗体片段。Fab、Fv和scFv抗体片段都可以在大肠杆菌或其他宿主细胞中表达并分泌,从而使得可以大量地制造这些片段。还可以从上文讨论的抗体噬菌体文库中分离出此类抗体片段。抗体片段还可以是例如美国专利第5,641,870号中所描述的线形抗体,并且可以是单特异性或双特异性的。可用于本发明的方法中的单链抗体可以按照例如美国专利4,946,778中的描述来制备。此外,可以调整方法来构建Fab表达文库(Huse等,Science 246:1275-1281(1989)),以使得可以快速且有效地鉴定出具有所需的针对NOTCH受体的特异性的单克隆Fab片段。抗体片段可以通过本领域中的技术来产生,包括但不限于:(a)通过抗体分子的胃蛋白酶

降解生成的 F(ab')2 片段 ;(b) 通过还原 F(ab')2 片段的二硫桥产生的 Fab 片段 ;(c) 通过用木瓜蛋白酶和还原剂处理抗体分子产生的 Fab 片段 ;和 (d)Fv 片段。对技术人员而言,制造抗体片段的其他技术将是显而易见的。

[0163] 还可能理想的是,特别是对于抗体片段的情况,修饰抗体以提高其血清半衰期。这可以通过例如下述方式来实现 :通过使抗体片段中的适当区域突变,或通过将表位整合至肽标签中并随后将该标签融合至抗体片段的任一末端或中部 (例如通过 DNA 合成或肽合成),从而将救助受体 (salvage receptor) 结合表位整合到抗体片段中。

[0164] 在某些实施方式中,可用于本发明的方法中的抗体是异源偶联抗体 (heteroconjugate antibody)。异源偶联抗体由两种共价接合的抗体构成。例如,已提出此类抗体能够将免疫细胞靶向不需要的细胞 (美国专利 4,676,980)。认为这些抗体可以使用合成蛋白化学中已知的方法体外制备,包括涉及交联剂的方法。例如,可以使用二硫键交换反应或通过形成硫醚键来构建免疫毒素。用于此目的的适合的试剂的实例包括亚氨基硫醇化物 (iminothiolate) 和甲基 -4- 巯基环丁酰亚胺。

[0165] 在本领域中已知恒定 Fc 区介导数种效应子功能。例如,补体的 C1 组分与抗体的结合激活补体系统。补体的激活在细胞病原体的调理作用和裂解中是重要的。补体的激活也刺激了炎症反应并且也可参与自身免疫过敏症。此外,抗体或可溶性受体可通过 Fc 区结合细胞,其中,抗体 Fc 区上的 Fc 受体位点结合至细胞上的 Fc 受体 (FcR)。存在许多对于不同类别的抗体有特异性的 Fc 受体,包括 IgG(γ 受体)、IgE(ε 受体)、IgA(α 受体) 和 IgM(μ 受体)。抗体与细胞表面上的 Fc 受体的结合触发了许多重要且多样的生物反应,包括抗体包被的颗粒的吞噬和破坏、免疫复合物的清除、杀伤细胞对抗体包被的靶细胞的裂解 (称作抗体依赖的细胞介导的细胞毒作用或 ADCC)、炎症介质的释放、胎盘转移和免疫球蛋白生成的控制。

[0166] 在某些实施方式中,可用于本发明的方法中的 NOTCH 拮抗剂多肽 (抗体和包含 Fc 的可溶性受体) 提供了改变的效应子功能,这进而影响了所施用的多肽的生物学特征。例如,恒定区结构域的 (通过点突变或其他方法造成的) 缺失或失活可以减少循环中的经修饰抗体与 Fc 受体的结合,从而提高肿瘤定位。在其他情况下可以是,恒定区修饰调节补体结合,因此降低血清半衰期并减少所偶联的细胞毒素的非特异性联结。可以使用对恒定区的其他修饰来消除二硫键或多糖部分,这使得能因抗原特异性或抗体柔性的提高而增强定位。类似地,使用完全在技术人员知识范围内的公知生物化学或分子工程技术,可以容易地做出对恒定区的修饰。

[0167] 在某些实施方式中,可用于本发明的方法中的包含 Fc 区的 NOTCH 拮抗剂多肽 (抗体和含 Fc 的可溶性受体) 不具有一个或多个效应子功能。例如,在一些实施方式中,所述多肽不具有抗体依赖的细胞介导的细胞毒性 (ADCC) 活性和 / 或不具有补体依赖的细胞毒性 (CDC) 活性。在某些实施方案中,所述多肽不结合 Fc 受体和 / 或补体因子。在某些实施方式中,抗体不具有效应子功能。

[0168] 本发明还涉及包含与细胞毒剂偶联的 NOTCH 拮抗剂多肽 (例如抗 NOTCH 抗体) 的免疫偶联物的应用。细胞毒剂包括化疗剂、生长抑制剂、毒素 (例如,源于细菌、真菌、植物或动物的具有酶活性的毒素或其片段)、放射性同位素 (即放射偶联物) 等。可用于产生此类免疫偶联物的化疗剂包括例如氨甲喋呤、阿霉素、多柔比星、美法仑、丝裂霉素 C、苯丁

酸氮芥、正定霉素或其他嵌入试剂。可以使用的具有酶活性的毒素及其片段包括白喉 A 链、白候毒素的非结合性活性片段、外毒素 A 链、蓖麻毒素 A 链、相思豆毒素 A 链、蒴莲根毒素 A 链、α－帚曲菌素、油桐蛋白、石竹素蛋白、美洲商陆蛋白 (PAPI、PAPII 和 PAP-S)、苦瓜抑制剂、麻风树毒素、巴豆毒素、肥皂草抑制剂、白树毒素、丝林霉素 (mitogellin)、局限曲菌素、酚霉素、依诺霉素和单端孢霉烯族毒素。多种放射性核素可用于产生放射偶联抗体，包括 ^{212}Bi、^{131}I、^{131}In、^{90}Y 和 ^{186}Re。抗体和细胞毒剂的偶联物是使用多种双功能蛋白偶合剂制成的，例如 N- 琥珀酰亚胺 -3-(2- 硫代吡啶) 丙酸酯 (SPDP)、亚氨基硫烷 (IT)、亚氨酸酯的双功能衍生物 (例如盐酸己二亚氨酸二甲酯)、活性酯 (例如辛二酸二琥珀酰亚氨酯)、醛 (例如戊二醛)、双叠氮化合物 (例如双 (对叠氮基苯甲酰基) 己二胺)、双重氮衍生物 (例如双 (对重氮基苯甲酰基) 乙二胺)、二异氰酸酯 (例如 2,4- 二异氰酸甲苯酯) 和双活性氟化合物 (例如 1,5- 二氟 -2,4- 二硝基苯)。还可以使用抗体和一种或多种小分子毒素 (例如卡奇霉素、美登醇、单端孢霉烯和 CC1065) 及这些毒素的具有毒素活性的衍生物的偶联物。

[0169] 偶联抗体由两种共价接合的抗体构成。例如，已提出此类抗体能够将免疫细胞靶向不需要的细胞 (美国专利 4,676,980)。认为这些抗体可以使用合成蛋白化学中已知的方法体外制备，包括涉及交联剂的方法。例如，可以使用二硫键交换反应或通过形成硫醚键来构建免疫毒素。用于此目的的适合的试剂的实例包括亚氨基硫醇化物和甲基 -4- 巯基环丁酰亚胺。

[0170] 不论获得了如何有用的量，可以以多种偶联形式 (即，免疫偶联物) 或未偶联形式中的任何一种形式来使用可用于本发明的方法中的 NOTCH 拮抗剂多肽 (抗体和可溶性受体)。作为另一选择，多肽可以以未偶联形式或“裸”形式使用。在某些实施方式中，以未偶联形式来使用本发明的多肽，从而控制受试对象的天然防御机制 (包括补体依赖的细胞毒作用 (CDC) 和抗体依赖的细胞介导的细胞毒作用 (ADCC)) 来清除恶性细胞。在一些实施方式中，使用多种公知的螯合剂中的任一种，或通过直接加标签，可以使多肽与放射性同位素偶联，例如 ^{90}Y、^{125}I、^{131}I、^{123}I、^{111}In、^{105}Rh、^{153}Sm、^{67}Cu、^{67}Ga、^{166}Ho、^{177}Lu、^{186}Re 和 ^{188}Re。在其他实施方式中，组合物可以包含与药物、前药或生物应答调节剂 (例如氨甲喋呤、阿霉素和淋巴因子 (例如干扰素)) 偶联的 NOTCH 拮抗剂多肽。另外的实施方式包括与特定生物毒素 (例如蓖麻蛋白或白喉毒素) 偶联的 NOTCH 拮抗剂多肽的应用。在另外一些实施方式中，NOTCH 拮抗剂多肽可以与其他具有免疫学活性的配体 (例如抗体或其片段) 复合，其中，所得的分子结合赘生性细胞和效应细胞 (例如 T 细胞)。选用何种偶联或未偶联的 NOTCH 拮抗剂多肽将取决于神经内分泌肿瘤的类型和阶段、对辅助治疗的使用 (例如，化疗或外部辐射) 和患者的状况。应认识到的是，鉴于本文的教导，本领域的技术人员可以容易地作出这种选择。

[0171] 可以进一步修饰多肽和类似物以包含通常不属于蛋白的一部分的额外化学部分。这些衍生出的部分可以改进蛋白的溶解性、生物学半衰期或吸收。这些部分还可以减少或消除蛋白的任何所需的副作用等。关于这些部分的概述可见于 Remington's Pharmaceutical Sciences(第 20 版), Mack Publishing Company, Easton, PA, 2000。

[0172] 最适合于衍生化的化学部分包括水溶性聚合物。水溶性聚合物是合乎需要的，因为与其连接的蛋白在水性环境 (例如生理环境) 中不沉淀。在一些实施方式中，所述聚合物对于制备治疗用产品或组合物而言是药学上可接受的。基于以下考虑，本领域技术人员

能够选择所需的聚合物：例如，考虑聚合物 / 蛋白偶联物是否将用于治疗，如果是，则考虑所需的剂量、循环时间、对蛋白分解的抗性，并考虑其他方面。所述衍生化的有效性可以通过以下方法来查明：以所需形式施用衍生物（即，通过渗透泵，或通过注射或输注，或进一步配制成用于口服、肺部或其他递送途径），并确定其有效性。适合的水溶性聚合物包括但不限于聚乙二醇 (PEG)、乙二醇 / 丙二醇共聚物、羧甲基纤维素、右旋糖酐、聚乙烯醇、聚乙烯吡咯烷酮、聚 (1,3- 二氧戊环）、聚 (1,3,6- 三噁烷）、乙烯 / 马来酸酐共聚物、聚氨基酸（均聚物或无规共聚物）、右旋糖酐、聚 (n- 乙烯基吡咯烷酮）- 聚乙二醇、聚丙二醇均聚物、聚氧化丙烯 / 氧化乙烯共聚物、聚氧乙基化多元醇（例如甘油）、聚乙烯醇及其混合物。聚乙二醇丙醛因其在水中的稳定性而在制造时可以具有优势。

[0173] 可以通过本领域已知的任何合适的方法产生可用于本发明的方法中的分离的多肽（例如抗体和可溶性受体）。这些方法从直接蛋白合成方法，到构建编码分离的多肽序列的 DNA 序列并在合适的转化宿主中表达这些序列。在一些实施方式中，使用重组技术通过分离或合成编码野生型目的蛋白的 DNA 序列来构建 DNA 序列。可选的是，可以通过位点特异性诱变来对该序列进行诱变从而提供其功能类似物。参见例如 Zoeller 等，Proc. Nat'l. Acad. Sci. USA 81:5662-5066(1984) 和美国专利 4,588,585。

[0174] 在一些实施方式中，编码目的多肽的 DNA 序列将通过使用寡核苷酸合成仪的化学合成来构建。可以基于所需多肽的氨基酸序列和选择在会产生重组目的多肽的宿主细胞中偏好的那些密码子来设计这些寡核苷酸。可以应用标准方法来合成编码分离的目的多肽的分离的多核苷酸序列。例如，可以使用完整氨基酸序列来构建回译基因。另外，可以合成含有编码特定分离的多肽的核苷酸序列的 DNA 寡聚物。例如，可以合成几种编码所需多肽的一部分的小寡核苷酸并然后连接。各寡核苷酸通常含有 5' 或 3' 延伸序列以用于互补组装。

[0175] 一旦进行了组装（通过合成、定点诱变或其他方法），则将编码特定的分离的目的多肽的多核苷酸序列插入表达载体并可操作地连接至适合在所需宿主中表达所述蛋白的表达控制序列。可以通过核苷酸测序、限制性酶切定位和在合适宿主中表达生物活性多肽来确认正确的组装。如本领域中众所周知的，为了在宿主中获得转染基因的高表达水平，必须将该基因可操作地连接至在所选表达宿主中具有功能的转录和翻译表达控制序列。

[0176] 在某些实施方式中，使用重组表达载体来扩增和表达 NOTCH 拮抗剂多肽（例如抗体或可溶性受体）。重组表达载体是可复制的 DNA 构建体，其具有编码目的多肽链的合成的或 cDNA 来源的 DNA 片段，所述 DNA 片段与源自哺乳动物、微生物、病毒或昆虫基因的合适的转录或翻译调控元件可操作地连接。转录单元通常包括以下组件的组装体：(1) 在基因表达中具有调控作用的遗传元件，例如，转录启动子或增强子，(2) 转录为 mRNA 并且翻译成蛋白的结构序列或编码序列，和 (3) 合适的转录和翻译起始序列和终止序列，这些在下文有详细描述。此类调控元件可以包括用于控制转录的操纵基因序列。在宿主中进行复制的能力通常由复制起点赋予，并且可以另外引入便于识别转化体的选择基因。当 DNA 区域在功能上相互相关时，这些 DNA 区域是“可操作地连接”的。例如，如果信号肽（分泌前导序列）的 DNA 表达为参与多肽分泌的前体，则该 DNA 与该多肽的 DNA 可操作地连接；如果启动子控制编码序列的转录，则启动子与该编码序列可操作地连接；或如果核糖体结合位点位于允许翻译的位置，则该核糖体结合位点与编码序列可操作地连接。意在在酵母表达体系中使用的结构元件包括能够使宿主细胞将翻译的蛋白分泌到胞外的前导序列。作为另一选择，

当表达不具有前导序列或转运序列的重组蛋白时，其可以包括 N 端甲硫氨酸残基。可以随后可选地从表达的重组蛋白中切下该残基以提供最终产物。

[0177] 表达控制序列和表达载体的选择将取决于对宿主的选择。可以采用多种表达宿主 / 载体组合。对于真核宿主有用的表达载体包括例如包含来自 SV40、牛乳头状瘤病毒、腺病毒和巨细胞病毒的表达控制序列的载体。对于细菌宿主有用的表达载体包括已知的细菌质粒，例如包括 pCR1、pBR322、pMB9 及其衍生物等在内的来自大肠杆菌的质粒，以及诸如 M13 和丝状单链 DNA 噬菌体等更广宿主范围的质粒。

[0178] 用于表达 NOTCH 拮抗剂多肽（例如抗体或可溶性受体）的合适的宿主细胞包括处于适当启动子控制下的原核生物、酵母、昆虫或高级真核细胞。原核生物包括革兰氏阴性生物体或革兰氏阳性生物体，例如大肠杆菌或芽孢杆菌。高级真核细胞包括如下所述的哺乳动物来源的已建立的细胞系。还可以使用无细胞翻译体系。用于细菌、真菌、酵母和哺乳动物细胞宿主的适合的克隆和表达载体见 Pouwels 等的描述 (Cloning Vectors:A Laboratory Manual,Elsevier,N.Y.,1985)，通过援引将其中的相关公开内容并入本文中。关于蛋白制造（包括抗体制造）方法的其他信息可见于美国专利公开 2008/0187954、美国专利 6,413,746 和 6,660,501 以及国际专利公开 WO 04009823，将其每个都通过援引完整并入本文。

[0179] 还有利地使用各种哺乳动物或昆虫细胞培养系统来表达重组多肽。可以在哺乳动物细胞中表达重组蛋白，因为所述蛋白通常正确地折叠、合适地修饰并具有完全功能。合适的哺乳动物宿主细胞系的实例包括 Gluzman 描述的猴肾细胞系 COS-7(Cell23:175,1981) 以及能够表达合适的载体的其他细胞系，包括例如 L 细胞、C127、3T3、中华仓鼠卵巢 (CHO)、HeLa 和 BHK 细胞系。哺乳动物表达载体可以包含非转录元件（例如与待表达的基因连接的复制起点、合适的启动子和增强子，以及其它 5’ 或 3’ 侧翼非转录序列）以及 5’ 或 3’ 非翻译序列（例如必需的核糖体结合位点、聚腺苷酸化位点、剪接供体和受体位点以及转录终止序列）。用于在昆虫细胞中产生异源蛋白的杆状病毒系统见综述 Luckow 和 Summers,Bio/Technology,6:47(1988)。

[0180] 可以根据任何合适的方法对由经转化宿主产生的蛋白进行纯化。这些标准方法包括色谱（例如离子交换色谱、亲和色谱和尺寸排阻柱色谱）、离心、差别溶解度法或通过用于蛋白纯化的任何其它标准技术。诸如六聚组氨酸、麦芽糖结合域、流感病毒衣壳序列和谷胱甘肽 -S- 转移酶等亲和标签可以与蛋白连接，从而允许通过合适的亲和柱容易地进行纯化。还可使用诸如蛋白水解、核磁共振和 X 射线晶体学等技术对分离的蛋白进行物理表征。

[0181] 例如，可以首先使用商购的蛋白浓缩过滤器（例如 Amicon 或 Millipore Pellicon 超滤装置）浓缩来自将重组蛋白分泌到培养基中的表达系统的上清液。浓缩步骤后，可将浓缩物施加到合适的纯化基质。作为另一选择，可以使用阴离子交换树脂，例如具有悬挂的二乙氨基乙基 (DEAE) 基团的基质或基底。所述基质可以是丙烯酰胺、琼脂糖、葡聚糖、纤维素或蛋白纯化中常用的其它种类。作为另一选择，可以使用阳离子交换步骤。合适的阳离子交换器包括各种含有磺丙基或羧甲基的不溶性基质。最后，可以使用一个或多个反相高效液相色谱 (RP-HPLC) 步骤来进一步纯化 NOTCH 拮抗剂多肽（例如抗体或可溶性受体），所述 RP-HPLC 步骤采用疏水性 RP-HPLC 介质，例如具有悬挂甲基或其它脂肪族基团的硅胶。还可以以各种组合使用上述纯化步骤中的一些或全部，从而提供均一的重组蛋白。

[0182] 细菌培养物中产生的重组蛋白可通过例如以下方式分离：从细胞团块中进行初步提取，然后是一次或多次浓缩、盐析、水性离子交换或尺寸排阻色谱步骤。对于最后的纯化步骤可以使用高效液相色谱(HPLC)。可以通过任何常规方法破碎用于表达重组蛋白的微生物细胞，包括冻融循环、超声处理、机械破碎或使用细胞裂解剂。

[0183] 本领域已知的用于纯化NOTCH拮抗剂多肽（例如抗体或可溶性受体）的方法还包括例如美国专利公布2008/0312425、2008/0177048和2009/0187005中描述的那些，将其各自通过引用整体并入本文中。

[0184] 6. 药物组合物

[0185] 可以用本领域中已知的任何适合的方法将NOTCH拮抗剂多肽（例如抗NOTCH抗体）配制成药物组合物。在某些实施方式中，所述药物组合物包含药学上可接受的载质。所述药物组合物用于抑制神经内分泌肿瘤的生长和治疗人患者的神经内分泌肿瘤。

[0186] 在某些实施方式中，通过将纯化的NOTCH拮抗剂（例如抗NOTCH抗体）与药学上可接受的载质（例如，载剂、赋形剂）合并，制备了用于储存和使用的制剂(Remington,The Science and Practice of Pharmacy(第20版),Mack Publishing,2000)。适合的药学上可接受的载质包括但不限于：无毒缓冲剂，例如磷酸盐、柠檬酸盐和其他有机酸；盐，例如氯化钠；抗氧化剂，包括抗坏血酸和甲硫氨酸；防腐剂（例如十八烷基二甲基苄基氯化铵；氯化六烃季铵；氯化苄烷铵；氯化苄乙氧铵；苯酚、丁醇或苄醇；对羟基苯甲酸烷基酯，例如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯；儿茶酚；间苯二酚；环己醇；3-戊醇；和间甲酚）；低分子量多肽（例如，小于约10个氨基酸残基）；蛋白，例如血清白蛋白、明胶或免疫球蛋白；亲水聚合物，例如聚乙烯吡咯烷酮；氨基酸，例如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸；碳水化合物，例如单糖、二糖、葡萄糖、甘露糖或糊精；螯合剂，例如EDTA；糖类，例如蔗糖、甘露醇、海藻糖或山梨醇；成盐反离子，例如钠；金属络合物（例如Zn-蛋白络合物）；和非离子表面活性剂，例如TWEEN或聚乙二醇(PEG)。

[0187] 在某些实施方式中，所述药物组合物是冷冻的。在某些替代性实施方式中，所述药物组合物是冻干的。

[0188] 可以以用于局部治疗或全身治疗的多种方式来施用本发明的药物组合物。施用可以是：局部施用（例如，至粘膜，包括阴道和直肠递送），例如经皮贴、软膏、洗剂、乳膏、凝胶、滴剂、栓剂、喷雾剂、液体和粉末；肺部施用（例如，通过吸入或吹入粉末或气雾剂，包括使用喷雾器；气管内、鼻内、表皮和经皮）；口服施用；或胃肠外施用，包括静脉内、动脉内、皮下、腹膜内或肌肉内注射或输注；或颅内施用（例如，鞘内或脑室内）。

[0189] 治疗性制剂可以是单位剂型。此类制剂包括：片剂、丸剂、胶囊、粉末、颗粒、水或非水性介质中的溶液或悬浮液或者栓剂，以用于口服施用、胃肠外施用、直肠施用或吸入式施用。在诸如片剂等固体组合物中，主要活性成分与药物载剂混合。常规的成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、磷酸二钙或树胶和其他稀释剂（例如水），从而形成包含本发明的化合物或无毒的其药物可接受的盐的均质混合物的固体预制组合物。随后将该固体预制组合物细分为上述类型的单位剂型。新型组合物的片剂、丸剂等可以经涂覆或以其他方式复合而提供具有延长作用的优点的剂型。例如，该片剂或丸剂可以包含经外部组分包被的内部组合物。此外，这两种组分可被由肠溶层分离，该肠溶层用来抵抗崩解并允许内部组分完整地通过胃或延迟释放。可将多种材料用于此类肠溶层或

涂层，所述材料包括多种聚合物酸和聚合物酸与诸如虫胶、鲸蜡醇和乙酸纤维素酯等材料的混合物。

[0190] 还可以将 NOTCH 拮抗剂（例如抗 NOTCH 抗体）封装在微胶囊中。通过例如凝聚技术或通过界面聚合来制备此类微胶囊，例如，分别为，在胶体药物递送系统（例如，脂质体、白蛋白微球体、微乳剂、纳米粒和纳米胶囊）或在粗乳剂（如 Remington, The Science and Practice of Pharmacy 第 20 版，Mack Publishing(2000) 中所述）中的羟甲基纤维素或明胶微胶囊和聚（甲基丙烯酸甲酯）微胶囊。

[0191] 在某些实施方式中，药物制剂包含与脂质体复合的 NOTCH 拮抗剂（例如抗 NOTCH 抗 体)(Epstein 等，1985, Proc. Natl. Acad. Sci USA 82:3688 ; Hwang 等，1980, Proc. Natl. Acad. Sci USA 77:4030 ；和美国专利第 4,485,045 和 4,544,545 号）。美国专利第 5,013,556 号中公开了具有增强的循环时间的脂质体。一些脂质体可以用含有磷脂酰胆碱、胆固醇和 PEG 衍生化的磷脂酰乙醇胺 (PEG-PE) 的液体组合物通过逆相蒸发来产生。将脂质体挤压通过具有确定孔径的滤器，从而产生具有所需直径的脂质体。

[0192] 此外，可以制备持续释放型制剂。持续释放型制剂的适合实例包括含有抗体的固体疏水聚合物的半透性基质，该基质为定形物形式（例如，膜或微胶囊）。持续释放型基质的实例包括聚酯、诸如聚（甲基丙烯酸 2- 羟乙酯）或聚乙烯醇等水凝胶、聚交酯（美国专利第 3,773,919 号）、L- 谷氨酸和 7- 乙基 -L- 谷氨酰胺的共聚物、不可降解的乙烯 - 乙酸乙烯酯、诸如 LUPRON DEPOTTM(由乳酸 - 乙醇酸共聚物和乙酸亮丙瑞林构成的可注射微球体）等可降解的乳酸 - 乙醇酸共聚物、蔗糖乙酸酯异丁酸酯和聚 D-(-)-3- 羟基丁酸。

[0193] 7. 试剂盒

[0194] 还提供了用于实施本发明方法的试剂盒。“试剂盒”是指包含至少一种用于特异性地检测样品（例如细胞、细胞系、肿瘤或组织）中的 NOTCH3 基因表达水平的试剂（例如核酸探针等）的任何制品（例如包装物或容器）。试剂盒可以作为用来实施本发明的方法的套件来促销、分售或销售。另外，试剂盒可以含有描述该试剂盒并且包含其使用说明资料的药品说明书。

[0195] 在一个实施方式中，提供了用于实施本发明方法的试剂盒。此类试剂盒与手动筛选和自动化筛选都兼容。对于 qRT-PCR 测定而言，试剂盒至少包含用于检测 NOTCH3 基因表达的本文公开的探针。所述试剂盒还可以包含用于 RNA 提取、逆转录和 / 或 PCR 扩增的试剂。在某些实施方式中，本发明的试剂盒包含至少一种寡核苷酸，所述寡核苷酸包含选自由 SEQ ID NO:35 ～ 43 组成的组的核苷酸序列。

[0196] 试剂盒中可以包括阳性和 / 或阴性对照来验证按照本发明使用的试剂的活性和正确使用。对照可以包括 ：已知对 NOTCH3mRNA 的存在呈阳性或阴性的样品，例如 RNA 制备物、福尔马林固定的组织等。对照的设计和使用是标准的，并且完全在本领域技术人员的常规能力范围内。

[0197] 还应意识到的是，本发明方法中的任何步骤或所有步骤都可以由人实施或以自动化方式进行。因此，身体样品的制备、样品的冷冻或固定、RNA 提取和 / 或检测 NOTCH3 转录本水平的步骤可以是自动化的。

[0198] 实施例

[0199] 应理解的是，本文描述的实施例和实施方式仅出于说明性目的，本领域技术人员

据此可以想到多种修改或改变，且这些修改和改变也包含在本申请的主旨和范围内。

[0200] 实施例 1

[0201] 使用作为单一试剂和与化疗剂组合的 OMP-59R5 抗 NOTCH2/3 受体抗体在体内防止肿瘤生长

[0202] 将 20,000 个 OMP-PN8 肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 22 天，直至它们达到 125mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 4 组，并用对照抗体、OMP-59R5（抗 NOTCH2/3）、吉西他滨、或 OMP-59R5 与吉西他滨的组合进行治疗。每隔一周以 40mg/kg 的剂量施用抗体。吉西他滨以每周 20mg/kg 的剂量施用。在所指示的治疗后天数测量肿瘤体积。作为单一试剂或与吉西他滨组合的 OMP-59R5 强烈地抑制了 OMP-PN8 肿瘤的生长（图 1A）。

[0203] 抗 NOTCH2/3OMP-59R5 抗体的抑制 OMP-PN17 胰腺肿瘤体内生长的能力使用基本相同的方法来确定。如图 1B 所示，作为单一试剂或与吉西他滨组合的 OMP-59R5 强烈地抑制了 OMP-PN17 肿瘤的生长。

[0204] 将 50,000 个 OMP-PN11 肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 21 天，直至它们达到 120mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 4 组，并用对照抗体、OMP-59R5（抗 NOTCH2/3）、吉西他滨、或 OMP-59R5 与吉西他滨的组合进行治疗。每隔一周以 40mg/kg 的剂量施用抗体。吉西他滨以每周 20mg/kg 的剂量施用。在所指示的治疗后天数测量肿瘤体积。如图 1C 所示，作为单一试剂或与吉西他滨组合的 OMP-59R5 均对 OMP-PN11 肿瘤的生长没有效果。

[0205] 将 20,000 个 UM-PE13 乳腺（NOTCH3 高表达）肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 37 天，直至它们达到 140mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 4 组，并用对照抗体、OMP-59R5、紫杉醇、或 OMP-59R5 与紫杉醇的组合进行治疗。每周以 20mg/kg 的剂量施用抗体。紫杉醇以每周 10mg/kg 的剂量施用。在所指示的治疗后天数测量肿瘤体积。如图 1D 所示，作为单一试剂或与紫杉醇组合的 OMP-59R5 强烈地抑制了 UM-PE13 肿瘤的生长。

[0206] 将 20,000 个 UM-T1 乳腺（NOTCH3 高表达）肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 28 天，直至它们达到 120mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 4 组，并用对照抗体、OMP-59R5 抗 NOTCH2/3 抗体、紫杉醇、或 OMP-59R5 与紫杉醇的组合进行治疗。每周以 20mg/kg 的剂量施用抗体。紫杉醇以每周 10mg/kg 的剂量施用。在所指示的治疗后天数测量肿瘤体积。如图 1E 所示，作为单一试剂或与紫杉醇组合的 OMP-59R5 对 UM-T1 肿瘤的生长没有效果。

[0207] 将 50,000 个 OMP-Lu40 肺（NOTCH3 低表达）肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 33 天，直至它们达到 140mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 4 组，并用对照抗体、OMP-59R5 抗 NOTCH2/3 抗体、紫杉醇、或 OMP-59R5 与紫杉醇的组合进行治疗。每周以 20mg/kg 的剂量施用抗体。紫杉醇以每周 10mg/kg 的剂量施用。在所指示的治疗后天数测量肿瘤体积。如图 1F 所示，与紫杉醇组合的 OMP-59R5 强烈地抑制了 OMP-Lu40 肿瘤的生长。

[0208] 将 50,000 个 OMP-Lu53 肺（NOTCH3 高表达）肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 33 天，直至它们达到 120mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 4 组，并用对照抗体、OMP-59R5 抗 NOTCH2/3 抗体、紫杉醇、或 OMP-59R5 与紫杉醇的组合进行治疗。每

隔一周以 40mg/kg 的剂量施用抗体。紫杉醇以每周 10mg/kg 的剂量施用。在所指示的治疗后天数测量肿瘤体积。如图 1G 所示，与紫杉醇组合的 OMP-59R5 对 OMP-Lu53 肿瘤的生长没有效果。

[0209] 实施例 2

[0210] 与吉西他滨组合的 OMP-59R5 的肿瘤生长抑制作用与胰腺肿瘤中的 NOTCH3 基因表达水平显著相关，但在乳腺肿瘤或肺肿瘤中却非如此。

[0211] 使用标准微阵列技术，在实施例 1 所述的体内异种移植测定中的胰腺肿瘤、乳腺肿瘤和肺肿瘤中确定了 NOTCH2 和 NOTCH3 基因表达水平。根据制造商的操作说明，使用 Affymetrix® U133 plus 2 阵列获得了表达数据。结果示于下表 1 至 3 中。这些表还包含了关于在实施例 1 所述的体内异种移植测定中特定肿瘤对与化疗剂联合的 OMP-59R5 抗 NOTCH2/3 抗体治疗的响应性的数据。对这些表中示出的 NOTCH2 和 NOTCH3 基因表达水平的分析是基于 500 的截断阈值。不过，当截断阈值在 300 ～ 1000 之间变化时，来自这些分析的总体结论始终是相同的。在乳腺肿瘤和肺肿瘤样品中，未观察到 NOTCH3 表达与体内治疗功效之间存在相关性：在 NOTCH3 基因高度表达的 14 个乳腺或肺肿瘤中，仅有 5 个有响应性。此外，在乳腺、肺或胰腺肿瘤样品中，未观察到 NOTCH2 表达与体内功效之间存在相关性。令人惊奇的是，在胰腺肿瘤中，在高水平的 NOTCH3 基因表达与 OMP-59R5/ 吉西他滨治疗的体内功效之间存在非常强的相关性：在 NOTCH3 基因高度表达的 10 个胰腺肿瘤中，9 个都对 OMP-59R5 和吉西他滨的治疗具有体内响应性。

[0212] 表 1. 胰腺肿瘤中的 NOTCH2 和 NOTCH3 基因表达水平。

[0213]

肿瘤	功效 (OMP-59R5 +吉西他滨)	N3 表达	N2 表达
PN4	+	高(1802)	高(4637)
PN7	-	低(274)	高(2140)
PN8	+	高(2484)	高(6909)
PN11	-	低(141)	高(4576)
PN13	-	低(23)	高(6848)
PN16	+	高(3318)	高(3812)
PN17	+	高(6106)	高(5904)
PN21	+	高(2776)	高(6203)
PN23	-	高(2978)	高(5166)
PN25	+	高(6600)	高(4383)

[0214] 表 2. 乳腺肿瘤中的 NOTCH2 和 NOTCH3 基因表达水平。

[0215]

肿瘤	功效 (OMP-59R5 +紫杉醇)	N3 表达	N2 表达
PE13	+	高(5616)	高(6283)
T1	-	高(11708)	高(7551)
B37	+	高(10217)	高(3231)
B40	-	高(11615)	高(10999)

[0216] 表 3. 肺肿瘤中的 NOTCH2 和 NOTCH3 基因表达水平。NSCLC- 非小细胞肺癌；SCLC- 小细胞肺癌。

[0217]

	肿瘤	功效 (OMP-59R5 +紫杉醇)	N3 表达	N2 表达
NSCLC	Lu15	-	低(440)	高(1995)
NSCLC	Lu24	-	高(5430)	高(3105)
NSCLC	Lu25	-	高(9768)	高(3225)
NSCLC	Lu53	-	高(12294)	高(7828)
SCLC	Lu40	+	低(423)	高(1040)
SCLC	Lu61	+	高 11732	高(1500)
SCLC	Lu65	+	低(269)	高(514)
SCLC	Lu66	+	低(9)	低(12)
SCLC	Lu67	-	高(682)	高(2214)
SCLC	Lu68	+	高(838)	高(3519)

[0218] 对胰腺肿瘤中高水平的 NOTCH3 基因表达与 OMP-59R5/ 吉西他滨联合治疗的体内功效之间的这种令人惊奇的相关性进行了进一步分析。使用标准多重转录本测序（例如 RNASeq），在 PN11、PN13、PN23、PN04、PN08、PN16、PN17、PN21 和 PN25 胰腺肿瘤细胞中确定了 NOTCH 基因表达水平。根据制造商的操作说明，使用 Illumina® HiSeq™ 2000 测序系统进行 RNASeq。图 2A 显示，在人胰腺异种移植物模型中，增加的 NOTCH3 基因表达与 OMP-59R5/ 吉西他滨联合治疗的体内肿瘤抑制作用显著相关 (0.823 ;p<0.021)。图 3 进一步显示，在响应性胰腺肿瘤中检测到的 NOTCH3 基因表达显著高于在非响应性胰腺肿瘤中检测到的表达水平。

[0219] 图 2B 显示了在对 OMP-59R5 抗 NOTCH2/3 抗体 - 吉西他滨联合治疗有响应的人胰腺肿瘤 (R ＝响应者 ;与吉西他滨单独治疗相比时 p 值 <0.05) 和经发现对 OMP-59R5 抗 NOTCH2/3 抗体 - 吉西他滨联合治疗无响应的异种移植物 (NR ＝无响应者 ;与吉西他滨单独治疗相比时 p 值 >0.05) 中检测到的 NOTCH3 基因表达的分布。非响应性胰腺肿瘤中的 NOTCH3 基因表达水平分布显示出与响应性胰腺肿瘤中的 NOTCH 基因表达水平分布的明显分离。

[0220] 基于通过 RNASeq 在胰腺癌中检测出的 NOTCH3 基因表达水平，使用逻辑回归（标准统计学模型）来预测特定胰腺癌对 OMP-59R5 与化疗剂（例如吉西他滨）的联合治疗的体内响应性 (Alan Agresti:An Introduction to Categorical Data Analysis, John Wiley and Sons, Inc. (1996))。分析结果示于图 4 中。NOTCH3 基因表达数据组的阳性预测值 (PPV)、阴性预测值 (NPV)、灵敏度 (SENS) 和特异性 (SPEC) 分别为 83％、75％、83％和 75％。

[0221] 通过在统计学分析中加入来自胰腺癌的 MAML2 基因表达数据，进一步提高了预测胰腺癌对 OMP-59R5 与吉西他滨的联合治疗的体内响应性的预测准确性。对 NOTCH3 和 MAML2 基因表达数据组实施逻辑回归所得到的结果示于图 5 中。NOTCH3 和 MAML2 基因表达数据组的阳性预测值 (PPV)、阴性预测值 (NPV)、灵敏度 (SENS) 和特异性 (SPEC) 为 100％。使用通过标准 RNASeq 法获得的基因表达数据对实验进行了交叉验证。

[0222] 实施例 3

[0223] 胰腺肿瘤样品中的 NOTCH3 蛋白表达

[0224] 进行了 NOTCH3 蛋白质印迹分析以确定人胰腺肿瘤中的 NOTCH3 蛋白表达（图 6A）。该分析中所用的抗 NOTCH3 抗体（细胞信号传导 #5276）检测到了全长 NOTCH3 (FL 约 250kDa)

以及 NOTCH3 的跨膜和细胞内区域 (TM 为约 98kDa)。

[0225] 图 6B 显示了在实施例 1 所述的异种移植物测定中的对 OMP-59R5 与吉西他滨的联合治疗有响应的人胰腺肿瘤 (R ＝响应者 ;与吉西他滨单独治疗相比时 p 值 <0.05) 和经发现对 OMP-59R5 与吉西他滨的联合治疗无响应的异种移植物 (NR ＝无响应者 ;与吉西他滨单独治疗相比时 p 值 >0.05) 中检测到的 NOTCH3 蛋白表达的分布。响应者与无响应者之间的 NOTCH3 蛋白表达分布上的分离度不如 NOTCH3 基因表达分布上的分离度明显。对胰腺癌中的 NOTCH3 蛋白表达数据进行了逻辑回归,以预测特定胰腺癌对 OMP-59R5 与吉西他滨的联合治疗的敏感性。在预测对 OMP-59R5+ 吉西他滨治疗的响应方面,NOTCH3 蛋白表达数据产生了与上述 NOTCH3 基因表达数据的表现相似的表现。

[0226] 实施例 4

[0227] 用 qRT-PCR 测量的转移胰腺肿瘤样品中的 NOTCH3 基因表达

[0228] 用标准的定量 qRT-PCR 在转移胰腺肿瘤样品中确定了 NOTCH3 基因表达。使用 NOTCH3RefSeq mRNA 序列 NM_000435.2 设计了测定探针。NOTCH3_A7 检测到了两种潜在的转录本中的一种,而 NOTCH3_A1 检测到了 Ensembl 数据库所预测的全部两种转录本。使用人新鲜冷冻 (FF) 人组织样品的和福尔马林固定的石蜡包埋 (FFPE) 的人组织样品检验了探针和 qRT-PCR 测定。

[0229] 表 3. 在 NOTCH3qRT-PCR 测定中使用的探针的核苷酸序列。

[0230]

NOTCH3_A1	正向	AGGCAGAGTGGCGACCTC (SEQ ID NO:35)
	反向	CGTCCACGTTCACTTCACAATTC (SEQ ID NO:36)
	探针	AACCCAGGAAGACAGGCACAGTCGT (SEQ ID NO:37)
NOTCH3_A9	正向	CTGGGTTTGAGGGTCAGAAT (SEQ ID NO:38)
	反向	GGGCACTGGCAGTTATAGGT (SEQ ID NO:39)
	探针	TGACGCCATCCACGCATGTC (SEQ ID NO:40)
NOTCH3_A7	正向	TGCAGGATAGCAAGGAGGAGAC (SEQ ID NO:41)
	反向	GCAGCTTGGCAGCCTCATAG (SEQ ID NO:42)
	探针	CTCGCGGGCGGCCAGGAATAGGG (SEQ ID NO:43)

[0231] 采集了约 100 份来自一线胰腺癌患者的福尔马林固定的石蜡包埋 (FFPE) 转移肿瘤组织,以确定该组中的 NOTCH3 表达水平和分布 (图 7)。使用标准的定量 RT-PCR 操作规程,用 NOTCH3_A7 引物 / 探针组确定了 NOTCH3 基因表达。进行了 ANOVA 统计学分析以确定 NOTCH3 水平是否与包括样品年龄、性别、患者年龄等在内的因素相关。除了肝脏转移部位显示出显著性和更宽的 NOTCH3 基因表达分布外,未发现 NOTCH3 水平与任何这些因素相关。图 7 显示了所检验的所有转移肿瘤样品的 NOTCH3 基因表达的第 10、第 25、第 50、第 75 和第 90 百分位数。

[0232] 对来自作为采集来源的人肝脏和淋巴结转移胰腺癌组织以及来自异种移植物测定中所用的原发性人胰腺肿瘤的 NOTCH3 基因表达进行了归一化,以对数据进行比较。在每个数据组中,减去数据平均值,并除以标准偏差。灰色 (浅色) 点表示实施例 1 中所述的异种移植物测定中的对 OMP-59R5 与吉西他滨的联合治疗无响应的人胰腺肿瘤,黑色 (深色) 点表示在异种移植物测定中有响应的人胰腺肿瘤 (图 8)。响应性肿瘤显示出的 NOTCH3 基因表达水平高于非响应性肿瘤,这表明 NOTCH3 基因表达可用于预测胰腺肿瘤对例如 OMP-59R5 与化疗剂的联合治疗的体内响应性。图 8 还显示了所检验的人肝脏和淋巴结转移

胰腺癌组织的 NOTCH3 基因表达的第 10、第 25、第 50、第 75 和第 90 百分位数。

[0233] 实施例 6

[0234] 与吉西他滨和 ABRAXANE™联用的 OMP-59R5 抗 NOTCH2/3 抗体抑制胰腺肿瘤的体外生长

[0235] 将 20,000 个 OMP-PN8(NOTCH3 高表达）肿瘤细胞注射至 NOD-SCID 小鼠内。使肿瘤生长 26 天，直至它们达到 110mm^{3}的平均体积。将带有肿瘤的小鼠随机分为 3 组（每组 n ＝ 9 只小鼠），并用对照抗体、吉西他滨 +ABRAXANE™（白蛋白结合的紫杉醇）、或 OMP-59R5 抗 NOTCH2/3 抗体与吉西他滨 +ABRAXANE™的组合进行治疗。每隔一周以 40mg/kg 的剂量施用 OMP-59R5。每周以 10mg/kg 的剂量施用吉西他滨，且每周以 30mg/kg 的剂量施用 ABRAXANE™。在所指示的治疗后天数测量肿瘤体积。与吉西他滨 +ABRAXANE™联用的 OMP-59R5 强烈地抑制了 OMP-PN8 肿瘤的生长，并且比仅用吉西他滨 +ABRAXANE™时活性更高（图 9）。上部图和下部图显示了从同一实验中获得的具有不同的比例尺的数据。下部图仅显示了从活性治疗组获得的数据，而不是从对照治疗动物获得的数据。结果表明，NOTCH3 表达水平可用于预测胰腺肿瘤对 OMP-59R5 抗体与多种化疗剂的联合治疗的体内响应性。

[0236] 出于所有目的将本文中所引用的所有出版物、专利、专利申请、互联网网站和登录号 / 数据库序列（包括多核苷酸和多肽序列）都通过援引完整地并入本文中，其程度如同具体地单独表明将各个单独的出版物、专利、专利申请、互联网网站和登录号 / 数据库序列都这样并入本文中。

[0237] 序列

[0238] SEQ ID NO:1

[0239] HKGAL

[0240] SEQ ID NO:1

[0241] HEDAI

[0242] SEQ ID NO:3:59R1 重链 CDR1

[0243] SSSGMS

[0244] SEQ ID NO:4:59R1 重链 CDR2

[0245] VIASSGSNTYYADSVKG

[0246] SEQ ID NO:5:59R1 重链 CDR3

[0247] GIFFAI

[0248] SEQ ID NO:6:59R1 轻链 CDR1

[0249] RASQSVRSNYLA

[0250] SEQ ID NO:7:59R1 轻链 CDR2

[0251] GASSRAT

[0252] SEQ ID NO:8:59R1 轻链 CDR3

[0253] QQYSNFPI

[0254] SEQ ID NO:9:59R5 重链 CDR3

[0255] SIFYTT

[0256] SEQ ID NO:10（重链 CDR3 共有序列）:

[0257] (G/S)(I/S)F(F/Y)(A/P)(I/T/S/N)

[0258] SEQ ID NO:11(替代性重链 CDR3)

[0259] SIFYPT

[0260] SEQ ID NO:12(替代性重链 CDR3)

[0261] SSFFAS

[0262] SEQ ID NO:13(替代性重链 CDR3)

[0263] SSFYAS

[0264] SEQ ID NO:14(替代性重链 CDR3)

[0265] SSFFAT

[0266] SEQ ID NO:15(替代性重链 CDR3)

[0267] SIFYPS

[0268] SEQ ID NO:16(替代性重链 CDR3)

[0269] SSFFAN

[0270] SEQ ID NO:17:59R5 重链可变区

[0271] EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYTTWGQGTLVTVSSAST

[0272] SEQ ID NO:18:59R1 IgG 抗体的 59R1 重链 VH

[0273] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

[0274] SEQ ID NO:19:59R1 重链 VH+ 哺乳动物信号序列(下划线)

[0275] MKHLWFFLLLVAAPRWVLSQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

[0276] SEQ ID NO:20: 变体 59R1 重链可变区

[0277] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYPTWGQGTLVTVSSA

[0278] SEQ ID NO:21: 变体 59R1 重链可变区

[0279] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFFASWGQGTLVTVSSA

[0280] SEQ ID NO:22: 变体 59R1 重链可变区

[0281] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFYASWGQGTLVTVSSA

[0282] SEQ ID NO:23: 变体 59R1 重链可变区

[0283] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFFATWGQGTLVTVSSA

[0284] SEQ ID NO:24: 变体 59R1 重链可变区

[0285] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYPSWGQGTLVTVSSA

[0286] SEQ ID NO:25: 变体 59R1 重链可变区

[0287] QVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFFANWGQGTLVTVSSA

[0288] SEQ ID NO:26:59RGV 抗体（59R1 的种系变体）的 59R1 重链 VH

[0289] EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSA

[0290] SEQ ID NO:27:59RGV 抗体（59R1 的种系变体）的 59R1 轻链 VL

[0291] EIVLTQSPATLSLSPGERATLSCRRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

[0292] SEQ ID NO:28:59R1 轻链 VL+ 哺乳动物信号序列（下划线）

[0293] MVLQTQVFISLLLWISGAYGDIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

[0294] SEQ ID NO:29:59R1 IgG 抗体的 59R1 轻链 VL

[0295] DIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKR

[0296] SEQ ID NO:30:59R5 重链

[0297] EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSIFYTTWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0298] SEQ ID NO:31: 抗 NOTCH2/3 59R1 IgG2 重链的预测蛋白序列 + 信号序列，信号序列如下划线所示

[0299] MKHLWFFLLLVAAPRWVLSQVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0300] SEQ ID NO:32: 抗 NOTCH2/3 59RGV（59R1 的种系变体）的重链的预测蛋白序列 + 信号序列，信号序列如下划线所示

[0301] MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSSGMSWVRQAPGKGLEWVSVIASSGSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIFFAIWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0302] SEQ ID NO:33: 抗 NOTCH2/3 59R1 轻链的预测蛋白序列 + 信号序列，信号序列如下划线所示

[0303] MVLQTQVFISLLLWISGAYGDIVLTQSPATLSLSPGERATLSCRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0304] SEQ ID NO:34:抗NOTCH2/3 59RGV抗体(59R1的种系变体)的轻链的预测蛋白序列+信号序列,信号序列如下划线所示

[0305] MVLQTQVFISLLLWISGAYGEIVLTQSPATLSLSPGERATLSCRRASQSVRSNYLAWYQQKPGQAPRLLIYGASSRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNFPITFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0306] SEQ ID NO:35

[0307] AGGCAGAGTGGCGACCTC

[0308] SEQ ID NO:36

[0309] CGTCCACGTTCACTTCACAATTC

[0310] SEQ ID NO:37

[0311] AACCCAGGAAGACAGGCACAGTCGT

[0312] SEQ ID NO:38

[0313] CTGGGTTTGAGGGTCAGAAT

[0314] SEQ ID NO:39

[0315] GGGCACTGGCAGTTATAGGT

[0316] SEQ ID NO:40

[0317] TGACGCCATCCACGCATGTC

[0318] SEQ ID NO:41

[0319] TGCAGGATAGCAAGGAGGAGAC

[0320] SEQ ID NO:42

[0321] GCAGCTTGGCAGCCTCATAG

[0322] SEQ ID NO:43

[0323] CTCGCGGGCGGCCAGGAATAGGG

[0001]

关于微生物保藏的说明

申请人或代理人档案号 2293.102PC01	国际申请号 PCT/US2014/026094

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 6 页，第 4.5 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ATCC 美国典型培养物保藏中心	
保藏单位地址 （包括邮政编码和国名） 美利坚合众国弗吉尼亚州(20110-2209)马纳萨斯市大学路 10801	
保藏日期 2008-10-15	保藏号 PTA-9547
C.补充说明（必要时）	更多信息在附加页中 ☐
无	
D.本说明是为下列指定国作的（如果说明不是为所有指定国而作的）	
所有指定国	
E.补充说明（必要时）	
下列说明将随后向国际局提供（写出说明的类别，例如：“保藏的编号”） 无	

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

[0002]

微生物保藏(2)	
A.对说明书第 27 页，第 8.9 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ATCC 美国典型培养物保藏中心	
保藏单位地址 （包括邮政编码和国名） 美利坚合众国弗吉尼亚州(20110-2209)马纳萨斯市大学路 10801	
保藏日期 2009-7-6	保藏号 PTA-10170
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	

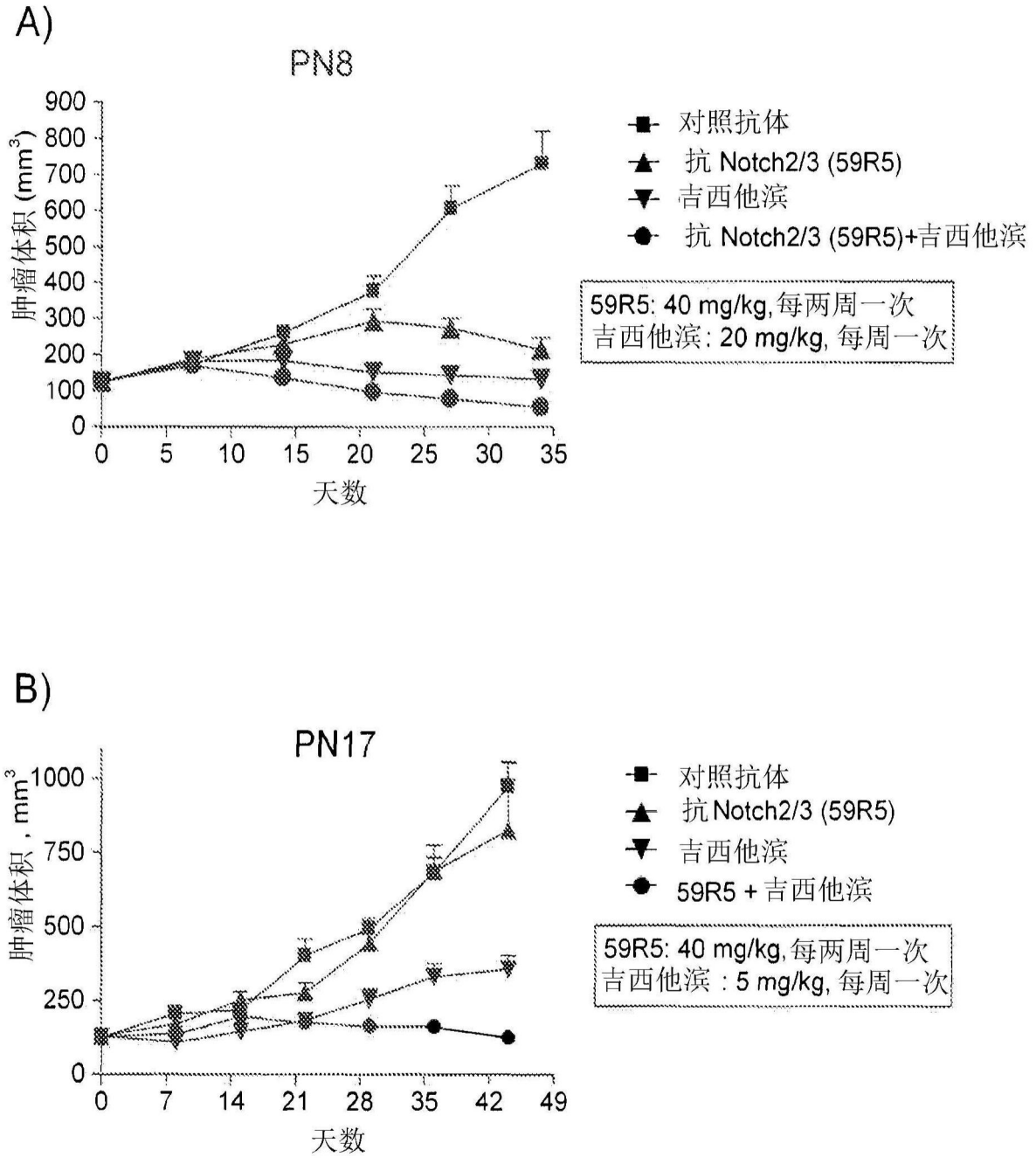

A)
PN8
肿瘤体积 (mm3)
900
800
700
600
500
400
300
200
100
0
0 5 10 15 20 25 30 35
天数
对照抗体
抗 Notch2/3 (59R5)
吉西他滨
抗 Notch2/3 (59R5)+吉西他滨
59R5: 40 mg/kg,每两周一次
吉西他滨: 20 mg/kg, 每周一次
B)
PN17
肿瘤体积，mm3
1000
750
500
250
0
0 7 14 21 28 35 42 49
天数
对照抗体
抗Notch2/3 (59R5)
吉西他滨
59R5 + 吉西他滨
59R5: 40 mg/kg,每两周一次
吉西他滨：5 mg/kg, 每周一次

图 1A-1B

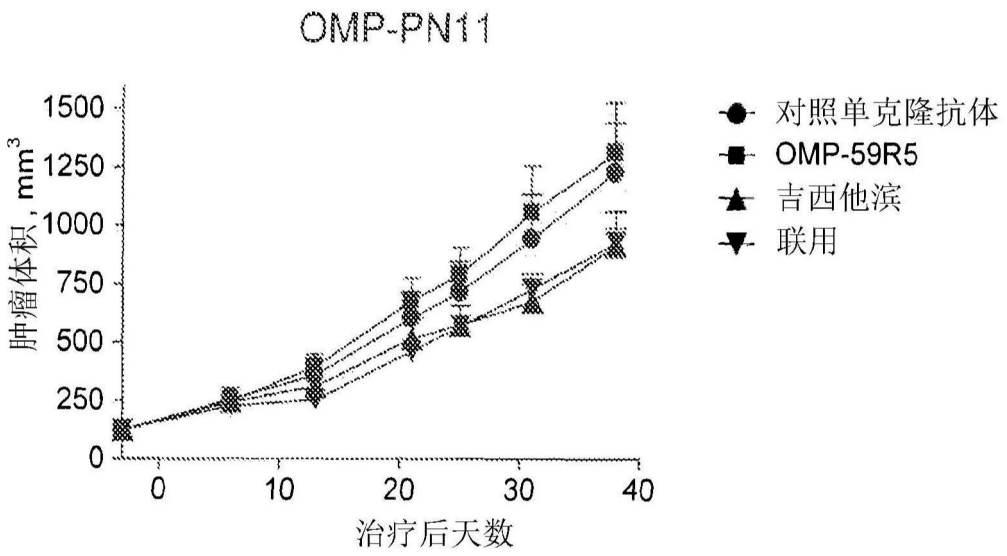

OMP-PN11
肿瘤体积, mm^3
1500
1250
1000
750
500
250
0
0
10
20
30
40
治疗后天数
对照单克隆抗体
OMP-59R5
吉西他滨
联用

图 1C

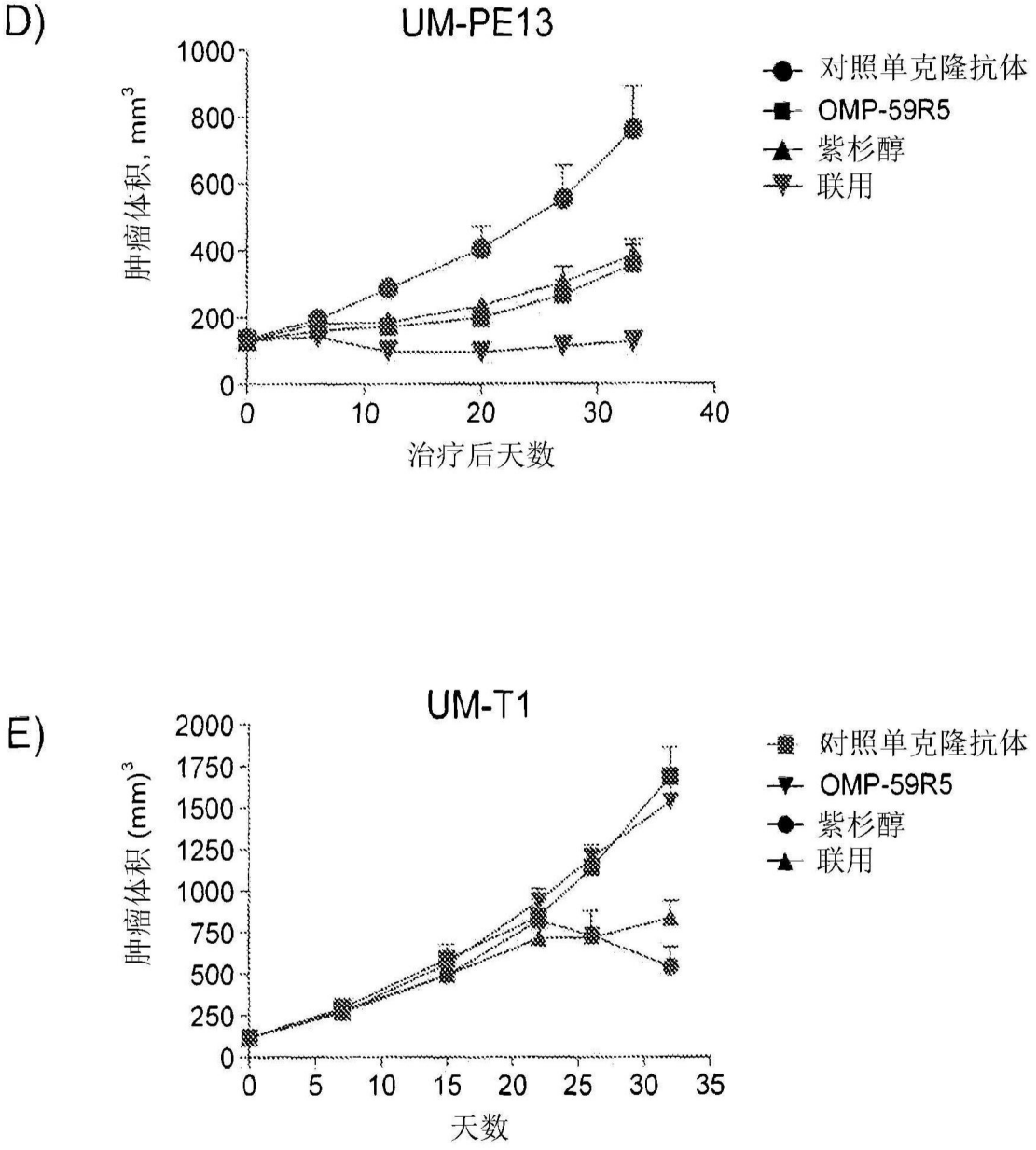

D)
UM-PE13
肿瘤体积, mm3
1000
800
600
400
200
0
0
10
20
30
40
治疗后天数
对照单克隆抗体
OMP-59R5
紫杉醇
联用
E)
UM-T1
肿瘤体积 (mm)3
2000
1750
1500
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35
天数
对照单克隆抗体
OMP-59R5
紫杉醇
联用

图 1D-1E

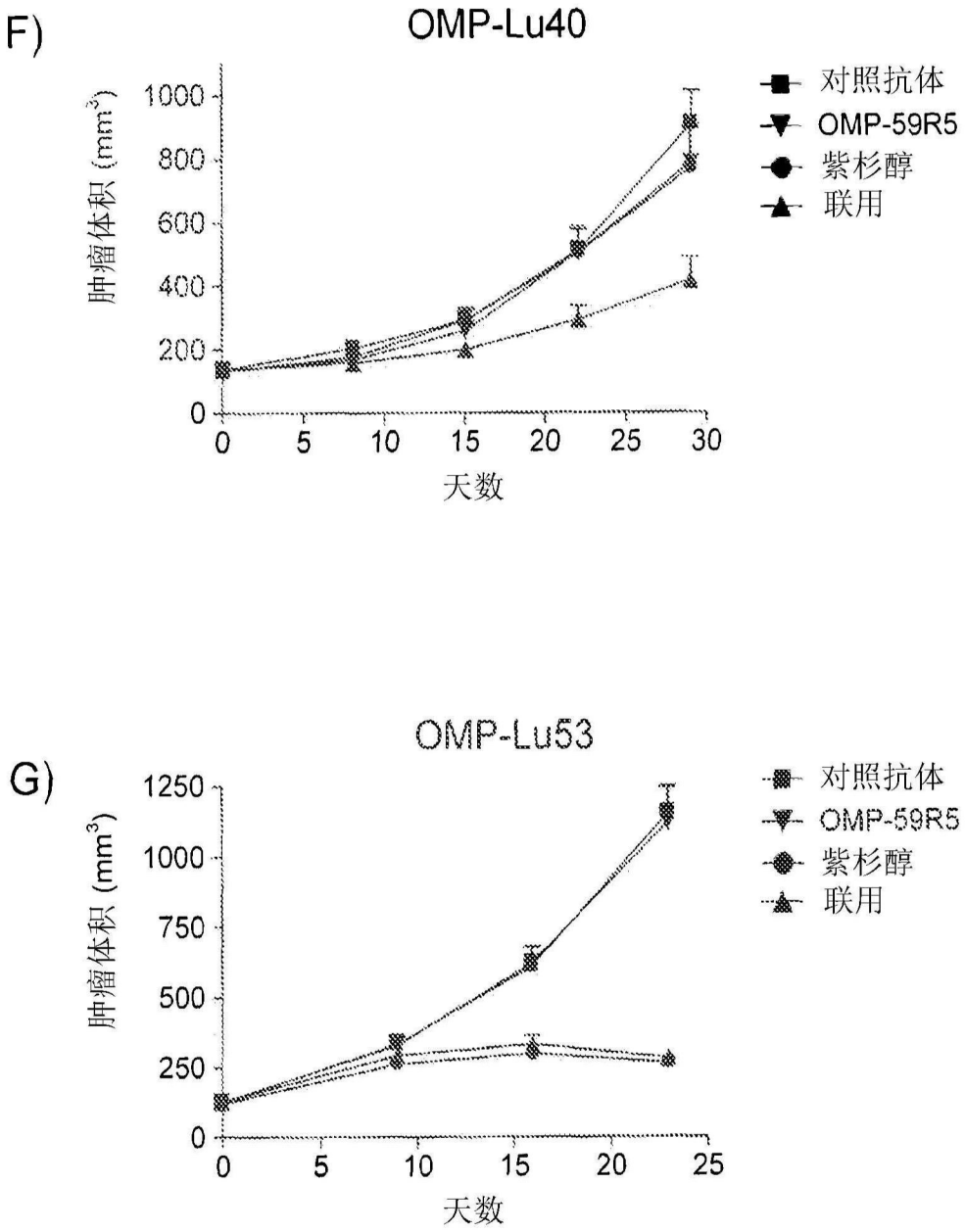

F)
OMP-Lu40
肿瘤体积 (mm³)
1000
800
600
400
200
0
0
5
10
15
20
25
30
天数
对照抗体
OMP-59R5
紫杉醇
联用
G)
OMP-Lu53
肿瘤体积 (mm³)
1250
1000
750
500
250
0
0
5
10
15
20
25
天数
对照抗体
OMP-59R5
紫杉醇
联用

图 1F-1G

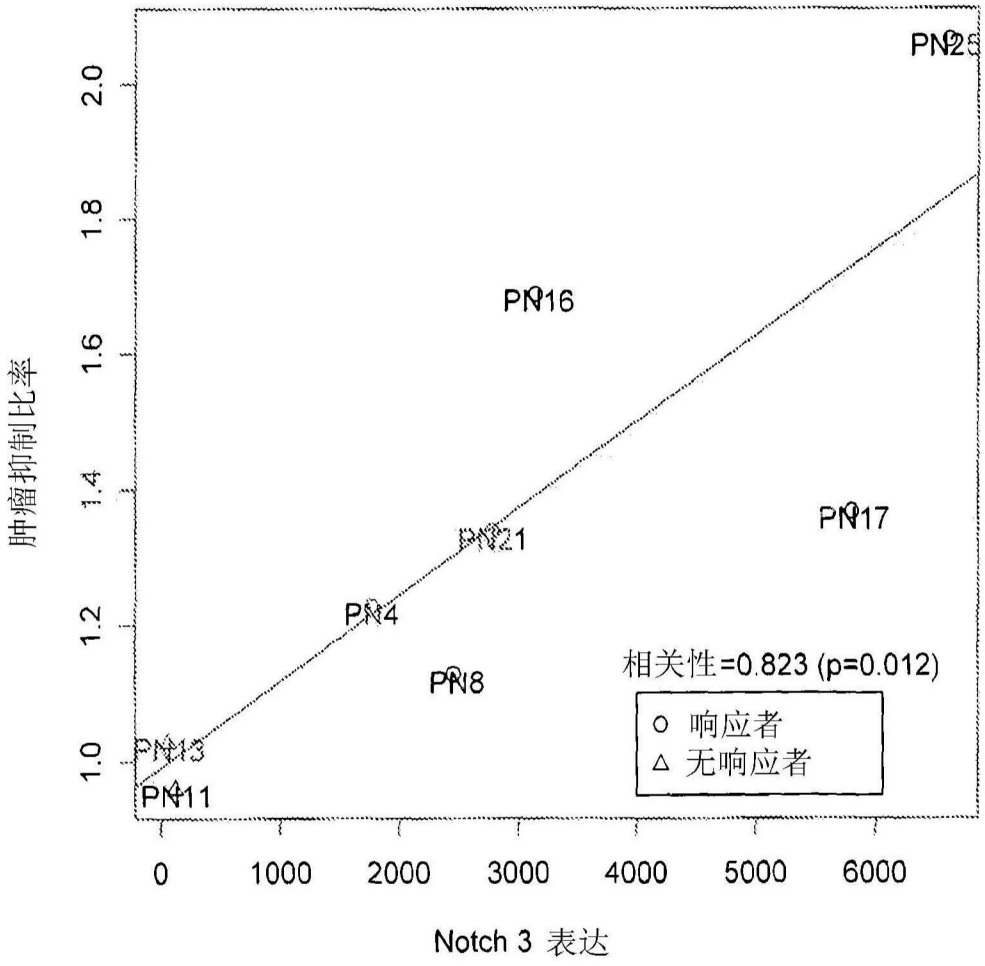

肿瘤抑制比率
2.0
1.8
1.6
1.4
1.2
1.0
0
1000
2000
3000
4000
5000
6000
Notch 3 表达
PN25
PN16
PN17
PN21
PN4
PN8
PN13
PN11
相关性=0.823 (p=0.012)
响应者
无响应者

图 2A

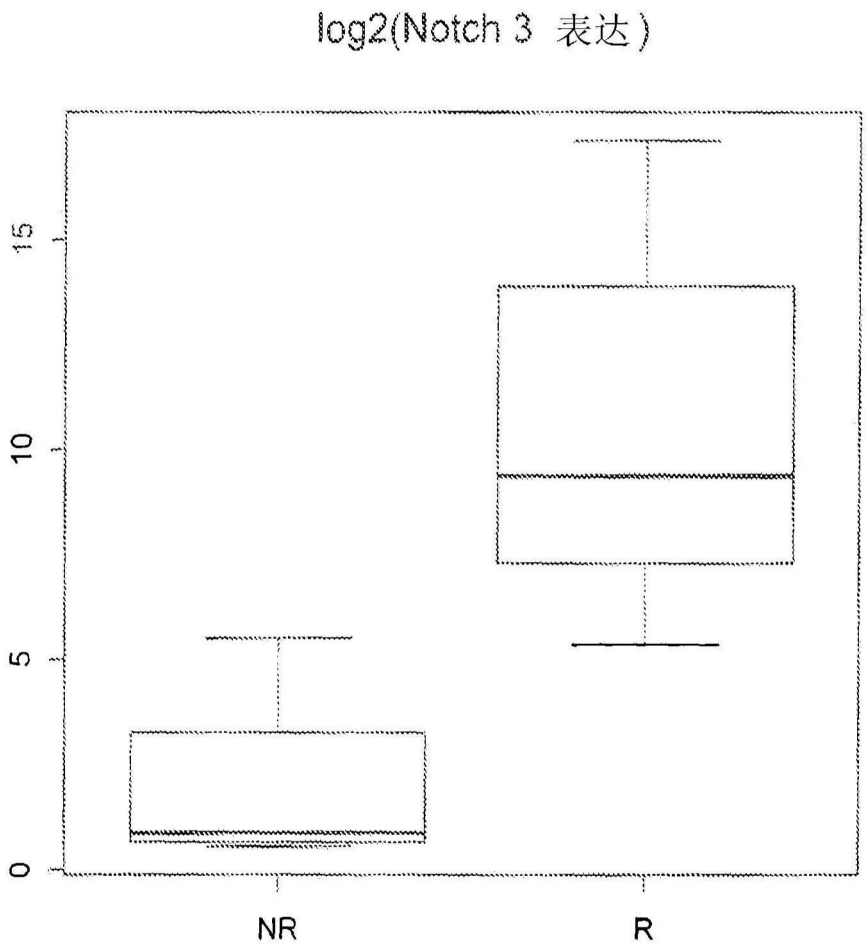
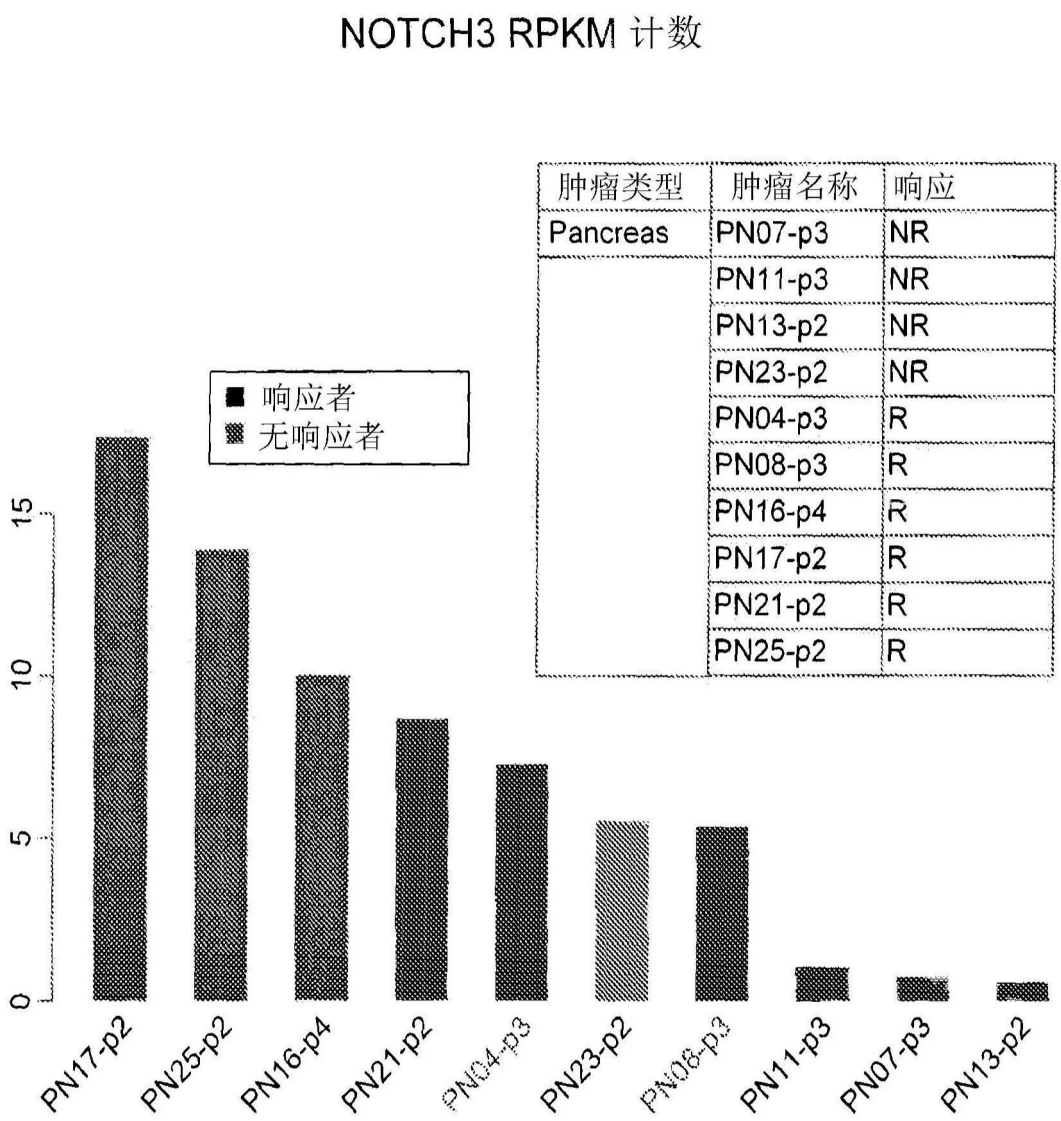


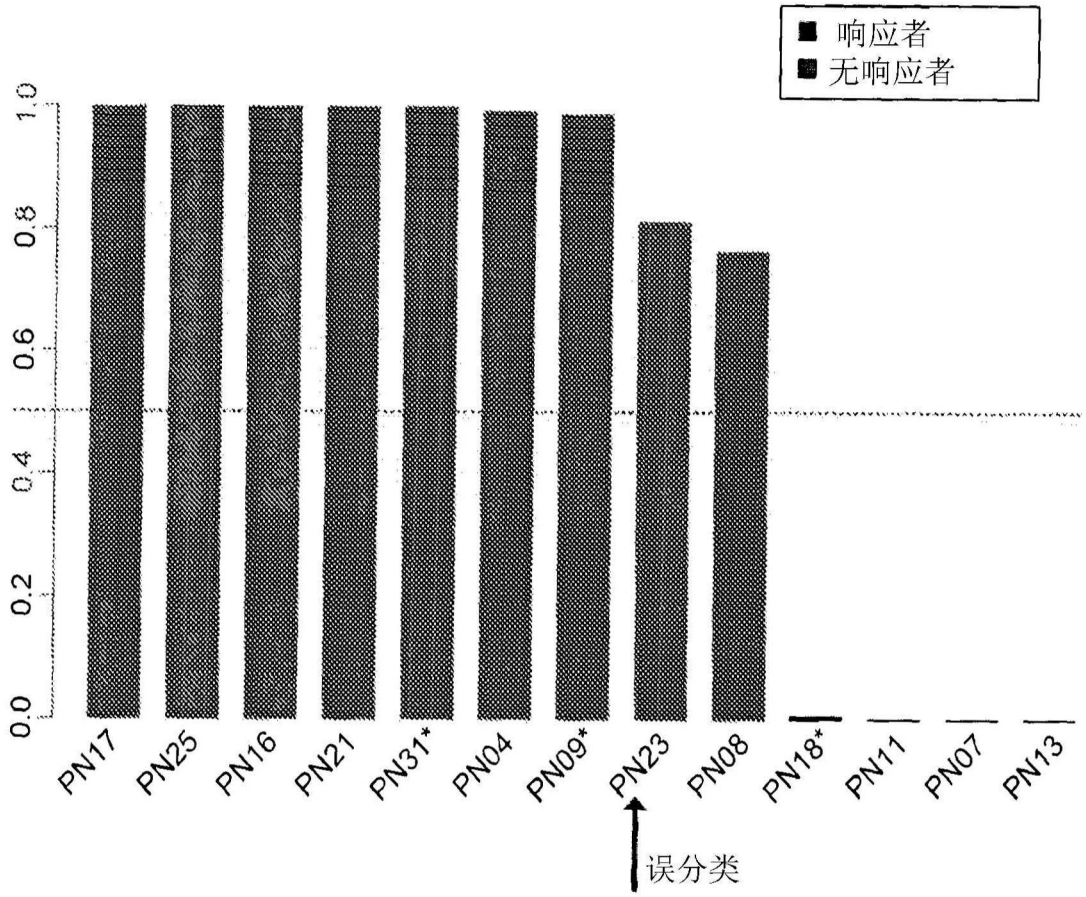
图 2B



肿瘤类型	肿瘤名称	响应
Pancreas	PN07-p3	NR
	PN11-p3	NR
	PN13-p2	NR
	PN23-p2	NR
	PN04-p3	R
	PN08-p3	R
	PN16-p4	R
	PN17-p2	R
	PN21-p2	R
	PN25-p2	R

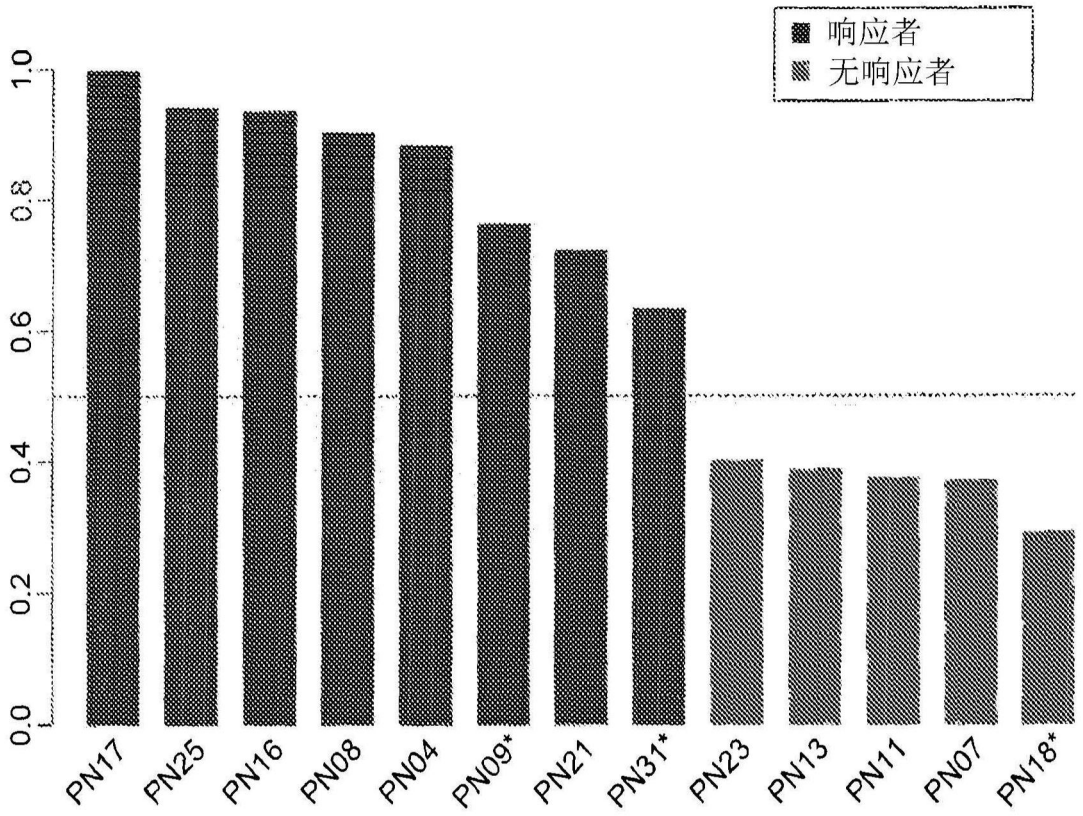
Notch3在R和NR之间的表达不同：p值=0.0086

图 3



分类器：逻辑回归
交叉验证的 PPV=83%, NPV=75%, SENS=83%, SPEC=75%
预测的响应者：PN31, PN09
预测的无响应者： PN18

图 4



预测的响应者：PN09, PN31
预测的无响应者：PN18
RNA-seq中交叉验证的PPV=NPV=SENS=SPEC=100%

图 5

胰腺肿瘤

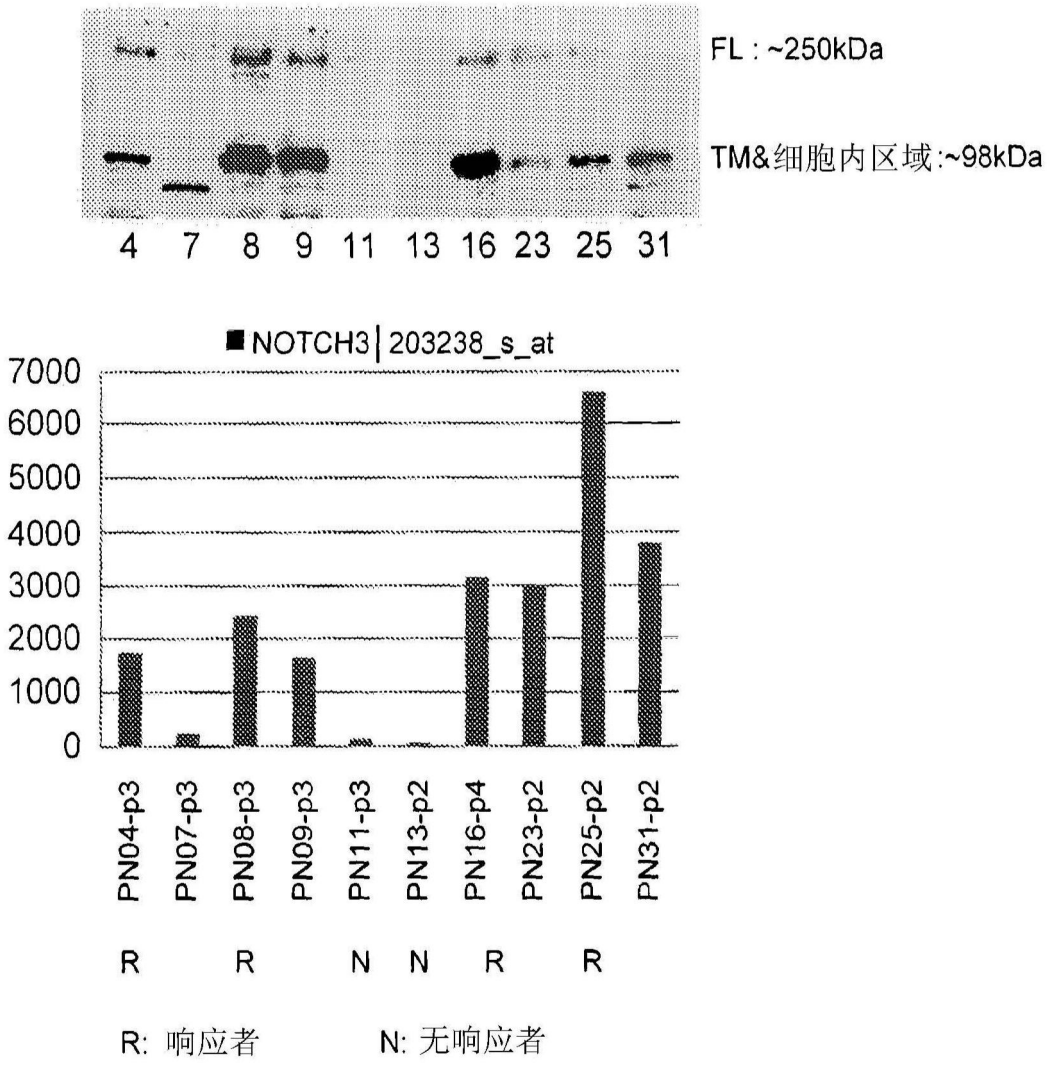

FL : ~250kDa
TM&细胞内区域:~98kDa
4 7 8 9 11 13 16 23 25 31
NOTCH3 | 203238_s_at
7000
6000
5000
4000
3000
2000
1000
0
PN04-p3
PN07-p3
PN08-p3
PN09-p3
PN11-p3
PN13-p2
PN16-p4
PN23-p2
PN25-p2
PN31-p2
R R N N R R
R: 响应者 N: 无响应者

图 6A

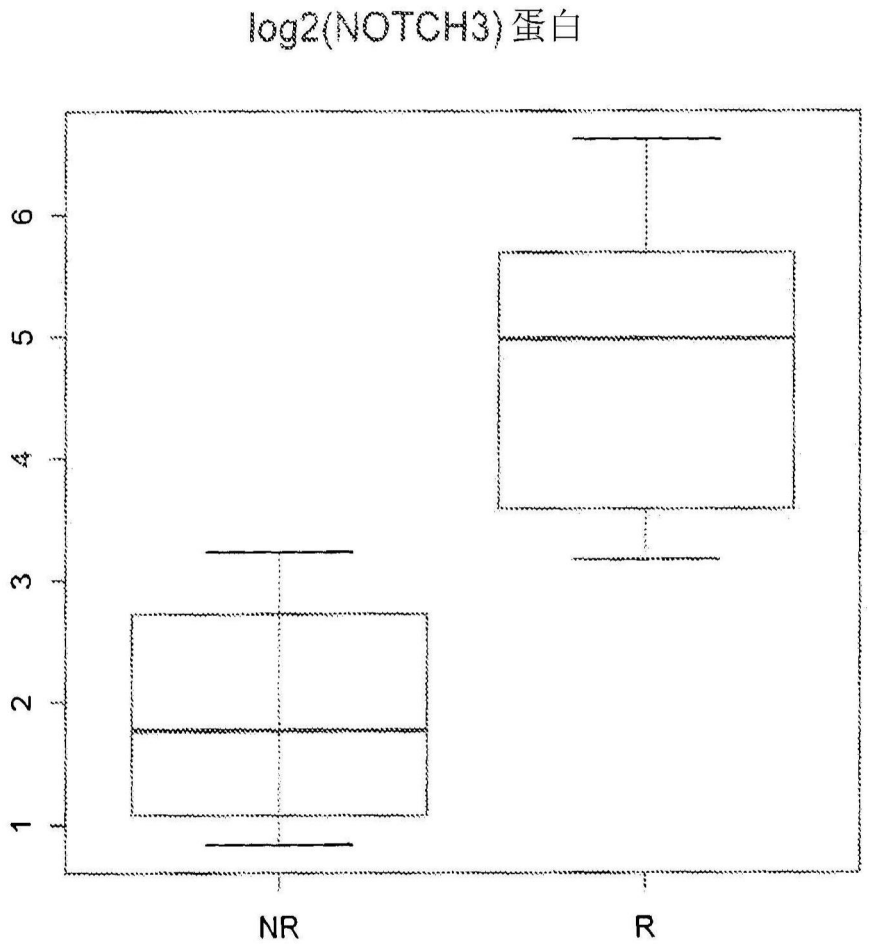


图 6B

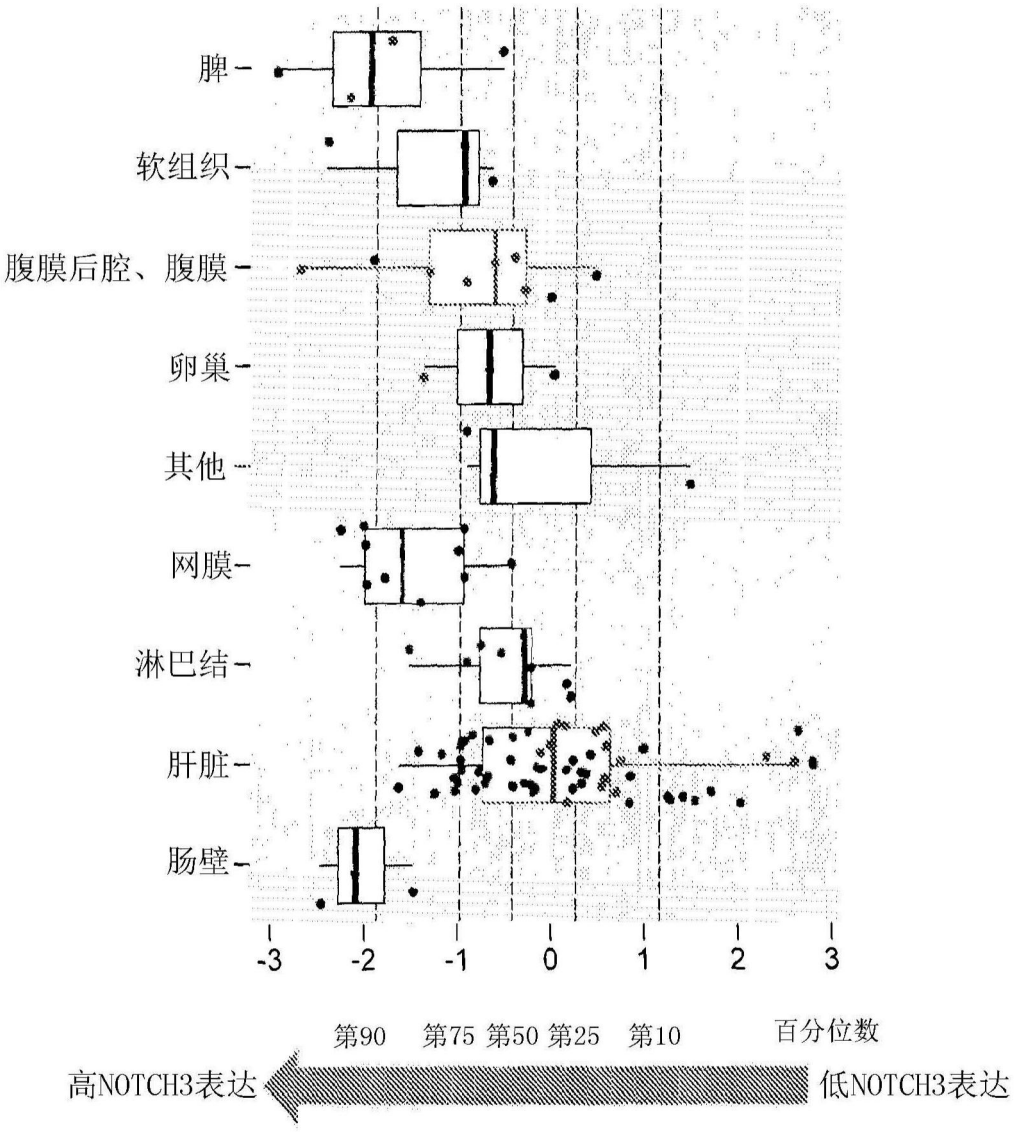

脾
软组织
腹膜后腔、腹膜
卵巢
其他
网膜
淋巴结
肝脏
肠壁
-3
-2
-1
0
1
2
3
第90
第75
第50
第25
第10
百分位数
高NOTCH3表达
低NOTCH3表达

图 7

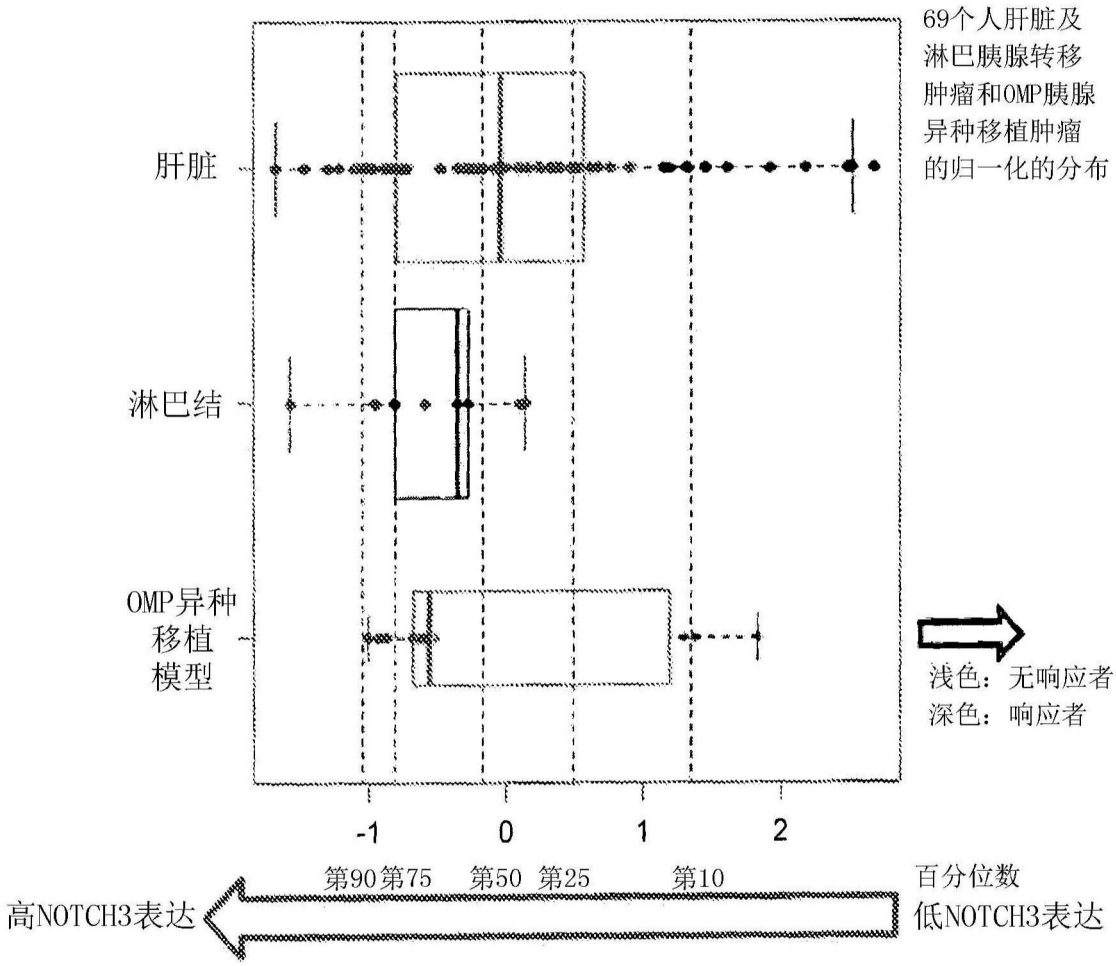
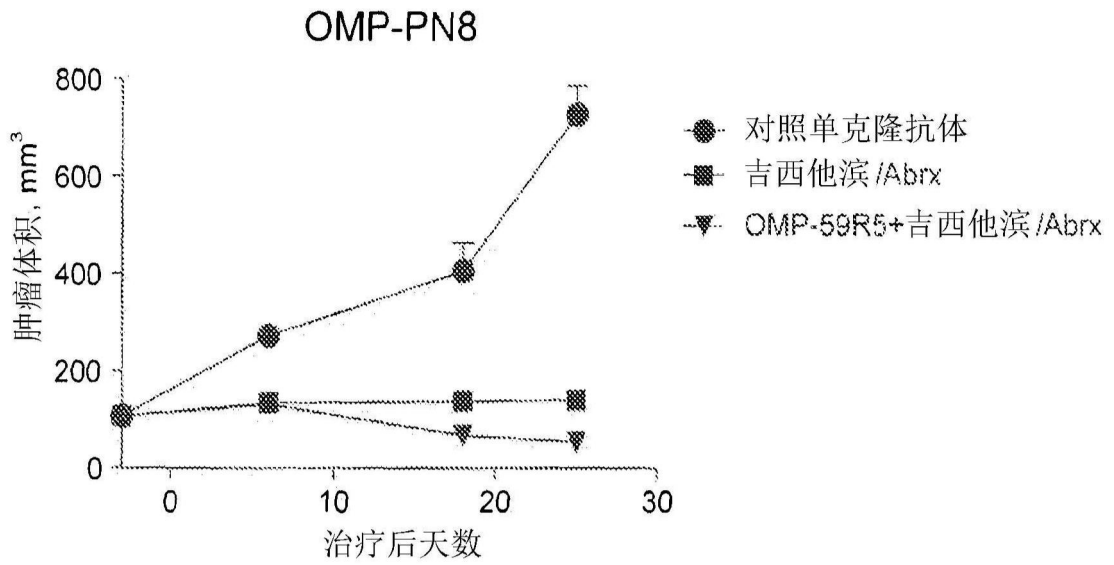

69个人肝脏及
淋巴胰腺转移
肿瘤和OMP胰腺
异种移植肿瘤
的归一化的分布
肝脏
淋巴结
OMP异种
移植
模型
浅色：无响应者
深色：响应者
-1
0
1
2
第90第75
第50
第25
第10
百分位数
高NOTCH3表达
低NOTCH3表达

图 8


OMP-PN8
肿瘤体积，mm3
800
600
400
200
0
0
10
20
30
治疗后天数
对照单克隆抗体
吉西他滨/Abrx
OMP-59R5+吉西他滨/Abrx

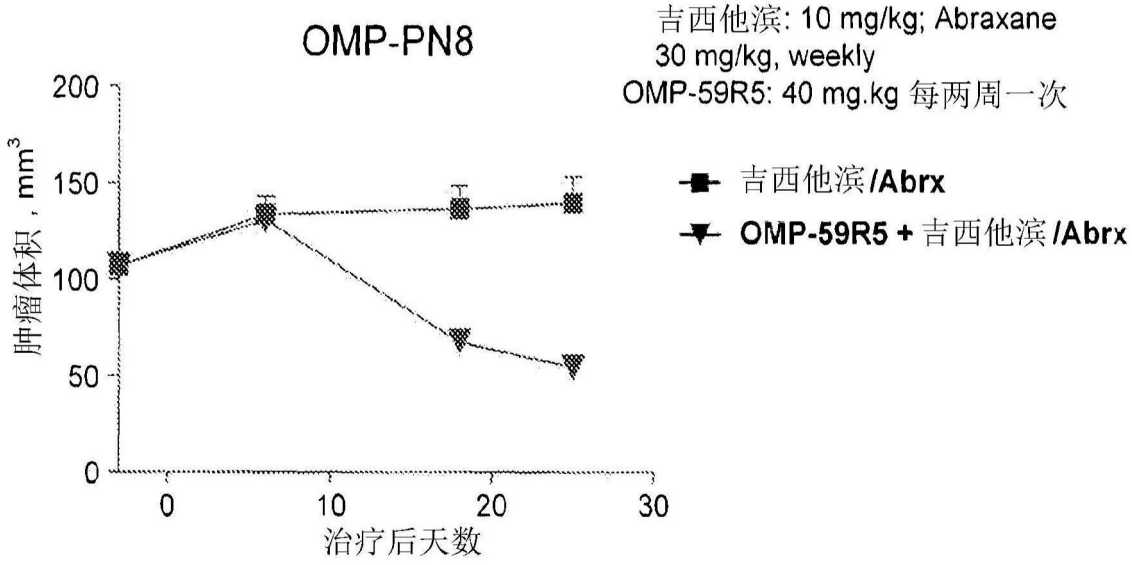

OMP-PN8
吉西他滨: 10 mg/kg; Abraxane
30 mg/kg, weekly
OMP-59R5: 40 mg.kg 每两周一次
肿瘤体积，mm3
200
150
100
50
0
0
10
20
30
治疗后天数
吉西他滨/Abrx
OMP-59R5 + 吉西他滨/Abrx

图 9

1.一种为了用NOTCH抑制剂进行治疗而选择胰腺癌患者的方法,所述方法包括:

(a)确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,和

(b)基于所述一种或多种生物标志物的表达水平选择患者。

2.一种确定被诊断为患有胰腺癌的患者是否可能对基于NOTCH抑制剂的疗法产生响应或者是否应当继续用NOTCH抑制剂进行治疗的方法,所述方法包括:确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3,并且所述一种或多种生物标志物的表达水平表明患者可能对疗法产生响应。

3.一种治疗患者的胰腺癌的方法,所述方法包括:

(a)确定来自所述患者的肿瘤细胞中的一种或多种生物标志物的表达水平,其中,所述一种或多种生物标志物包含NOTCH3;和

(b)向所述患者施用治疗有效量的NOTCH抑制剂。

4.如权利要求1～3中任一项所述的方法,其中,每种生物标志物经确定都以高于该生物标志物的参照水平的水平表达。

5.如权利要求1～4中任一项所述的方法,其中,一种或多种生物标志物的表达水平通过确定生物标志物mRNA或生物标志物蛋白的水平来确定。

6.如权利要求5所述的方法,其中,所述生物标志物mRNA水平通过定量聚合酶链式反应或通过阵列杂交来确定。

7.如权利要求6所述的方法,其中,所述生物标志物是NOTCH3且所述mRNA水平使用以下(a)、(b)和/或(c)来确定:

(a)核苷酸序列选自由SEQ ID NO:35、SEQ ID NO:38和SEQ ID NO:41组成的组的正向引物;

(b)核苷酸序列选自由SEQ ID NO:36、SEQ ID NO:39和SEQ ID NO:42组成的组的反向引物;和/或

(c)包含寡核苷酸的探针,所述寡核苷酸的核苷酸序列选自由SEQ ID NO:37、SEQ ID NO:40和SEQ ID NO:43组成的组。

8.如权利要求7所述的方法,其中,所述NOTCH3 mRNA水平使用以下(a)、(b)或(c)来确定:

(a)序列为SEQ ID NO:35的正向引物,序列为SEQ ID NO:36的反向引物,和包含序列为SEQ ID NO:37的寡核苷酸的探针;

(b)序列为SEQ ID NO:38的正向引物,序列为SEQ ID NO:39的反向引物,和包含序列为SEQ ID NO:40的寡核苷酸的探针;或

(c)序列为SEQ ID NO:41的正向引物,序列为SEQ ID NO:42的反向引物,和包含序列为SEQ ID NO:43的寡核苷酸的探针。

9.如权利要求1～8中任一项所述的方法,其中,所述一种或多种生物标志物还包括MAML2,并且MAML2的表达水平经确定高于MAML2表达的参照水平。

10.如权利要求1～9中任一项所述的方法,其中,生物标志物的参照水平是预定值或者该生物标志物在对照样品中的表达水平。

11.如权利要求1～10中任一项所述的方法,其中,NOTCH3的参照表达水平是胰腺癌

或胰腺癌亚类中的NOTCH3表达的第25、第30、第40、第50、第60、第70、第75或第80百分位数。

12.如权利要求1～11中任一项所述的方法，所述方法还包括从所述患者获得样品。

13.如权利要求12所述的方法，其中，所述样品是全血、血浆、血清或组织。

14.如权利要求12或13所述的方法，其中，所述样品是胰腺肿瘤样品。

15.如权利要求12～14中任一项所述的方法，其中，所述样品是福尔马林固定的石蜡包埋(FFPE)的组织。

16.如权利要求1、2或4～15中任一项所述的方法，所述方法还包括向所述患者施用NOTCH抑制剂。

17.如权利要求1～16中任一项所述的方法，其中，所述NOTCH抑制剂是γ分泌酶抑制剂或抗NOTCH抗体。

18.如权利要求17所述的方法，其中，所述抗NOTCH抗体特异性地结合人NOTCH2和/或人NOTCH3。

19.如权利要求18所述的方法，其中，所述抗NOTCH抗体由以PTA-9547保藏在ATCC的多核苷酸所编码。

20.如权利要求17所述的方法，其中，所述抗NOTCH抗体特异性地结合人NOTCH2和/或人NOTCH3，其中，所述抗体包含：

(a)包含SSSGMS(SEQ ID NO:3)的重链CDR1，包含VIASSGSNTYYADSVKG(SEQ ID NO:4)的重链CDR2，和包含SIFYTT(SEQ ID NO:9)或GIFFAI(SEQ ID NO:5)的重链CDR3；以及包含RASQSVRSNYLA(SEQ ID NO:6)的轻链CDR1，包含GASSRAT(SEQ ID NO:7)的轻链CDR2，和包含QQYSNFPI(SEQ ID NO:8)的轻链CDR3；

(b)与SEQ ID NO:17、SEQ ID NO:18或SEQ ID NO:26具有至少约90％序列同一性的重链可变区；以及与SEQ ID NO:29或SEQ ID NO:27具有至少约90％序列同一性的轻链可变区。

21.如权利要求17所述的方法，其中，所述抗NOTCH抗体与选自由以下抗体组成的组的抗体竞争对人NOTCH2和/或人NOTCH3的特异性结合：

(a)包含含有SEQ ID NO:17或SEQ ID NO:18的重链可变区和含有SEQ ID NO:29的轻链可变区的抗体；

(b)包含含有SSSGMS(SEQ ID NO:3)的重链CDR1、含有VIASSGSNTYYADSVKG(SEQ ID NO:4)的重链CDR2和含有SIFYTT(SEQ ID NO:9)的重链CDR3以及含有RASQSVRSNYLA(SEQ ID NO:6)的轻链CDR1、含有GASSRAT(SEQ ID NO:7)的轻链CDR2和含有QQYSNFPI(SEQ ID NO:8)的轻链CDR3的抗体；和

(c)由以PTA-9547保藏在ATCC的多核苷酸所编码的抗体。

22.如权利要求17～21中任一项所述的方法，其中，所述抗NOTCH抗体是嵌合抗体、人源化抗体、人抗体或抗体片段。

23.如权利要求3或16～22中任一项所述的方法，所述方法还包括施用第二治疗剂；可选地，其中所述第二治疗剂是化疗剂、核苷类似物或有丝分裂抑制剂。

24.一种包含分离的多核苷酸的诊断用组合物，所述多核苷酸包含选自由SEQ ID NO:35～43组成的组的序列。

25.如权利要求24所述的诊断用组合物，所述诊断用组合物包含：

(a)序列为SEQ ID NO:35的多核苷酸，序列为SEQ ID NO:36的多核苷酸，和序列为SEQ ID NO:37的多核苷酸；

(b)序列为SEQ ID NO:38的多核苷酸，序列为SEQ ID NO:39的多核苷酸，和序列为SEQ ID NO:40的多核苷酸；或

(c)序列为SEQ ID NO:41的多核苷酸，序列为SEQ ID NO:42的多核苷酸，和序列为SEQ ID NO:43的多核苷酸。

26.一种检测样品中的NOTCH3 mRNA的方法，所述方法包括使所述样品接触包含选自由SEQ ID NO:35～43组成的组的序列的多核苷酸。

27.如权利要求26所述的方法，所述方法包括使所述样品接触：

(a)序列为SEQ ID NO:35的正向引物，序列为SEQ ID NO:36的反向引物，和包含序列为SEQ ID NO:37的寡核苷酸的探针；

(b)序列为SEQ ID NO:38的正向引物，序列为SEQ ID NO:39的反向引物，和包含序列为SEQ ID NO:40的寡核苷酸的探针；或

(c)序列为SEQ ID NO:41的正向引物，序列为SEQ ID NO:42的反向引物，和包含序列为SEQ ID NO:43的寡核苷酸的探针。

28.一种用于检测样品中的NOTCH3 mRNA的试剂盒，所述试剂盒包含：包含选自由SEQ ID NO:35～43组成的组的序列的多核苷酸。

29.如权利要求28所述的试剂盒，所述试剂盒包含：

(a)序列为SEQ ID NO:35的多核苷酸，序列为SEQ ID NO:36的多核苷酸，和序列为SEQ ID NO:37的多核苷酸；

(b)序列为SEQ ID NO:38的多核苷酸，序列为SEQ ID NO:39的多核苷酸，和序列为SEQ ID NO:40的多核苷酸；或

(c)序列为SEQ ID NO:41的多核苷酸，序列为SEQ ID NO:42的多核苷酸，和序列为SEQ ID NO:43的多核苷酸。

30.一种引物，其序列选自由SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:38、SEQ ID NO:39、SEQ ID NO:41和SEQ ID NO:42组成的组。

31.一种包含寡核苷酸的探针，所述寡核苷酸的序列选自由SEQ ID NO:37、SEQ ID NO:40和SEQ ID NO:43组成的组。

Abstract

Novel methods of treating pancreatic cancer are provided. In one embodiment, the method comprises determining NOTCH mRNA expression levels in pancreatic cancer cells. In another embodiment, the method further comprises administering to a subject in need thereof a therapeutically effective dose of a NOTCH antagonist..