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Compositions and methods for diagnosis and treatment of hepatic cancers

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(54) Title: COMPOSITIONS AND METHODS FOR DIAGNOSIS AND TREATMENT OF HEPATIC CANCERS

(57) Abstract: The present invention relates to methods of treating liver cancer using a Notch signaling inhibitor. Compositions and
methods for the treatment of liver cancers are also provided.

COMPOSITIONS AND METHODS FOR DIAGNOSIS AND TREATMENT OF HEPATIC CANCERS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/789,475,

5   filed March 15, 2013, the disclosure of which is incorporated herein by reference as if set forth

in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which is hereby incorporated by

reference in its entirety.

FIELD OF THE INVENTION

10

The present invention relates generally to the field of molecular biology. More

specifically, the invention relates to methods of treatment of pathological hepatic conditions,

such as cancer.

BACKGROUND

15   Liver cancer is the fifth most common form of cancer. Each year, approximately

750,000 cases are diagnosed and about 700,000 people die from the disease each year, making

it the third most common cause of cancer death in the world (Ferlay et al., Int. J. Cancer

127:2893-2917 (2010)). In the United States, the incidence of primary liver cancer has been

rising, and while some progress has been made in detecting and treating localized disease, the

20   five year survival rate for late stage liver cancer is still well below 10% (American-Cancer-

Society. 2012. Cancer Facts & Figures 2012. Atlanta: American Cancer Society).

Established treatments for liver cancer include surgical removal of the part of the liver

containing the tumor (partial hepatectomy), liver transplantation, transcatheter arterial

chemoembolization (TACE), in situ tumor destruction by various methods such as

25   radiofrequency ablation (RFA) or cryosurgery and administration of Sorafenib. Treatment

options for late stage liver patients are limited. Thus, effective treatments of liver cancer

remains a significant unmet medical need.

The role of Notch signaling in liver cancer is not well understood. Qi et al. report that

Notch1 signaling inhibits growth of human hepatocellular carcinoma cells in vitro and in

30   vivo by inducing cell cycle arrest and apoptosis (Qi et al., Cancer Res. 63:8323 (2003))

and Viatour et al. report that expression of Notch1 intracellular domain decreased

proliferation and induced apoptosis in murine and human HCC cells (Viatour et al., J. Exp. Med. 208(10):1963 (2011)). Others report that Notch1 small interfering RNA (siRNA) reduced cell invasion and migration but not viability (Zhou et al. Dig. Dis. Sci.). Yet others report that inhibition of individual Notch pathway family members had no effect. Taken together, the Notch pathway's role in liver cancer was not well understood.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

SUMMARY

Use of Notch2 signaling inhibitors for the treatment of patients having or at risk of having proliferative disorders of the liver is disclosed.

A first aspect provides a method of treating a liver cancer in an individual in need thereof, the method comprising the step of administering to the individual an effective amount of a Notch2 signaling inhibitor, wherein the liver cancer comprises cells that express EpCAM and/or alphafetoprotein (AFP), and wherein the Notch2 signaling inhibitor is selected from an anti-Jag1 antibody and an anti-Notch2 antibody.

A second aspect provides use of a Notch2 signaling inhibitor in the manufacture of a medicament for treating a liver cancer in an individual in need thereof, wherein the liver cancer comprises cells that express EpCAM and/or alphafetoprotein (AFP), and wherein the Notch2 signaling inhibitor is selected from an anti-Jag1 antibody and an anti-Notch2 antibody.

Also disclosed are methods of treating a liver cancer in an individual in need thereof, comprising the step of administering to the individual an effective amount of a Notch2 signaling inhibitor. In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma, or metastatic liver cancer. In some embodiments, the hepatocellular carcinoma comprises progenitor-like or cholangiocarcinoma-like liver tumor. In some embodiments, the liver cancer is a refractory cancer.

In some embodiments, the method further comprises administering at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to chemotherapeutic agent and an antibody.

In some embodiments, the liver cancer comprises cells that express EpCAM, AFP, Notch2, Jag1, Notch2 and Jag1, nuclear Notch2 ICD, Sox9, CK19, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1, Aurkb, Wnt2, Axin2, or Glul, or any combination thereof. In specific

embodiments, the liver cancer comprises cells that are AFP^+ $EpCAM^+$. In some embodiments, the liver cancer comprises cells that are AFP^+ $EpCAM^-$, AFP^+ $EpCAM^+$ $SPP1^+$, AFP^- $EpCAM^+$, AFP^- $EpCAM^+$ $Notch2^+$, AFP^+ $EpCAM^-$ $Notch2^+$, AFP^+ $EpCAM^+$ $Sox9^+$ and AFP^+ $EpCAM^+$ $Sox9^+$ or AFP^- $EpCAM^+$ $SPP1^+$. Liver cancers that comprise cells with alternative combinations of marker expression are specifically contemplated.

In some embodiments, at least one of EpCAM, AFP, Notch2, Jag1, Sox9, CK19, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1, Aurkb, Wnt2, Axin2, or Glul protein expression was determined in a sample from the individual using immunohistochemistry (IHC). In some embodiments, expression is nucleic acid expression. In some embodiments, expression is determined by a method selected from the group consisting of RNAseq, microarray analysis, immunohistochemistry, enzyme-linked immunosorbent assay, gene expression profiling, polymerase chain reaction, SAGE, MassARRAY technique, fluorescent in situ hybridization and Western blotting.

In some embodiments, administering the Notch2 signaling inhibitor results in a decrease in the expression in the liver cancer of at least one of EpCAM, AFP, Notch2, Notch2 ICD, Jag1, Prom1, Spp1, FoxM1, Plk1, ccnb1 and Aurkb. In some embodiments, administering the Notch2 signaling inhibitor results in an increase in the expression in the liver cancer of at least one of Wnt2, Axin2 and Glul. In some embodiments, expression is determined by RNAseq, microarray analysis, immunohistochemistry, enzyme-linked immunosorbent assay, and Western blotting.

Any of the antibodies of the above embodiments may be a full-length IgG1 or IgG2a antibody. In some embodiments, the antibody causes cancer cell death, e.g., liver cancer cell death. Any of the antibodies in the above embodiments may be conjugated to a growth inhibitory agent, e.g., a cytotoxic agent. Examples of cytotoxic agent include, but are not limited to, toxins, antibiotics, radioactive isotopes and nucleolytic enzymes. Any of the antibodies in the above embodiments may be produced by known methods in the art, e.g., in bacteria or in CHO cells.

Also disclosed are methods for preventing liver cancer in an individual at risk of having liver cancer, comprising the step of administering to the individual an effective amount of a Notch2 signaling inhibitor. In some embodiments, the individual has a liver condition selected from the group consisting of hepatitis B or C, cirrhosis of the liver, non-viral/non-alcoholic steatohepatitis, benign liver tumors, hemangiomas, hepatic adenomas, and focal nodular hyperplasia. In some embodiments, the Notch2 signaling inhibitor is an anti-Jag1 antibody, e.g., an anti-Jag1 antagonist antibody.

Also disclosed are methods for inhibiting growth of a cell that expresses secreted phosphoprotein1 (SPP1), comprising contacting the cell with a Notch2 signaling inhibitor, thereby inhibiting growth of the cell. In one embodiment, SPP1 protein comprises the amino acid sequence shown in FIG. 11. In one embodiment, contacting the cell with the Notch2 signaling inhibitor reduces SPP1 expression in the cell. For example, contacting the cell with the Notch2 signaling inhibitor reduces SPP1 expression in the cell by at least about 50%, 60%, 70%, 80%, 90%, or 100%. The expression of SPP1 mRNA or protein can be determined by any method in the art. In some embodiments, the Notch2 signaling inhibitor is an anti-Notch2 antibody, e.g., an anti-Notch2 negative regulatory region (NRR) antibody, such as any anti-Notch2 NRR antibody disclosed herein. In some embodiments, the antibody is an anti-Jag1 antibody, such as any anti-Jag1 antibody disclosed herein. In some embodiments, the cell is a liver cancer cell. In some embodiments, the liver cancer cell expresses EpCAM, AFP, AFP and EpCAM, Notch2, Jag1, Notch2 and Jag1, nuclear Notch2 ICD, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1, Aurkb, Wnt2, Axin2, or Glul, or any combination thereof. In some embodiments, contacting the cell with the Notch2 signaling inhibitor results in a decrease in the expression in the cell of at least one of EpCAM, AFP, Notch2, Notch2 ICD, Jag1, Prom1, Spp1, FoxM1, Plk1, ccnb1 and Aurkb. In some embodiments, administering the Notch2 signaling inhibitor results in an increase in the expression in the cell of at least one of Wnt2, Axin2 and Glul. In some embodiments, expression is determined by RNAseq, microarray analysis, immunohistochemistry, enzyme-linked immunosorbent assay, and Western blotting.

Also disclosed are methods for inhibiting proliferation of a cell that expresses secreted phosphoprotein1 (SPP1), comprising contacting the cell with a Notch2 signaling inhibitor, thereby inhibiting proliferation of the cell.

Also disclosed are methods for treating a mammal having a liver cancer comprising cells that express a Spp1 gene encoding a peptide comprising an amino acid sequence having at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to the polypeptide shown in FIG. 11, comprising administering to the mammal an effective amount of a Notch2 signaling inhibitor, thereby effectively treating the mammal. In some embodiments, the cells express a SPP1 protein comprising an amino acid sequence having at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to the polypeptide shown in FIG. 11.

In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma, or metastatic liver cancer. In some embodiments, the liver cancer is a refractory cancer. Any of the

antibodies herein may be a full-length IgG1 or IgG2a antibody. In some embodiments, the antibody causes cancer cell death, e.g., liver cell death. Any of the herein may be conjugated to a growth inhibitory agent, e.g., a cytotoxic agent. Examples of cytotoxic agent include, but are not limited to, toxins, antibiotics, radioactive isotopes and nucleolytic enzymes. Any of the antibodies herein may be produced by known methods in the art, e.g., in bacteria or in CHO cells.

In some embodiments, the method further comprises administering at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to chemotherapeutic agent and an antibody.

In some embodiments, the liver cancer comprises cells that express EpCAM, AFP, AFP and EpCAM, Notch2, Jag1, Notch2 and Jag1, nuclear Notch2 ICD, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1, Aurkb, Wnt2, Axin2, or Glul, or any combination thereof. In some embodiments, administering the Notch2 signaling inhibitor results in a decrease in the expression in the liver cancer of at least one of EpCAM, AFP, Notch2, Notch2 ICD, Jag1, Prom1, Spp1, FoxM1, Plk1, ccnb1 and Aurkb. In some embodiments, administering the Notch2 signaling inhibitor results in an increase in the expression in the liver cancer of at least one of Wnt2, Axin2 and Glul. In some embodiments, expression is determined by RNAseq, microarray analysis, immunohistochemistry, enzyme-linked immunosorbent assay, and Western blotting.

Also disclosed are methods for treating a liver cell proliferative disorder associated with increased expression or activity of a protein having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% amino acid sequence identity to the polypeptide shown in FIG. 8C, comprising administering to an individual in need of such treatment an effective amount of an anti-Jag1 antagonist antibody, thereby effectively treating the liver cell proliferative disorder. In some embodiments, the cell proliferative disorder is a cancer, such as liver cancer. In some embodiments the individual has a liver condition selected from the group consisting of hepatitis B or C, cirrhosis of the liver, benign liver tumors, hemangiomas, hepatic adenomas, and focal nodular hyperplasia.

In certain embodiments, the anti-Jag1 antibody is any of the anti-Jag1 antibodies described herein. In certain embodiments, the anti-Jag1 antibody is a human, humanized, or chimeric antibody. In certain embodiments, any of the antibodies of the above embodiments is an antibody fragment.

Also disclosed are methods for treating a liver cell proliferative disorder associated with increased expression or activity of a protein having at least 90% amino acid sequence identity to the polypeptide shown in FIG. 11, comprising administering to an individual in need of such

treatment an effective amount of an anti-Jag1 antagonist antibody, thereby effectively treating the liver cell proliferative disorder. In some embodiments, the cell proliferative disorder is a cancer, such as liver cancer. In some embodiments the individual has a liver condition selected from the group consisting of hepatitis B or C, cirrhosis of the liver, benign liver tumors, hemangiomas, hepatic adenomas, and focal nodular hyperplasia.

Also disclosed are methods for reducing serum SPP1 protein levels in an individual, the method comprising administering to the individual an effective amount of a Notch2 signaling inhibitor thereby reducing serum SPP1 levels in the individual. In one embodiment, the individual has a liver cancer. In one embodiment, the serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor to the individual are at least about 80 ng/ml. In certain embodiments, the serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor to the individual are between about 80 ng/ml and about 500 ng/ml, between about 86 ng/ml and about 250 ng/ml, between about 120 ng/ml and about 170 ng/ml, or about 165 ng/ml. In some embodiments, administering the Notch2 signaling inhibitor to the individual results in serum SPP1 protein levels of less than 80 ng/ml. In specific embodiments, serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor are 24 hours prior to administering the Notch2 signaling inhibitor. Serum SPP1 protein levels prior to or following administration of the Notch2 signaling inhibitor may be determined by any appropriate method, such as enzyme-linked immunosorbent assay. In specific embodiments, serum SPP1 protein levels are reduced about one, two, three, six or 12 month after administering the Notch2 signaling inhibitor. In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma or metastatic liver cancer. In some embodiments, the Notch2 signaling inhibitor is an siRNA, small-molecule inhibitor or antibody. In some embodiments, the antibody is an antagonist antibody.

In certain embodiments, any of the antibodies of the above embodiments is a monoclonal antibody. In certain embodiments, any of the antibodies of the above embodiments is a human, humanized, or chimeric antibody. In certain embodiments, any of the antibodies of the above embodiments is an antibody fragment.

In some embodiments, the method further comprises administering at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to chemotherapeutic agent and an antibody. In some embodiments, Sorafenib is an additional therapeutic agent.

In some embodiments, the liver cancer comprises cells that express EpCAM, AFP, AFP and EpCAM, Notch2, Jag1, Notch2 and Jag1, nuclear Notch2 ICD, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1, Aurkb, Wnt2, Axin2, or Glul, or any combination thereof. In some embodiments, Ras is a mutant Ras. In some embodiments, administering the Notch2 signaling inhibitor results in a decrease in the expression in the liver cancer of at least one of EpCAM, AFP, Notch2, Notch2 ICD, Jag1, Prom1, Spp1, FoxM1, Plk1, ccnb1 and Aurkb. In some embodiments, administering the Notch2 signaling inhibitor results in an increase in the expression in the liver cancer of at least one of Wnt2, Axin2 and Glul. In some embodiments, expression is determined by RNAseq, microarray analysis, immunohistochemistry, enzyme-linked immunosorbent assay, and Western blotting.

Also disclosed are methods for treating a liver tumor in a mammal, wherein the growth of the liver tumor is at least in part dependent upon a growth potentiating effect of Notch2 signaling, comprising contacting the tumor with an antibody that binds to Notch2 or Jag1. In one embodiment, binding of the antibody to the tumor antagonizes the growth-potentiating activity of Notch2. In some embodiments, the mammal is a human.

Also disclosed are methods for treating of liver cancer comprising administering to an individual who has elevated serum SPP1 protein levels an effective amount of a Notch2 signaling inhibitor. In one embodiment, the serum SPP1 protein levels of the individual are at least about 80 ng/ml. In certain embodiments, the serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor to the individual are between about 80 ng/ml and about 500 ng/ml, between about 86 ng/ml and about 250 ng/ml, between about 120 ng/ml and about 170 ng/ml, or about 165 ng/ml. In some embodiments, administering the Notch2 signaling inhibitor to the individual results in serum SPP1 protein levels of less than 80 ng/ml. In specific embodiments, serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor are 24 hours prior to administering the Notch2 signaling inhibitor. Serum SPP1 protein levels prior to or following administration of the Notch2 signaling inhibitor may be determined by any appropriate method, such as enzyme-linked immunosorbent assay. In specific embodiments, serum SPP1 protein levels are reduced in the individual about one, two, three, six or 12 month after administering the Notch2 signaling inhibitor. In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma or metastatic liver cancer. In some embodiments, the Notch2 signaling inhibitor is an siRNA, small-molecule inhibitor or antibody. In some embodiments, the antibody is an antagonist antibody, such as an anti-Notch2 antagonist antibody or an anti-Jag1 antagonist antibody.

Also disclosed are methods for treating an individual having a liver cancer, comprising the steps of administering to the individual a Notch2 signaling inhibitor; and determining Notch2 signaling, wherein a decrease in Notch2 signaling following treatment, compared to Notch2 signaling prior to treatment, is indicative of reduction of liver cancer in the individual. In some embodiments, Notch2 signaling is determined by measuring Notch2 ICD nuclear localization, e.g., by immunohistochemical analysis of a liver cancer sample from the individual. In some embodiments, Notch2 signaling is determined by measuring expression of a gene selected from the group consisting of Notch2, Jag1, Hes and Hey1. Expression can be determined by any method, e.g., RT-PCR, microarray, and RNAseq analysis. In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma, and metastatic liver cancer. In some embodiments, the Notch2 signaling inhibitor is an siRNA, small-molecule inhibitor or antibody. In some embodiments, the antibody is an antagonist antibody, such as an anti-Notch2 antagonist antibody or an anti-Jag1 antagonist antibody.

Also disclosed are methods for inhibiting cellular proliferation comprising treating mammalian liver cancer cells with an antibody to Notch2 or Jag1, whereby proliferation of the liver cancer cell is inhibited. In certain embodiments, the cell is in a patient. In certain embodiments, the cell is in a culture medium. In certain embodiments, the cell is a liver cancer cell. In certain embodiments, the antibody is an anti-Notch2 or anti-Jag1 antagonist antibody is as described herein. In certain embodiments, the antibody is a human, humanized, or chimeric antibody. In certain embodiments, any of the antibodies of the above embodiments is an antibody fragment.

In any of the methods herein, Notch2 signaling inhibitors may be the following inhibitors. In some embodiments, the Notch2 signaling inhibitor is a siRNA, small-molecule inhibitor or antibody. In some embodiments, the antibody is an antagonist antibody. In some embodiments, the antibody is an anti-Notch2 antibody, e.g., an anti-Notch2 negative regulatory region (NRR) antibody. In some embodiments, the antibody does not significantly bind to a Notch family member other than Notch2. In some embodiments, the antibody binds to mouse Notch2 NRR and human Notch2 NRR, e.g., with a Kd of $\leq$ 10 nM. In some embodiments, the antibody comprises:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;

(d) an HVR-L1 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:9;

(e) an HVR-L2 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:14; and

(f) an HVR-L3 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:19.

In further embodiments, the antibody is an anti-Jag1 antibody. In some embodiments, the antibody comprises at least one, two, three, four, five, or six HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) HVR-H2 comprising an amino acid sequence of SEQ ID NO:84; (c) HVR-H3 comprising an amino acid sequence of SEQ ID NO:87; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:92. In one embodiment, the antibody comprises: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90.

In one embodiment, the antibody comprises: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:86; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:91. In one embodiment, the antibody comprises: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:83; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90.

In certain embodiments, any of the antibodies of the embodiments herein is a monoclonal antibody. In certain embodiments, any of the antibodies of the above embodiments is a human, humanized, or chimeric antibody. In certain embodiments, any of the antibodies of the above embodiments is an antibody fragment.

In a further embodiment, any antibody of the embodiments herein further comprises light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 of FIG. 18.

5   In a further embodiment, any antibody of the embodiments herein further comprises a heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 of FIG. 18.

In a further embodiment, any antibody of the embodiments herein further comprises a

10   light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 of FIG. 19.

In a further embodiment, any antibody of the embodiments herein further comprises a heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4

15   comprising, in order, the amino acid sequence of huMAb4D5-8 heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 of FIG. 19.

In certain embodiments, the antibody is an isolated antibody that binds to Jag1, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:94; (b) a VL sequence having at least 95% sequence identity to the

20   amino acid sequence of SEQ ID NO:97; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO:94. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO:97. In some embodiments, the antibody comprises a VH sequence of SEQ ID NO:94 and a VL sequence of SEQ ID NO:97. In some embodiments, the antibody comprises (a) a VH sequence having at

25   least 95% sequence identity to the amino acid sequence of SEQ ID NO:95; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:98; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO:95. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO:98. In some embodiments, the antibody comprises a VH

30   sequence of SEQ ID NO:95 and a VL sequence of SEQ ID NO:98.

In certain embodiments, the antibody is an isolated antibody that binds to Jag1, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:93; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:96; or (c) a VH sequence as in (a) and a VL sequence as in

(b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO:93. In some embodiments, the antibody comprises a VL sequence of SEQ ID NO:96. In some embodiments, the antibody comprises a VH sequence of SEQ ID NO:93 and a VL sequence of SEQ ID NO:96.

5   Also disclosed is an article of manufacture comprising (a) a container; (b) a composition of matter contained within the container comprising an anti-Notch2 antibody or an anti-Jagged1 antibody and a carrier for the treatment of liver cancer; and (c) a label affixed to the container, or a package insert included with the container, referring to the use of the composition of matter for the therapeutic treatment of or the diagnostic detection of a liver

10   cancer.

Also disclosed is an anti-Notch2 antibody for use in the treatment of a liver cancer. In certain embodiments, the liver cancer is hepatocellular carcinoma. In certain embodiments, the antibody is an anti-Notch2 NRR antagonist antibody. Also disclosed is an anti-Jag1 antibody for use in the treatment of a liver cancer. In certain embodiments, the liver cancer is

15   hepatocellular carcinoma. In certain embodiments, the antibody is an anti-Jag1 antagonist antibody.

Also disclosed is use of an anti-Notch2 antibody in the preparation of a medicament for the therapeutic treatment of a liver cancer.   Also disclosed is use of an anti-Jagged1 antibody in the preparation of a medicament for the therapeutic treatment of a liver cancer.

20   Also disclosed is use of an article of manufacture described herein in the preparation of a medicament for the therapeutic treatment of a liver cancer. Also disclosed is use of an article of manufacture as described herein in the preparation of a medicament for treatment or prevention of a liver cell proliferative disorder.

25   **BRIEF DESCRIPTION OF THE FIGURES**

FIGs. 1A-F illustrate characterization of liver cancer marker expression in the AKT/Ras HTV liver cancer model. FIGs. 1A-C depict immunohistochemical staining of tumors that are AFP$^+$EpCAM$^-$(FIG. 1A), AFP$^+$EpCAM$^+$(FIG. 1B) and AFP$^-$EpCAM$^+$(FIG. 1C). FIG. 1D depicts the prevalence of marker expression, expressed in percentage of total cells. FIG. 1F

30   depicts immunohistochemical staining of activation of Notch2 signaling indicated by localization of the Notch2 protein in the nucleus. FIG. 1E depicts the percent cells with nuclear Notch2 staining.

FIGs. 2A-D illustrate reduced tumor burden in Ras/AKT HTV mice treated with anti-Notch2 or anti-Jag1 antagonist antibodies. FIG. 2A depicts isolated livers from HTV mice treated with an isotype control antibody (upper left), an anti-Notch2 antibody (upper right) or an anti-Jag1 antibody (lower left) beginning on the day of the HTV. FIG. 2B depicts Ras/AKT HTV mice liver weight following antibody treatment administered at the time of HTV injection, expressed in percent body weight. FIG. 2C depicts Ras/AKT HTV mice liver weight

following antibody treatment administered two weeks after HTV injection, expressed in percent body weight (p<0.05, n>8). FIG. 2D depicts Ras/AKT HTV mice liver weight following antibody treatment (p<0.02, n>6).

FIGs. 3A-H illustrate treatment of AKT/Ras HTV tumor-bearing mice with Notch inhibitory antibodies impedes the development of a broad range of tumor types. FIG. 3A depicts immunofluorescence analysis of AFP and EpCAM expression in livers of AKT/Ras HTV mice treated with anti-Notch2, anti-Jag1 or isotype control antibody. FIGs. 3B and C depict a decrease in EpCAM$^+$ (FIG. 3B; p<0.007, n≥7) and AFP$^+$ (FIG. 3C; p<0.03, n≥7) area following anti-Notch2 and anti-Jag1 treatment. FIG. 3D depicts immunofluorescence analysis of AFP and EpCAM expression in livers of AKT/Ras HTV mice treated with anti-Notch1 antibody. FIG. 3E depicts the percentage of EpCAM$^+$ cells following AKT/Ras HTV mice treatment with an anti-Notch1, anti-Notch2, anti-Notch3, anti-Jag1 or isotype control antibody. FIG. 3F depicts relative expression of Cytokeratin 19 (CK19) following AKT/Ras HTV mice treatment with an anti-Notch1, anti-Notch2, anti-Notch3, anti-Jag1 or isotype control antibody. FIG. 3G depicts RNA and FIG. 3H depicts protein levels of Sox9 following AKT/Ras HTV mice treatment with an anti-Notch1, anti-Notch2, anti-Notch3, anti-Jag1 or isotype control antibody.

FIGs. 4A-E illustrate that treatment of AKT/Ras HTV mice with Notch inhibitory antibodies reduces Notch pathway activation in tumor-bearing livers. FIG. 4A depicts Notch2 nuclear immunohistochemical staining expressed in number of cells. FIG. 4B depicts relative expression of Notch2, determined by QRTPCR. FIG. 4C depicts immunohistochemical staining for Hes1 in AKT/Ras HTV tumor-bearing livers. FIG.4D depicts the fraction of Hes1$^+$ cells, determined by immunohistochemistry. FIG. 4E depicts relative expression of HeyL, determined by QRTPCR.

FIGs. 5A-I illustrate the effect of Notch inhibitory antibodies on expression of Notch signaling pathway components in the AKT/Ras model of liver cancer. Mice subjected to AKT/Ras HTV were treated with antagonistic antibodies to Notch1, Notch2, Notch3, or Jag1, or an anti-Ragweed negative control antibody and quantitative real-time PCR was performed on the isolated RNA from livers after 5 weeks for Notch1 (FIG.5A), Notch2 (FIG.5B), Notch3 (FIG.5C), Notch4 (FIG.5D), Jag1 (FIG.5E), Jag2 (FIG.5F), DLL1 (FIG. 5G), DLL3 (FIG.5H), DLL4 (FIG.5I).

FIGs. 6A-I illustrate results from RNAseq analysis of livers from AKT/Ras HTV mice treated with isotype control, anti-Notch2 or anti-Jag1 antibody. Depicted are normalized counts for Prom1 (FIG. 6A), Spp1 (FIG. 6B), FoxM1 (FIG. 6C), Plk1 (FIG. 6D), ccnb1 (FIG.

6E), Aurkb (FIG. 6F), Wnt2 (FIG. 6G), Axin2 (FIG. 6H) and glutamine synthetase (Glul, FIG. 6I).

FIGs. 7A-B illustrate expression of Notch2 in human HCC. FIG. 7A depicts Notch1, Notch2 and Notch3 expression, determined by RT-PCR, in cultured human HCC cell lines. FIG. 7B depicts immunohistochemical staining of human HCC tumors for Notch2, Jag1 and Hes1.

FIGs. 8A-D show exemplary amino acid sequences of human (C) and murine (D) Notch2 protein and human (A) and murine (B) Notch2 negative regulatory region (NRR).

FIG. 9 shows exemplary amino acid sequences of human and murine Jagged 1 protein.

FIGs. 10A-B show the amino acid sequences of peptides used for phage antibody library screening and selection. All proteins were expressed as a secreted protein in BEVS cells and their sequences are listed in the N-terminal to C-terminal direction. FIG. 10A shows the amino acid sequence of expressed protein murine Jagged 1-DSL-EGF1-4 (Q34-D377). The bold font at the N-terminus represents a short linker sequence (ADLGS (SEQ ID NO: 2)). The bold font at the C-terminus represents a short linker sequence (EFG), a thrombin cleavage site (LVPRGS (SEQ ID NO: 26)), a G spacer and the 6-His tag (SEQ ID NO: 27). FIG. 10B shows the amino acid sequence of expressed protein human Jag1-DSL-EGF1-4. Only the Jag1 sequence is shown although the antigen also contained a TEV protease cleavage site and 6-His tag (SEQ ID NO: 27) at the C-terminus.

FIG. 11 shows an exemplary amino acid sequences of human secreted phosphoprotein1 (SPP1).

FIG. 12 shows the H1, H2, and H3 heavy chain hypervariable region (HVR) sequences of anti-Notch2 NRR antagonist antibodies. Amino acid positions are numbered according to the Kabat numbering system as described below.

FIG. 13 shows the L1, L2, and L3 light chain HVR sequences of anti-Notch2 NRR antagonist antibodies. Amino acid positions are numbered according to the Kabat numbering system as described below.

FIGs. 14A-B show an alignment of the amino acid sequences for the heavy chain variable domains of anti-Notch2 antibodies. Amino acid positions of the complementarity determining regions (CDRs) are indicated.

FIGs. 15A-B show an alignment of the amino acid sequences for the light chain variable domains of anti-Notch2 antibodies. Amino acid positions of the complementarity determining regions (CDRs) are indicated.

FIGs. 16A-B show exemplary acceptor human variable heavy (VH) consensus framework sequences for use in practicing the instant invention. Sequence identifiers are as follows:

- human VH subgroup I consensus framework "A" minus Kabat CDRs (SEQ ID

5   NOs:32, 33, 34, 35).

- human VH subgroup I consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:36, 37, 34, 35; SEQ ID NOs:36, 37, 38, 35; and SEQ ID NOs:36, 37, 39, 35).

- human VH subgroup II consensus framework "A" minus Kabat CDRs (SEQ ID

10   NOs:40, 41, 42, 35).

- human VH subgroup II consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:43, 44, 42, 35; SEQ ID NOs:43, 44, 45, 35; and SEQ ID NOs:43, 44, 46, and 35).

- human VH subgroup III consensus framework "A" minus Kabat CDRs (SEQ ID

15   NOs:47, 48, 49, 35).

- human VH subgroup III consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:50, 51, 49, 35; SEQ ID NOs:50, 51, 52, 35; and SEQ ID NOs:50, 51, 53, 35).

- human VH acceptor framework "A" minus Kabat CDRs (SEQ ID NOs:54, 48, 55,

20   35).

- human VH acceptor frameworks "B" and "C" minus extended hypervariable regions (SEQ ID NOs:50, 51, 55, 35; and SEQ ID NOs:50, 51, 56, 35).

- human VH acceptor 2 framework "A" minus Kabat CDRs (SEQ ID NOs:54, 48, 57, 35).

25   - human VH acceptor 2 framework "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:50, 51, 57, 35; SEQ ID NOs:50, 51, 58, 35; and SEQ ID NOs:50, 51, 59, 35).

FIG. 17 shows exemplary acceptor human variable light (VL) consensus framework sequences for use in practicing the instant invention. Sequence identifiers are as follows:

30   - human VL kappa subgroup I consensus framework (κv1): SEQ ID NOs:60, 61, 62, 63

- human VL kappa subgroup II consensus framework (κv2): SEQ ID NOs:64, 65, 66, 63

- human VL kappa subgroup III consensus framework (κv3): SEQ ID NOs:67, 68, 69, 63

- human VL kappa subgroup IV consensus framework (κv4): SEQ ID NOs:70, 71, 72, 63.

FIG. 18 depicts framework region sequences of huMAb4D5-8 light and heavy chains (SEQ ID NOS 60, 61, 30, 63, 50, 51, 59 and 35, respectively, in order of appearance). Numbers in superscript indicate amino acid positions according to Kabat.

FIG. 19 depicts modified/variant framework region sequences of huMAb4D5-8 light and heavy chains (SEQ ID NOS 60, 61, 62, 31, 50, 51, 53 and 35, respectively, in order of appearance). Numbers in superscript indicate amino acid positions according to Kabat.

FIG. 20 shows the H1, H2, and H3 heavy chain hypervariable region (HVR) sequences of anti- Jagged antibodies, as described in the Example s. Amino acid positions are numbered according to the Kabat numbering system as described below.

FIG. 21 shows the L1, L2, and L3 light chain HVR sequences of anti-Jagged antibodies, as described in the Examples. Amino acid positions are numbered according to the Kabat numbering system as described below.

FIG. 22 shows light and heavy chain variable domain framework sequences of anti-Jagged antibodies (SEQ ID NOS 60, 61, 62, 77, 50, 99, 57 and 35, respectively, in order of appearance). Numbers in superscript indicate amino acid positions according to Kabat.

FIGs. 23A-B show an alignment of the amino acid sequences for the heavy chain variable domains of antibodies to Notch2 (SEQ ID NO: 100) (B?), Notch1 (SEQ ID NO: 101) (Y), Notch3 (SEQ ID NO: 102) (W) and Jag1 (SEQ ID NO: 103) (A-2) described in the Examples.

FIGs. 24A-B show an alignment of the amino acid sequences for the light chain variable domains of antibodies to Notch2 (SEQ ID NO: 104), Notch1 (SEQ ID NO: 105), Notch3 (SEQ ID NO: 106) and Jag1 (SEQ ID NO: 107) used in the Examples.

FIGs. 25A-B show an alignment of the amino acid sequences for the heavy (FIG. 25A) and light (FIG. 25B) chain variable domains of anti-Jag1 antibodies. Amino acid positions of the complementarity determining regions (CDRs) are indicated.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

In the claims which follow and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the word

"comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

5 An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain

variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

The terms "anti-Jag1 antibody" and "an antibody that binds to Jag1" refer to an antibody that is capable of binding Jag1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Jag1. In one embodiment, the extent of binding of an anti Jag1 antibody to an unrelated, non-Jag1 protein is less than about 10% of the binding of the antibody to Jag1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to Jag1 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti- Jag1 antibody binds to an epitope of Jag1 that is conserved among Jag1 from different species.

An "anti-Jag1 antagonist antibody" is an anti-Jag1antibody that effects decreased Jag1-mediated signaling, e.g., Jag1-mediated Notch2 signaling.

The terms "anti-Notch2 antibody" and "an antibody that binds to Notch2" refer to an antibody that is capable of binding Notch2 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Notch2. In one embodiment, the

extent of binding of an anti-Notch2 antibody to an unrelated, non- Notch2 protein is less than about 10% of the binding of the antibody to Notch2 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to Notch2 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M

5   or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-Notch2 antibody binds to an epitope of Notch2 that is conserved among Notch2 from different species.

       An "anti-Notch2 antagonist antibody" is an anti-Notch2 antibody (including an anti-Notch2 NRR antibody) that effects decreased Notch2 signaling, as defined below.

10   The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

       An "antibody fragment" refers to a molecule other than an intact antibody that

15   comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')$_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

       An "antibody that binds to the same epitope" as a reference antibody refers to an

20   antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

       A "blocking" antibody or an "antagonist" antibody is one which significantly inhibits

25   (either partially or completely) a biological activity of the antigen it binds.

       The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

       The terms "cancer" and "cancerous" refer to or describe the physiological condition in

30   mammals that is typically characterized by unregulated cell growth/proliferation. Examples of liver cancer include, but are not limited to, hepatocellular carcinoma, hepatoma, hepatoblastoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, sarcoma, lymphoma and hepatic angiosarcoma. Liver cancer also includes cancer that originated in the liver and has metastasized to another part of the body.

The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

A "chemotherapeutic agent" refers to a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omega1I (see, e.g., Nicolaou *et al.*, *Angew. Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); CDP323, an oral alpha-4 integrin inhibitor; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), liposomal doxorubicin TLC D-99 (MYOCET®), peglylated liposomal doxorubicin (CAELYX®), and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin,

zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2'-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoid, *e.g.*, paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin (e.g., ELOXATIN®), and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovorin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1

inhibitor (*e.g.*, LURTOTECAN®); rmRH (*e.g.*, ABARELIX®); BAY439006 (Sorafenib; Bayer); SU-11248 (sunitinib, SUTENT®, Pfizer); perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); serine-threonine kinase inhibitors such as rapamycin (sirolimus, RAPAMUNE®); farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Chemotherapeutic agents as defined herein include "anti-hormonal agents" or "endocrine therapeutics" which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. They may be hormones themselves, including, but not limited to: anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrazole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors include vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, and 4(5)-imidazoles; lutenizing hormone-releaseing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and tripterelin; sex steroids, including progestines such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretionic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG,

and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

5   "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass), which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid

10   sequences (polypeptides with a variant Fc region) and increased or decreased C1q binding capability are described, e.g., in US Patent No. 6,194,551 B1 and WO 1999/51642. See also, e.g., Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not

15   limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as

20   small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

The term "cytostatic agent" refers to a compound or composition which arrests growth of a cell either in vitro or in vivo. Thus, a cytostatic agent may be one which significantly

25   reduces the percentage of cells in S phase. Further examples of cytostatic agents include agents that block cell cycle progression by inducing G0/G1 arrest or M-phase arrest. The humanized anti-Her2 antibody trastuzumab (HERCEPTIN®) is an example of a cytostatic agent that induces G0/G1 arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin,

30   daunorubicin, etoposide, and bleomycin. Certain agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled

"Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), *e.g.*, p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®,

5   Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions

10   include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired

15   therapeutic or prophylactic result.

The term "expression" refers to conversion of the information encoded in a gene into messenger RNA (mRNA), and then to the protein.

Herein, a sample or cell that "expresses" a protein of interest is one in which mRNA encoding the protein, or the protein, including fragments thereof, is determined to be present in

20   the sample or cell.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the

25   heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

30   "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable

loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

In one embodiment, HVR residues comprise those identified in Figures 12, 13, 20 and 21.

Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "individual at risk of having liver cancer" refers to an individual having a higher than average propensity of acquiring liver cancer. Examples of individuals at risk of having liver cancer include, without limitation, individuals having hepatitis, e.g., hepatitis B or C, cirrhosis of the liver, benign liver tumors, hemangiomas, hepatic adenomas, and focal nodular hyperplasias.

The term "inhibit" means to decrease or reduce an activityu, function, and/or amount as compared to a reference.

"Inhibiting cell growth or proliferation" means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-Jag1 antibody" or "isolated nucleic acid encoding an anti-Notch2 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "Jagged" or "Jag," as used herein, refers to any native Jagged from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed Jag as well as any form of Jagged that results from processing in the cell. The term also encompasses naturally occurring variants of Jagged, e.g., splice variants or allelic variants.

The term "Jagged1" or "Jag1," as used herein, refers to any native Jag1 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed Jag1 as well as any form of Jag1 that results from processing in the cell. The term also encompasses naturally occurring variants of Jag1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human and murine Jag1 is shown in FIG. 9.

The term "level of expression" or "expression level" as used herein, refers to the amount of a polynucleotide, mRNA, or an amino acid product or protein in a biological sample.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "Notch," as used herein, refers to any native Notch from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed Notch as well as any form of Notch that results from processing in the cell. The term also encompasses naturally occurring variants of Notch, e.g., splice variants or allelic variants.

The term "Notch2," as used herein, refers to any native Notch2 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed Notch2 as well as any form of Notch2 that results from processing in the cell. The term also encompasses naturally occurring variants of Notch2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human Notch2 is shown in FIG.8.

The term "Notch2 signaling inhibitor" refers to an agent that effects decreased Notch2 signaling, as defined above. Notch2 signaling inhibitors include Notch2-specific antagonists and Jag1-specific antagonists. A Notch2-specific antagonist decreases Notch2 signaling and does not significantly affect signaling by another Notch receptor (Notch1, 3, or 4 in mammals). Examples of Notch2-specific antagonist include agents that block Notch2 binding to a Notch2 ligand. A Jag1-specific antagonist decreases Jag1-mediated signaling. Examples of Jag1-specific antagonists include agents that bind Notch2. Pan-Notch inhibitors, such as gamma secretase inhibitors, are explicitly excluded from Notch2 signaling inhibitors defined herein.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be

compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

"Relapsed" refers to the regression of the patient's illness back to its former diseased state, especially the return of symptoms following an apparent recovery or partial recovery. Unless otherwise indicted, relapsed state refers to the process of returning to or the return to illness before the previous treatment including, but not limited to, chemotherapies and stem cell transplantation treatments.

"Refractory" refers to the resistance or non-responsiveness of a disease or condition to a treatment (e.g., the number of neoplastic plasma cells increases even though treatment if given). Unless otherwise indicated, the term "refractory" refers a resistance or non-

responsiveness to any previous treatment including, but not limited to, chemotherapies and stem cell transplantation treatments.

The term "secreted phosphoprotein1" or "SPP1" or "osteopontin," as used herein, refers to any native SPP1 from any vertebrate source, including mammals such as primates (e.g.

5   humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed SPP1 as well as any form of SPP1 that results from processing in the cell. The term also encompasses naturally occurring variants of SPP1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human SPP1 is shown in FIG.11.

The phrase "substantially reduced" or "substantially different," as used herein, refers to

10 a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values).

15 The term "substantially similar" or "substantially the same," as used herein, refers to a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological

20 characteristic measured by said values (*e.g.*, Kd values).

The term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

25 As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or

30 indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II.   COMPOSITIONS AND METHODS

In one aspect, the invention is based, in part, on inhibition of Notch pathway components for the treatment of liver cancer.

In one aspect, methods of treating a liver cancer in an individual in need thereof are provided, comprising the step of administering to the individual an effective amount of a Notch2 signaling inhibitor. In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma, or metastatic liver cancer. In some embodiments, the liver cancer is a refractory cancer.

Examples of Notch2 signaling inhibitors are known in the art and some are exemplified herein, including, but not limited to, soluble Notch receptors, soluble Notch ligand variants, *e.g.,* dominant negative ligand variants, aptamers or oligopeptides that bind Notch2 or Jag1, organic or inorganic molecules that interfere specifically with Notch2 signaling, anti-Notch2 antagonist antibodies and anti-Jag1 antagonist antibodies. Examples of Notch2-specfic antagonists include those described in U.S. Patent Application Publication No. US 2010/0111958 and Sjölund et al., J. Clin. Invest. 118(1):217–228 (2008).

In certain embodiments, the Notch2 signaling inhibitor is an anti-Notch2 antagonist antibody. In one such embodiment, the anti-Notch2 antagonist antibody is an antibody that

binds to the extracellular domain of Notch2 and effects decreased Notch2 signaling. In one such embodiment, the anti-Notch2 antagonist antibody is an anti-Notch2 NRR antibody. Anti-Notch2 NRR antibodies include, but are not limited to, any anti-Notch2 NRR antibodies disclosed in International Application Publication No. WO2010039832, which is expressly
5   incorporated by reference herein in its entirety. Such antibodies include, but are not limited to anti-Notch2 NRR antibodies that bind to the LNR-A and HD-C domains of Notch2 NRR. Exemplary anti-Notch2 NRR antibodies are monoclonal antibodies designated herein as Antibody B, Antibody B-1, Antibody B-2, and Antibody B-3. Antibody B that binds to Notch2 NRR was isolated from a phage library. That antibody was affinity matured to generate
10   Antibody B-1, Antibody B-2, and Antibody B-3. The sequences of the heavy chain and light chain hypervariable regions (HVRs) of Antibody B, Antibody B-1, Antibody B-2, and Antibody B-3 are shown in FIGs. 12 and 13, respectively. The sequences of the heavy and light chain variable domains of Antibody B, Antibody B-1, Antibody B-2, and Antibody B-3 are shown in FIGs. 14 and 15. Further embodiments of anti-Notch2 NRR antibodies are
15   provided as follows.

In one aspect, an antagonist antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises at least one, two, three, four, five, or six HVRs selected from:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;
20   (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;

(d) an HVR-L1 comprising an amino acid sequence that conforms to the consensus
    sequence of SEQ ID NO:9;

(e) an HVR-L2 comprising an amino acid sequence that conforms to the consensus
25   sequence of SEQ ID NO:14; and

(f) an HVR-L3 comprising an amino acid sequence that conforms to the consensus
    sequence of SEQ ID NO:19.

In a further aspect, the antibody comprises an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4 and at least one, two, three, four, or five HVRs selected from (a),
30   (b), (d), (e), and (f) above. In a further aspect, the antibody comprises (a), (b), (c), (d), (e), and (f) above. With respect to (a), (d), (e), and (f), any one or more of the following embodiments are contemplated: HVR-H1 comprises an amino acid sequence of SEQ ID NO:1; HVR-L1 comprises an amino acid sequence selected from SEQ ID NOs:5-8; HVR-L2 comprises an

amino acid sequence selected from SEQ ID NOs:10-13; and HVR-L3 comprises an amino acid sequence selected from SEQ ID NOs:15-18.

In another aspect, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4.

In another aspect, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises an HVR-L1 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:9, an HVR-L2 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:14, and an HVR-L3 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:19. The following embodiments are contemplated in any combination: HVR-L1 comprises an amino acid sequence selected from SEQ ID NOs:5-8; HVR-L2 comprises an amino acid sequence selected from SEQ ID NOs:10-13; and HVR-L3 comprises an amino acid sequence selected from SEQ ID NOs:15-18. In one embodiment, an antibody that binds to Notch2 NRR comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:10; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:15. In another embodiment, an antibody that binds to Notch2 NRR comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:6; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:11; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:16. In another embodiment, an antibody that binds to Notch2 NRR comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:17. In another embodiment, an antibody that binds to Notch2 NRR comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:8; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

In one embodiment, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:5;

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:10; and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:15.

In another embodiment, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:6;

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:11; and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:16.

In another embodiment, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7;

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:17.

In another embodiment, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:8;

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

In certain embodiments, any of the above antibodies further comprises at least one framework selected from a VH subgroup III consensus framework and a VL subgroup I consensus framework.

In certain embodiments, an anti-Notch2 NRR antibody is affinity matured. For example, any one or more of the following substitutions in the indicated HVR positions (Kabat numbered) may be made in any combination:

- in HVR-L1 (SEQ ID NO:5): S28N; I29N or V; S30R or K; S31R; Y32F
- in HVR-L2 (SEQ ID NO:10): G50R; S53I or T; A55E
- in HVR-L3 (SEQ ID NO:15): S93I or R; L96S or H

The specific antibodies disclosed herein, Antibody B as well as affinity matured forms of Antibody B (B-1, B-2, and B-3), may undergo further affinity maturation. Accordingly, affinity matured forms of any of the antibodies described herein are provided.

In certain embodiments, the Notch2 signaling inhibitor is an anti-Jag1 antagonist antibody. In one such embodiment, the anti-Jag1 antagonist antibody is an antibody that binds to the extracellular domain of Jag1 and effects decreased Notch2 signaling. In one such embodiment, the anti-Jag1 antagonist antibody is an anti-Jag1 EGF1-4 antibody. Anti-Jag1 antibodies include, but are not limited to, any anti-Jag1 antibodies disclosed herein.

In further embodiments, the antibody is an anti-Jag1 antibody. In some embodiments, the antibody comprises at least one, two, three, four, five, or six HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) HVR-H2 comprising an amino acid sequence of SEQ ID NO:84; (c) HVR-H3 comprising an amino acid sequence of SEQ ID NO:87; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) HVR-L3 comprising an amino acid sequence of SEQ ID NO:92. In one embodiment, the antibody comprises: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90.

In one embodiment, the antibody comprises: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:86; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:91. In one embodiment, the antibody comprises: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:83; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90.

In certain embodiments, an anti-Notch2 NRR antibody or anti-Jag1 antibody having any of the above HVR sequences can further comprise any suitable framework variable domain sequence, provided binding activity to Notch2 NRR and Jag1, respectively, is substantially

retained. In certain embodiments, an anti-Notch2 NRR antibody or anti-Jag1 antibody comprises a human variable heavy (VH) consensus framework sequence, as in any of the VH consensus framework sequences shown in FIGs. 16A and 16B. In one embodiment, the VH consensus framework sequence comprises a human subgroup III heavy chain framework

5   consensus sequence, e.g., as shown in FIGs. 16A and 16B. In another embodiment, the VH consensus framework sequence comprises an "Acceptor 2" framework sequence, e.g., as shown in FIGs. 16A and 16B. In a particular embodiment, the VH framework consensus sequence comprises FR1-FR4 of Acceptor 2B or Acceptor 2D, wherein the FR4 comprises SEQ ID NO:35 (FIGs. 16A and 16B), with the last residue of SEQ ID NO:35 (S11) optionally

10   being substituted with alanine. In a further particular embodiment, the VH framework consensus sequence comprises the sequences of SEQ ID NOs:50; 51; 57 or 59; and 35, wherein S11 of SEQ ID NO:35 is optionally substituted with alanine.

In certain embodiments, an anti-Notch2 NRR antibody or anti-Jag1 antibody having any of the above HVR sequences can further comprise a human variable light (VL) consensus

15   framework sequence as shown in FIGs. 17. In one embodiment, the VL consensus framework sequence comprises a human VL kappa subgroup I consensus framework (κv1) sequence, e.g., as shown in FIGs. 17. In another embodiment, the VL framework consensus sequence comprises FR1-FR4 of huMAb4D5-8 as shown in FIGs. 18 or 19. In a particular embodiment, the VL framework consensus sequence comprises the sequences of SEQ ID NOs:60, 61, 62,

20   and 63.

In another aspect, an anti-Notch2 NRR antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NOs:20. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

25   98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-Notch2 NRR antibody comprising that sequence retains the ability to bind to Notch2 NRR. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of SEQ ID NOs:20. In certain embodiments, substitutions, insertions, or deletions occur in regions

30   outside the HVRs (i.e., in the FRs). In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4.

In another aspect, an antibody that specifically binds to Notch2 NRR is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs:22-25. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-Notch2 NRR antibody comprising that sequence retains the ability to bind to Notch2 NRR. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in an amino acid sequence selected from SEQ ID NOs:22-25. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:9; (b) an HVR-L2 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:14; and (c) an HVR-L3 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:19. In one such embodiment, the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:5-8; (b) an HVR-L2 comprising an amino acid sequence selected from SEQ ID NOs:10-13; and (c) an HVR-L3 comprising an amino acid sequence selected from SEQ ID NOs:15-18. In one such embodiment, the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:10; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:15. In another such embodiment, the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:6; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:11; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:16. In another such embodiment, the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:17. In another such embodiment, the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:8; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

In certain embodiments of the variant VH and VL sequences provided above, substitutions, insertions, or deletions may occur within the HVRs. In such embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example,

5   conservative alterations that do not substantially reduce binding affinity may be made in HVRs. In certain instances, alterations in HVRs may actually improve antibody affinity. Such alterations may be made in HVR "hotspots" (i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process) in order to increase antibody affinity. (See, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196, 2008.) In certain

10   embodiments of the variant VH and VL sequences provided above, each HVR either is conserved (unaltered), or contains no more than a single amino acid substitution, insertion or deletion.

In another aspect, an antibody that specifically binds Notch2 NRR is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any

15   of the embodiments provided above. In one embodiment, the antibody comprises a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:20, and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:22. In one such embodiment, the VH comprises one, two or three HVRs selected

20   from: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4, and the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:10; and (c) an HVR-L3 comprising the

25   amino acid sequence of SEQ ID NO:15. In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:20, and a VL comprising the amino acid sequence of SEQ ID NO:22.

In another embodiment, an anti-Notch2 NRR antibody that specifically binds Notch2 NRR comprises a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,

30   or 100% sequence identity to the amino acid sequence of SEQ ID NO:20, and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs:23-25. In one such embodiment, the VH comprises one, two or three HVRs selected from: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID

NO:3, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4, and the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:6-8; (b) an HVR-L2 comprising an amino acid sequence selected from SEQ ID NOs:11-13; and (c) an HVR-L3 comprising an amino acid sequence selected from SEQ ID NOs:16-18. In particular embodiments, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:20 and a VL comprising an amino acid sequence selected from SEQ ID NOs:23-25.

In another aspect, an antibody that specifically binds Jag1 is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs:93-95, and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs:96-98. In one such embodiment, the VH comprises one, two or three HVRs selected from: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85, and the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90. In a particular embodiment, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:93, and a VL comprising the amino acid sequence of SEQ ID NO:96.

In another embodiment, an anti-Jag1 antibody that specifically binds Jag1 comprises a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:94, and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:97. In one such embodiment, the VH comprises one, two or three HVRs selected from: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:86, and the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:91. In particular embodiments,

the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:94 and a VL comprising the amino acid sequence of SEQ ID NO:97.

In another embodiment, an anti-Jag1 antibody that specifically binds Jag1 comprises a VH having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%

5   sequence identity to the amino acid sequence of SEQ ID NO:95, and a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:98. In one such embodiment, the VH comprises one, two or three HVRs selected from: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:83, and (c) an

10   HVR-H3 comprising the amino acid sequence of SEQ ID NO:85, and the VL comprises one, two or three HVRs selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90. In particular embodiments, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:95 and a VL

15   comprising the amino acid sequence of SEQ ID NO:98.

In certain embodiments, an affinity-matured form of any of the above antibodies is provided. In further embodiments, a recombinant protein that specifically binds Notch2 NRR or Jag1 is provided, wherein the recombinant protein comprises an antigen binding site(s) of any of the above antibodies. In one such embodiment, a recombinant protein comprises any

20   one or more of the HVRs provided above.

In certain embodiments, a polynucleotide encoding any of the above antibodies is provided. In one embodiment, a vector comprising the polynucleotide is provided. In one embodiment, a host cell comprising the vector is provided. In one embodiment, the host cell is eukaryotic. In one embodiment, the host cell is a CHO cell. In one embodiment, a method of

25   making an anti-Notch2 NRR antibody is provided, wherein the method comprises culturing the host cell under conditions suitable for expression of the polynucleotide encoding the antibody, and isolating the antibody.

In another embodiment, an isolated antibody is provided that binds to the same epitope as an antibody provided herein. In one embodiment, an isolated anti-Notch2 NRR antibody is

30   provided that binds to the same epitope as an antibody selected from Antibody B, Antibody B-1, Antibody B-2, and Antibody B-3. In another embodiment, the invention provides an anti-Notch2 NRR antibody that competes for binding with an antibody selected from Antibody B, Antibody B-1, Antibody B-2, and Antibody B-3. In another embodiment, an isolated antibody is provided that binds to at least one domain selected from the LNR-A domain and the HD-C

domain of Notch2. In one such embodiment, the antibody binds to both the LNR-A domain and the HD-C domain. In another such embodiment, the antibody further binds to the LNR-B and/or HD-N domains.

In one embodiment, an isolated anti-Jag1 antibody is provided that binds to the same epitope as an antibody selected from Antibody A, Antibody A-1, and Antibody A-2. In another embodiment, the invention provides an anti-Jag1 antibody that competes for binding with an antibody selected from Antibody A, Antibody A-1, and Antibody A-2. In another embodiment, an isolated antibody is provided that binds to at least one domain selected from the DSL domain and the EGF domain of Jag1. In one such embodiment, the antibody binds to EGF1-4 of Jag1.

Any of the Notch2 signaling inhibitors provided herein may be used in the methods described herein.

The invention also provides methods for selecting a therapeutic treatment for a patient having a liver cancer, the method comprising determining expression of one or more of Notch2, Jag1 and SPP1 in a sample obtained from the patient. In some embodiments, the patient is selected for treatment with a Notch2 signaling inhibitor if expression of one or more of Notch2, Jag1 and SPP1 is detected in the patient sample. In some embodiments, elevated expression of one or more of Notch2, Jag1 and Spp1 in the sample obtained from the patient, relative to a control, identifies the patient as suitable for receiving treatment with a Notch2 signaling inhibitor, as described herein. In some embodiments, additional parameters, such as, *e.g.*, examination by a physician, histologic evaluation of a biopsy, determination of serum levels characterizing the liver cancer, are employed to identify the patient for receiving the Notch2 signaling inhibitor treatment.

In some embodiments, a sample or biopsy from the patient is analyzed for mRNA expression of one or more of Notch2, Jag1 and Spp1 using methods well known in the art, such as, *e.g.*, quantitative PCR analysis, and compared to expression of the same gene or genes in a biopsy obtained from a control individual or compared to a reference value. In some embodiments, expression is determined using enzyme linked immunosorbent assay (ELISA). In some embodiments, a sample or biopsy from the patient is analyzed for Notch2 activation, for example by detection of the activated form of Notch2 as described herein.

In one aspect, methods are provided for preventing liver cancer in an individual at risk of having liver cancer, comprising the step of administering to the individual an effective amount of a Notch2 signaling inhibitor. In some embodiments, the individual has a liver condition selected from the group consisting of hepatitis B or C, cirrhosis of the liver, benign

liver tumors, hemangiomas, hepatic adenomas, and focal nodular hyperplasia. In some

embodiments, the method further comprises administering at least one additional therapeutic

agent. Examples of additional therapeutic agents include growth inhibitory agents, such as

cytotoxic agents, peptides, small-molecules and antibodies.

5   In another aspect, methods are provided for inhibiting the growth of a cell that

expresses secreted phosphoprotein1 (SPP1), comprising contacting the cell with a Notch2

signaling inhibitor, thereby inhibiting growth of the cell. In one embodiment, SPP1 protein

comprises the amino acid sequence shown in FIG. 11. In one embodiment, contacting the cell

with the Notch2 signaling inhibitor reduces SPP1 expression in the cell. For example,

10   contacting the cell with the Notch2 signaling inhibitor reduces SPP1 expression in the cell by

at least about 50%, 60%, 70%, 80%, 90%, or 90%. The expression of SPP1 mRNA or protein

can be determined by any method in the art. In some embodiments, the cell is a liver cancer

cell. In some embodiments, the liver cancer cell expresses EpCAM, AFP, AFP and EpCAM,

Notch2, Jag1, Notch2 and Jag1, nuclear Notch2 ICD, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1,

15   Aurkb, Wnt2, Axin2, or Glul, or any combination thereof. In some embodiments, contacting

the cell with the Notch2 signaling inhibitor results in a decrease in the expression in the cell of

at least one of EpCAM, AFP, Notch2, Notch2 ICD, Jag1, Prom1, Spp1, FoxM1, Plk1, ccnb1

and Aurkb. In some embodiments, administering the Notch2 signaling inhibitor results in an

increase in the expression in the cell of at least one of Wnt2, Axin2 and Glul. In some

20   embodiments, expression is determined by RNAseq, microarray analysis,

immunohistochemistry, enzyme-linked immunosorbent assay, and Western blotting.

              In another aspect, methods are provided for therapeutically treating a mammal having a

liver cancer comprising cells that express an Spp1 gene encoding a peptide comprising an

amino acid sequence having at least 90% identity to the polypeptide shown in FIG. 11,

25   comprising administering to the mammal a therapeutically effective amount of a Notch2

signaling inhibitor, thereby effectively treating the mammal.

              In another aspect, methods are provided for treating or preventing a liver cell

proliferative disorder associated with increased expression or activity of a protein having at

least 90% amino acid sequence identity to the polypeptide shown in FIG. 8C, comprising

30   administering to an individual in need of such treatment an effective amount of an anti-Jag1

antagonist antibody, thereby effectively treating or preventing the liver cell proliferative

disorder. In some embodiments, the cell proliferative disorder is a cancer, such as liver cancer.

In some embodiments the individual has a liver condition selected from the group consisting of

hepatitis B or C, cirrhosis of the liver, benign liver tumors, hemangiomas, hepatic adenomas, and focal nodular hyperplasia.

In one aspect, methods are provided for reducing serum SPP1 protein levels in an individual, the method comprising administering to the individual an effective amount of a Notch2 signaling inhibitor thereby reducing serum SPP1 levels in the individual. In some embodiments, reducing is relative to serum SPP1 levels in the individual prior to administering the Notch2 signaling inhibitor. In some embodiments, reducing is relative to a reference level. In one embodiment, the individual has a liver cancer. In one embodiment, the serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor to the individual are at least about 80 ng/ml. In certain embodiments, the serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor to the individual are between about 80 ng/ml and about 500 ng/ml, between about 86 ng/ml and about 250 ng/ml, between about 120 ng/ml and about 170 ng/ml, or about 165 ng/ml. In some embodiments, administering the Notch2 signaling inhibitor to the individual results in serum SPP1 protein levels of less than 80 ng/ml. In specific embodiments, serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor are 24 hours prior to administering the Notch2 signaling inhibitor. Serum SPP1 protein levels prior to or following administration of the Notch2 signaling inhibitor may be determined by any appropriate method, such as enzyme-linked immunosorbent assay. In specific embodiments, serum SPP1 protein levels are reduced about one, two, three, six or 12 month after administering the Notch2 signaling inhibitor.

In one aspect, methods are provided for therapeutically treating a liver tumor in a mammal, wherein the growth of the liver tumor is at least in part dependent upon a growth potentiating effect of Notch2 signaling, comprising contacting the tumor with an antibody that binds to Notch2 or Jag1, thereby effectively treating the tumor. In one embodiment, binding of the antibody to the tumor antagonizes the growth-potentiating activity of Notch2.

In one aspect, methods are provided for preventing recurrence of liver cancer comprising administering to an individual who has been treated for liver cancer and who has elevated serum SPP1 protein levels an effective amount of a Notch2 signaling inhibitor. In one embodiment, the serum SPP1 protein levels of the individual are at least about 80 ng/ml. In certain embodiments, the serum SPP1 protein levels prior to administering the Notch2 signaling inhibitor to the individual are between about 80 ng/ml and about 500 ng/ml, between about 86 ng/ml and about 250 ng/ml, between about 120 ng/ml and about 170 ng/ml, or about 165 ng/ml. In some embodiments, administering the Notch2 signaling inhibitor to the individual results in serum SPP1 protein levels of less than 80 ng/ml.

In some aspects, methods are provided for treating an individual having a liver cancer, comprises the steps of administering to the individual a Notch2 signaling inhibitor; and determining Notch2 signaling, wherein a decrease in Notch2 signaling following treatment, compared to Notch2 signaling prior to treatment, is indicative of reduction of liver cancer in the individual. In some embodiments, Notch2 signaling is determined by measuring Notch2 ICD nuclear localization, e.g., by immunohistochemical analysis. In some embodiments, Notch2 signaling is determined by measuring expression of a gene selected from the group consisting of Notch2, Jag1, Hes and Hey1. In some embodiments, the liver cancer is hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma, and metastatic liver cancer. In some embodiments, the Notch2 signaling inhibitor is an siRNA, small-molecule inhibitor or antibody. In some embodiments, the antibody is an antagonist antibody, such as an anti-Notch2 antagonist antibody or an anti-Jag1 antagonist antibody.

In some aspects, methods for inhibiting cellular proliferation comprising treating mammalian liver cancer cells with an antibody to Notch2 or Jag1, whereby proliferation of the liver cancer cell is inhibited. In certain embodiments, the antibody is an anti-Notch2 or anti-Jag1 antagonist antibody is as described herein. In certain embodiments, the antibody is a human, humanized, or chimeric antibody. In certain embodiments, any of the antibodies of the above embodiments is an antibody fragment. In certain embodiments, the cells are in a patient. In certain embodiments, the cells are in a culture medium.

Notch2 signaling inhibitors of the invention, such as anti-Notch2 and anti-Jag1 antibodies, can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antagonist of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Notch2 signaling inhibitors of the invention can also be used in combination with radiation therapy.

The antagonist can be administered to a human patient by any known method, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The Notch2 signaling inhibitor might be

administered as a protein or as a nucleic acid encoding a protein (see, for example, International Application Publication No. WO96/07321). Other therapeutic regimens may be combined with the administration of the Notch2 signaling inhibitor. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. In some embodiments, such combined therapy results in a synergistic therapeutic effect.

The dosage and mode of administration will be chosen by the physician according to known criteria. The appropriate dosage of antibody, or other Notch2 signaling inhibitor, will depend on the type of disease to be treated, the severity and course of the disease, whether the antibody, oligopeptide or organic molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the Notch2 signaling inhibitor, and the discretion of the attending physician. The Notch2 signaling inhibitor can be administered to the patient at one time or over a series of treatments.

Success of treatment of liver cancer can be monitored by assessing parameters of liver function and recovery. Such parameters include, but are not limited to, improved liver function tests, (*e.g.,* assessing serum albumin, bilirubin, bile acids, total protein, clotting times), liver enzymes (*e.g.,* alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyl transpeptidase), histologic appearance (*e.g.,* needle biopsy showing improved hepatic architecture), and imaging modalities (*e.g.,* ultrasound, magnetic resonance imaging for fibrosis and liver size). Success of treatment can also be monitored by measuring serum levels of SPP1 protein, wherein a decrease in serum levels in a treated patient, compared to pre-treatment levels, indicate successful treatment.

In a further aspect, a Notch2 signaling inhibitor is an antibody used in any of the above embodiments that incorporates any of the features, singly or in combination, as described in Sections 1-7 below.

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its

antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6

$M^{-1} s^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see,* e.g., U.S. Patent No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al.*, Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit,

or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

5   In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human

10   antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

15   Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan,

20   *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

           Human framework regions that may be used for humanization include but are not

25   limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline

30   framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001)

5   and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous

10   immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No.

15   5,770,429 describing HuMab® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

20   Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies

25   generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is

30   also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5   **5. *Library-Derived Antibodies***

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in

10   Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol.*

15   *Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*,

20   12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as

25   described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include,

30   for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for Jag1 and the other is for any other antigen. In certain embodiments, one of the binding specificities is for Notch2 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Jag1. In certain embodiments, bispecific antibodies may bind to two different epitopes of Notch2. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Jag1 and/or Notch2. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see, e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to Jag1 or Notch2, as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or

other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a)   Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

5   (3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes

10   for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the

15   parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding

20   affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen,

25   with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the

variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR

5   residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to

10 bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

15   A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the

20 interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be

25 screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other

30 insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) <u>Glycosylation variants</u>

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US

Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

5   Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody

10   variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c)   Fc region variants

15   In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

20   In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities.

25   For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-

30   limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively,

non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells.

5   Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may

10   be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

15   Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

20   Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

          In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or

25   334 of the Fc region (EU numbering of residues).

          In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

30   Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants

include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of 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), and dextran or 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 may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not

limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B.   Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-Jag1 antibody described herein is provided. In one embodiment, isolated nucleic acid encoding an anti-Notch2 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-Jag1 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium). In one embodiment, a method of making an anti-Notch2 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-Jag1 antibody or an anti-Notch2 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary

tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C.   Assays

The antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1.   Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with Antibody A, A-1 or A-2 for binding to Jag1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by Antibody A, A-1 or A-2. In another aspect, competition assays may be used to identify an antibody that competes with Antibody B, B-1, B-2 or B-3 for binding to Notch2. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by Antibody B, B-1, B-2 or B-3. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In an exemplary competition assay, immobilized Jag1 is incubated in a solution comprising a first labeled antibody that binds to Jag1 (e.g., Antibody A, A-1 or A-2) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to Jag1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized Jag1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to Jag1, excess unbound antibody is removed, and the amount of label associated with immobilized Jag1 is measured. If the amount of label associated with

immobilized Jag1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to Jag1. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). In another exemplary competition assay, immobilized

5   Notch2 is incubated in a solution comprising a first labeled antibody that binds to Notch2 (e.g., Antibody B, B-1, B-2 or B3) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to Notch2. The second antibody may be present in a hybridoma supernatant. As a control, immobilized Notch2 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation

10 under conditions permissive for binding of the first antibody to Notch2, excess unbound antibody is removed, and the amount of label associated with immobilized Notch2 is measured. If the amount of label associated with immobilized Jag1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to Notch2.

15   ## 2. *Activity assays*

In one aspect, assays are provided for identifying Notch2 signaling inhibitor antibodies, such as anti-Jag1 antibodies and anti-Notch2 antibodies, having biological activity. Biological activity may include, e.g., inhibition or reduction of Notch2 activity, e.g., Notch2 signaling, inhibition or reduction of Jag1-mediated Notch signaling, e.g., Jag1-mediated Notch2

20 signaling. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, an antibody of the invention is tested for such biological activity. In certain embodiments, an antibody of the invention is tested for its ability to inhibit reduce Spp1 expression. An exemplary assay is provided in the Examples. In certain other embodiments, an antibody of the invention is tested for its ability to inhibit expression of a

25 reporter gene that is responsive to Notch2 signaling. In certain other embodiments, an antibody of the invention is tested for its ability to inhibit expression of a reporter gene that is responsive to Jag1-mediated signaling, e.g., Jag1-mediated Notch2 signaling. In one exemplary assay, NIH-3T3 cells stably transfected with Notch 2 or transiently transfected with plasmids containing other Notch receptors are co-transfected with a Notch-responsive TP-I (12X CSL)

30 Firefly luciferase reporter and a constitutively active Renilla Luciferase reporter (pRL-CMV, Promega) to control for transfection efficiency. Cells are allowed to recover from the transfection from 6 hours to overnight. Treatments of antibodies and NIH-3T3 cells stably transfected with ligand are used to stimulate the receptor cells. After 20 hours, firefly and

Renilla luciferase are measured with Dual Glo Luciferase Assay system (Promega). Replicates are analyzed for each condition by dividing the Firefly signal by the Renilla signal to control for transfection efficiency. The mean and standard deviation are calculated and values are normalized to calculated values for co-culture stimulated with NIH-3T3 cells without ligand transfected.

In certain embodiments, an antibody of the invention is tested for its ability to inhibit cell growth or proliferation in vitro. Assays for inhibition of cell growth or proliferation are well known in the art. Certain assays for cell proliferation, exemplified by the "cell killing" assays described herein, measure cell viability. One such assay is the CellTiter-Glo[TM] Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. *See* Crouch et al (1993) *J. Immunol. Meth.* 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). *See* Cree et al (1995) *AntiCancer Drugs* 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

Another assay for cell proliferation is the "MTT" assay, a colorimetric assay that measures the oxidation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan by mitochondrial reductase. Like the CellTiter-Glo[TM] assay, this assay indicates the number of metabolically active cells present in a cell culture. *See, e.g.,* Mosmann (1983) *J. Immunol. Meth.* 65:55-63, and Zhang et al. (2005) *Cancer Res.* 65:3877-3882.

In one aspect, an antibody of the invention is tested for its ability to induce cell death in vitro. Assays for induction of cell death are well known in the art. In some embodiments, such assays measure, e.g., loss of membrane integrity as indicated by uptake of propidium iodide (PI), trypan blue (*see* Moore et al. (1995) *Cytotechnology*, 17:1-11), or 7AAD. In an exemplary PI uptake assay, cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. Thus, the assay is performed in the absence of complement and immune effector cells. Cells are seeded at a density of 3×10^6 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is removed and replaced with fresh medium alone or medium

containing various concentrations of the antibody or immunoconjugate. The cells are incubated for a 3-day time period. Following treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4 °C, the pellet resuspended in 3 ml cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12 x 75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Antibodies which induce statistically significant levels of cell death as determined by PI uptake are thus identified.

In one aspect, an antibody of the invention is tested for its ability to induce apoptosis (programmed cell death) in vitro. An exemplary assay for antibodies or immunconjugates that induce apoptosis is an annexin binding assay. In an exemplary annexin binding assay, cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is removed and replaced with fresh medium alone or medium containing 0.001 to 10 μg/ml of the antibody or immunoconjugate. Following a three-day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca^{2+} binding buffer, and aliquoted into tubes as discussed in the preceding paragraph. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 μg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (BD Biosciences). Antibodies that induce statistically significant levels of annexin binding relative to control are thus identified. Another exemplary assay for antibodies or immunconjugates that induce apoptosis is a histone DNA ELISA colorimetric assay for detecting internucleosomal degradation of genomic DNA. Such an assay can be performed using, e.g., the Cell Death Detection ELISA kit (Roche, Palo Alto, CA).

Cells for use in any of the above in vitro assays include cells or cell lines that naturally express Notch2and/or Jag1 or that have been engineered to express Notch2and/or Jag1. Such cells include tumor cells that overexpress Notch2and/or Jag1 relative to normal cells of the same tissue origin. Such cells also include cell lines (including tumor cell lines) that express Notch2and/or Jag1 and cell lines that do not normally express Notch2and/or Jag1 but have been transfected with nucleic acid encoding Notch2and/or Jag1. Exemplary cell lines provided herein for use in any of the above in vitro assays include NIH-3T3 cells.

In one aspect, an antibody of the invention is tested for its ability to inhibit cell growth or proliferation in vivo. In certain embodiments, an anti-Jag1 antibody thereof is tested for its

ability to inhibit tumor growth in vivo. In certain embodiments, an anti-Notch2 antibody thereof is tested for its ability to inhibit tumor growth in vivo. In vivo model systems, such as xenograft models, can be used for such testing. In an exemplary xenograft system, human tumor cells are introduced into a suitably immunocompromised non-human animal, e.g., an

5   athymic "nude" mouse. An antibody of the invention is administered to the animal. The ability of the antibody to inhibit or decrease tumor growth is measured. In certain embodiments of the above xenograft system, the human tumor cells are tumor cells from a human patient. Such xenograft models are commercially available from Oncotest GmbH (Frieberg, Germany). In certain embodiments, the human tumor cells are cells from a human

10 tumor cell line, such as HepG2, Hep3B, PCL/PRF/5, Snu387, Snu398, Snu423, Snu449, Snu475, Huh-7, HLE, HLF, JHH1, JHH4, JHH5 and JHH7. In certain embodiments, the human tumor cells are introduced into a suitably immunocompromised non-human animal by subcutaneous injection or by transplantation into a suitable site, such as a mammary fat pad.

It is understood that any of the above assays may be carried out using an

15 immunoconjugate of the invention in place of or in addition to an Notch2 signaling inhibitor.

D.   Immunoconjugates

The invention also provides immunoconjugates comprising an anti-Notch2 antibody or anti-Jag1 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins,

20 enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235

25 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline

30 such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med.*

Chem. 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises an antibody as described
5 herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), 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,
10 gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the
15 radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

20   Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido
25 compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-
30 DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-
5   SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E.   Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the antibodies provided herein is useful for detecting the presence of a Notch2 or fragment thereof, or a Jag1 or fragment thereof, in a biological sample.
10   The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as hepatocyte, liver cancer cell and liver tumor tissue.

In one embodiment, an anti-Notch2 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of Notch2 in a
15   biological sample is provided. In certain embodiments, a method of detecting the presence of Notch2 intracellular domain (ICD) in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-Notch2 antibody as described herein under conditions permissive for binding of the anti-Notch2 antibody to Notch2, and detecting whether a complex is formed between the anti-Notch2 antibody and
20   Notch2. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-Notch2 antibody is used to select subjects eligible for therapy with an anti-Notch2 antibody, as described above, e.g. where Notch2, in particular activated Notch2, is a biomarker for selection of patients.

In one embodiment, an anti-Jag1 antibody for use in a method of diagnosis or detection
25   is provided. In a further aspect, a method of detecting the presence of Jag1 in a biological sample is provided. In certain embodiments, a method of detecting the presence of Jag1 intracellular domain (ICD) in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-Jag1 antibody as described herein under conditions permissive for binding of the anti-Jag1 antibody to Jag1, and detecting
30   whether a complex is formed between the anti-Jag1 antibody and Jag1. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-Jag1antibody is used to select subjects eligible for therapy with an anti-Jag1 antibody, as described above, e.g. where Jag1 is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention include liver cancer, specifically, hepatocellular carcinoma.

In certain embodiments, labeled anti-Notch2 or anti-Jag1 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F.   Pharmaceutical Formulations

Pharmaceutical formulations of an anti-Notch2 or anti-Jag1 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as 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 (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including 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/or non-ionic surfactants such as polyethylene glycol (PEG).
Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug
dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for
example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®,
5   Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including
rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In
one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such
as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958.
10   Aqueous antibody formulations include those described in US Patent No. 6,171,586 and
WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary
for the particular indication being treated, preferably those with complementary activities that
do not adversely affect each other. For example, it may be desirable to further provide a
15   chemotherapeutic agent or another therapeutic antibody with the anti-Notch2 or anti-
Jag1antibody. In some embodiments, a formulation may contain an anti-Notch2 antibody and
an anti-Jag1 antibody. Such active ingredients are suitably present in combination in amounts
that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by
20   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. Such techniques are
disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

25   Sustained-release preparations may 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.

The formulations to be used for *in vivo* administration are generally sterile. Sterility
30   may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G.   Therapeutic Compositions

Also provided herein are article of manufacture is provided comprising (a) a container;
(b) a composition of matter contained within the container comprising an anti-Notch2 antibody

or an anti-Jagged1 antibody and a carrier for the treatment of liver cancer; and (c) a label affixed to the container, or a package insert included with the container, referring to the use of the composition of matter for the therapeutic treatment of or the diagnostic detection of a liver cancer.

5   Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a chemotherapeutic agent.

Such combination therapies noted above encompass combined administration (where

10   two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-Notch2 or Jag1 antibody and administration of an additional therapeutic agent occur within about one month, or within

15   about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other. Antibodies of the invention can also be used in combination with radiation therapy.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular,

20   intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

25   Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical

30   practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages

described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-Notch2 or anti-Jag1 antibody.

H.   Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. For example, an article of manufacture is provided comprising (a) a container; (b) a composition of matter contained within the container comprising an anti-Notch2 antibody or an anti-Jagged1 antibody and a carrier for the treatment of liver cancer; and (c) a label affixed to the container, or a package insert included with the container, referring to the use of the composition of matter for the therapeutic treatment of or the diagnostic detection of a liver cancer.

Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a Notch2 signaling inhibitor, e.g., an anti-Notch2 antibody or anti-Jag1 antibody. The label or

package insert indicates that the composition is used for treating a proliferative disorder of the liver, such as liver cancer. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein,

5   wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a proliferative disorder of the liver, such as liver cancer. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such

10   as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-Notch2 or anti-Jag1

15   antibody.

III.   **EXAMPLES**

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description

20   provided above.

<u>Example 1</u>

<u>Murine model of liver cancer</u>

FVB-N mice (Charles River, Hollister) were subjected to hydrodynamic tail vein injection of Ras, AKT, and Sleeping Beauty transposase encoding plasmids as previously

25   described (Ho et al., C., Hepatology 55:833-845 (2012)). Briefly, 10ug of pT3-CAGGS-NRasV12, 10ug of pT3-EF1A-AKT, and 0.8ug CMV-SB (Ho et al., Hepatology 55:833-845 (2012); Yant et al., Mol. Cell. Biol. 24:9239-9247 (2004)) were diluted in approximately 2mL of Saline Solution (0.9% NaCl) and injected into the lateral tail vein of FVB-N mice in 5 to 8 seconds.

30   To model the development of liver cancer, mice were subjected to hydrodynamic tail vein injection of plasmids encoding oncogenic Ras and constitutively active AKT along with Sleeping Beauty transposase, as described above and as previously described (Ho et al., C., Hepatology 55:833-845 (2012)). This model allows for efficient and stable transfection of

hepatocytes and reliable expression of the transfected oncogenes. Within 5 weeks following hydrodynamic tail vein injection, mice developed numerous intrahepatic tumor masses. Much of the normal liver parenchyma was displaced by tumor epithelium and livers of these mice expanded to as much as ten times their original size. Consistent with previous reports, the

5   tumors that developed in these mice comprised a wide spectrum of liver tumor types, including hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). Approximately 80% of tumor nodules met the histopathological criteria for hepatocellular carcinoma and 20% met histopathological criteria for identification as cholangiocarcinoma.

Each liver comprised dozens of tumors each of which expressed a given marker or

10   combination of markers, such as AFP (FIG. 1A) and EpCAM (FIG. 1C). Some tumors express both AFP and EpCAM (FIG. 1B). Tumors in this model varied in their expression of HCC- and CC-specific tumor markers (FIG. 1A). Expression of alpha-Fetoprotein (AFP), specific for HCC, was detected in about 12.8% of cells in tumor-bearing livers (FIG. 1D), compared to less than 1% in normal non-tumor-bearing livers. Expression of EpCAM (Fig. 1B), a marker of

15   cholangiocarcinoma, was less prevalent in this model, detected in an average of about 5% of all liver cells, compared to about 1% in normal non-tumor-bearing livers. Tumors expressing both HCC- and CC-specific markers were also observed (FIG. 1C). These combined HCC-CC (cHCC-CC) tumors are characterized by particularly aggressive clinical features (American-Cancer-Society. 2012. Cancer Facts & Figures 2012. Atlanta: American Cancer Society) and

20   share gene expression patterns with liver progenitor cells (Coulouarn et al., Carcinogenesis 33:1791-1796 (2012)).

Example 2
Activation of Notch2 signaling

To determine if liver cancer is associated with Notch2 activation, immunofluorescense

25   analysis was performed on tumors arising in the livers of AKT/Ras HTV mice. Liver tissues were embedded and frozen in O.C.T.™ freezing medium (TISSUE-TEK®), and cryosectioned at 8μm. Sections were fixed in 4% paraformaldehyde (PFA) and stained using primary antibodies for Notch2 (Cell Signaling Technology), EpCAM (BioLegend), and AFP (R&D Systems). For image analysis, immunofluorescence-stained slides were scanned using an Ariol

30   slide scanning system (Leica).

High levels of Notch2 activation, as determined by immunofluorescence detection of nuclear Notch2, was observed in AFP[+]/EpCAM[+] tumors (FIG. 1E) and to a lesser extent in EpCAM[+] tumors (FIG. 1E, FIG.1F). Less prominent staining for activated Notch2 was observed in other tumor cell types (FIG. 1E).

<u>Example 3</u>

<u>Generation of antibodies</u>

To determine if Notch2 signaling was important for driving the development or growth of liver cancer, specifically in double positive tumors, mice were subjected to hydrodynamic tail vain injection with the AKT/Ras construct as described in Example 1, and treated with an anti-Notch2 antibody, anti-Jag1 antibody or isotype control (anti-Ragweed) antibody.

a. Library Sorting and Screening to Identify anti-Jagged 1 Antibodies

Human phage antibody libraries with synthetic diversities in the selected complementarity determining regions, mimicking the natural diversity of human IgG repertoire, were used for panning Fab fragments displayed on the surface of M13 bacteriophage particles. Human Jag1-DSL-EGF1-4 (FIG. 10) was used as antigen for library sorting. Nunc 96 well Maxisorp immunoplates were coated overnight at 4°C with target antigen (10μg/ml) and were blocked for 1 hour at room temperature with phage blocking buffer PBST (phosphate-buffered saline (PBS) and 1% (w/v) bovine serum albumin (BSA) and 0.05% (v/v) tween-20). Antibody phage libraries VH (see, e.g., Lee et al., J. Immunol. Meth. 284:119-132, 2004) and VH/VL (see Liang et al., JMB. 366: 815-829, 2007) were added to antigen plates separately and incubated overnight at room temperature. The following day antigen-coated plates were washed ten times with PBT (PBS with 0.05% Tween-20), and bound phage were eluted with 50mM HCl and 500mM NaCl for 30 minutes and neutralized with an equal volume of 1 M Tris base (pH7.5). Recovered phages were amplified in *E. coli* XL-1 Blue cells. During the subsequent selection rounds, incubation of antibody phage with the antigen-coated plates was reduced to 2-3 hours, and the stringency of plate washing was gradually increased.

After 4 rounds of panning, significant enrichment was observed. 96 clones were picked each from VH and VH/VL library sorting to determine whether they specifically bound to human Jagged 1. The variable regions of these clones were PCR sequenced to identify unique sequence clones. The affinities of phage antibodies were ranked using spot competition ELISA. The phage antibody IC50 values were further determined using competitive phage-binding ELISA. Unique phage antibodies that bind specifically to human Jagged 1 (and not Jagged 2), or to both Jagged 1 and Jagged 2 were chosen and reformatted to full-length IgGs for evaluation in *in vitro* cell assays.

Clones of interest were reformatted into IgGs by cloning V_L and V_H regions of individual clones into a pRK mammalian cell expression vector (pRK.LPG3.HumanKappa) containing the human kappa constant domain, and expression vector (pRK.LPG4.HumanHC) encoding the full-length human IgG1 constant domain, respectively (Shields et al., *J Biol Chem*

2000; 276: 6591-6604). The antibodies were then transiently expressed in mammalian CHO cells, and purified with a protein A column.

   b. *Construction of libraries for affinity improvement of clones derived from the V_H or*
      $V_H V_L$ libraries

5   Phagemid pW0703, derived from phagemid pV0350-2b (Lee et al., *J. Mol. Biol* 340, 1073-1093 (2004), containing stop codon (TAA) in all CDR-L3 positions and displaying monovalent Fab on the surface of M13 bacteriophage) served as the library templates for grafting heavy chain variable domains (V_H) of clones of interest from the V_H library for affinity maturation. Both hard and soft randomization strategies were used for affinity maturation. For
10 hard randomization, one light chain library with selected positions of the three light chain CDRs was randomized using amino acids designed to mimic natural human antibodies and the designed DNA degeneracy was as described in Lee et al. (*J. Mol. Biol* 340, 1073-1093 (2004)). To achieve the soft randomization conditions, which introduced the mutation rate of approximately 50% at the selected positions, the mutagenic DNA was synthesized with 70-10-
15 10-10 mixtures of bases favoring the wild type nucleotides (Gallop et al., *J.Med. Chem.* 37:1233-1251 (1994)). For soft randomization, residues at positions 91-96 of CDR-L3, 30-33, 35 of CDR-H1, 50, 52, 53-54, and 56 of CDR-H2, 95-98 of CDR-H3 were targeted; and three different combinations of CDR loops, H1/L3, H2/L3, and H3/L3, were selected for randomization.

20   For clones originated from $V_H V_L$ library, phagemids containing 4 stop codons (TAA) in each CDR and displaying monovalent Fab on the surface of M13 bacteriophage were generated individually, and served as the templates for kunkel mutagenesis for the construction of affinity maturation libraries. Only soft randomization strategy was used for clones derived from $V_H V_L$ library, as diversity of CDR-L3 was built into the naïve library. To achieve the soft
25 randomization conditions, residues at positions 28-31 of CDR-L1, 50, 53-55 of CDR-L2, 91-96 of CDR-L3, 30-35 of CDR-H1, 50-56 of CDR-H2, 95-100 of CDR-H3 were targeted; and four different combinations of CDR loops, H1/L3*, H2/L3*, and H3/L3* and L1/L2/L3* (where * denotes the position of stop codons on the template), were selected for randomization.

   c. *Phage Sorting Strategy to Generate Affinity Improvement*

30   For affinity improvement selection, Jag1 antigens were first biotinylated under limiting reagent condition. Phage libraries were subjected to one round of plate sorting and five rounds of solution sorting with increasing stringency. For the first round of plate sorting, 10ug/ml antigen was first coated on Maxisorp plate and preblocked with blocking buffer (1% BSA and 0.05% Tween20 in PBS). 3 O.D./ml in blocking buffer of phage input were incubated to

antigen plates for 3 hours. The wells were washed with PBS-0.05% Tween20 ten times. Bound phage was eluted with 150ul/well 50mM HCl, 500mM KCl for 30 minutes, and subsequently neutralized by 50ul/well of 1M Tris pH8, titered, and propagated for the next round. For subsequent rounds, panning of the phage libraries was done in solution phase, where phage

5   library was incubated with 100 nM biotinylated target protein (the concentration is based on parental clone phage IC50 value) in 100μl buffer containing 1% Superblock (Pierce Biotechnology) and 0.05% Tween20 for 2 hours at room temperature. The mixture was further diluted 10X with 1% Superblock, and 100μl/well was applied to neutravidin-coated wells (10μg/ml) for 30 minutes at room temperature with gentle shaking. To determine background

10   binding, control wells containing phage were captured on neutravidin-coated plates. Bound phage was then washed, eluted and propagated as described for first round. Five more rounds of solution sorting were carried out together with increasing selection stringency. The first couple rounds of which is for on-rate selection by decreasing biotinylated target protein concentration from 100nM to 0.1nM, and the last two rounds of which is for off-rate selection

15   by adding excess amounts of non-biotinylated target protein (300 to 1000 fold more) to compete off weaker binders at room temperature.

   d. High Throughput Affinity Screening ELISA (Single Spot Competition)

   Colonies were picked from the sixth round of screening. Colonies were grown overnight at 37°C in 150μl/well of 2YT media with 50μg/ml carbenicillin and 1x 10^{10}/ml

20   M13KO7 in 96-well plate (Falcon). From the same plate, a colony of XL-1 infected parental phage was picked as control. 96-well Nunc Maxisorp plates were coated with 100μl/well of either Jag1 or Jag2 (0.5μg/ml) in PBS at 4°C overnight. The plates were blocked with 150μl of 1% BSA and 0.05% Tween20 in PBS 20 for 1 hour.

   35μl of the phage supernatant was diluted with 75μl of ELISA (enzyme linked

25   immunosorbent assay) buffer (PBS with 0.5% BSA, 0.05% Tween20) with or without 5nM Jag1 or Jag2 and let incubate for 1 hour at room temperature in an F plate (NUNC). 95μl of mixture was transferred side by side to the antigen coated plates. The plate was gently shaken for 15 min and was washed ten times with PBS-0.05% Tween 20. The binding was quantified by adding horseradish peroxidase (HRP)-conjugated anti-M13 antibody in ELISA buffer

30   (1:2500) and incubated for 30 minutes at room temperature. The plates were washed with PBS-0.05% Tween 20 ten times. Next, 100μl/well of Peroxidase substrate was added to the well and incubated for 5 minutes at room temperature. The reaction was stopped by adding 100μl 0.1M Phosphoric Acid (H_3PO_4) to each well and allowed to incubate for 5 minutes at room temperature. The O.D. (optical density) of the yellow color in each well was determined

using a standard ELISA plate reader at 450 nm. In comparison to the OD_{450nm} reduction (%) of the well of parental phage (100%), clones that had the OD_{450nm} reduction (%) lower than 50% were picked for sequence analysis. Unique clones were selected for phage preparation to determine binding affinity (phage IC50) against Jag1 by comparison to respective parental clones. Then the most affinity-improved clones were reformatted into human IgG1 for antibody production.

Parent antibody A and affinity matured antibodies A-1 and A-2 specifically bound to human and murine Jag1, specifically, to Jag1 DSL-EGF1-4, but not human or murine Jag 2.

The generation and characterization of certain anti-Notch2 NRR antibodies have been previously described. See PCT Application No. PCT/US09/059028.

Example 4

Treatment with Notch2 signaling inhibitor reduces tumor burden

AKT/Ras HTV mice as described in Example 1 were treated with an anti-Notch2 antibody (15mg/kg, 1x/week), anti-Jag1 antibody (10mg/kg, 1x/week) or isotype control antibody beginning the day of the hydrodynamic tail vain injection. Livers were imaged and weighed at necropsy on a standard laboratory balance. Mice treated with the control antibody developed a heavy tumor burden (FIG. 2A) five weeks following hydrodynamic tail vain injection, with their livers increasing in size to about 8.9g or approximately 31% of body weight, up from 1.2g or 5.8% of body weight (FIG. 2B) in normal, non-tumor-bearing mice. Treatment with either anti-Notch2 or anti-Jag1 antibody significantly impeded tumor development (FIG. 2A and B; p<0.0001, n>8). Mice treated with anti-Notch2 antibody developed a significantly smaller tumor burden with their livers growing to an average of 5.1g or 19.3% of body weight (FIG. 2B). Anti-Jag1 treatment had an even greater effect. In these mice final liver weights averaged 4.3g or 15.8% of body weight (FIG. 2B).

EpCAM$^+$ and AFP$^+$/EpCAM$^+$ subsets of tumors (FIG. 1E), in which Notch2 signaling was more highly activated than in EpCAM$^-$ tumors as determined by detection of nuclear Notch2 by immunofluorescence, were highly susceptible to Notch2 pathway inhibition. EpCAM$^+$ tumors (AFP$^-$/EpCAM$^+$ and AFP$^+$/EpCAM$^+$ tumors) were significantly reduced in area following treatment with either anti-Notch2 antibody or anti-Jag1 antibody (FIG. 3A, FIG. 3B). Notch2 signaling was not as highly activated in AFP$^+$/EpCAM$^-$ tumors (FIG. 1E), suggesting that these tumors might not be affected by Notch2 pathway inhibition. However, contrary to expectation, both anti-Notch2 treatment and anti-Jag1 treatment led to significant reduction in AFP$^+$ tumor area (FIG. 3C). Taken together, these results demonstrate that anti-Notch2 or anti-Jag1 antibody treatment blocks the development of a broad range of liver

tumors in this model of liver cancer. Successful treatment with anti-Notch2 and anti-Jag1 antibodies resulted in reduction of overall tumor burden including both HCC-like and cholangiocarcinoma-like tumors as indicated by significant reductions in AFP and EpCAM staining following anti-Notch2 and anti-Jag1 antibody treatment.

5   Example 5

                                        Inhibition of Notch1 and Notch3

        Inhibition of Jag1 had a similar effect as inhibition of Notch2, suggesting that Jag1 and Notch2 are acting in the same pathway, specifically, that Jag1 acts as the ligand for Notch2 in supporting tumor formation. To determine if inhibition of other Notch receptors could also

10   reduce liver cancer formation or growth, mice were subjected to hydrodynamic tail vain injection with the Ras/AKT construct as described in Example 1 and treated with an anti-Notch1 antagonist antibody (10mg/kg, 1x/week) or an anti-Notch3 antagonist antibody (30mg/kg, 3x/week). Treatment with the anti-Notch1 antibody reduced liver weight in Ras/AKT HTV mice, compared to isotype controls, while treatment with an anti-Notch3

15   antibody did not significantly affect liver weight (FIG. 2D; $p<0.02$, $n \geq 7$). While anti-Notch2 or anti-Jag1 treatment decreased the level of EpCAM transcript (FIG. 3E, $p<0.005$, $n \geq 7$), inhibition of Notch1 increased the cross-sectional area of EpCAM positive tumors in the liver (FIG. 3D, FIG. 3E, $p<0.02$, $n \geq 7$) and increased the expression of the cholangiocarcinoma marker Cytokeratin 19 (CK19; FIG. 3F). Treatment with antagonist antibodies to anti-Notch1

20   or anti-Notch3 did not affect expression of Sox9, a liver progenitor cell and progenitor cell-like tumor marker and cholangiocarcinoma-like tumor marker, while treatment with an anti-Notch2 or anti-Jag1 antibody drastically decreased Sox9 at both the mRNA (FIG. 3G) and protein level (FIG. 3H).

        Thus, treatment with anti-Notch1 or anti-Notch3 antibodies did not significantly

25   decrease tumor burden. In fact, inhibition of Notch1 caused an increase in the number and cross-sectional area occupied by EpCAM$^+$ cholangiocarcinoma-like tumors. These results, taken together with the observation of increased cholangiocarcinoma-like lesions following anti-Notch1 treatment, further supported the conclusion that there are opposing roles for Notch2 and Notch1 in liver cancer.

30   Example 6

                    Anti-Notch2 or anti-Jag1 antibody treatment reduces Notch2 activation

        For immunohistochemical analysis, tissues were fixed in 10% Neutral Buffered Formalin, embedded in paraffin, and sectioned. 4 μm-thick formalin-fixed paraffin embedded human tissues were subject to staining. For Jag1 IHC staining, all steps were carried out on the

Ventana Discovery XT autostainer using Ventana detection reagents (Ventana Medical Systems, Tucson, AZ). Tissue sections were deparaffinized in EZPrep solution and pretreatment was done with Cell Conditioner 1 using standard incubation time. Tissue sections were then incubated with goat polyclonal anti-Jag1 primary antibody (Santa Cruz

5   Biotechnology Inc, Santa Cruz, CA; Cat# sc-6011) at 0.2 μg/ml for 32 minutes at room temperature followed by incubation with biotinylated rabbit anti-goat IgG antibody (Vector Labs, Burlingame, CA) at 7.5 μg/ml for 32 minutes at room temperature. Both primary and secondary antibodies were diluted in 10% normal human serum (Jackson ImmunoResearch) in 3% BSA. The sections were subsequently incubated with anti-Rabbit OmniMAP-HRP reagent

10   for 16 minutes at room temperature.

For Notch 2 IHC, all steps were carried out on the Ventana Discovery XT Platform utilizing Ventana detection reagents (Ventana Medical Systems, Tuscon, AZ). Sections were deparaffinized using EZ Prep and pretreatment was accomplished with Cell Conditioner 1 using standard incubation time. Sections were then incubated with rabbit monoclonal anti-

15   Notch2 primary antibody (Clone D76A6, Cell Signaling Technologies, Beverley, MA) at 8 μg/ml for 60 minutes at 37 °C, followed by incubation with anti-Rabbit OMNIMAP- HRP reagent for 32 minutes.

For Hes1, all steps were carried out on the Dako autostainer using DAKO wash buffer and DAKO Target Retrieval Solution. Sections were deparaffinized, re-hydrated then

20   incubated with DAKO Target Retrieval Solution at 99 °C for 20 min, quenched with 3%H2O2 for 4 minutes then blocked with Avidin Biotin Blocking Kit (Vector Laboratories: cat#sp-2001). Sections were incubated 45 min at RT with 1ug/ml anti-HES-1 (clone NM1; MBL International) then 5ug/ml secondary antibody Bt-Dk anti-Rat (JacksonImmunoResearch) 15 minutes followed by Biotinylated-Tyramide (1:50) in amplification diluent for 3 minutes.

25   Sections were subsequently incubated with DAB and Hematoxylin II reagents for chromogenic detection and counterstaining. Slides were dehydrated, cleared in xylenes and coverslipped. All Sections were subsequently incubated with DAB and Hematoxylin II reagents for chromogenic detection and counterstaining. Slides were dehydrated, cleared in xylenes and coverslipped. For image analysis, immunohistochemistry slides were scanned using the

30   Nanozoomer slide scanning system (Hamamatsu).

Quantitative real-time PCR (QRTPCR) was performed using the TaqMan One-Step RT-PCR Kit for one step reactions using the 7900 HT RT-PCR system (Applied Biosystems) with TaqMan probes (Applied Biosystems). Probes used were Notch1 (Mm00435245_m1, Hs01062014_m1), Notch2 (Mm00803077_m1, Hs01050719_m1), Notch3 (Mm00435270_m1,

Hs01128541_m1), Notch4 (Mm00440525), Jag1 (Mm00496902_m1), Jag2 (Mm01325629_m1), DLL1 (Mm01279269_m1), DLL3 (Mm00432854_m1), DLL4 (Mm00444619), Hey1 (Mm00516555_m1), CK19 (Mm00492980_m1) and Sox9 (Mm00448840_m1).

5   In keeping with the broader effect of Notch2 and Jag1 inhibition on tumor formation, Notch2 signaling, as determined by detection of nuclear Notch2 protein by immunofluorescence, was significantly reduced throughout the tumor-bearing livers following treatment with either anti-Notch2 or anti-Jag1 (FIG. 4A; $p<0.05$, $n \geq 7$). This reduction appeared to be due to a direct effect on activation of the Notch2 protein, as overall levels of

10   Notch2 expression, as determined by quantitative RT-PCR, did not change. Consistent with anti-Notch2 or anti-Jag1 antibody treatment blocking Notch2 activation, immunostaining was reduced for Hes1, a downstream transcriptional target of the Notch2 signaling pathway. Control-treated Ras/AKT HTV livers showed high levels of Hes1 staining in (FIG. 4D). However, treatment with anti-Notch2 or anti-Jag1 antibody significantly reduced Hes1

15   staining, consistent with effective blockade of Notch2 signaling in the antibody- treated livers (FIG. 4C, FIG. 4D, $p \leq 0.0001$, $n > 10$). Confirming that Notch2 signaling was blocked, quantitative RT-PCR analysis revealed that the Notch pathway target gene HeyL was also strongly decreased with either anti-Notch2 or anti-Jag1 antibody treatment (FIG. 4E, $p<0.0001$, $n>7$).

20   In each case, anti-Jag1 antibody treatment had the same effect as anti-Notch2 antibody treatment, further supporting the conclusion that Jag1 is acting primarily through, i.e. as a ligand for, Notch2 in supporting liver cancer formation and growth. Taken together, these results demonstrate that treatment with either anti-Notch2 or anti-Jag1 antibody resulted in a reduction of Notch2 activation.

25   Example 7

          Effect of Notch inhibitory antibodies on expression of Notch signaling pathway
                                              components

          Mice subjected to AKT/Ras HTV were treated with antagonistic antibodies to Notch1 (10mg/kg, 1x/week), Notch2 (10mg/kg, 2x/week), Notch3 (30mg/kg, 3x/week), or Jag1

30   (10mg/kg, 1x/week). An anti-Ragweed antibody was administered at 30mg/kg, 3x/week as a negative control. After 5 weeks, livers were harvested and quantitative real-time PCR was performed on the isolated RNA to determine the effect of treatment on transcripts of Notch signaling pathway components. Inhibition of individual Notch family receptors did not lead to a compensatory increase in the expression of other Notch receptor family members.

As described above, inhibition of individual Notch receptors, Notch1, Notch2, and Notch3, had distinct effects in the AKT/Ras model of liver tumor development. This result suggests that the individual Notch receptors do not necessarily compensate for one another in liver cancer. To determine the effect of inhibition of individual Notch receptors and ligands on expression of Notch receptor family members, expression was assessed following treatment of mice injection with the AKT/Ras construct as described in Example 1 with either an anti-Notch1, anti-Notch2, anti-Notch3, anti-Jag1 antibody or isotype control antibody. No increase in receptor transcript expression for any of the individual Notch receptors was observed upon inhibition with any of the three inhibitory antibodies (FIG. 5 A-C). On the contrary Notch2 inhibition with a specific inhibitory antibody led to a significant decrease in expression levels of both Notch3 and Notch4 ($p<0.05$, $n \geq 7$). Inhibition of Notch3 also led to a decrease in its own expression ($p<0.005$, $n \geq 7$). This is consistent with previous observations that both Notch2 and Notch3 control the transcription of Notch3 (Wang et al., PLoS ONE 7:e37365 (2012); Liu et al., Circulation Research 107:860-870 (2010)). The effect of treatment with the antagonist antibodies was even greater with respect to expression of Notch ligands Jag1 and DLL1. Notch2 inhibition led to a significant decrease in Jag1 expression (FIG. 5E), likely as a result of the decrease in Jag1-expressing cholangiocarcinoma and progenitor-like tumors in these livers. Jag1 inhibition had a similar effect, further confirming that Jag1 and Notch2 are acting together in this tumor model. In contrast inhibition of Notch1 significantly increased the expression of the Notch ligands Jag2 and DLL1 (FIG. 5 F, FIG. 5G). This result might, at least in part, help to explain the observed increase in cholangiocarcinoma-like lesions in livers treated with an anti-Notch1 antagonist antibody.

In summary, a compensatory increase in expression of other Notch receptors upon Notch2 inhibition was not observed, suggesting that successful treatment with anti-Notch2 or anti-Jag1 antibodies will not lead to resistance through upregulation of alternative Notch signaling components. In fact, treatment with either Notch2 or Jag1 antagonist antibodies actually led to a decrease in Jag1 ligand expression, and treatment with anti-Notch2 led to a decrease in Notch3 expression.

Example 8

Anti-Notch2 or anti-Jag1 antibody treatment blocks progenitor-like and cholangiocarcinoma-like liver tumor growth

To address the mechanisms by which Notch2 inhibition is leading to decreased tumor burden, high throughput RNA sequencing analysis was performed. Mice subjected to AKT/Ras HTV were treated with antagonistic antibodies to Notch2 (10mg/kg, 2x/week), Jag1

(10mg/kg, 1x/week), or anti-Ragweed control (30mg/kg, 3x/week). After 5 weeks, livers were harvested and RNA was subjected to high-throughput sequencing.

Progenitor-cell and cholangiocarcinoma-like HCC expression signature gene expression was down-regulated in tumor-bearing livers following anti-Notch2 and anti-Jag1

5   antibody treatment. EpCAM and CK19 expression was significantly down-regulated, as was expression of CD133/Prom1 (FIG. 6A and data not shown) and Spp1 (FIG. 6B), both markers of liver progenitor cells. Because FoxM1 has previously been shown to play a role in HCC proliferation in general (Xia et al., Carcinogenesis 33:2250-2259 (2012)) and in the Ras/Akt model of liver cancer specifically (Ho et al., Hepatology 55:833-845 (2012)), we examined its

10   expression and were able to show that both Notch2 and Jag1 inhibition lead to a decrease in FoxM1 expression(FIG. 6C). Moreover, FoxM1 target genes (Laoukili et al., Nat Cell Biol 7:126-136 (2005)), PLK1 (FIG. 6D), Ccnb1 (FIG. 6E), and Aurkb (FIG. 6F) were also decreased in tumor-bearing livers treated with either anti-Notch2 or anti-Jag1 antibody compared to controls. Markers of Wnt signaling were increased upon Notch2 or Jag1

15   inhibition. Specifically, Wnt2 ligand was increased (FIG. 6G) as was the Wnt-pathway target geneAxin2 (FIG. 6H). The expression of Glutamine synthetase (Glul; FIG. 6I), a marker of a subset of terminally differentiated hepatocytes, was also increased in tumor-bearing livers treated with either anti-Notch2 or anti-Jag1 antibody compared to controls. These observations are consistent with Notch2 inhibition inducing a decrease in tumor cell proliferation through

20   downregulation of FoxM1 and inducing a differentiated hepatocyte fate through induction of Wnt-signaling (Boulter et al., Nat. Med. 18(4):572 (2012)). It is possible that Notch2 signaling inhibition in liver cancer effects terminal differentiation of tumor cells into hepatocytes. Consistent with this hypothesis, Notch2 and Jag1 inhibition led to an increase in transcriptional markers of terminally differentiated hepatocytes as well as Wnt signaling, which is known to

25   be important in differentiation of hepatocytes from progenitor cells (Boulter et al., Nat. Med. 18(4):572 (2012)). Notch2 and Jag1 inhibition may also be acting by decreasing proliferation and increasing tumor cell death.

Example 9

Expression and activation of Notch2 in human hepatocellular carcinoma

30   Human HCC cell lines and primary human HCC tumors were analyzed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) for expression of Notch signaling components. HCC cell lines HepG2, Hep3B, PCL/PRF/5, Snu387, Snu398, Snu423, Snu449, Snu475 were acquired from ATCC (Manassas, Virginia). HCC cell lines Huh-7, HLE, HLF, JHH1, JHH4, JHH5, JHH7 were acquired from the Japanese Collection of

Bioresources Cell Bank (Osaka, Japan). Whole stained tissue sections were analyzed using Definiens software.

Notch2 was expressed at higher levels than either Notch1 or Notch3 in 15 of 16 cultured HCC cell lines (FIG. 7A). In many cases, Notch2 expression exceeded that of the other Notch family members by more than 10 fold when expression was normalized to a reference gene (RPL19, FIG. 7A). Consistent with this result, prominent expression of Notch2 was observed in 28 of 76 (37%) human primary HCC samples as determined by IHC. In 15 of these 28 human primary HCC samples (54%) Notch2 showed varying degrees of nuclear localization (FIG. 7B) indicating Notch2 pathway activation. Jag1 expression, evaluated by IHC, was observed in 34 of 59 (57%) human primary HCC samples examined (FIG. 7B). Of the 56 human primary HCC samples evaluated for both Notch2 and Jag1, 15 (27%) were found to have expression of both Notch2 and Jag1. Of those 15 tissues with overlapping expression, 11 (73%) showed some degree of Notch2 nuclear localization indicating active Notch2 signaling.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1.   A method of treating a liver cancer in an individual in need thereof, the method comprising the step of administering to the individual an effective amount of a Notch2 signaling inhibitor, wherein the liver cancer comprises cells that express EpCAM and/or alphafetoprotein (AFP), and wherein the Notch2 signaling inhibitor is selected from an anti-Jag1 antibody and an anti-Notch2 antibody.

2.   Use of a Notch2 signaling inhibitor in the manufacture of a medicament for treating a liver cancer in an individual in need thereof, wherein the liver cancer comprises cells that express EpCAM and/or alphafetoprotein (AFP), and wherein the Notch2 signaling inhibitor is selected from an anti-Jag1 antibody and an anti-Notch2 antibody.

3.   The method of claim 1 or use of claim 2, wherein the liver cancer is selected from the group consisting of hepatocellular carcinoma, hepatoma, cholangiocarcinoma, hepatoblastoma, hepatic carcinoma, hepatic angiosarcoma, and metastatic liver cancer.

4.   The method or use of any one of claims 1 to 3, wherein the antibody is a monoclonal antibody.

5.   The method or use of claim 4, wherein the antibody is an antagonist antibody.

6.   The method or use of claim 4, wherein the antibody is an anti-Notch2 antibody.

7.   The method or use of claim 6, wherein the antibody is an anti-Notch2 NRR antibody.

8.   The method or use of claim 6, wherein the antibody does not significantly bind to a Notch family member other than Notch2.

9.   The method or use of claim 7, wherein the antibody binds to mouse Notch2 NRR and human Notch2 NRR.

10.   The method or use of claim 7, wherein the antibody binds to a Notch2 NRR with a Kd of $\leq$ 10 nM.

11.   The method or use of claim 7, wherein the antibody comprises:
        (a)   an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1;
        (b)   an HVR-H2 comprising the amino acid sequence of SEQ ID NO:3;
        (c)   an HVR-H3 comprising the amino acid sequence of SEQ ID NO:4;
        (d)   an HVR-L1 comprising the amino acid sequence of SEQ ID NO:9;
        (e)   an HVR-L2 comprising the amino acid sequence of SEQ ID NO:14; and

(f)   an HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

12.   The method or use of claim 4, wherein the antibody is an anti-Jag1 antibody.

13.   The method or use of claim 12, wherein the anti-Jag1 antibody comprises:

(i)

     (a)   HVR-H1 comprising the amino acid sequence of SEQ ID NO:81;

     (b)   HVR-H2 comprising an amino acid sequence of SEQ ID NO:84;

     (c)   HVR-H3 comprising an amino acid sequence of SEQ ID NO:87;

     (d)   HVR-L1 comprising the amino acid sequence of SEQ ID NO:88;

     (e)   HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and

     (f)   HVR-L3 comprising an amino acid sequence of SEQ ID NO:92; or

(ii)

     (a)   an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81;

     (b)   an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82;

     (c)   an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85;

     (d)   an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88;

     (e)   an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and

     (f)   an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90; or

(iii)

     (a)   an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81;

     (b)   an HVR-H2 comprising the amino acid sequence of SEQ ID NO:82;

     (c)   an HVR-H3 comprising the amino acid sequence of SEQ ID NO:86;

     (d)   an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88;

     (e)   an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and

     (f)   an HVR-L3 comprising the amino acid sequence of SEQ ID NO:91; or

(iv)

     (a)   an HVR-H1 comprising the amino acid sequence of SEQ ID NO:81;

     (b)   an HVR-H2 comprising the amino acid sequence of SEQ ID NO:83;

     (c)   an HVR-H3 comprising the amino acid sequence of SEQ ID NO:85;

     (d)   an HVR-L1 comprising the amino acid sequence of SEQ ID NO:88;

     (e)   an HVR-L2 comprising the amino acid sequence of SEQ ID NO:89; and

     (f)   an HVR-L3 comprising the amino acid sequence of SEQ ID NO:90.

14.   The method or use of claim 13, wherein the anti-Jag1 antibody further comprises:

(a)   light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 of FIG. 18;

(b)   heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 of FIG. 18;

(c)   light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 light chain variable domain framework LC-FR1, LC-FR2, LC-FR3, and LC-FR4 of FIG. 19; or

(d)   heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 comprising, in order, the amino acid sequence of huMAb4D5-8 heavy chain variable domain framework HC-FR1, HC-FR2, HC-FR3, and HC-FR4 of FIG. 19.

15.   The method or use of claim 13, wherein the anti-Jag1 antibody comprises:

(a)   a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:93;

(b)   a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:96; or

(c)   a VH sequence as in (a) and a VL sequence as in (b).

16.   The method or use of claim 15, wherein the anti-Jag1 antibody comprises:

(a)   a VH sequence of SEQ ID NO:93;

(b)   a VL sequence of SEQ ID NO:96; or

(c)   a VH sequence of SEQ ID NO:93 and a VL sequence of SEQ ID NO:96.

17.   The method or use of claim 13, wherein the anti-Jag1 antibody comprises:

(a)   a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:94;

(b)   a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:97; or

(c)   a VH sequence as in (a) and a VL sequence as in (b).

18.   The method or use of claim 17, wherein the anti-Jag1 antibody comprises:

(a)   a VH sequence of SEQ ID NO:94;

(b)   a VL sequence of SEQ ID NO:97; or

(c)   a VH sequence of SEQ ID NO:94 and a VL sequence of SEQ ID NO:97.

19.   The method or use of claim 13, wherein the anti-Jag1 antibody comprises:

(a)   a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:95;

(b)   a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:98; or

(c)   a VH sequence as in (a) and a VL sequence as in (b).

20.   The method or use of claim 19, wherein the anti-Jag1 antibody comprises:

(a)   a VH sequence of SEQ ID NO:95;

(b)   a VL sequence of SEQ ID NO:98; or

(c)   a VH sequence of SEQ ID NO:95 and a VL sequence of SEQ ID NO:98.

21.   The method or use of any one of claims 1 to 20, wherein the antibody is:

(a) a full-length IgG1 or IgG2a antibody;

(b) an antibody fragment;

(c) a human, humanized or chimeric antibody;

(d) conjugated to a growth inhibitory agent; or

(e) conjugated to a cytotoxic agent.

22.   The method or use of claim 21, wherein the cytotoxic agent is selected from the group consisting of toxins, antibiotics, radioactive isotopes and nucleolytic enzymes.

23.   The method or use of any one of claims 1 to 22, wherein the antibody causes cancer cell death.

24.   The method of any one of claims 1 or 3 to 23, further comprising administering to the individual at least one additional therapeutic agent or the use of any one of claims 2 to 23 wherein the individual is administered at least one additional therapeutic agent.

25.   The method or use of claim 24, wherein the additional therapeutic agent is a chemotherapeutic agent.

26.   The method or use of any one of claims 1 to 25, wherein the liver cancer comprises cells that express at least one of Notch2, Jag1, Sox9, CK19, Ras, Prom1, Spp1, FoxM1, Plk1, ccnb1, Aurkb, Wnt2, Axin2, or Glul.

27.   The method or use of claim 26, wherein the liver cancer comprises:

(a)   cells having nuclear Notch2; and/or

(b)   cells having activated Ras.

28.   The method or use of any one of claims 1 to 27, wherein the liver cancer comprises cells that express EpCAM and wherein administering the Notch2 signaling inhibitor results in a decrease in EpCAM expression in the cells.

29.   The method or use of any one of claims 1 to 28, wherein the liver cancer comprises cells that express AFP and wherein administering the Notch2 signaling inhibitor results in a decrease in AFP expression in the cells.

30.   The method or use of claim 26, wherein administering the Notch2 signaling inhibitor results in a decrease in expression, compared to expression prior to administering the inhibitor, in the liver cancer of at least one of Prom1, Spp1, FoxM1, Plk1, ccnb1 and Aurkb.

31.   The method or use of claim 26, wherein administering the Notch2 signaling inhibitor results in an increase in expression in the liver cancer of at least one of Wnt2, Axin2 and Glul.

FIG. 1B
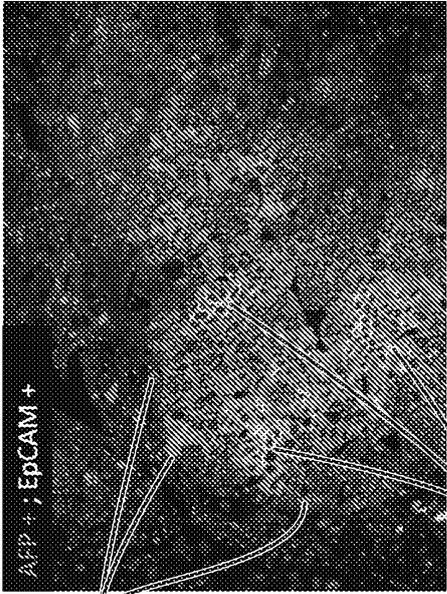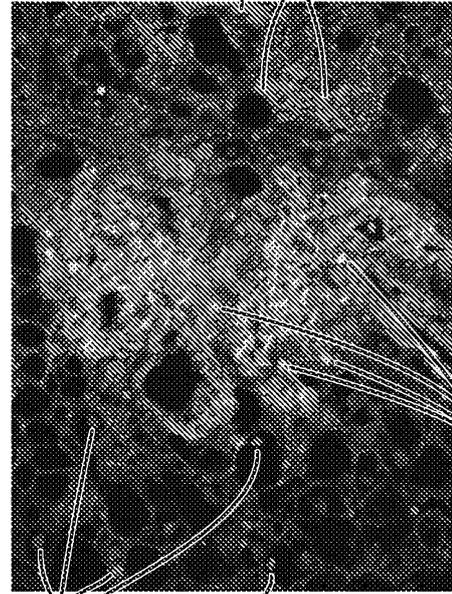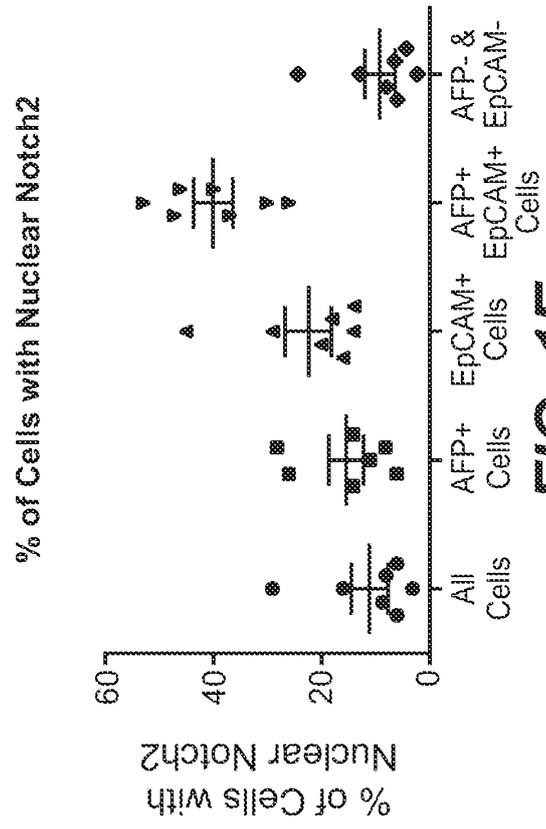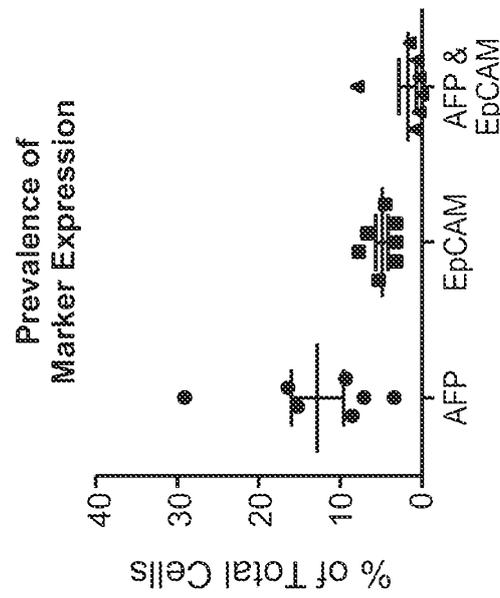
FIG. 1F

FIG. 1A

FIG. 1C

FIG. 1E

FIG. 1D

FIG. 2A

Ras/AKT HTV Liver Weights

FIG. 2B

Ras/AKT HTV Liver Weights: Intervention

FIG. 2C

Ras/AKT HTV Liver Weights

FIG. 2D

FIG. 3A

FIG. 3D

Total EpCAM+ Area

FIG. 3B

AFP

FIG. 3C

% of Cells EpCAM+
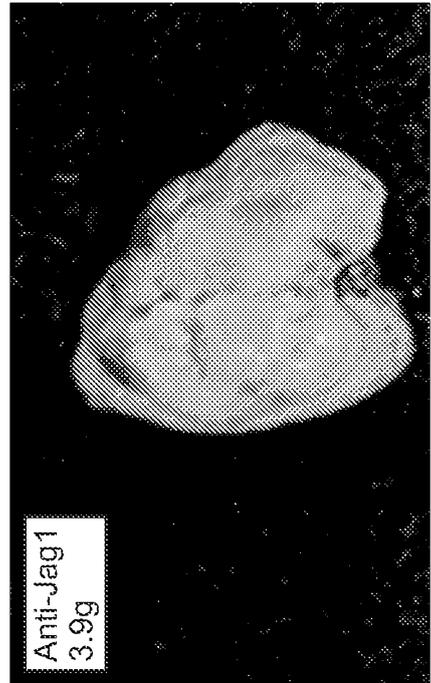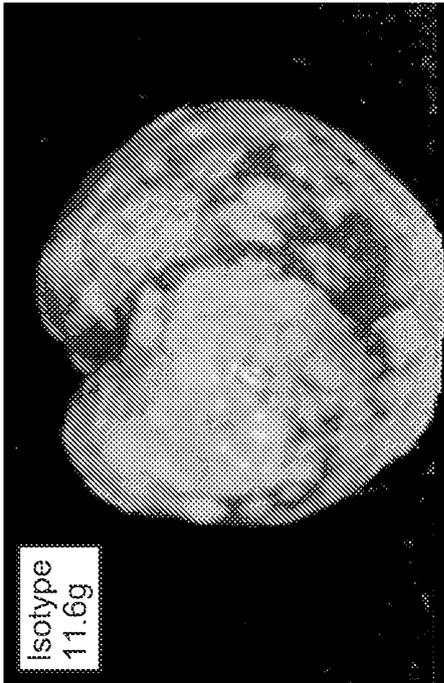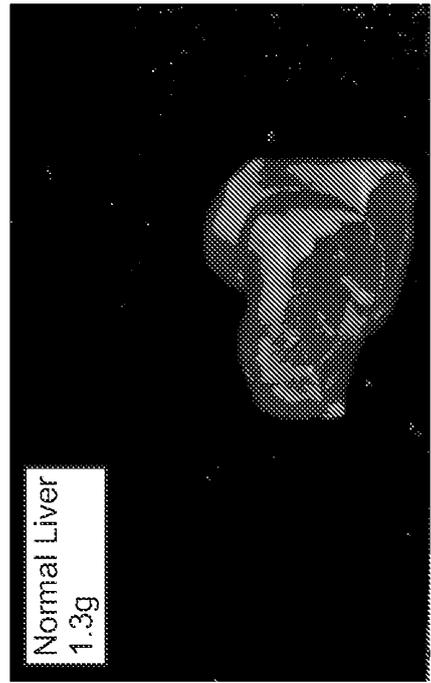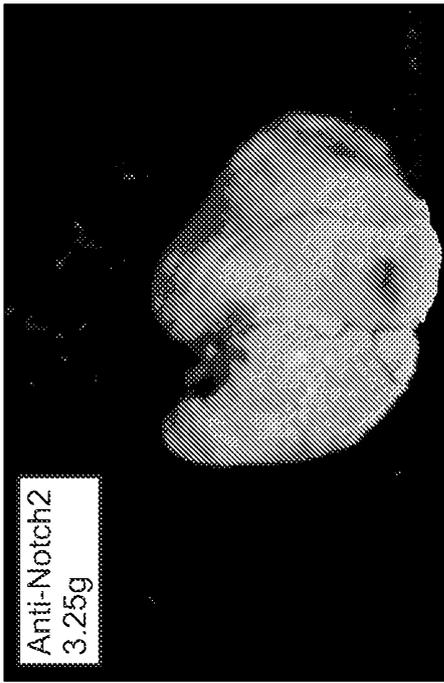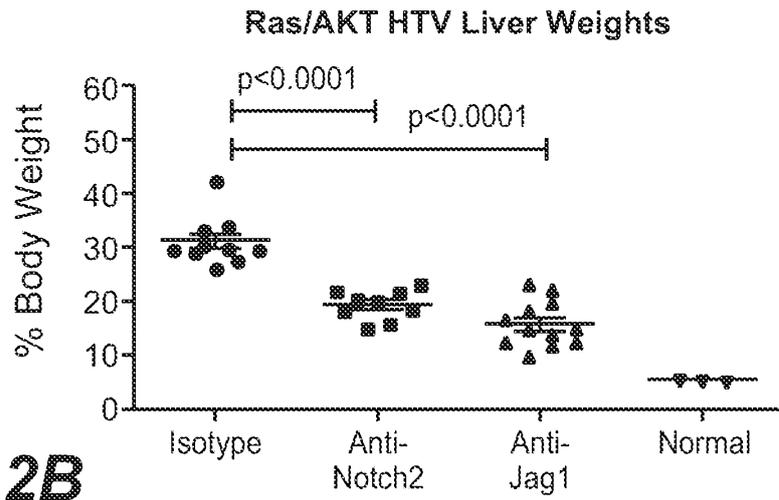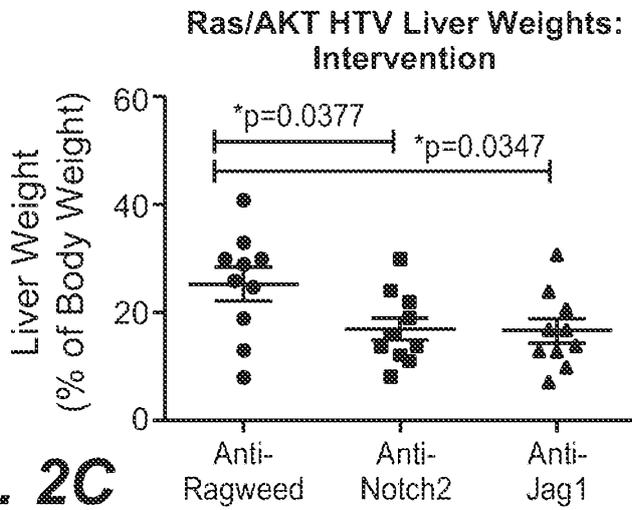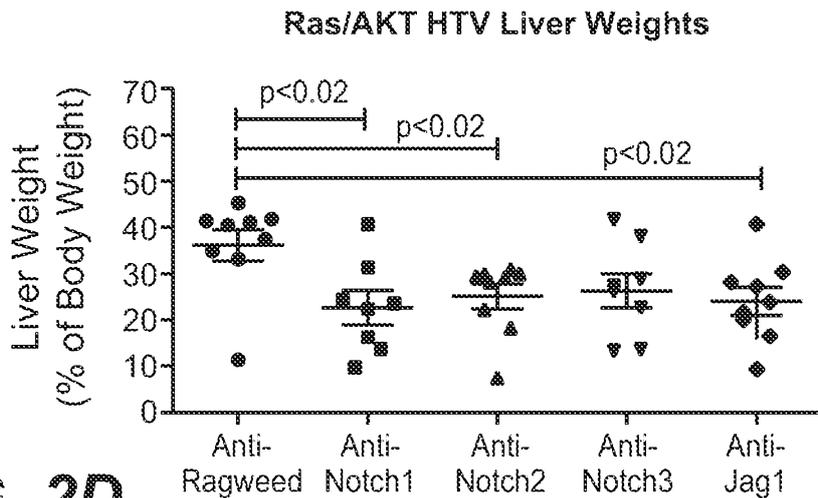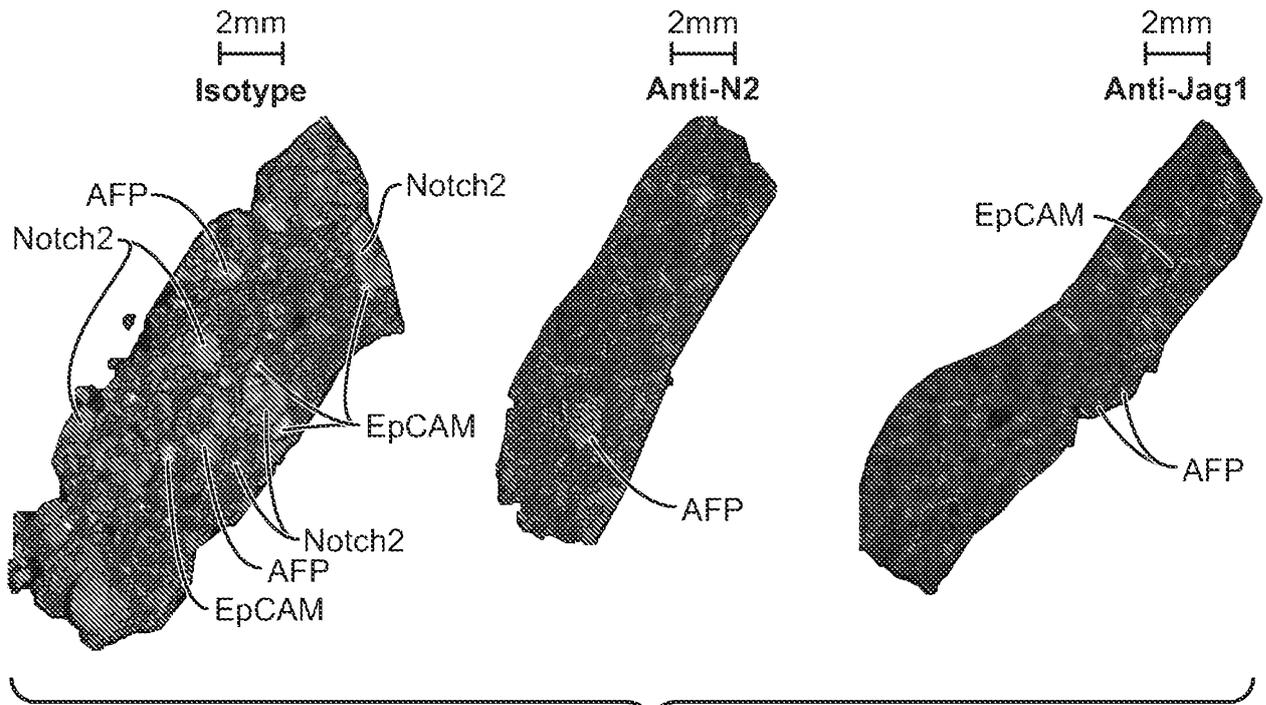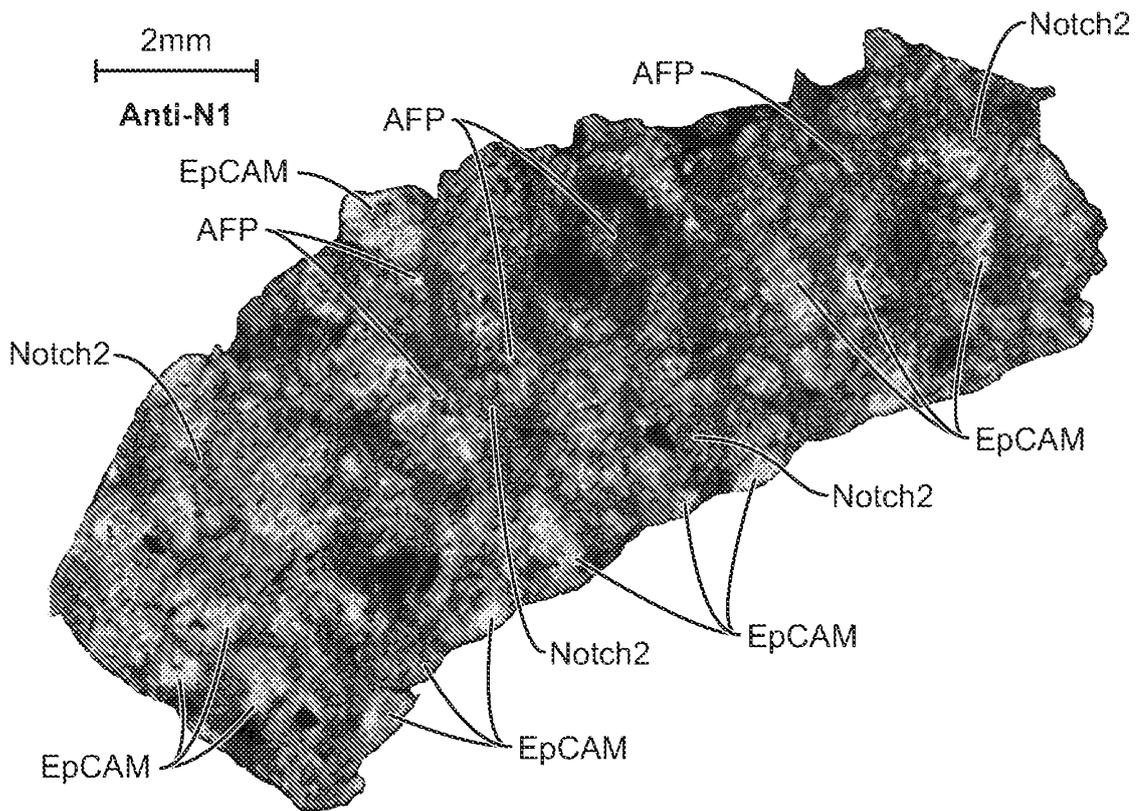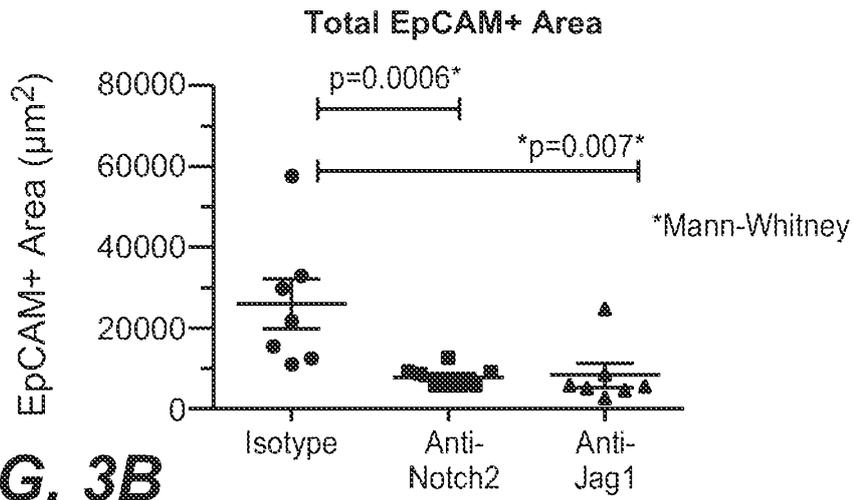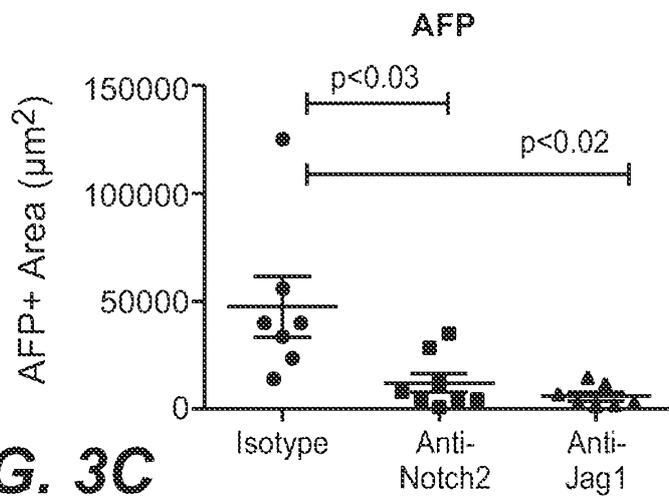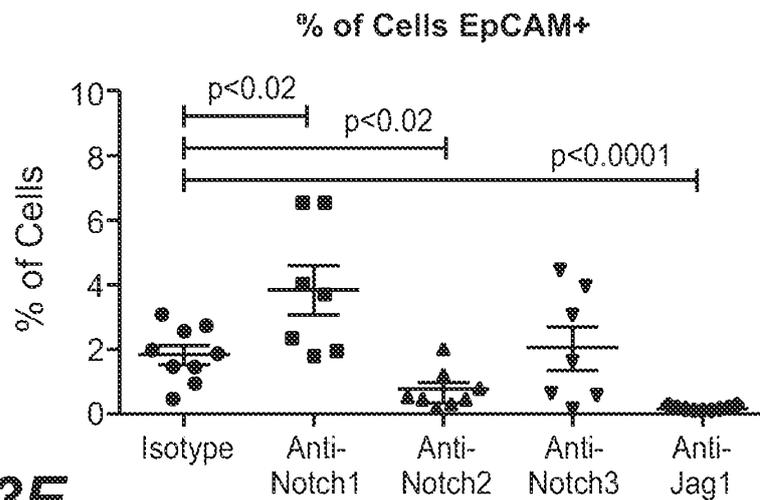
FIG. 3E
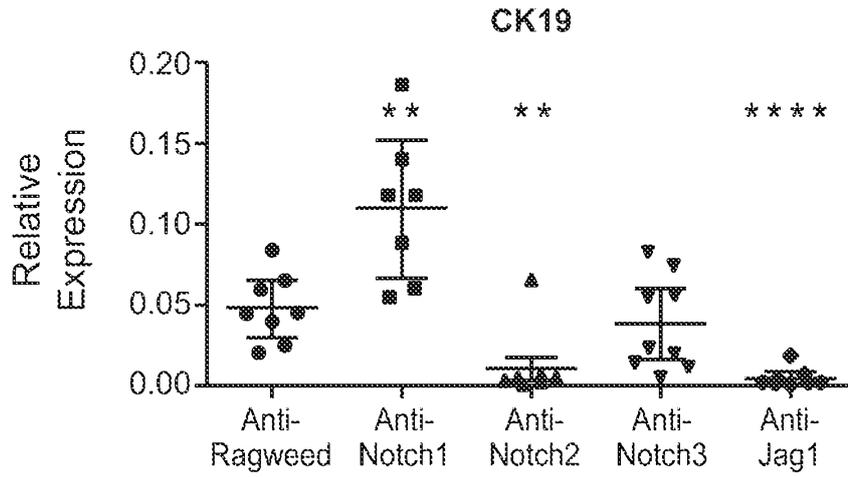
FIG. 3F
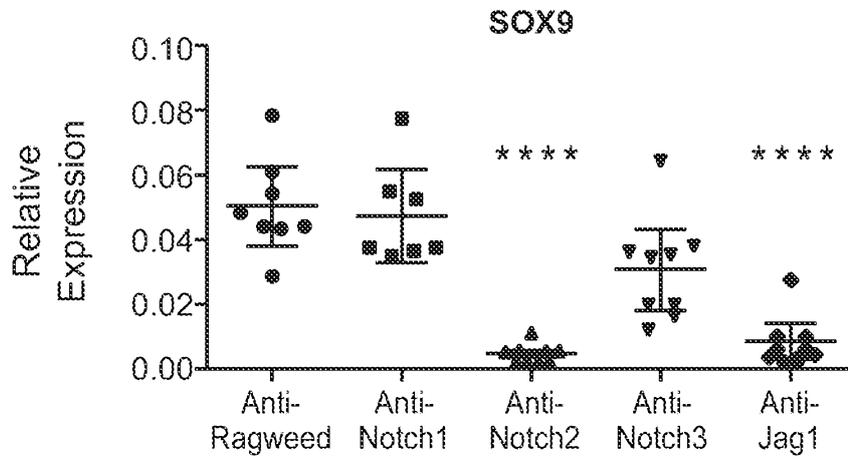
FIG. 3G
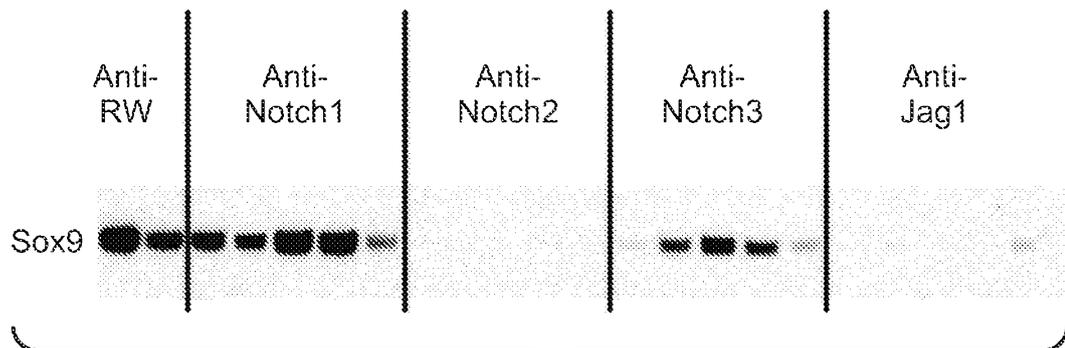
FIG. 3H
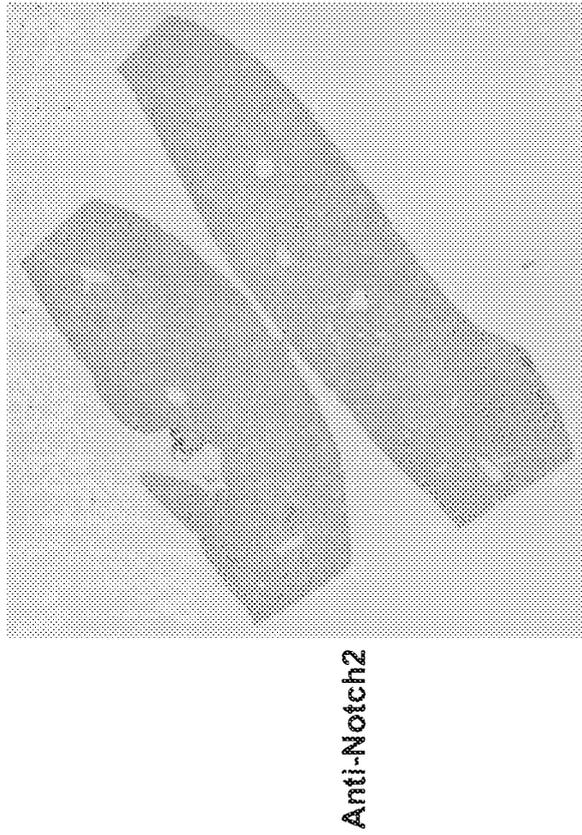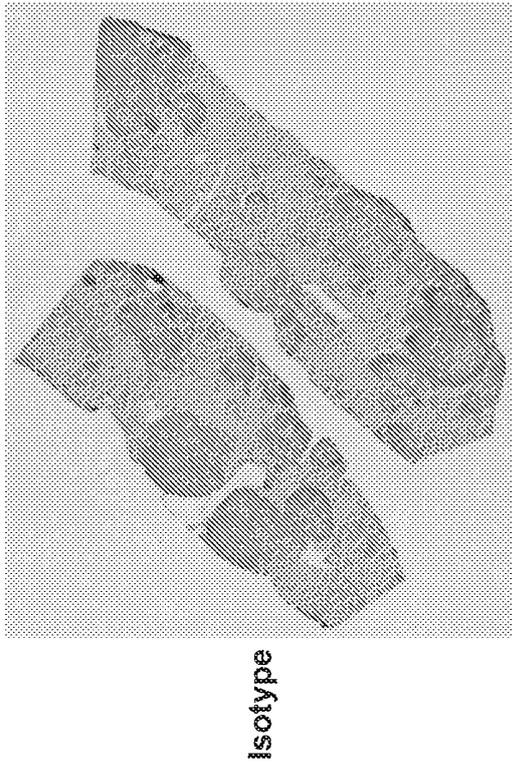
FIG. 4C
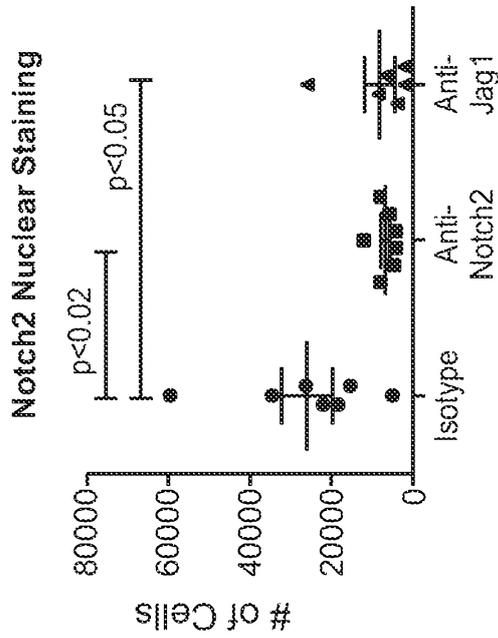
FIG. 4A
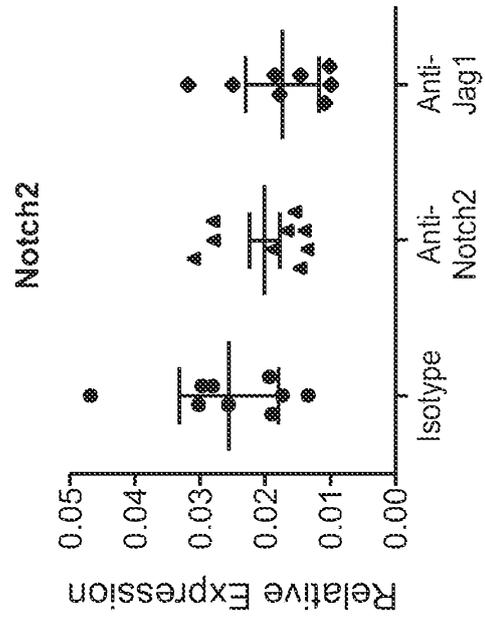
FIG. 4B
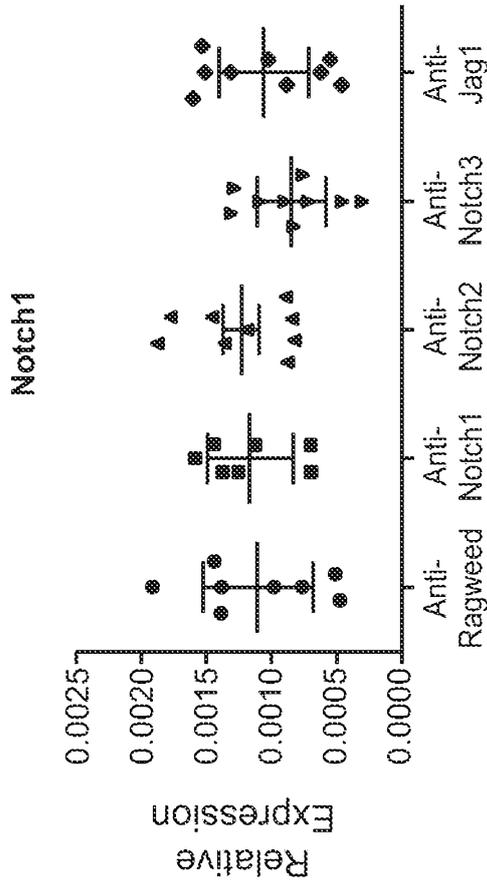
FIG. 5A
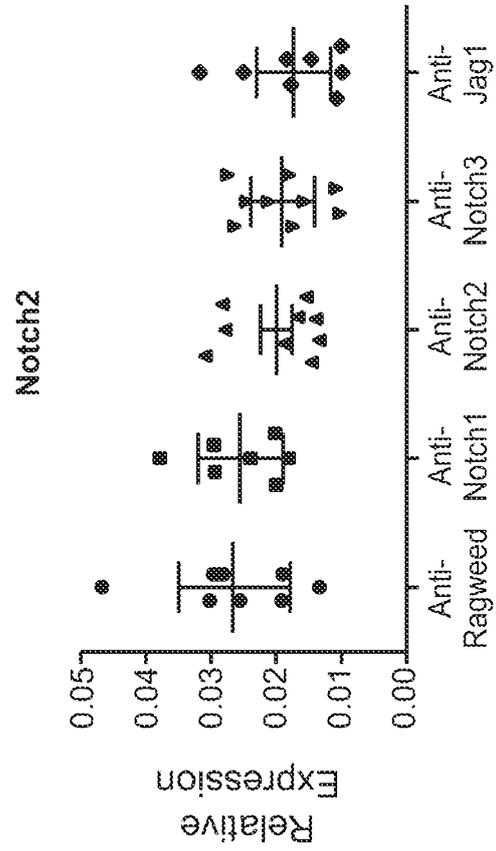
FIG. 5B
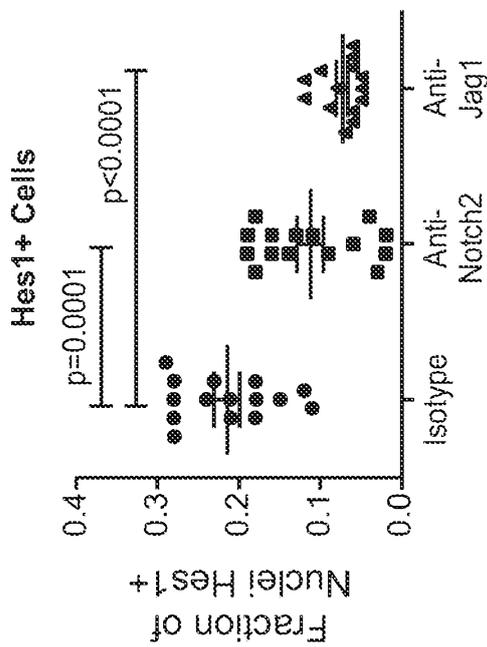
FIG. 4D
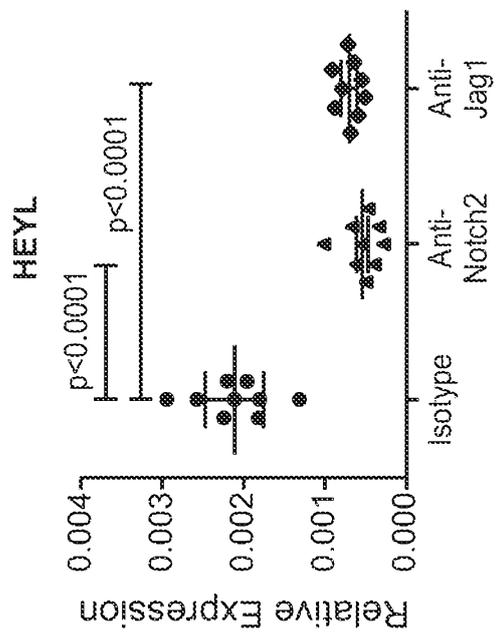
FIG. 4E
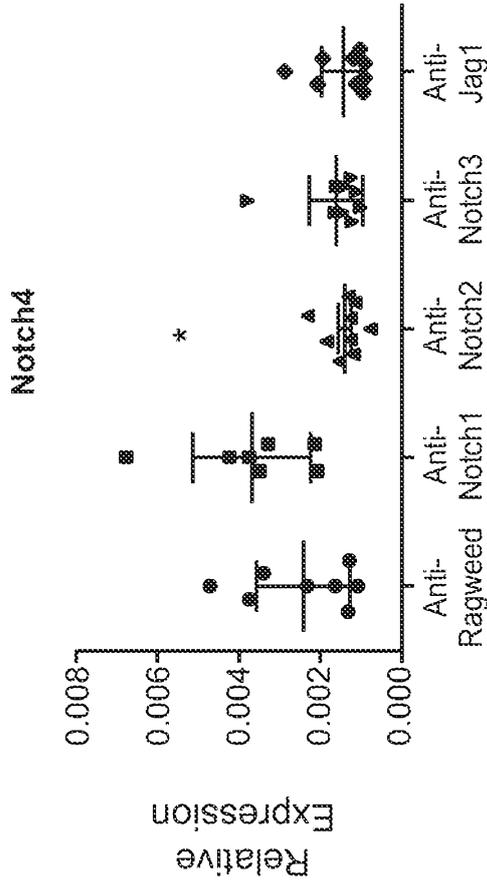
FIG. 5D
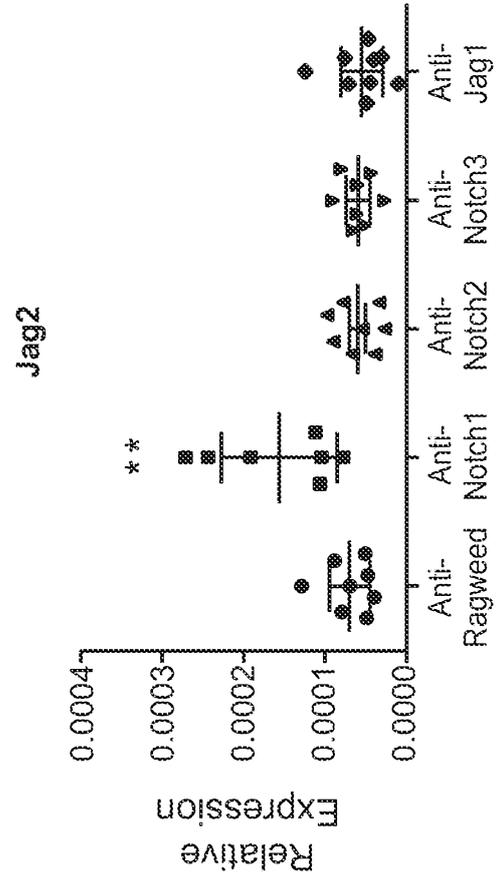
FIG. 5F
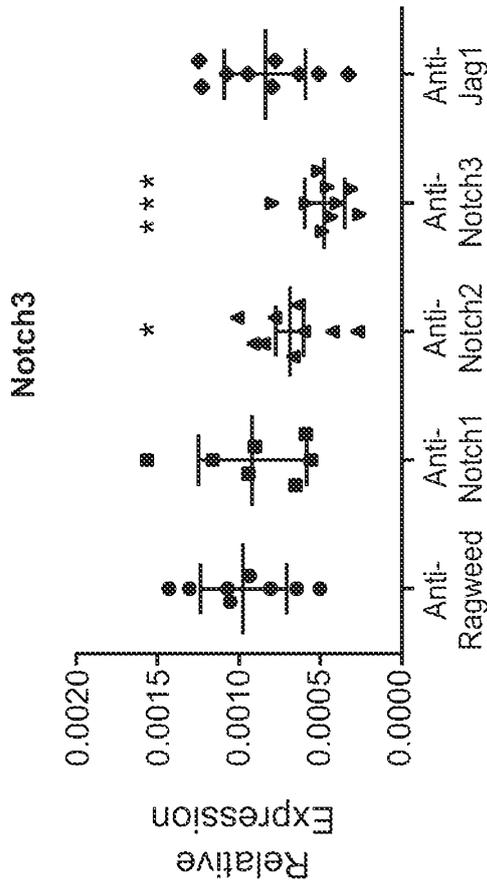
FIG. 5C
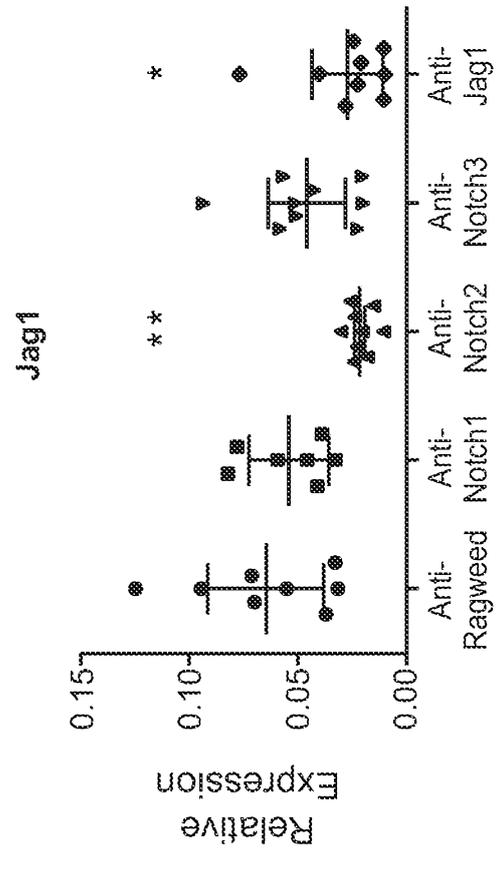
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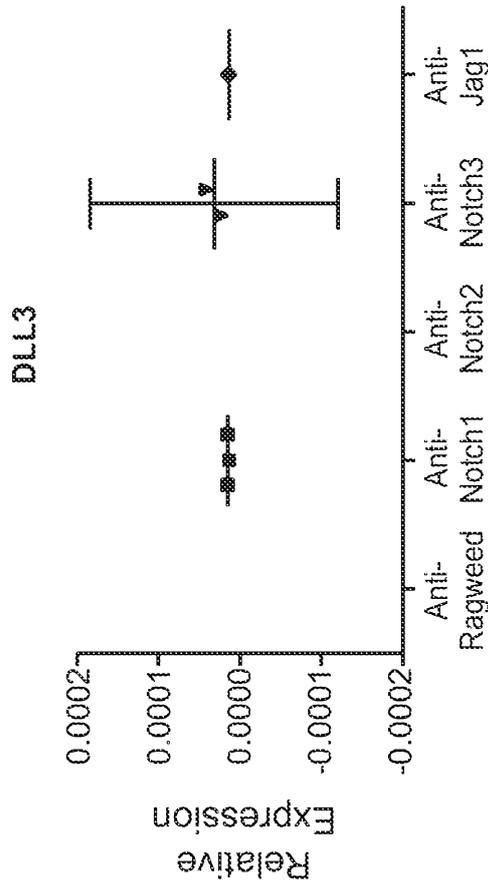
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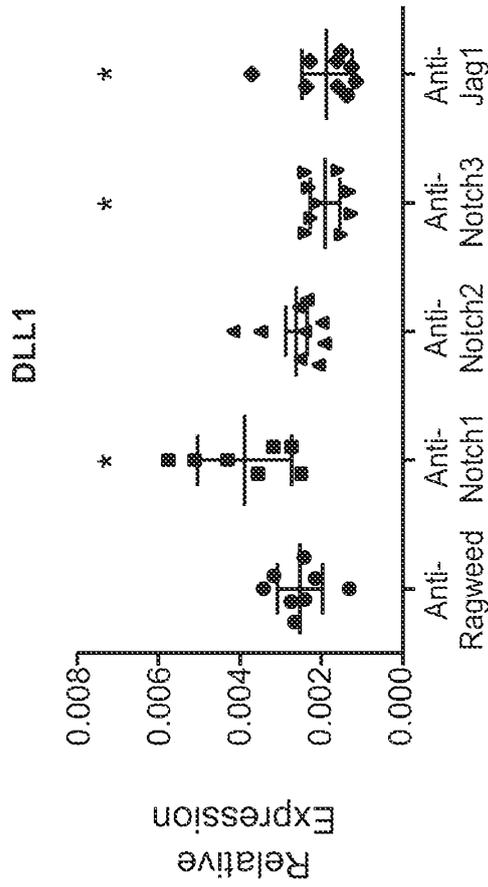
FIG. 6A

FIG. 5G
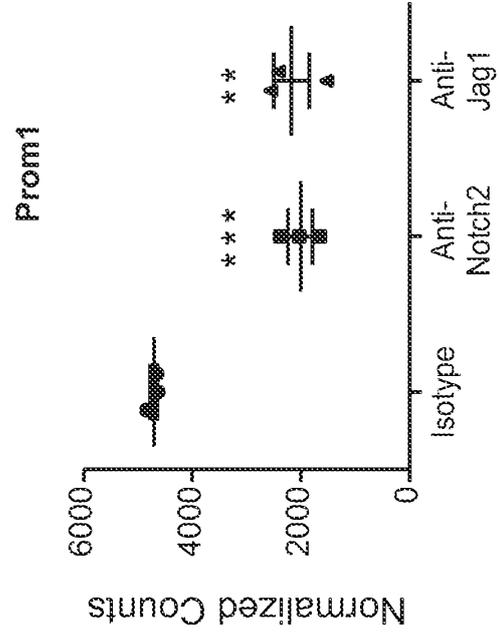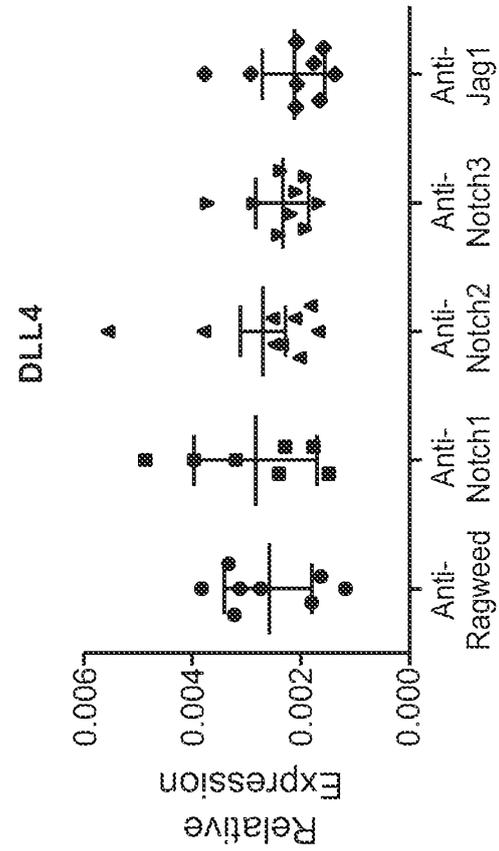
FIG. 5I
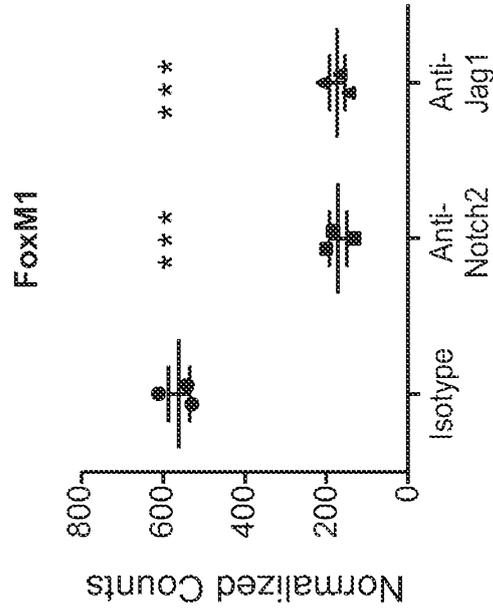
FIG. 6B

FIG. 6C
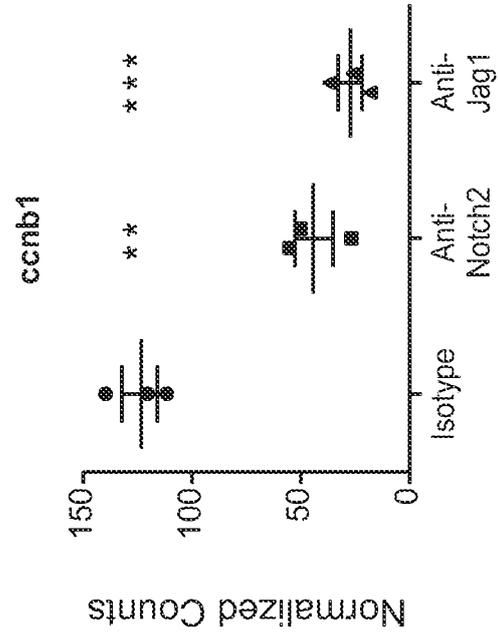
FIG. 6D

FIG. 6E
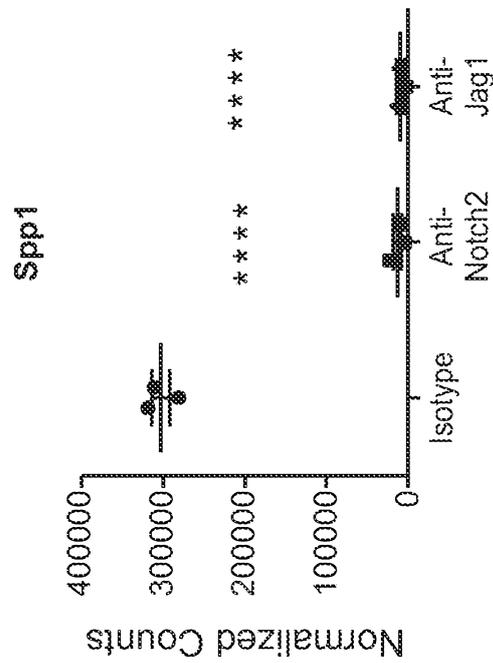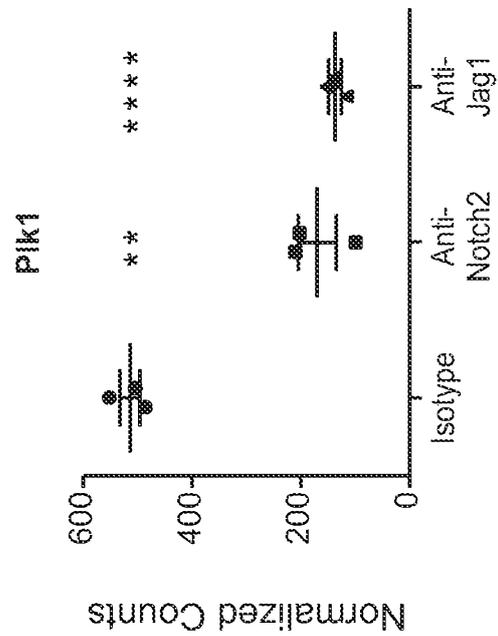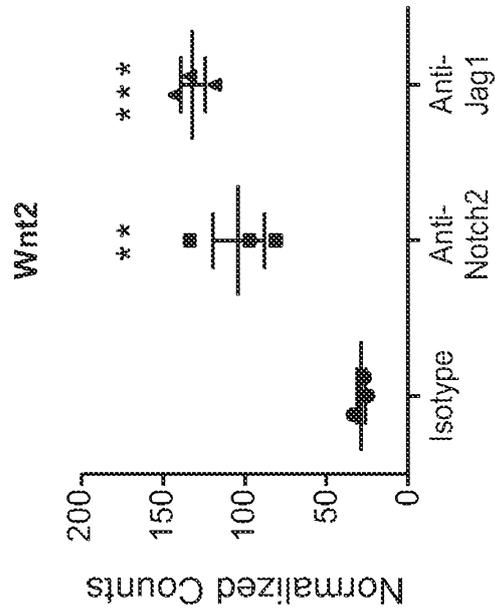
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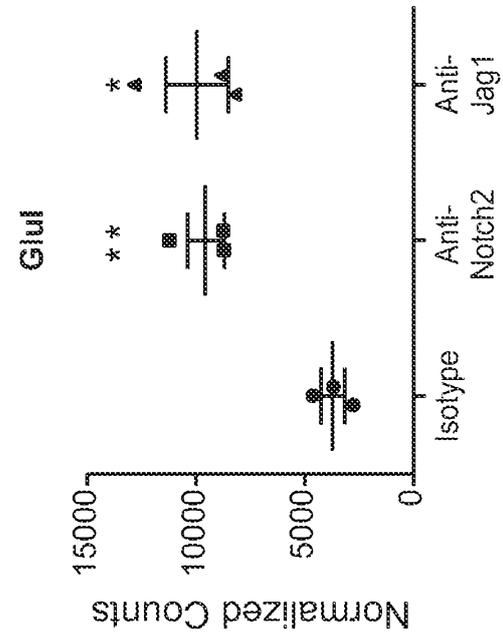
FIG. 6I

FIG. 6F
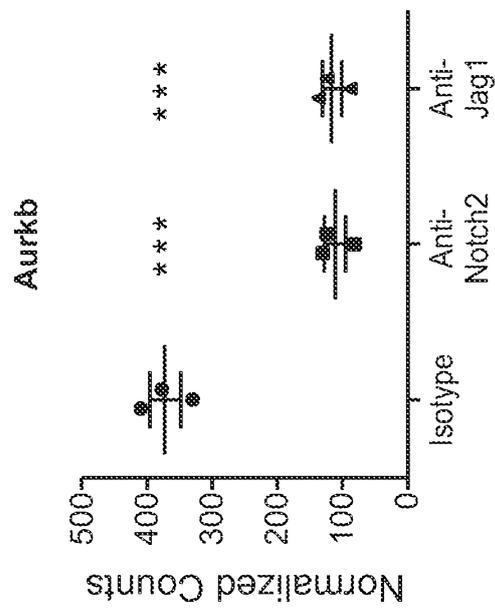
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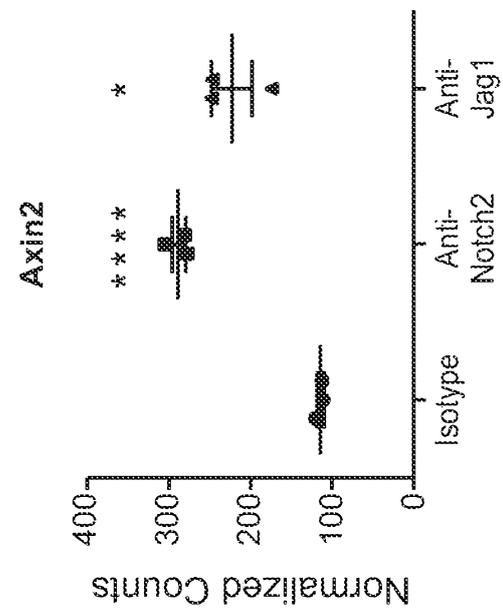
FIG. 7A
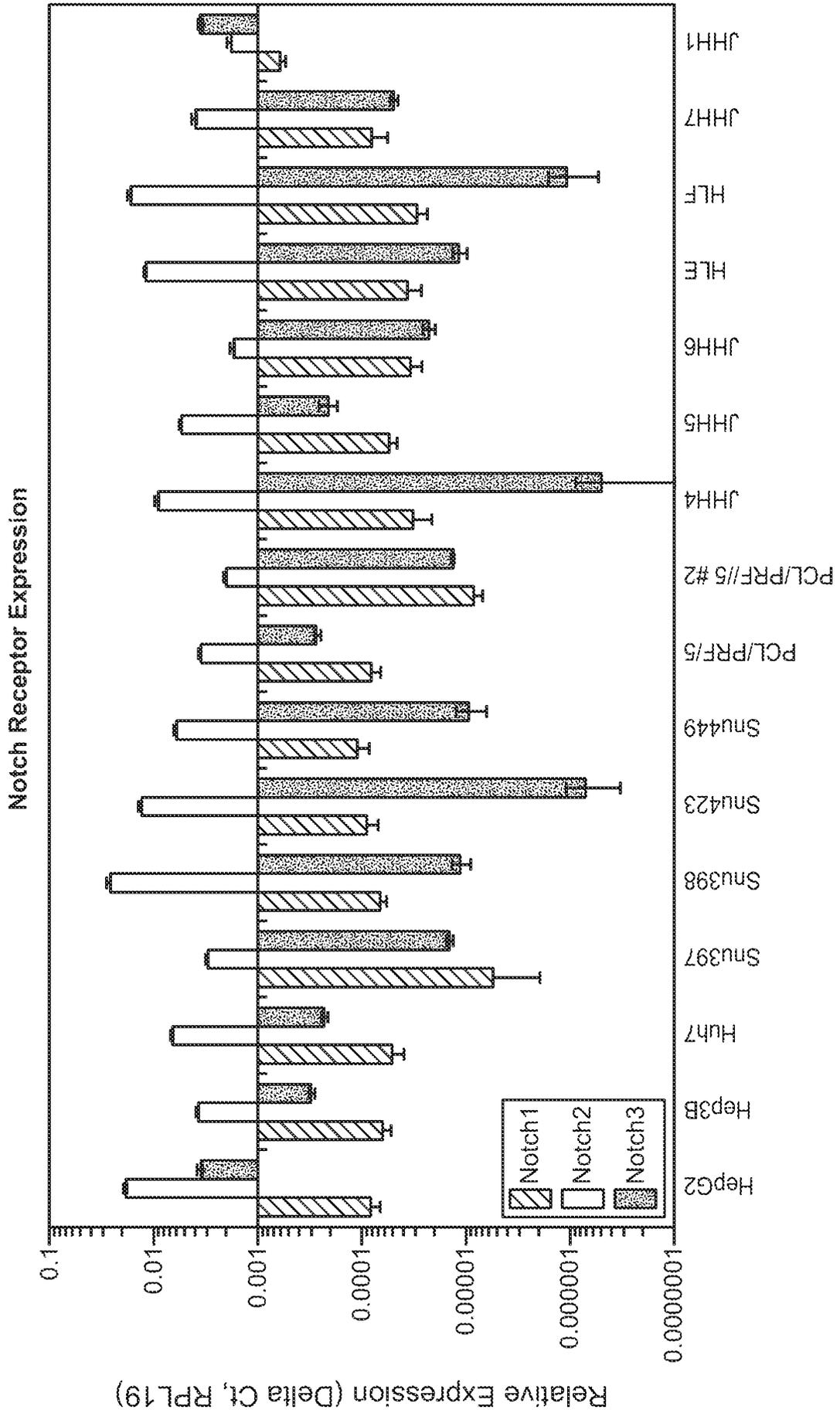
Jag1: 34/59 HCCs (57%)   15/56 (27%)
Notch2: 28/76 (37%)   Overlapping Jag1 and
Nuclear Notch2 15/76 (20%)   Nuclear Notch2

Notch2   Jag1   Hes1
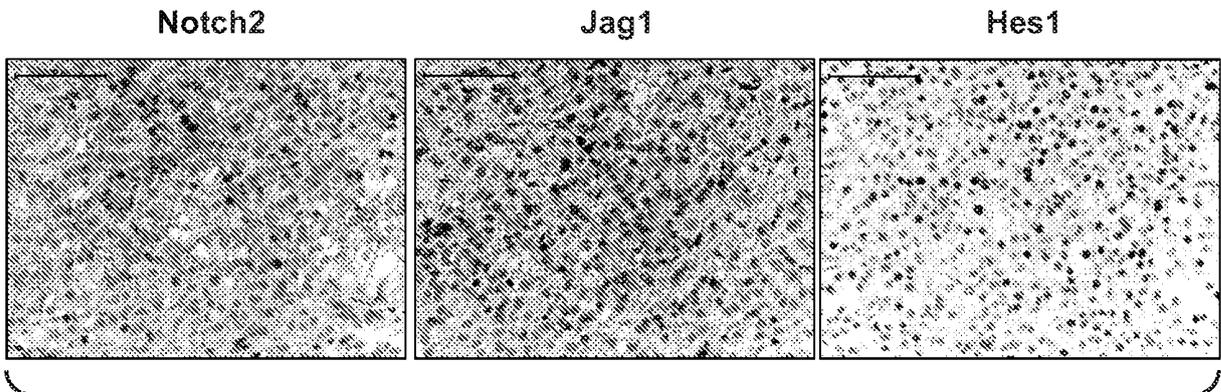
FIG. 7B

Human Notch2 NRR (SEQ ID NO: 73)

PATCLSQYCADKARDGVCDEACNSHACQWDGGDCSLTMENPWANCSSPLPCWDYI
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LDCAADQPENLAEGTLVIVVLMPPEQLLQDARSFLRALGTLLHTNLRIKRDSQGELMVY
PYYGEKSAAMKKQRMTRRSLPGEQEQEVAGSKVFLEIDNRQCVQDSDHCFKNTDAA
AALLASHAIQGTLSYPLVSVVSESLTPERTQ

FIG. 8A

Mouse Notch2 NRR (SEQ ID NO: 74)

PATCQSQYCADKARDGICDEACNSHACQWDGGDCSLTMEDPWANCTSTLRCWEYIN
NQCDEQCNTAECLFDNFECQRNSKTCKYDKYCADHFKDNHCDQGCNSEECGWDGL
DCASDQPENLAEGTLIIVVLLPPEQLLQDSRSFLRALGTLLHTNLRIKQDSQGALMVYPY
FGEKSAAMKKQKMTRRSLPEEQEQEQEVIGSKIFLEIDNRQCVQDSDQCFKNTDAAAA
LLASHAIQGTLSYPLVSVFSELESPRNAQ

FIG. 8B

HUMAN Notch2 (SEQ ID NO: 75)

MPALRPALLWALLALWLCCAAPAHALQCRDGYEPCVNEGMCVTYHNGTGYCKCPEGFLGEY
CQHRDPCEKNRCQNGGTCVAQAMLGKATCRCASGFTGEDCQYSTSHPCFVSRPCLNGGTC
HMLSRDTYECTCQVGFTGKECQWTDACLSHPCANGSTCTTVANQFSCKCLTGFTGQKCETD
VNECDIPGHCQHGGTCLNLPGSYQCQCPQGFTGQYCDSLYVPCAPSPCVNGGTCRQTGDFT
FECNCLPGFEGSTCERNIDDCPNHRCQNGGVCVDGVNTYNCRCPPQWTGQFCTEDVDECLL
QPNACQNGGTCANRNGGYGCVCVNGWSGDDCSENIDDCAFASCTPGSTCIDRVASFSCMCP
EGKAGLLCHLDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTEDVDECAMANSNPC
EHAGKCVNTDGAFHCECLKGYAGPRCEMDINECHSDPCQNDATCLDKIGGFTCLCMPGFKGV
HCELEINECQSNPCVNNGQCVDKVNRFQCLCPPGFTGPVCQIDIDDCSSTPCLNGAKCIDHPN
GYECQCATGFTGVLCEENIDNCDPDPCHHGQCQDGIDSYTCICNPGYMGAICSDQIDECYSSP
CLNDGRCIDLVNGYQCNCQPGTSGVNCEINFDDCASNPCIHGICMDGINRYSCVCSPGFTGQR
CNIDIDECASNPCRKGATCINGVNGFRCICPEGPHHPSCYSQVNECLSNPCIHGNCTGGLSGY
KCLCDAGWVGINCEVDKNECLSNPCQNGGTCDNLVNGYRCTCKKGFKGYNCQVNIDECASN
PCLNQGTCFDDISGYTCHCVLPYTGKNCQTVLAPCSPNPCENAAVCKESPNFESYTCLCAPG
WQGQRCTIDIDECISKPCMNHGLCHNTQGSYMCECPPGFSGMDCEEDIDDCLANPCQNGGS
CMDGVNTFSCLCLPGFTGDKCQTDMNECLSEPCKNGGTCSDYVNSYTCKCQAGFDGVHCEN
NINECTESSCFNGGTCVDGINSFSCLCPVGFTGSFCLHEINECSSHPCLNEGTCVDGLGTYRC
SCPLGYTGKNCQTLVNLCSRSPCKNKGTCVQKKAESQCLCPSGWAGAYCDVPNVSCDIAASR
RGVLVEHLCQHSGVCINAGNTHYCQCPLGYTGSYCEEQLDECASNPCQHGATCSDFIGGYRC
ECVPGYQGVNCEYEVDECQNQPCQNGGTCIDLVNHFKCSCPPGTRGLLCEENIDDCARGPH
CLNGGQCMDRIGGYSCRCLPGFAGERCEGDINECLSNPCSSEGSLDCIQLTNDYLCVCRSAFT
GRHCETFVDVCPQMPCLNGGTCAVASNMPDGFICRCPPGFSGARCQSSCGQVKCRKGEQC
VHTASGPRCFCPSPRDCESGCASSPCQHGGSCHPQRQPPYYSCQCAPPFSGSRCELYTAPP
STPPATCLSQYCADKARDGVCDEACNSHACQWDGGDCSLTMENPWANCSSPLPCWDYINN
QCDELCNTVECLFDNFECQGNSKTCKYDKYCADHFKDNHCDQGCNSEECGWDGLDCAADQ
PENLAEGTLVIVVLMPPEQLLQDARSFLRALGTLLHTNLRIKRDSQGELMVYPYYGEKSAAMKK
QRMTRRSLPGEQEQEVAGSKVFLEIDNRQCVQDSDHCFKNTDAAAALLASHAIQGTLSYPLVS
VVSESLTPERTQLLYLLAVAVVIILFIILLGVIMAKRKRKHGSLWLPEGFTLRRDASNHKRREPVG
QDAVGLKNLSVQVSEANLIGTGTSEHWVDDEGPQPKKVKAEDEALLSEEDDPIDRRPWTQQH
LEAADIRRTPSLALTPPQAEQEVDVLDVNRGPDGCTPLMLASLRGGSSDLSDEDEDAEDSSA
NIITDLVYQGASLQAQTDRTGEMALHLAARYSRADAAKRLLDAGADANAQDNMGRCPLHAAVA
ADAQGVFQILIRNRVTDLDARMNDGTTPLILAARLAVEGMVAELINCQADVNAVDDHGKSALHW
AAAVNNVEATLLLLKNGANRDMQDNKEETPLFLAAREGSYEAAKILLDHFANRDITDHMDRLPR
DVARDRMHHDIVRLLDEYNVTPSPPGTVLTSALSPVICGPNRSFLSLKHTPMGKKSRRPSAKST
MPTSLPNLAKEAKDAKGSRRKKSLSEKVQLSESSVTLSPVDSLESPHTYVSDTTSSPMITSPGIL
QASPNPMLATAAPPAPVHAQHALSFSNLHEMQPLAHGASTVLPSVSQLLSHHHIVSPGSGSAG
SLSRLHPVPVPADWMNRMEVNETQYNEMFGMVLAPAEGTHPGIAPQSRPPEGKHITTPREPL
PPIVTFQLIPKGSIAQPAGAPQPQSTCPPAVAGPLPTMYQIPEMARLPSVAFPTAMMPQQDGQ
VAQTILPAYHPFPASVGKYPTPPSQHSYASSNAAERTPSHSGHLQGEHPYLTPSPESPDQWSS
SSPHSASDWSDVTTSPTPGGAGGGQRGPGTHMSEPPHNNMQVYA

FIG. 8C

HUMAN Notch2 (SEQ ID NO: 76)

MPDLRPAALRALLWLWLCGAGPAHALQCRGGQEPCVNEGTCVTYHNGTGFCRCPEGFLGEY
CQHRDPCEKNRCQNGGTCVPQGMLGKATCRCAPGFTGEDCQYSTSHPCFVSRPCQNGGTC
HMLSRDTYECTCQVGFTGKQCQWTDACLSHPCENGSTCTSVASQFSCKCPAGLTGQKCEAD
INECDIPGRCQHGGTCLNLPGSYRCQCGQGFTGQHCDSPYVRGLPCVNGGTCRQTGDFTLE
CNCLPGFEGSTCERNIDDCPNHKCQNGGVCVDGVNTYNCRCPPQWTGQFCTEDVDECLLQP
NACQNGGTCTNRNGGYGCVCVNGWSGDDCSENIDDCAYASCTPGSTCIDRVASFSCLCPEG
KAGLLCHLDDACISNPCHKGALCDTNPLNGQYICTCPQGYKGADCTEDVDECAMANSNPCEH
AGKCVNTDGAFHCECLKGYAGPRCEMDINECHSDPCQNDATCLDKIGGFTCLCMPGFKGVHC
ELEVNECQSNPCVNNGQCVDKVNRFQCLCPPGFTGPVCQIDIDDCSSTPCLNGAKCIDHPNG
YECQCATGFTGILCDENIDNCDPDPCHHGQCQDGIDSYTCICNPGYMGAICSDQIDECYSSPCL
NDGRCIDLVNGYQCNCQPGTSGLNCEINFDDCASNPCMHGVCVDGINRYSCVCSPGFTGQR
CNIDIDECASNPCRKGATCINDVNGFRCICPEGPHHPSCYSQVNECLSNPCIHGNCTGGLSGY
KCLCDAGWVGVNCEVDKNECLSNPCQNGGTCNNLVNGYRCTCKKGFKGYNCQVNIDECASN
PCLNQGTCFDDVSGYTCHCMLPYTGKNCQTVLAPCSPNPCENAAVCKEAPNFESFSCLCAPG
WQGKRCTVDVDECISKPCMNNGVCHNTQGSYVCECPPGFSGMDCEEDINDCLANPCQNGG
SCVDHVNTFSCQCHPGFIGDKCQTDMNECLSEPCKNGGTCSDYVNSYTCTCPAGFHGVHCE
NNIDECTESSCFNGGTCVDGINSFSCLCPVGFTGPFCLHDINECSSNPCLNAGTCVDGLGTYR
CICPLGYTGKNCQTLVNLCSRSPCKNKGTCVQEKARPHCLCPPGWDGAYCDVLNVSCKAAAL
QKGVPVEHLCQHSGICINAGNTHHCQCPLGYTGSYCEEQLDECASNPCQHGATCNDFIGGYR
CECVPGYQGV

FIG. 8D

HUMAN Jag1 (SEQ ID NO: 78)

MRSPRTRGRSGRPLSLLLALLCALRAKVCGASGQFELEILSMQNVNGELQNGNCCGGARN
PGDRKCTRDECDTYFKVCLKEYQSRVTAGGPCSFGSGSTPVIGGNTFNLKASRGNDRNRI
VLPFSFAWPRSYTLLVEAWDSSNDTVQPDSIIEKASHSGMINPSRQWQTLKQNTGVAHFE
YQIRVTCDDYYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPECNRAICRQGCSP
KHGSCKLPGDCRCQYGWQGLYCDKCIPHPGCVHGICNEPWQCLCETNWGGQLCDKDLNYC
GTHQPCLNGGTCSNTGPDKYQCSCPEGYSGPNCEIAEHACLSDPCHNRGSCKETSLGFEC
ECSPGWTGPTCSTNIDDCSPNNCSHGGTCQDLVNGFKCVCPPQWTGKTCQLDANECEAKP
CVNAKSCKNLIASYYCDCLPGWMGQNCDININDCLGQCQNDASCRDLVNGYRCICPPGYA
GDHCERDIDECASNPCLNGGHCQNEINRFQCLCPTGFSGNLCQLDIDYCEPNPCQNGAQC
YNRASDYFCKCPEDYEGKNCSHLKDHCRTTPCEVIDSCTVAMASNDTPEGVRYISSNVCG
PHGKCKSQSGGKFTCDCNKGFTGTYCHENINDCESNPCRNGGTCIDGVNSYKCICSDGWE
GAYCETNINDCSQNPCHNGGTCRDLVNDFYCDCKNGWKGKTCHSRDSQCDEATCNNGGTC
YDEGDAFKCMCPGGWEGTTCNIARNSSCLPNPCHNGGTCVVNGESFTCVCKEGWEGPICA
QNTNDCSPHPCYNSGTCVDGDNWYRCECAPGFAGPDCRININECQSSPCAFGATCVDEIN
GYRCVCPPGHSGAKCQEVSGRPCITMGSVIPDGAKWDDDCNTCQCLNGRIACSKVWCGPR
PCLLHKGHSECPSGQSCIPILDDQCFVHPCTGVGECRSSSLQPVKTKCTSDSYYQDNCAN
ITFTFNKEMMSPGLTTEHICSELRNLNILKNVSAEYSIYIACEPSPSANNEIHVAISAED
IRDDGNPIKEITDKIIDLVSKRDGNSSLIAAVAEVRVQRRPLKNRTDFLVPLLSSVLTVA
WICCLVTAFYWCLRKRRKPGSHTHSASEDNTTNNVREQLNQIKNPIEKHGANTVPIKDYE
NKNSKMSKIRTHNSEVEEDDMDKHQQKARFAKQPAYTLVDREEKPPNGTPTKHPNWTNKQ
DNRDLESAQSLNRMEYIV

MURINE Jag1 (SEQ ID NO:79)

MRSPRTRGRPGRPLSLLLALLCALRAKVCGASGQFELEILSMQNVNGELQNGNCCGGVRN
PGDRKCTRDECDTYFKVCLKEYQSRVTAGGPCSFGSGSTPVIGGNTFNLKASRGNDRNRI
VLPFSFAWPRSYTLLVEAWDSSNDTIQPDSIIEKASHSGMINPSRQWQTLKQNTGIAHFE
YQIRVTCDDHYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPDCNKAICRQGCSP
KHGSCKLPGDCRCQYGWQGLYCDKCIPHPGCVHGTCNEPWQCLCETNWGGQLCDKDLNYC
GTHQPCLNRGTCSNTGPDKYQCSCPEGYSGPNCEIAEHACLSDPCHNRGSCKETSSGFEC
ECSPGWTGPTCSTNIDDCSPNNCSHGGTCQDLVNGFKCVCPPQWTGKTCQLDANECEAKP
CVNARSCKNLIASYYCDCLPGWMGQNCDININDCLGQCQNDASCRDLVNGYRCICPPGYA
GDHCERDIDECASNPCLNGGHCQNEINRFQCLCPTGFSGNLCQLDIDYCEPNPCQNGAQC
YNRASDYFCKCPEDYEGKNCSHLKDHCRTTTCEVIDSCTVAMASNDTPEGVRYISSNVCG
PHGKCKSQSGGKFTCDCNKGFTGTYCHENINDCESNPCKNGGTCIDGVNSYKCICSDGWE
GAHCENNINDCSQNPCHYGGTCRDLVNDFYCDCKNGWKGKTCHSRDSQCDEATCNNGGTC
YDEVDTFKCMCPGGWEGTTCNIARNSSCLPNPCHNGGTCVVNGDSFTCVCKEGWEGPICT
QNTNDCSPHPCYNSGTCVDGDNWYRCECAPGFAGPDCRININECQSSPCAFGATCVDEIN
GYQCICPPGHSGAKCHEVSGRSCITMGRVILDGAKWDDDCNTCQCLNGRVACSKVWCGPR
PCRLHKSHNECPSGQSCIPVLDDQCFVRPCTGVGECRSSSLQPVKTKCTSDSYYQDNCAN
ITFTFNKEMMSPGLTTEHICSELRNLNILKNVSAEYSIYIACEPSLSANNEIHVAISAED
IRDDGNPVKEITDKIIDLVSKRDGNSSLIAAVAEVRVQRRPLKNRTDFLVPLLSSVLTVA
WVCCLVTAFYWCVRKRRKPSSHTHSAPEDNTTNNVREQLNQIKNPIEKHGANTVPIKDYE
NKNSKMSKIRTHNSEVEEDDMDKHQQKVRFAKQPVYTLVDREEKAPSGTPTKHPNWTNKQ
DNRDLESAQSLNRMEYIV

FIG. 9

Expressed protein murine Jag1-DSL-EGF1-4 (mouse Jag1 antigen)
(SEQ ID NO: 80)

ADLGSQFELEILSMQNVNGELQNGNCCGGVRNPGDRKCTRDECDTYFKVCLKEYQSRVTAG
GPCSFGSGSTPVIGGNTFNLKASRGNDRNRIVLPFSFAWPRSYTLLVEAWDSSNDTIQPDSIIE
KASHSGMINPSRQWQTLKQNTGIAHFEYQIRVTCDDHYYGFGCNKFCRPRDDFFGHYACDQN
GNKTCMEGWMGPDCNKAICRQGCSPKHGSCKLPGDCRCQYGWQGLYCDKCIPHPGCVHGT
CNEPWQCLCETNWGGQLCDKDLNYCGTHQPCLNRGTCSNTGPDKYQCSCPEGYSGPNCEI
AEHACLSDPCHNRGSCKETSSGFECECSPGWTGPTCSTNIDD**EFGLVPRGSGHHHHHH**

FIG. 10A

Expressed protein human Jag1-DSL-EGF1-4 (human Jag1 antigen)
(SEQ ID NO: 28)

QFELEILSMQNVNGELQNGNCCGGARNPGDRKCTRDECDTYFKVCLKEYQSRVTAGGPCSF
GSGSTPVIGGNTFNLKASRGNDRNRIVLPFSFAWPRSYTLLVEAWDSSNDTVQPDSIIEKASHS
GMINPSRQWQTLKQNTGVAHFEYQIRVTCDDYYYGFGCNKFCRPRDDFFGHYACDQNGNKT
CMEGWMGPECNRAICRQGCSPKHGSCKLPGDCRCQYGWQGLYCDKCIPHPGCVHGICNEP
WQCLCETNWGGQLCDKDLNYCGTHQPCLNGGTCSNTGPDKYQCSCPEGYSGPNCEIAEHA
CLSDPCHNRGSCKETSLGFECECSPGWTGPTCSTNIDD

FIG. 10B

Human Secreted Phosphoprotein1 (SPP1)
(SEQ ID NO: 29)

MRIAVICFCLLGITCAIPVKQADSGSSEEKQLYNKYPDAVATWLNPDPSQKQNLLAPQNA
VSSEETNDFKQETLPSKSNESHDHMDDMDDEDDDDHVDSQDSIDSNDSDDVDDTDDSHQS
DESHHSDESDELVTDFPTDLPATEVFTPVVPTVDTYDGRGDSVVYGLRSKSKKFRRPDIQ
YPDATDEDITSHMESEELNGAYKAIPVAQDLNAPSDWDSRGKDSYETSQLDDQSAETHSH
KQSRLYKRKANDESNEHSDVIDSQELSKVSREFHSHEFHSHEDMLVVDPKSKEEDKHLKF
RISHELDSASSEVN

FIG. 11

HVR-H1 Sequences - Antibody B and Affinity Matured Antibodies Derived from Antibody B

Antibody	SEQ ID NO:	Kabat Number									
		26	27	28	29	30	31	32	33	34	35
B, B-1, B-2, B-3	1	G	Y	S	F	T	S	Y	G	M	S

HVR-H2 Sequence - Antibody B and Affinity Matured Antibodies Derived from Antibody B

Antibody #	SEQ ID NO:	Kabat Number																	
		49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65
B, B-1, B-2, B-3	3	S	Y	I	Y	P	Y	S	G	A	T	Y	Y	A	D	S	V	K	G

HVR-H3 Sequence - Antibody B and Affinity Matured Antibodies Derived from Antibody B

Antibody #	SEQ ID NO:	Kabat Number												
		95	96	97	98	99	100	100A	100B	100C	100D	100K	101	102
B, B-1, B-2, B-3	4	H	S	G	Y	Y	R	I	S	S	A	M	D	V

FIG. 12

HVR-L1 Sequences - Antibody B and Affinity Matured Antibodies Derived from Antibody B

Antibody #	SEQ ID NO:	Kabat Number										
		24	25	26	27	28	29	30	31	32	33	34
B	5	R	A	S	Q	S	I	S	S	Y	L	A
B-1	6	R	A	S	Q	S	N	R	R	F	L	A
B-2	7	R	A	S	Q	S	V	R	S	F	L	A
B-3	8	R	A	S	Q	N	I	K	R	F	L	A
Consensus	9	R	A	S	Q	S/N	I/N/V	S/R/K	S/R	Y/F	L	A

HVR-L2 Sequences - Antibody B and Affinity Matured Antibodies Derived from Antibody B

Antibody #	SEQ ID NO:	Kabat Number						
		50	51	52	53	54	55	56
B	10	G	A	S	S	R	A	S
B-1	11	G	A	S	R	R	A	S
B-2	12	R	A	S	I	R	A	S
B-3	13	G	A	S	T	R	E	S
Consensus	14	G/R	A	S	S/R/I/T	R	A/E	S

HVR-L3 Sequences - Antibody B and Affinity Matured Antibodies Derived from Antibody B

Antibody #	SEQ ID NO:	Kabat Number								
		89	90	91	92	93	94	95	96	97
B	15	Q	Q	Y	Y	S	S	P	L	T
B-1	16	Q	Q	Y	Y	I	S	P	L	T
B-2	17	Q	Q	Y	Y	I	S	P	W	T
B-3	18	Q	Q	Y	Y	R	S	P	H	T
Consensus	19	Q	Q	Y	Y	S/I/R	S	P	L/H/S	T

FIG. 13

Kabat Numbering

Kabat Numbering	SEQ ID NO:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Antibody B	20	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A
Antibody B-1	20	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A
Antibody B-2	20	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A
Antibody B-3	20	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A

Kabat - CDR H1

	SEQ ID NO:	25	26	27	28	29	30	31	32	33	34	35	35A	35B	36	37	38	39	40	41	42	43	44	45	46
Antibody B	20	S	G	Y	S	F	T	S	Y	G	M	S	.	.	W	V	R	Q	A	P	G	K	G	L	E
Antibody B-1	20	S	G	Y	S	F	T	S	Y	G	M	S	.	.	W	V	R	Q	A	P	G	K	G	L	E
Antibody B-2	20	S	G	Y	S	F	T	S	Y	G	M	S	.	.	W	V	R	Q	A	P	G	K	G	L	E
Antibody B-3	20	S	G	Y	S	F	T	S	Y	G	M	S	.	.	W	V	R	Q	A	P	G	K	G	L	E

Kabat - CDR H2

	SEQ ID NO:	47	48	49	50	51	52	52A	52B	52C	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
Antibody B	20	W	V	S	Y	I	Y	P	.	.	Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F
Antibody B-1	20	W	V	S	Y	I	Y	P	.	.	Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F
Antibody B-2	20	W	V	S	Y	I	Y	P	.	.	Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F
Antibody B-3	20	W	V	S	Y	I	Y	P	.	.	Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F

	SEQ ID NO:	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82A	82B	82C	83	84	85	86	87	88
Antibody B	20	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A
Antibody B-1	20	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A
Antibody B-2	20	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A
Antibody B-3	20	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A

FIG. 14A

Kabat Numbering

Kabat - CDR H3

	SEQ ID NO:	89	90	91	92	93	94	95	96	97	98	99	100	100A	100B	100C	100D	100E	100F	100G	100H	100I	100J	100K	100L
Antibody B	20	V	Y	Y	C	A	R	H	S	G	Y	Y	R	I	S	S	A	.	.	.	.	.	.	.	.
Antibody B-1	20	V	Y	Y	C	A	R	H	S	G	Y	Y	R	I	S	S	A	.	.	.	.	.	.	.	.
Antibody B-2	20	V	Y	Y	C	A	R	H	S	G	Y	Y	R	I	S	S	A	.	.	.	.	.	.	.	.
Antibody B-3	20	V	Y	Y	C	A	R	H	S	G	Y	Y	R	I	S	S	A	.	.	.	.	.	.	.	.

	SEQ ID NO:	100M	101	102	103	104	105	106	107	108	109	110	111	112	113
Antibody B	20	W	D	V	W	G	Q	G	T	L	V	T	V	S	S
Antibody B-1	20	W	D	V	W	G	Q	G	T	L	V	T	V	S	S
Antibody B-2	20	W	D	V	W	G	Q	G	T	L	V	T	V	S	S
Antibody B-3	20	W	D	V	W	G	Q	G	T	L	V	T	V	S	S

FIG. 14B

Kabat Numbering

Kabat Numbering	SEQ ID NO:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Antibody B	22	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
Antibody B-1	23	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
Antibody B-2	24	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
Antibody B-3	25	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C

Kabat - CDR L1

Kabat Numbering	SEQ ID NO:	24	25	26	27	27A	27B	27C	27D	27E	27F	28	29	30	31	32	33	34	35	36	37	38	39	40
Antibody B	22	R	A	S	Q	.	.	.	.	.	.	S	I	S	S	Y	L	A	W	Y	Q	Q	K	P
Antibody B-1	23	R	A	S	Q	.	.	.	.	.	.	S	N	R	R	F	L	A	W	Y	Q	Q	K	P
Antibody B-2	24	R	A	S	Q	.	.	.	.	.	.	S	V	R	S	F	L	A	W	Y	Q	Q	K	P
Antibody B-3	25	R	A	S	Q	.	.	.	.	.	.	N	I	K	K	F	L	A	W	Y	Q	Q	K	P

Kabat - CDR L2

Kabat Numbering	SEQ ID NO:	41	42	43	44	45	46	47	48	49	50	51	52	53	54	54A	54B	54C	54D	54E	55	56	57	58
Antibody B	22	G	K	A	P	K	L	L	I	Y	G	A	S	S	R	.	.	.	.	.	A	S	G	V
Antibody B-1	23	G	K	A	P	K	L	L	I	Y	G	A	S	R	R	.	.	.	.	.	A	S	G	V
Antibody B-2	24	G	K	A	P	K	L	L	I	Y	R	A	S	I	R	.	.	.	.	.	A	S	G	V
Antibody B-3	25	G	K	A	P	K	L	L	I	Y	G	A	S	T	R	.	.	.	.	.	E	S	G	V

Kabat Numbering	SEQ ID NO:	59	60	61	62	63	64	65	66	66A	66B	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Antibody B	22	P	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
Antibody B-1	23	P	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
Antibody B-2	24	P	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
Antibody B-3	25	P	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P

FIG. 15A

Kabat Numbering

																Kabat - CDR L3							
	SEQ ID NO:	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	95A	95B	95C	95D	95E	95F	96	97
Antibody B	22	E	D	F	A	T	Y	Y	C	Q	Q	Y	Y	S	S	P	.	.	.	.	.	.	L	T
Antibody B-1	23	E	D	F	A	T	Y	Y	C	Q	Q	Y	Y	I	S	P	.	.	.	.	.	.	L	T
Antibody B-2	24	E	D	F	A	T	Y	Y	C	Q	Q	Y	Y	I	S	P	.	.	.	.	.	.	S	T
Antibody B-3	25	E	D	F	A	T	Y	Y	C	Q	Q	Y	Y	R	S	P	.	.	.	.	.	.	H	T

	98	99	100	101	102	103	104	105	106	107
Antibody B	F	G	Q	G	T	K	V	E	I	K
Antibody B-1	F	G	Q	G	T	K	V	E	I	K
Antibody B-2	F	G	Q	G	T	K	V	E	I	K
Antibody B-3	F	G	Q	G	T	K	V	E	I	K

FIG. 15B

FR1　　　　　　　　　　　　　　　　　　　FR2

I
A　QVQLVQSGAEVKKPGASVKVSCKASGYTFT　-H1-　WVRQAPGQGLEWMG　-H2-　RVTIT
B　QVQLVQSGAEVKKPGASVKVSCKAS　-H1-　WVRQAPGQGLEWM　-H2-　RVTIT
C　QVQLVQSGAEVKKPGASVKVSCKAS　-H1-　WVRQAPGQGLEWM　-H2-　RVTIT
D　QVQLVQSGAEVKKPGASVKVSCKAS　-H1-　WVRQAPGQGLEWM　-H2-　RVTIT

II
A　QVQLQESGPGLVKPSQTLSLTCTVSGGSVS　-H1-　WIRQPPGKGLEWIG　-H2-　RVTIS
B　QVQLQESGPGLVKPSQTLSLTCTVS　-H1-　WIRQPPGKGLEWI　-H2-　RVTIS
C　QVQLQESGPGLVKPSQTLSLTCTVS　-H1-　WIRQPPGKGLEWI　-H2-　RVTIS
D　QVQLQESGPGLVKPSQTLSLTCTVS　-H1-　WIRQPPGKGLEWI　-H2-　RVTIS

III
A　EVQLVESGGGLVQPGGSLRLSCAASGFTFS　-H1-　WVRQAPGKGLEWVS　-H2-　RFTIS
B　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS
C　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS
D　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS

Acceptor - 1
A　EVQLVESGGGLVQPGGSLRLSCAASGFNIK　-H1-　WVRQAPGKGLEWVS　-H2-　RFTIS
B　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS
C　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS

Acceptor - 2
A　EVQLVESGGGLVQPGGSLRLSCAASGFNIK　-H1-　WVRQAPGKGLEWVS　-H2-　RFTIS
B　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS
C　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS
D　EVQLVESGGGLVQPGGSLRLSCAAS　-H1-　WVRQAPGKGLEWV　-H2-　RFTIS

FIG. 16A

	FR3	FR4	SEQ ID NOs of FR1, FR2, FR3, FR4
I			
A	ADTSTSTAYMELSSLRSEDTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 32, 33, 34, 35
B	ADTSTSTAYMELSSLRSEDTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 36, 37, 34, 35
C	ADTSTSTAYMELSSLRSEDTAVYYCA	-H3- WGQGTLVTVSS	SEQ ID NO.: 36, 37, 38, 35
D	ADTSTSTAYMELSSLRSEDTAVYYC	-H3- WGQGTLVTVSS	SEQ ID NO.: 36, 37, 39, 35
II			
A	VDTSKNQFSLKLSSVTAADTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 40, 41, 42, 35
B	VDTSKNQFSLKLSSVTAADTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 43, 44, 42, 35
C	VDTSKNQFSLKLSSVTAADTAVYYCA	-H3- WGQGTLVTVSS	SEQ ID NO.: 43, 44, 45, 35
D	VDTSKNQFSLKLSSVTAADTAVYYC	-H3- WGQGTLVTVSS	SEQ ID NO.: 43, 44, 46, 35
III			
A	RDNSKNTLYLQMNSLRAEDTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 47, 48, 49, 35
B	RDNSKNTLYLQMNSLRAEDTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 49, 35
C	RDNSKNTLYLQMNSLRAEDTAVYYCA	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 52, 35
D	RDNSKNTLYLQMNSLRAEDTAVYYC	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 53, 35
Acceptor - 1			
A	ADTSKNTAYLQMNSLRAEDTAVYYCSR	-H3- WGQGTLVTVSS	SEQ ID NO.: 54, 48, 55, 35
B	ADTSKNTAYLQMNSLRAEDTAVYYCSR	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 55, 35
C	ADTSKNTAYLQMNSLRAEDTAVYYCS	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 56, 35
Acceptor - 2			
A	ADTSKNTAYLQMNSLRAEDTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 54, 48, 57, 35
B	ADTSKNTAYLQMNSLRAEDTAVYYCAR	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 57, 35
C	ADTSKNTAYLQMNSLRAEDTAVYYCA	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 58, 35
D	ADTSKNTAYLQMNSLRAEDTAVYYC	-H3- WGQGTLVTVSS	SEQ ID NO.: 50, 51, 59, 35

FIG. 16B

FR1

kv1 DIQMTQSPSSLSASVGDRVTITC -L1-

kv2 DIVMTQSPLSLPVTPGEPASISC -L1-

kv3 EIVLTQSPGTLSLSPGERATLSC -L1-

kv4 DIVMTQSPDSLAVSLGERATINC -L1-

FR2

WYQQKPGKAPKLLIY -L2-

WYLQKPGQSPQLLIY -L2-

WYQQKPGQAPRLLIY -L2-

WYQQKPGQPPKLLIY -L2-

FR3

GVPSRFSGSGSGTDFTLTISSLQP

GVPDRFSGSGSGTDFTLKISRVEA

GIPDRFSGSGSGTDFTLTISRLEP

GVPDRFSGSGSGTDFTLTISSLQA

FR4
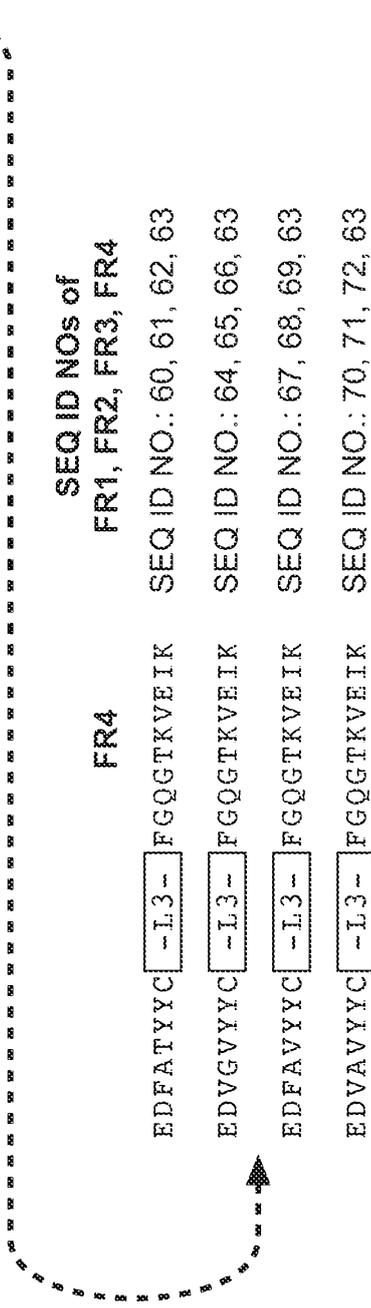
EDFATYYC -L3- FGQGTKVEIK

EDVGVYYC -L3- FGQGTKVEIK

EDFAVYYC -L3- FGQGTKVEIK

EDVAVYYC -L3- FGQGTKVEIK

SEQ ID NOs of FR1, FR2, FR3, FR4

SEQ ID NO.: 60, 61, 62, 63

SEQ ID NO.: 64, 65, 66, 63

SEQ ID NO.: 67, 68, 69, 63

SEQ ID NO.: 70, 71, 72, 63

FIG. 17

Framework Sequences of huMAb4D5-8 Light Chain Variable Domain

LC-FR1 ^{1}Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys^{23}

LC-FR2 ^{35}Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr^{49}

LC-FR3 ^{57}Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys^{88}

LC-FR4 ^{98}Phe Gly Gln Gly Thr Lys Val Glu Ile Lys^{107}

Framework Sequences of huMAb4D5-8 Heavy Chain Variable Domain

HC-FR1 ^{1}Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser^{25}

HC-FR2 ^{36}Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val^{48}

HC-FR3 ^{66}Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn^{83} Ser^{83a} Leu^{83b} Arg^{83c} Ala Glu Asp Thr Ala Val Tyr Tyr Cys^{92}

HC-FR4 ^{103}Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser^{113}

FIG. 18

Framework Sequences of huMAb4D5-8 Light Chain Variable Domain Modified at Positions 66 and 99 (Underlined)

LC-FR1 ^{1}Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys^{23}

LC-FR2 ^{35}Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr^{49}

LC-FR3 ^{57}Gly Val Pro Ser Arg Phe Ser Gly Ser <u>Gly</u> Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys^{88}

LC-FR4 ^{98}Phe <u>Arg</u> Gln Gly Thr Lys Val Glu Ile Lys^{107}

Framework Sequences of huMAb4D5-8 Heavy Chain Variable Domain Modified at Postions 71, 73 and 78 (Underlined)

HC-FR1 ^{1}Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser^{25}

HC-FR2 ^{36}Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val^{48}

HC-FR3 ^{66}Arg Phe Thr Ile Ser <u>Arg</u> Asp <u>Asn</u> Ser Lys Asn Thr <u>Leu</u> Tyr Leu Gln Met Asn83 Ser83a Leu83b Arg83c Ala Glu Asp Thr Ala Val Tyr Tyr Cys^{92}

HC-FR4 ^{103}Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser^{113}

FIG. 19

HVR-H1 Sequences - Antibody A and Affinity Matured Antibodies Derived from Antibody A

Antibody #	SEQ ID NO:	Kabat Number									
		26	27	28	29	30	31	32	33	34	35
A, A-1, A-2	81	G	F	T	F	S	N	Y	G	I	H

HVR-H2 Sequences - Antibody A and Affinity Matured Antibodies Derived from Antibody A

Antibody #	SEQ ID NO:	Kabat Number																
		50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	65
A, A-1	82	W	I	T	P	D	G	G	Y	T	D	Y	A	D	S	V	K	G
A-2	83	W	I	T	G	N	G	G	Y	S	D	Y	A	D	S	V	K	G
Consensus	84	W	I	T	P/G	D/N	G	G	Y	T/S	D	Y	A	D	S	V	K	G

HVR-H3 Sequences - Antibody A and Affinity Matured Antibodies Derived from Antibody A

Antibody #	SEQ ID NO:	Kabat Number						
		95	96	97	98	100k	101	102
A, A-2	85	A	G	S	W	F	A	Y
A-1	86	A	G	S	L	F	A	Y
Consensus	87	A	G	S	W/L	F	A	Y

FIG. 20

HVR-L1 Sequences - Antibody A and Affinity Matured Antibodies Derived from Antibody A

Antibody #	SEQ ID NO:	Kabat Number										
		24	25	26	27	28	29	30	31	32	33	34
A, A-1, A-2	88	R	A	S	Q	D	V	S	T	A	V	A

HVR-L2 Sequences - Antibody A and Affinity Matured Antibodies Derived from Antibody A

Antibody #	SEQ ID NO:	Kabat Number						
		50	51	52	53	54	55	56
A, A-1, A-2	89	S	A	S	F	L	Y	S

HVR-L3 Sequences - Antibody A and Affinity Matured Antibodies Derived from Antibody A

Clone #	SEQ ID NO:	Kabat Number								
		89	90	91	92	93	94	95	96	97
A, A-2	90	Q	Q	S	Y	T	T	P	P	T
A-1	91	Q	Q	Y	Y	T	T	A	T	T
Consensus	92	Q	Q	S/Y	Y	T	T	P/A	P/T	T

FIG. 21

<u>Framework Sequences of Antibodies A, A-1, A-2 Light Chain Variable Domain</u>

LC-FR1 ^{1}Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
Arg Val Thr Ile Thr Cys^{23}

LC-FR2 ^{35}Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr^{49}

LC-FR3 ^{57}Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
Tyr Cys^{88}

LC-FR4 ^{98}Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg^{108}

<u>Framework Sequences of Antibodies A, A-1, A-2 Heavy Chain Variable Domain</u>

HC-FR1 ^{1}Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
Leu Arg Leu Ser Cys Ala Ala Ser^{25}

HC-FR2 ^{36}Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly^{49}

HC-FR3 ^{66}Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met
Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
Ala Arg^{94}

HC-FR4 ^{103}Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser^{113}

FIG. 22

Light Chain Sequences

Antibody	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Anti-Notch2	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
Anti-Notch1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
Anti-Notch3	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
Anti-Jag1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C

Kabat - CDR L1

Antibody	24	25	26	27	27A	27B	27C	27D	27E	27F	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Anti-Notch2	R	A	S	Q	.	.	.	.	.	.	N	I	K	R	F	L	A	W	Y	Q	Q	K	P	G
Anti-Notch1	R	A	S	Q	.	.	.	.	.	.	D	V	S	T	A	V	A	W	Y	Q	Q	K	P	G
Anti-Notch3	R	A	S	Q	.	.	.	G	.	.	G	I	S	S	Y	V	A	W	Y	Q	Q	K	P	G
Anti-Jag1	R	A	S	Q	.	.	.	.	.	.	D	V	S	T	A	V	A	W	Y	Q	Q	K	P	G

Kabat - CDR L2

Antibody	42	43	44	45	46	47	48	49	50	51	52	53	54	54A	54B	54C	54D	54E	55	56	57	58	59
Anti-Notch2	K	A	P	K	L	L	I	Y	G	A	S	T	R	.	.	.	.	.	E	S	G	V	P
Anti-Notch1	K	A	P	K	L	L	I	Y	S	A	S	F	L	.	.	.	.	.	Y	S	G	V	P
Anti-Notch3	K	A	P	K	L	L	I	Y	D	A	S	S	L	.	.	.	.	.	E	S	G	V	P
Anti-Jag1	K	A	P	K	L	L	I	Y	S	A	S	F	L	.	.	.	.	.	Y	S	G	V	P

Antibody	60	61	62	63	64	65	66	66A	66B	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
Anti-Notch2	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
Anti-Notch1	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
Anti-Notch3	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
Anti-Jag1	S	R	F	S	G	S	G	.	.	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E

FIG. 23A

Kabat - CDR L3

Antibody	Light Chain Sequences																							
	82	83	84	85	86	87	88	89	90	91	92	93	94	95	95A	95B	95C	95D	95E	95F	96	97	98	99
Anti-Notch2	D	F	A	T	Y	Y	C	Q	Q	Y	Y	R	S	P	.	.	.	.	.	.	H	T	F	G
Anti-Notch1	D	F	A	T	Y	Y	C	Q	Q	F	Y	T	T	P	.	.	.	.	.	.	S	T	F	G
Anti-Notch3	D	F	A	T	Y	Y	C	Q	Q	W	N	S	Y	P	.	.	.	.	.	.	F	T	F	G
Anti-Jag1	D	F	A	T	Y	Y	C	Q	Q	S	Y	T	T	P	.	.	.	.	.	.	P	T	F	G

Antibody	100	101	102	103	104	105	106	107
Anti-Notch2	Q	G	T	K	V	E	I	K
Anti-Notch1	Q	G	T	K	V	E	I	K
Anti-Notch3	Q	G	T	K	V	E	I	K
Anti-Jag1	Q	G	T	K	V	E	I	K

FIG. 23B

Heavy Chain Sequences

Antibody	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Anti-Notch2	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A
Anti-Notch1	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A
Anti-Notch3	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A
Anti-Jag1	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A

Kabat - CDR H1 (positions 31–35B)

Antibody	24	25	26	27	28	29	30	31	32	33	34	35	35A	35B
Anti-Notch2	A	S	G	Y	S	F	T	S	Y	G	M	S	.	.
Anti-Notch1	A	S	G	F	T	F	S	S	Y	W	I	H	.	.
Anti-Notch3	A	S	G	F	T	F	S	N	Y	W	M	S	.	.
Anti-Jag1	A	S	G	F	T	F	S	S	Y	G	I	H	.	.

Kabat - CDR H2 (positions 50–65)

Antibody	45	46	47	48	49	50	51	52	52A	52B	52C	53	54	55	56	57	58	59	60	61	62	63	64	65
Anti-Notch2	L	E	W	V	S	Y	I	Y	P	.	.	Y	S	G	A	T	Y	Y	A	D	S	V	K	G
Anti-Notch1	L	E	W	V	A	R	I	N	P	S	.	.	N	R	S	N	Q	Y	A	D	S	V	K	G
Anti-Notch3	L	E	W	V	G	A	I	S	S	.	.	S	G	S	S	T	Y	Y	A	D	S	V	K	G
Anti-Jag1	L	E	W	V	G	W	I	T	G	G	.	N	G	Y	Y	S	D	Y	A	D	S	V	K	G

Antibody	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82A	82B	82C	83	84	85	86
Anti-Notch2	R	F	T	I	S	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D
Anti-Notch1	R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D
Anti-Notch3	R	F	T	I	S	R	D	N	S	K	N	T	L	V	L	Q	M	N	S	L	R	A	E	D
Anti-Jag1	R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D

FIG. 24A

Heavy Chain Sequences

Kabat – CDR H3 (spanning positions 93–100J)

Antibody	87	88	89	90	91	92	93	94	95	96	97	98	99	100	100A	100B	100C	100D	100E	100F	100G	100H	100I	100J
Anti-Notch2	T	A	V	Y	Y	C	A	R	H	S	G	Y	Y	R	I	S	S	A	.	.	.	.	.	.
Anti-Notch1	T	A	V	Y	Y	C	A	R	G	S	G	F	R	W	V	.	.	.	.	.	.	.	.	.
Anti-Notch3	T	A	V	Y	Y	C	A	R	Q	Y	Y	R	D	.	.	.	.	.	.	.	.	.	.	.
Anti-Jag1	T	A	V	Y	Y	C	A	R	A	G	S	W	.	.	.	.	.	.	.	.	.	.	.	.

Antibody	100K	100L	100M	101	102	103	104	105	106	107	108	109	110	111	112	113
Anti-Notch2	.	M	D	V	W	G	Q	G	T	L	V	T	V	S	S	
Anti-Notch1	.	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S	
Anti-Notch3	.	M	D	V	W	G	Q	G	T	L	V	T	V	S	S	
Anti-Jag1	.	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	

FIG. 24B

Kabat# 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

A	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	Y	G	I	H	W	V	R	Q	A
A-1	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	Y	G	I	H	W	V	R	Q	A
A-2	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	N	Y	G	I	H	W	V	R	Q	A

Kabat - CDR H1
Chothia - CDR H1
Contact - CDR H1

Kabat# 41 42 43 44 45 46 47 48 49 50 51 52 A B C 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78

A	P	G	K	G	L	E	W	V	G	W	I	T	P	.	.	.	D	G	G	Y	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A
A-1	P	G	K	G	L	E	W	V	G	W	I	T	P	.	.	.	D	G	G	Y	T	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A
A-2	P	G	K	G	L	E	W	V	G	W	I	T	G	.	.	.	N	G	G	Y	S	D	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A

Kabat - CDR H2
Chothia - CDR H2
Contact - CDR H2

Kabat# 79 80 81 82 A B C 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 A B C D E F G H K 101 102 103 104 105 106 107 108 109 110 111 112 113

A	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	A	G	S	W	—	—	—	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:93
A-1	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	A	G	S	L	—	—	—	F	A	L	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:94
A-2	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	A	G	S	W	—	—	—	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:95

Kabat - CDR H3
Chothia - CDR H3
Contact - CDR H3

FIG. 25A

Block 1 — Kabat positions 1–37 (CDR L1 region)

Kabat#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	A	B	C	D	E	F	28	29	30	31	32	33	34	35	36	37
A	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	·	·	·	·	·	·	D	V	S	T	A	V	A	W	Y	Q
A-1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	·	·	·	·	·	·	D	V	S	T	A	V	A	W	Y	Q
A-2	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	·	·	·	·	·	·	D	V	S	T	A	V	A	W	Y	Q

Kabat – CDR L1
Chothia – CDR L1
Contact – CDR L1

Block 2 — Kabat positions 38–80 (CDR L2 region)

Kabat#	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
A	Q	K	P	G	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
A-1	Q	K	P	G	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
A-2	Q	K	P	G	K	A	P	K	L	L	I	Y	S	A	S	F	L	Y	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P

Kabat – CDR L2
Chothia – CDR L2
Contact – CDR L2

Block 3 — Kabat positions 81–107 (CDR L3 region)

Kabat#	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107		
A	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	T	T	P	P	F	G	Q	G	T	K	V	E	I	K			SEQ ID NO: 96
A-1	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	T	T	P	P	F	G	Q	G	T	K	V	E	I	K			SEQ ID NO: 97
A-2	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	T	T	P	P	F	G	Q	G	T	K	V	E	I	K			SEQ ID NO: 98

Kabat – CDR L3
Chothia – CDR L3
Contact – CDR L3

FIG. 25B

<110> GENENTECH, INC. ET AL.

<120> COMPOSITIONS AND METHODS FOR DIAGNOSIS AND TREATMENT OF HEPATIC
      CANCERS

<130> P5570R1-WO

<140>
<141>

<150> 61/789,475
<151> 2013-03-15

<160> 107

<170> PatentIn version 3.5

<210> 1
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 1
Gly Tyr Ser Phe Thr Ser Tyr Gly Met Ser
1   5   10

<210> 2
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 2
Ala Asp Leu Gly Ser
1   5

<210> 3
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 3
Ser Tyr Ile Tyr Pro Tyr Ser Gly Ala Thr Tyr Tyr Ala Asp Ser Val
1   5   10   15

Lys Gly

<210> 4
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
       peptide"

<400> 4
His Ser Gly Tyr Tyr Arg Ile Ser Ser Ala Met Asp Val
1   5   10

<210> 5
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
       peptide"

<400> 5
Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Ala
1   5   10

<210> 6
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
       peptide"

<400> 6
Arg Ala Ser Gln Ser Asn Arg Arg Phe Leu Ala
1   5   10

<210> 7
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
       peptide"

<400> 7
Arg Ala Ser Gln Ser Val Arg Ser Phe Leu Ala
1   5   10

<210> 8
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

peptide"

<400> 8
Arg Ala Ser Gln Asn Ile Lys Arg Phe Leu Ala
1   5   10

<210> 9
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<220>
<221> VARIANT
<222> (5)..(5)
<223> /replace="Asn

<220>
<221> VARIANT
<222> (6)..(6)
<223> /replace="Asn" or "Val"

<220>
<221> VARIANT
<222> (7)..(7)
<223> /replace="Arg" or "Lys"

<220>
<221> VARIANT
<222> (8)..(8)
<223> /replace="Arg"

<220>
<221> VARIANT
<222> (9)..(9)
<223> /replace="Phe"

<220>
<221> misc_feature
<222> (1)..(11)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 9
Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Ala
1   5   10

<210> 10
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 10
Gly Ala Ser Ser Arg Ala Ser
1   5

<210> 11
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
     peptide"

<400> 11
Gly Ala Ser Arg Arg Ala Ser
1   5

<210> 12
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
     peptide"

<400> 12
Arg Ala Ser Ile Arg Ala Ser
1   5

<210> 13
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
     peptide"

<400> 13
Gly Ala Ser Thr Arg Glu Ser
1   5

<210> 14
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
     peptide"

<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Arg"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Arg" or "Ile" or "Thr"

```
<220>
<221> VARIANT
<222> (6)..(6)
<223> /replace="Glu"

<220>
<221> misc_feature
<222> (1)..(7)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 14
Gly Ala Ser Ser Arg Ala Ser
1               5


<210> 15
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 15
Gln Gln Tyr Tyr Ser Ser Pro Leu Thr
1               5


<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 16
Gln Gln Tyr Tyr Ile Ser Pro Leu Thr
1               5


<210> 17
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 17
Gln Gln Tyr Tyr Ile Ser Pro Ser Thr
1               5


<210> 18
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
```

<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 18
Gln Gln Tyr Tyr Arg Ser Pro His Thr
1   5

<210> 19
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<220>
<221> VARIANT
<222> (5)..(5)
<223> /replace="Ile" or "Arg"

<220>
<221> VARIANT
<222> (8)..(8)
<223> /replace="Ser" or "His"

<220>
<221> misc_feature
<222> (1)..(9)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 19
Gln Gln Tyr Tyr Ser Ser Pro Leu Thr
1   5

<210> 20
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 20
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20   25   30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35   40   45

Ser Tyr Ile Tyr Pro Tyr Ser Gly Ala Thr Tyr Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg His Ser Gly Tyr Tyr Arg Ile Ser Ser Ala Met Asp Val Trp
            100   105   110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            115   120

<210> 21

<400> 21
000

<210> 22
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 22
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Gly Ala Ser Ser Arg Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Ser Pro Leu
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 23
<211> 107
<212> PRT
<213> Artificial Sequence

<220>

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 23
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Asn Arg Arg Phe
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Gly Ala Ser Arg Arg Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ile Ser Pro Leu
            85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 24
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 24
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Arg Ser Phe
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Arg Ala Ser Ile Arg Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ile Ser Pro Ser
            85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 25
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 25
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asn Ile Lys Arg Phe
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Arg Ser Pro His
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 26
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 26
Leu Val Pro Arg Gly Ser
1   5

<210> 27
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      6xHis tag"

```
<400> 27
His His His His His His
1               5


<210> 28
<211> 344
<212> PRT
<213> Homo sapiens

<400> 28
Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly Glu Leu
1               5               10              15


Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp Arg Lys
            20              25              30


Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu Lys Glu
            35              40              45


Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly Ser Gly
        50              55              60


Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala Ser Arg
65              70              75              80


Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala Trp Pro
                85              90              95


Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn Asp Thr
            100             105             110


Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly Met Ile
        115             120             125


Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly Val Ala
    130             135             140


His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr Tyr Gly
145             150             155             160


Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe Gly His
                165             170             175


Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly Trp Met
            180             185             190


Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly Cys Ser Pro Lys
        195             200             205


His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr Gly Trp
    210             215             220


Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys Val His
```

225   230   235   240

Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn Trp Gly
                245   250   255

Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His Gln Pro
                260   265   270

Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys Tyr Gln
                275   280   285

Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile Ala Glu
    290   295   300

His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys Lys Glu
305   310   315   320

Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr Gly Pro
                325   330   335

Thr Cys Ser Thr Asn Ile Asp Asp
                340

<210> 29
<211> 314
<212> PRT
<213> Homo sapiens

<400> 29
Met Arg Ile Ala Val Ile Cys Phe Cys Leu Leu Gly Ile Thr Cys Ala
1   5   10   15

Ile Pro Val Lys Gln Ala Asp Ser Gly Ser Ser Glu Glu Lys Gln Leu
                20   25   30

Tyr Asn Lys Tyr Pro Asp Ala Val Ala Thr Trp Leu Asn Pro Asp Pro
                35   40   45

Ser Gln Lys Gln Asn Leu Leu Ala Pro Gln Asn Ala Val Ser Ser Glu
    50   55   60

Glu Thr Asn Asp Phe Lys Gln Glu Thr Leu Pro Ser Lys Ser Asn Glu
65   70   75   80

Ser His Asp His Met Asp Asp Met Asp Asp Glu Asp Asp Asp Asp His
                85   90   95

Val Asp Ser Gln Asp Ser Ile Asp Ser Asn Asp Ser Asp Asp Val Asp
                100   105   110

Asp Thr Asp Asp Ser His Gln Ser Asp Glu Ser His His Ser Asp Glu
                115   120   125

```
Ser Asp Glu Leu Val Thr Asp Phe Pro Thr Asp Leu Pro Ala Thr Glu
    130             135             140
```

```
Val Phe Thr Pro Val Val Pro Thr Val Asp Thr Tyr Asp Gly Arg Gly
145             150             155             160
```

```
Asp Ser Val Val Tyr Gly Leu Arg Ser Lys Ser Lys Lys Phe Arg Arg
                165             170             175
```

```
Pro Asp Ile Gln Tyr Pro Asp Ala Thr Asp Glu Asp Ile Thr Ser His
            180             185             190
```

```
Met Glu Ser Glu Glu Leu Asn Gly Ala Tyr Lys Ala Ile Pro Val Ala
        195             200             205
```

```
Gln Asp Leu Asn Ala Pro Ser Asp Trp Asp Ser Arg Gly Lys Asp Ser
    210             215             220
```

```
Tyr Glu Thr Ser Gln Leu Asp Asp Gln Ser Ala Glu Thr His Ser His
225             230             235             240
```

```
Lys Gln Ser Arg Leu Tyr Lys Arg Lys Ala Asn Asp Glu Ser Asn Glu
                245             250             255
```

```
His Ser Asp Val Ile Asp Ser Gln Glu Leu Ser Lys Val Ser Arg Glu
            260             265             270
```

```
Phe His Ser His Glu Phe His Ser His Glu Asp Met Leu Val Val Asp
        275             280             285
```

```
Pro Lys Ser Lys Glu Glu Asp Lys His Leu Lys Phe Arg Ile Ser His
    290             295             300
```

```
Glu Leu Asp Ser Ala Ser Ser Glu Val Asn
305             310
```

```
<210> 30
<211> 32
<212> PRT
<213> Artificial Sequence
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```
<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"
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```
<400> 30
Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr
1               5               10              15
```

```
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
                20              25              30
```

<210> 31
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 31
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1   5   10

<210> 32
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 32
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
            20   25   30

<210> 33
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 33
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
1   5   10

<210> 34
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 34
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1   5   10   15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
            20   25   30

```
<210> 35
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 35
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1               5                   10


<210> 36
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 36
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser
            20                  25


<210> 37
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 37
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
1               5                   10


<210> 38
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 38
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1               5                   10                  15


Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
            20                  25                  30
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<210> 39
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 39
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1               5                   10                  15


Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                20                  25                  30


<210> 40
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 40
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser
                20                  25                  30


<210> 41
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 41
Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly
1               5                   10


<210> 42
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 42
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1               5                   10                  15
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Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
            20   25   30

<210> 43
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 43
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser
            20   25

<210> 44
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 44
Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
1   5   10

<210> 45
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 45
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1   5   10   15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
            20   25   30

<210> 46
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 46

Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1   5   10   15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
            20   25   30

<210> 47
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 47
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
            20   25   30

<210> 48
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 48
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1   5   10

<210> 49
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 49
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
            20   25   30

<210> 50
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
        peptide"

<400> 50
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser
            20   25

<210> 51
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        peptide"

<400> 51
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1   5   10

<210> 52
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        polypeptide"

<400> 52
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
            20   25   30

<210> 53
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        polypeptide"

<400> 53
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
            20   25   30

<210> 54
<211> 30
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 54
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
            20   25   30

<210> 55
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 55
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg
            20   25   30

<210> 56
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 56
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser
            20   25   30

<210> 57
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 57
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
            20   25   30

<210> 58
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 58
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
            20   25   30

<210> 59
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 59
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1   5   10   15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
            20   25   30

<210> 60
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 60
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys
            20

<210> 61
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 61
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1   5   10   15

<210> 62
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 62
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1   5   10   15

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

<210> 63
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 63
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1   5   10

<210> 64
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 64
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1   5   10   15

Glu Pro Ala Ser Ile Ser Cys
            20

<210> 65
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<210> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 65
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1   5   10   15

<210> 66
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 66
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1   5   10   15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
                20   25   30

<210> 67
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 67
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1   5   10   15

Glu Arg Ala Thr Leu Ser Cys
                20

<210> 68
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 68
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1   5   10   15

<210> 69
<211> 32
<212> PRT
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 69
Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1   5   10   15

Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
            20   25   30

<210> 70
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 70
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1   5   10   15

Glu Arg Ala Thr Ile Asn Cys
            20

<210> 71
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 71
Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr
1   5   10   15

<210> 72
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 72
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1   5   10   15

Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys
            20   25   30

<210> 73
<211> 256

<212> PRT
<213> Homo sapiens

<400> 73
Pro Ala Thr Cys Leu Ser Gln Tyr Cys Ala Asp Lys Ala Arg Asp Gly
1   5   10   15

Val Cys Asp Glu Ala Cys Asn Ser His Ala Cys Gln Trp Asp Gly Gly
                20   25   30

Asp Cys Ser Leu Thr Met Glu Asn Pro Trp Ala Asn Cys Ser Ser Pro
            35   40   45

Leu Pro Cys Trp Asp Tyr Ile Asn Asn Gln Cys Asp Glu Leu Cys Asn
        50   55   60

Thr Val Glu Cys Leu Phe Asp Asn Phe Glu Cys Gln Gly Asn Ser Lys
65   70   75   80

Thr Cys Lys Tyr Asp Lys Tyr Cys Ala Asp His Phe Lys Asp Asn His
                85   90   95

Cys Asp Gln Gly Cys Asn Ser Glu Glu Cys Gly Trp Asp Gly Leu Asp
                100   105   110

Cys Ala Ala Asp Gln Pro Glu Asn Leu Ala Glu Gly Thr Leu Val Ile
            115   120   125

Val Val Leu Met Pro Pro Glu Gln Leu Leu Gln Asp Ala Arg Ser Phe
    130   135   140

Leu Arg Ala Leu Gly Thr Leu Leu His Thr Asn Leu Arg Ile Lys Arg
145   150   155   160

Asp Ser Gln Gly Glu Leu Met Val Tyr Pro Tyr Tyr Gly Glu Lys Ser
                165   170   175

Ala Ala Met Lys Lys Gln Arg Met Thr Arg Arg Ser Leu Pro Gly Glu
                180   185   190

Gln Glu Gln Glu Val Ala Gly Ser Lys Val Phe Leu Glu Ile Asp Asn
            195   200   205

Arg Gln Cys Val Gln Asp Ser Asp His Cys Phe Lys Asn Thr Asp Ala
    210   215   220

Ala Ala Ala Leu Leu Ala Ser His Ala Ile Gln Gly Thr Leu Ser Tyr
225   230   235   240

Pro Leu Val Ser Val Val Ser Glu Ser Leu Thr Pro Glu Arg Thr Gln
                245   250   255

<210> 74
<211> 258
<212> PRT
<213> Mus sp.

<400> 74

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Pro Ala Thr Cys Gln Ser Gln Tyr Cys Ala Asp Lys Ala Arg Asp Gly
1               5                   10                  15


Ile Cys Asp Glu Ala Cys Asn Ser His Ala Cys Gln Trp Asp Gly Gly
            20                  25                  30


Asp Cys Ser Leu Thr Met Glu Asp Pro Trp Ala Asn Cys Thr Ser Thr
        35                  40                  45


Leu Arg Cys Trp Glu Tyr Ile Asn Asn Gln Cys Asp Glu Gln Cys Asn
    50                  55                  60


Thr Ala Glu Cys Leu Phe Asp Asn Phe Glu Cys Gln Arg Asn Ser Lys
65                  70                  75                  80


Thr Cys Lys Tyr Asp Lys Tyr Cys Ala Asp His Phe Lys Asp Asn His
            85                  90                  95


Cys Asp Gln Gly Cys Asn Ser Glu Glu Cys Gly Trp Asp Gly Leu Asp
            100                 105                 110


Cys Ala Ser Asp Gln Pro Glu Asn Leu Ala Glu Gly Thr Leu Ile Ile
            115                 120                 125


Val Val Leu Leu Pro Pro Glu Gln Leu Leu Gln Asp Ser Arg Ser Phe
    130                 135                 140


Leu Arg Ala Leu Gly Thr Leu Leu His Thr Asn Leu Arg Ile Lys Gln
145                 150                 155                 160


Asp Ser Gln Gly Ala Leu Met Val Tyr Pro Tyr Phe Gly Glu Lys Ser
                165                 170                 175


Ala Ala Met Lys Lys Gln Lys Met Thr Arg Arg Ser Leu Pro Glu Glu
                180                 185                 190


Gln Glu Gln Glu Gln Glu Val Ile Gly Ser Lys Ile Phe Leu Glu Ile
            195                 200                 205


Asp Asn Arg Gln Cys Val Gln Asp Ser Asp Gln Cys Phe Lys Asn Thr
    210                 215                 220


Asp Ala Ala Ala Ala Leu Leu Ala Ser His Ala Ile Gln Gly Thr Leu
225                 230                 235                 240


Ser Tyr Pro Leu Val Ser Val Phe Ser Glu Leu Glu Ser Pro Arg Asn
                245                 250                 255
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Ala Gln

<210> 75
<211> 2471
<212> PRT
<213> Homo sapiens

<400> 75
Met Pro Ala Leu Arg Pro Ala Leu Leu Trp Ala Leu Leu Ala Leu Trp
1   5   10   15

Leu Cys Cys Ala Ala Pro Ala His Ala Leu Gln Cys Arg Asp Gly Tyr
            20   25   30

Glu Pro Cys Val Asn Glu Gly Met Cys Val Thr Tyr His Asn Gly Thr
        35   40   45

Gly Tyr Cys Lys Cys Pro Glu Gly Phe Leu Gly Glu Tyr Cys Gln His
    50   55   60

Arg Asp Pro Cys Glu Lys Asn Arg Cys Gln Asn Gly Gly Thr Cys Val
65   70   75   80

Ala Gln Ala Met Leu Gly Lys Ala Thr Cys Arg Cys Ala Ser Gly Phe
                85   90   95

Thr Gly Glu Asp Cys Gln Tyr Ser Thr Ser His Pro Cys Phe Val Ser
            100   105   110

Arg Pro Cys Leu Asn Gly Gly Thr Cys His Met Leu Ser Arg Asp Thr
        115   120   125

Tyr Glu Cys Thr Cys Gln Val Gly Phe Thr Gly Lys Glu Cys Gln Trp
    130   135   140

Thr Asp Ala Cys Leu Ser His Pro Cys Ala Asn Gly Ser Thr Cys Thr
145   150   155   160

Thr Val Ala Asn Gln Phe Ser Cys Lys Cys Leu Thr Gly Phe Thr Gly
                165   170   175

Gln Lys Cys Glu Thr Asp Val Asn Glu Cys Asp Ile Pro Gly His Cys
            180   185   190

Gln His Gly Gly Thr Cys Leu Asn Leu Pro Gly Ser Tyr Gln Cys Gln
        195   200   205

Cys Pro Gln Gly Phe Thr Gly Gln Tyr Cys Asp Ser Leu Tyr Val Pro
    210   215   220

Cys Ala Pro Ser Pro Cys Val Asn Gly Gly Thr Cys Arg Gln Thr Gly
225   230   235   240

Asp Phe Thr Phe Glu Cys Asn Cys Leu Pro Gly Phe Glu Gly Ser Thr
            245   250   255

Cys Glu Arg Asn Ile Asp Asp Cys Pro Asn His Arg Cys Gln Asn Gly
            260   265   270

Gly Val Cys Val Asp Gly Val Asn Thr Tyr Asn Cys Arg Cys Pro Pro
        275   280   285

Gln Trp Thr Gly Gln Phe Cys Thr Glu Asp Val Asp Glu Cys Leu Leu
    290   295   300

Gln Pro Asn Ala Cys Gln Asn Gly Gly Thr Cys Ala Asn Arg Asn Gly
305   310   315   320

Gly Tyr Gly Cys Val Cys Val Asn Gly Trp Ser Gly Asp Asp Cys Ser
            325   330   335

Glu Asn Ile Asp Asp Cys Ala Phe Ala Ser Cys Thr Pro Gly Ser Thr
            340   345   350

Cys Ile Asp Arg Val Ala Ser Phe Ser Cys Met Cys Pro Glu Gly Lys
        355   360   365

Ala Gly Leu Leu Cys His Leu Asp Asp Ala Cys Ile Ser Asn Pro Cys
    370   375   380

His Lys Gly Ala Leu Cys Asp Thr Asn Pro Leu Asn Gly Gln Tyr Ile
385   390   395   400

Cys Thr Cys Pro Gln Gly Tyr Lys Gly Ala Asp Cys Thr Glu Asp Val
            405   410   415

Asp Glu Cys Ala Met Ala Asn Ser Asn Pro Cys Glu His Ala Gly Lys
            420   425   430

Cys Val Asn Thr Asp Gly Ala Phe His Cys Glu Cys Leu Lys Gly Tyr
        435   440   445

Ala Gly Pro Arg Cys Glu Met Asp Ile Asn Glu Cys His Ser Asp Pro
    450   455   460

Cys Gln Asn Asp Ala Thr Cys Leu Asp Lys Ile Gly Gly Phe Thr Cys
465   470   475   480

Leu Cys Met Pro Gly Phe Lys Gly Val His Cys Glu Leu Glu Ile Asn
            485   490   495

Glu Cys Gln Ser Asn Pro Cys Val Asn Asn Gly Gln Cys Val Asp Lys
            500   505   510

Val Asn Arg Phe Gln Cys Leu Cys Pro Pro Gly Phe Thr Gly Pro Val
            515   520   525

Cys Gln Ile Asp Ile Asp Asp Cys Ser Ser Thr Pro Cys Leu Asn Gly
    530   535   540

Ala Lys Cys Ile Asp His Pro Asn Gly Tyr Glu Cys Gln Cys Ala Thr
545   550   555   560

Gly Phe Thr Gly Val Leu Cys Glu Glu Asn Ile Asp Asn Cys Asp Pro
            565   570   575

Asp Pro Cys His His Gly Gln Cys Gln Asp Gly Ile Asp Ser Tyr Thr
        580   585   590

Cys Ile Cys Asn Pro Gly Tyr Met Gly Ala Ile Cys Ser Asp Gln Ile
            595   600   605

Asp Glu Cys Tyr Ser Ser Pro Cys Leu Asn Asp Gly Arg Cys Ile Asp
    610   615   620

Leu Val Asn Gly Tyr Gln Cys Asn Cys Gln Pro Gly Thr Ser Gly Val
625   630   635   640

Asn Cys Glu Ile Asn Phe Asp Asp Cys Ala Ser Asn Pro Cys Ile His
            645   650   655

Gly Ile Cys Met Asp Gly Ile Asn Arg Tyr Ser Cys Val Cys Ser Pro
            660   665   670

Gly Phe Thr Gly Gln Arg Cys Asn Ile Asp Ile Asp Glu Cys Ala Ser
            675   680   685

Asn Pro Cys Arg Lys Gly Ala Thr Cys Ile Asn Gly Val Asn Gly Phe
    690   695   700

Arg Cys Ile Cys Pro Glu Gly Pro His His Pro Ser Cys Tyr Ser Gln
705   710   715   720

Val Asn Glu Cys Leu Ser Asn Pro Cys Ile His Gly Asn Cys Thr Gly
            725   730   735

Gly Leu Ser Gly Tyr Lys Cys Leu Cys Asp Ala Gly Trp Val Gly Ile
            740   745   750

Asn Cys Glu Val Asp Lys Asn Glu Cys Leu Ser Asn Pro Cys Gln Asn
        755   760   765

Gly Gly Thr Cys Asp Asn Leu Val Asn Gly Tyr Arg Cys Thr Cys Lys
    770   775   780

Lys Gly Phe Lys Gly Tyr Asn Cys Gln Val Asn Ile Asp Glu Cys Ala
785   790   795   800

Ser Asn Pro Cys Leu Asn Gln Gly Thr Cys Phe Asp Asp Ile Ser Gly
            805   810   815

Tyr Thr Cys His Cys Val Leu Pro Tyr Thr Gly Lys Asn Cys Gln Thr
            820   825   830

Val Leu Ala Pro Cys Ser Pro Asn Pro Cys Glu Asn Ala Ala Val Cys
        835   840   845

Lys Glu Ser Pro Asn Phe Glu Ser Tyr Thr Cys Leu Cys Ala Pro Gly
    850   855   860

Trp Gln Gly Gln Arg Cys Thr Ile Asp Ile Asp Glu Cys Ile Ser Lys
865   870   875   880

Pro Cys Met Asn His Gly Leu Cys His Asn Thr Gln Gly Ser Tyr Met
            885   890   895

Cys Glu Cys Pro Pro Gly Phe Ser Gly Met Asp Cys Glu Glu Asp Ile
            900   905   910

Asp Asp Cys Leu Ala Asn Pro Cys Gln Asn Gly Gly Ser Cys Met Asp
            915   920   925

Gly Val Asn Thr Phe Ser Cys Leu Cys Leu Pro Gly Phe Thr Gly Asp
    930   935   940

Lys Cys Gln Thr Asp Met Asn Glu Cys Leu Ser Glu Pro Cys Lys Asn
945   950   955   960

Gly Gly Thr Cys Ser Asp Tyr Val Asn Ser Tyr Thr Cys Lys Cys Gln
            965   970   975

Ala Gly Phe Asp Gly Val His Cys Glu Asn Asn Ile Asn Glu Cys Thr
            980   985   990

Glu Ser Ser Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser
    995   1000   1005

Phe Ser Cys Leu Cys Pro Val Gly Phe Thr Gly Ser Phe Cys Leu
    1010   1015   1020

His Glu Ile Asn Glu Cys Ser Ser His Pro Cys Leu Asn Glu Gly
    1025   1030   1035

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Thr Cys Val Asp Gly Leu Gly Thr Tyr Arg Cys Ser Cys Pro Leu
    1040            1045            1050

Gly Tyr Thr Gly Lys Asn Cys Gln Thr Leu Val Asn Leu Cys Ser
    1055            1060            1065

Arg Ser Pro Cys Lys Asn Lys Gly Thr Cys Val Gln Lys Lys Ala
    1070            1075            1080

Glu Ser Gln Cys Leu Cys Pro Ser Gly Trp Ala Gly Ala Tyr Cys
    1085            1090            1095

Asp Val Pro Asn Val Ser Cys Asp Ile Ala Ala Ser Arg Arg Gly
    1100            1105            1110

Val Leu Val Glu His Leu Cys Gln His Ser Gly Val Cys Ile Asn
    1115            1120            1125

Ala Gly Asn Thr His Tyr Cys Gln Cys Pro Leu Gly Tyr Thr Gly
    1130            1135            1140

Ser Tyr Cys Glu Glu Gln Leu Asp Glu Cys Ala Ser Asn Pro Cys
    1145            1150            1155

Gln His Gly Ala Thr Cys Ser Asp Phe Ile Gly Gly Tyr Arg Cys
    1160            1165            1170

Glu Cys Val Pro Gly Tyr Gln Gly Val Asn Cys Glu Tyr Glu Val
    1175            1180            1185

Asp Glu Cys Gln Asn Gln Pro Cys Gln Asn Gly Gly Thr Cys Ile
    1190            1195            1200

Asp Leu Val Asn His Phe Lys Cys Ser Cys Pro Pro Gly Thr Arg
    1205            1210            1215

Gly Leu Leu Cys Glu Glu Asn Ile Asp Asp Cys Ala Arg Gly Pro
    1220            1225            1230

His Cys Leu Asn Gly Gly Gln Cys Met Asp Arg Ile Gly Gly Tyr
    1235            1240            1245

Ser Cys Arg Cys Leu Pro Gly Phe Ala Gly Glu Arg Cys Glu Gly
    1250            1255            1260

Asp Ile Asn Glu Cys Leu Ser Asn Pro Cys Ser Ser Glu Gly Ser
    1265            1270            1275

Leu Asp Cys Ile Gln Leu Thr Asn Asp Tyr Leu Cys Val Cys Arg
    1280            1285            1290
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Ser Ala Phe Thr Gly Arg His Cys Glu Thr Phe Val Asp Val Cys
    1295   1300   1305

Pro Gln Met Pro Cys Leu Asn Gly Gly Thr Cys Ala Val Ala Ser
    1310   1315   1320

Asn Met Pro Asp Gly Phe Ile Cys Arg Cys Pro Pro Gly Phe Ser
    1325   1330   1335

Gly Ala Arg Cys Gln Ser Ser Cys Gly Gln Val Lys Cys Arg Lys
    1340   1345   1350

Gly Glu Gln Cys Val His Thr Ala Ser Gly Pro Arg Cys Phe Cys
    1355   1360   1365

Pro Ser Pro Arg Asp Cys Glu Ser Gly Cys Ala Ser Ser Pro Cys
    1370   1375   1380

Gln His Gly Gly Ser Cys His Pro Gln Arg Gln Pro Pro Tyr Tyr
    1385   1390   1395

Ser Cys Gln Cys Ala Pro Pro Phe Ser Gly Ser Arg Cys Glu Leu
    1400   1405   1410

Tyr Thr Ala Pro Pro Ser Thr Pro Pro Ala Thr Cys Leu Ser Gln
    1415   1420   1425

Tyr Cys Ala Asp Lys Ala Arg Asp Gly Val Cys Asp Glu Ala Cys
    1430   1435   1440

Asn Ser His Ala Cys Gln Trp Asp Gly Gly Asp Cys Ser Leu Thr
    1445   1450   1455

Met Glu Asn Pro Trp Ala Asn Cys Ser Ser Pro Leu Pro Cys Trp
    1460   1465   1470

Asp Tyr Ile Asn Asn Gln Cys Asp Glu Leu Cys Asn Thr Val Glu
    1475   1480   1485

Cys Leu Phe Asp Asn Phe Glu Cys Gln Gly Asn Ser Lys Thr Cys
    1490   1495   1500

Lys Tyr Asp Lys Tyr Cys Ala Asp His Phe Lys Asp Asn His Cys
    1505   1510   1515

Asp Gln Gly Cys Asn Ser Glu Glu Cys Gly Trp Asp Gly Leu Asp
    1520   1525   1530

Cys Ala Ala Asp Gln Pro Glu Asn Leu Ala Glu Gly Thr Leu Val
    1535   1540   1545

```
Ile Val  Val Leu Met Pro Pro  Glu Gln Leu Leu Gln  Asp Ala Arg
    1550             1555              1560
```

```
Ser Phe  Leu Arg Ala Leu Gly  Thr Leu Leu His Thr  Asn Leu Arg
    1565             1570              1575
```

```
Ile Lys  Arg Asp Ser Gln Gly  Glu Leu Met Val Tyr  Pro Tyr Tyr
    1580             1585              1590
```

```
Gly Glu  Lys Ser Ala Ala Met  Lys Lys Gln Arg Met  Thr Arg Arg
    1595             1600              1605
```

```
Ser Leu  Pro Gly Glu Gln Glu  Gln Glu Val Ala Gly  Ser Lys Val
    1610             1615              1620
```

```
Phe Leu  Glu Ile Asp Asn Arg  Gln Cys Val Gln Asp  Ser Asp His
    1625             1630              1635
```

```
Cys Phe  Lys Asn Thr Asp Ala  Ala Ala Ala Leu Leu  Ala Ser His
    1640             1645              1650
```

```
Ala Ile  Gln Gly Thr Leu Ser  Tyr Pro Leu Val Ser  Val Val Ser
    1655             1660              1665
```

```
Glu Ser  Leu Thr Pro Glu Arg  Thr Gln Leu Leu Tyr  Leu Leu Ala
    1670             1675              1680
```

```
Val Ala  Val Val Ile Ile Leu  Phe Ile Ile Leu Leu  Gly Val Ile
    1685             1690              1695
```

```
Met Ala  Lys Arg Lys Arg Lys  His Gly Ser Leu Trp  Leu Pro Glu
    1700             1705              1710
```

```
Gly Phe  Thr Leu Arg Arg Asp  Ala Ser Asn His Lys  Arg Arg Glu
    1715             1720              1725
```

```
Pro Val  Gly Gln Asp Ala Val  Gly Leu Lys Asn Leu  Ser Val Gln
    1730             1735              1740
```

```
Val Ser  Glu Ala Asn Leu Ile  Gly Thr Gly Thr Ser  Glu His Trp
    1745             1750              1755
```

```
Val Asp  Asp Glu Gly Pro Gln  Pro Lys Lys Val Lys  Ala Glu Asp
    1760             1765              1770
```

```
Glu Ala  Leu Leu Ser Glu Glu  Asp Asp Pro Ile Asp  Arg Arg Pro
    1775             1780              1785
```

```
Trp Thr  Gln Gln His Leu Glu  Ala Ala Asp Ile Arg  Arg Thr Pro
    1790             1795              1800
```

```
Ser Leu  Ala Leu Thr Pro Pro  Gln Ala Glu Gln Glu  Val Asp Val
    1805                 1810                1815

Leu Asp  Val Asn Val Arg Gly  Pro Asp Gly Cys Thr  Pro Leu Met
    1820                 1825                1830

Leu Ala  Ser Leu Arg Gly Gly  Ser Ser Asp Leu Ser  Asp Glu Asp
    1835                 1840                1845

Glu Asp  Ala Glu Asp Ser Ser  Ala Asn Ile Ile Thr  Asp Leu Val
    1850                 1855                1860

Tyr Gln  Gly Ala Ser Leu Gln  Ala Gln Thr Asp Arg  Thr Gly Glu
    1865                 1870                1875

Met Ala  Leu His Leu Ala Ala  Arg Tyr Ser Arg Ala  Asp Ala Ala
    1880                 1885                1890

Lys Arg  Leu Leu Asp Ala Gly  Ala Asp Ala Asn Ala  Gln Asp Asn
    1895                 1900                1905

Met Gly  Arg Cys Pro Leu His  Ala Ala Val Ala Ala  Asp Ala Gln
    1910                 1915                1920

Gly Val  Phe Gln Ile Leu Ile  Arg Asn Arg Val Thr  Asp Leu Asp
    1925                 1930                1935

Ala Arg  Met Asn Asp Gly Thr  Thr Pro Leu Ile Leu  Ala Ala Arg
    1940                 1945                1950

Leu Ala  Val Glu Gly Met Val  Ala Glu Leu Ile Asn  Cys Gln Ala
    1955                 1960                1965

Asp Val  Asn Ala Val Asp Asp  His Gly Lys Ser Ala  Leu His Trp
    1970                 1975                1980

Ala Ala  Ala Val Asn Asn Val  Glu Ala Thr Leu Leu  Leu Leu Lys
    1985                 1990                1995

Asn Gly  Ala Asn Arg Asp Met  Gln Asp Asn Lys Glu  Glu Thr Pro
    2000                 2005                2010

Leu Phe  Leu Ala Ala Arg Glu  Gly Ser Tyr Glu Ala  Ala Lys Ile
    2015                 2020                2025

Leu Leu  Asp His Phe Ala Asn  Arg Asp Ile Thr Asp  His Met Asp
    2030                 2035                2040

Arg Leu  Pro Arg Asp Val Ala  Arg Asp Arg Met His  His Asp Ile
    2045                 2050                2055
```

```
Val Arg Leu Leu Asp Glu Tyr  Asn Val Thr Pro Ser  Pro Pro Gly
    2060            2065               2070

Thr Val Leu Thr Ser Ala Leu  Ser Pro Val Ile Cys  Gly Pro Asn
    2075            2080               2085

Arg Ser Phe Leu Ser Leu Lys  His Thr Pro Met Gly  Lys Lys Ser
    2090            2095               2100

Arg Arg Pro Ser Ala Lys Ser  Thr Met Pro Thr Ser  Leu Pro Asn
    2105            2110               2115

Leu Ala Lys Glu Ala Lys Asp  Ala Lys Gly Ser Arg  Arg Lys Lys
    2120            2125               2130

Ser Leu Ser Glu Lys Val Gln  Leu Ser Glu Ser Ser  Val Thr Leu
    2135            2140               2145

Ser Pro Val Asp Ser Leu Glu  Ser Pro His Thr Tyr  Val Ser Asp
    2150            2155               2160

Thr Thr Ser Ser Pro Met Ile  Thr Ser Pro Gly Ile  Leu Gln Ala
    2165            2170               2175

Ser Pro Asn Pro Met Leu Ala  Thr Ala Ala Pro Pro  Ala Pro Val
    2180            2185               2190

His Ala Gln His Ala Leu Ser  Phe Ser Asn Leu His  Glu Met Gln
    2195            2200               2205

Pro Leu Ala His Gly Ala Ser  Thr Val Leu Pro Ser  Val Ser Gln
    2210            2215               2220

Leu Leu Ser His His His Ile  Val Ser Pro Gly Ser  Gly Ser Ala
    2225            2230               2235

Gly Ser Leu Ser Arg Leu His  Pro Val Pro Val Pro  Ala Asp Trp
    2240            2245               2250

Met Asn Arg Met Glu Val Asn  Glu Thr Gln Tyr Asn  Glu Met Phe
    2255            2260               2265

Gly Met Val Leu Ala Pro Ala  Glu Gly Thr His Pro  Gly Ile Ala
    2270            2275               2280

Pro Gln Ser Arg Pro Pro Glu  Gly Lys His Ile Thr  Thr Pro Arg
    2285            2290               2295

Glu Pro Leu Pro Pro Ile Val  Thr Phe Gln Leu Ile  Pro Lys Gly
    2300            2305               2310
```

```
Ser Ile  Ala Gln Pro Ala Gly  Ala Pro Gln Pro Gln  Ser Thr Cys
    2315             2320             2325


Pro Pro  Ala Val Ala Gly Pro  Leu Pro Thr Met Tyr  Gln Ile Pro
    2330             2335             2340


Glu Met  Ala Arg Leu Pro Ser  Val Ala Phe Pro Thr  Ala Met Met
    2345             2350             2355


Pro Gln  Gln Asp Gly Gln Val  Ala Gln Thr Ile Leu  Pro Ala Tyr
    2360             2365             2370


His Pro  Phe Pro Ala Ser Val  Gly Lys Tyr Pro Thr  Pro Pro Ser
    2375             2380             2385


Gln His  Ser Tyr Ala Ser Ser  Asn Ala Ala Glu Arg  Thr Pro Ser
    2390             2395             2400


His Ser  Gly His Leu Gln Gly  Glu His Pro Tyr Leu  Thr Pro Ser
    2405             2410             2415


Pro Glu  Ser Pro Asp Gln Trp  Ser Ser Ser Ser Pro  His Ser Ala
    2420             2425             2430


Ser Asp  Trp Ser Asp Val Thr  Thr Ser Pro Thr Pro  Gly Gly Ala
    2435             2440             2445


Gly Gly  Gly Gln Arg Gly Pro  Gly Thr His Met Ser  Glu Pro Pro
    2450             2455             2460


His Asn  Asn Met Gln Val Tyr  Ala
    2465             2470
```

<210> 76
<211> 1180
<212> PRT
<213> Homo sapiens

<400> 76
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Met Pro Asp Leu Arg Pro Ala Ala Leu Arg Ala Leu Leu Trp Leu Trp
1               5                   10                  15


Leu Cys Gly Ala Gly Pro Ala His Ala Leu Gln Cys Arg Gly Gly Gln
                20              25              30


Glu Pro Cys Val Asn Glu Gly Thr Cys Val Thr Tyr His Asn Gly Thr
        35              40              45


Gly Phe Cys Arg Cys Pro Glu Gly Phe Leu Gly Glu Tyr Cys Gln His
    50              55              60


Arg Asp Pro Cys Glu Lys Asn Arg Cys Gln Asn Gly Gly Thr Cys Val
65              70              75              80
```

Pro Gln Gly Met Leu Gly Lys Ala Thr Cys Arg Cys Ala Pro Gly Phe
              85   90   95

Thr Gly Glu Asp Cys Gln Tyr Ser Thr Ser His Pro Cys Phe Val Ser
            100   105   110

Arg Pro Cys Gln Asn Gly Gly Thr Cys His Met Leu Ser Arg Asp Thr
            115   120   125

Tyr Glu Cys Thr Cys Gln Val Gly Phe Thr Gly Lys Gln Cys Gln Trp
    130   135   140

Thr Asp Ala Cys Leu Ser His Pro Cys Glu Asn Gly Ser Thr Cys Thr
145   150   155   160

Ser Val Ala Ser Gln Phe Ser Cys Lys Cys Pro Ala Gly Leu Thr Gly
            165   170   175

Gln Lys Cys Glu Ala Asp Ile Asn Glu Cys Asp Ile Pro Gly Arg Cys
            180   185   190

Gln His Gly Gly Thr Cys Leu Asn Leu Pro Gly Ser Tyr Arg Cys Gln
            195   200   205

Cys Gly Gln Gly Phe Thr Gly Gln His Cys Asp Ser Pro Tyr Val Arg
    210   215   220

Gly Leu Pro Cys Val Asn Gly Gly Thr Cys Arg Gln Thr Gly Asp Phe
225   230   235   240

Thr Leu Glu Cys Asn Cys Leu Pro Gly Phe Glu Gly Ser Thr Cys Glu
            245   250   255

Arg Asn Ile Asp Asp Cys Pro Asn His Lys Cys Gln Asn Gly Gly Val
            260   265   270

Cys Val Asp Gly Val Asn Thr Tyr Asn Cys Arg Cys Pro Pro Gln Trp
            275   280   285

Thr Gly Gln Phe Cys Thr Glu Asp Val Asp Glu Cys Leu Leu Gln Pro
    290   295   300

Asn Ala Cys Gln Asn Gly Gly Thr Cys Thr Asn Arg Asn Gly Gly Tyr
305   310   315   320

Gly Cys Val Cys Val Asn Gly Trp Ser Gly Asp Asp Cys Ser Glu Asn
            325   330   335

Ile Asp Asp Cys Ala Tyr Ala Ser Cys Thr Pro Gly Ser Thr Cys Ile
            340   345   350

Asp Arg Val Ala Ser Phe Ser Cys Leu Cys Pro Glu Gly Lys Ala Gly
        355   360   365

Leu Leu Cys His Leu Asp Asp Ala Cys Ile Ser Asn Pro Cys His Lys
    370   375   380

Gly Ala Leu Cys Asp Thr Asn Pro Leu Asn Gly Gln Tyr Ile Cys Thr
385   390   395   400

Cys Pro Gln Gly Tyr Lys Gly Ala Asp Cys Thr Glu Asp Val Asp Glu
            405   410   415

Cys Ala Met Ala Asn Ser Asn Pro Cys Glu His Ala Gly Lys Cys Val
        420   425   430

Asn Thr Asp Gly Ala Phe His Cys Glu Cys Leu Lys Gly Tyr Ala Gly
        435   440   445

Pro Arg Cys Glu Met Asp Ile Asn Glu Cys His Ser Asp Pro Cys Gln
    450   455   460

Asn Asp Ala Thr Cys Leu Asp Lys Ile Gly Gly Phe Thr Cys Leu Cys
465   470   475   480

Met Pro Gly Phe Lys Gly Val His Cys Glu Leu Glu Val Asn Glu Cys
            485   490   495

Gln Ser Asn Pro Cys Val Asn Asn Gly Gln Cys Val Asp Lys Val Asn
            500   505   510

Arg Phe Gln Cys Leu Cys Pro Pro Gly Phe Thr Gly Pro Val Cys Gln
        515   520   525

Ile Asp Ile Asp Asp Cys Ser Ser Thr Pro Cys Leu Asn Gly Ala Lys
    530   535   540

Cys Ile Asp His Pro Asn Gly Tyr Glu Cys Gln Cys Ala Thr Gly Phe
545   550   555   560

Thr Gly Ile Leu Cys Asp Glu Asn Ile Asp Asn Cys Asp Pro Asp Pro
            565   570   575

Cys His His Gly Gln Cys Gln Asp Gly Ile Asp Ser Tyr Thr Cys Ile
            580   585   590

Cys Asn Pro Gly Tyr Met Gly Ala Ile Cys Ser Asp Gln Ile Asp Glu
            595   600   605

Cys Tyr Ser Ser Pro Cys Leu Asn Asp Gly Arg Cys Ile Asp Leu Val
    610   615   620

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Asn Gly Tyr Gln Cys Asn Cys Gln Pro Gly Thr Ser Gly Leu Asn Cys
625   630   635   640

Glu Ile Asn Phe Asp Asp Cys Ala Ser Asn Pro Cys Met His Gly Val
             645   650   655

Cys Val Asp Gly Ile Asn Arg Tyr Ser Cys Val Cys Ser Pro Gly Phe
             660   665   670

Thr Gly Gln Arg Cys Asn Ile Asp Ile Asp Glu Cys Ala Ser Asn Pro
         675   680   685

Cys Arg Lys Gly Ala Thr Cys Ile Asn Asp Val Asn Gly Phe Arg Cys
    690   695   700

Ile Cys Pro Glu Gly Pro His His Pro Ser Cys Tyr Ser Gln Val Asn
705   710   715   720

Glu Cys Leu Ser Asn Pro Cys Ile His Gly Asn Cys Thr Gly Gly Leu
             725   730   735

Ser Gly Tyr Lys Cys Leu Cys Asp Ala Gly Trp Val Gly Val Asn Cys
             740   745   750

Glu Val Asp Lys Asn Glu Cys Leu Ser Asn Pro Cys Gln Asn Gly Gly
         755   760   765

Thr Cys Asn Asn Leu Val Asn Gly Tyr Arg Cys Thr Cys Lys Lys Gly
    770   775   780

Phe Lys Gly Tyr Asn Cys Gln Val Asn Ile Asp Glu Cys Ala Ser Asn
785   790   795   800

Pro Cys Leu Asn Gln Gly Thr Cys Phe Asp Asp Val Ser Gly Tyr Thr
             805   810   815

Cys His Cys Met Leu Pro Tyr Thr Gly Lys Asn Cys Gln Thr Val Leu
         820   825   830

Ala Pro Cys Ser Pro Asn Pro Cys Glu Asn Ala Ala Val Cys Lys Glu
         835   840   845

Ala Pro Asn Phe Glu Ser Phe Ser Cys Leu Cys Ala Pro Gly Trp Gln
    850   855   860

Gly Lys Arg Cys Thr Val Asp Val Asp Glu Cys Ile Ser Lys Pro Cys
865   870   875   880

Met Asn Asn Gly Val Cys His Asn Thr Gln Gly Ser Tyr Val Cys Glu
             885   890   895

Cys Pro Pro Gly Phe Ser Gly Met Asp Cys Glu Glu Asp Ile Asn Asp
            900   905   910

Cys Leu Ala Asn Pro Cys Gln Asn Gly Gly Ser Cys Val Asp His Val
            915   920   925

Asn Thr Phe Ser Cys Gln Cys His Pro Gly Phe Ile Gly Asp Lys Cys
    930   935   940

Gln Thr Asp Met Asn Glu Cys Leu Ser Glu Pro Cys Lys Asn Gly Gly
945   950   955   960

Thr Cys Ser Asp Tyr Val Asn Ser Tyr Thr Cys Thr Cys Pro Ala Gly
            965   970   975

Phe His Gly Val His Cys Glu Asn Asn Ile Asp Glu Cys Thr Glu Ser
            980   985   990

Ser Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser Phe Ser
        995   1000   1005

Cys Leu Cys Pro Val Gly Phe Thr Gly Pro Phe Cys Leu His Asp
    1010   1015   1020

Ile Asn Glu Cys Ser Ser Asn Pro Cys Leu Asn Ala Gly Thr Cys
    1025   1030   1035

Val Asp Gly Leu Gly Thr Tyr Arg Cys Ile Cys Pro Leu Gly Tyr
    1040   1045   1050

Thr Gly Lys Asn Cys Gln Thr Leu Val Asn Leu Cys Ser Arg Ser
    1055   1060   1065

Pro Cys Lys Asn Lys Gly Thr Cys Val Gln Glu Lys Ala Arg Pro
    1070   1075   1080

His Cys Leu Cys Pro Pro Gly Trp Asp Gly Ala Tyr Cys Asp Val
    1085   1090   1095

Leu Asn Val Ser Cys Lys Ala Ala Ala Leu Gln Lys Gly Val Pro
    1100   1105   1110

Val Glu His Leu Cys Gln His Ser Gly Ile Cys Ile Asn Ala Gly
    1115   1120   1125

Asn Thr His His Cys Gln Cys Pro Leu Gly Tyr Thr Gly Ser Tyr
    1130   1135   1140

Cys Glu Glu Gln Leu Asp Glu Cys Ala Ser Asn Pro Cys Gln His
    1145   1150   1155

Gly Ala Thr Cys Asn Asp Phe Ile Gly Gly Tyr Arg Cys Glu Cys
    1160   1165   1170

Val Pro Gly Tyr Gln Gly Val
    1175   1180

<210> 77
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

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Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1   5   10

<210> 78
<211> 1218
<212> PRT
<213> Homo sapiens

<400> 78
Met Arg Ser Pro Arg Thr Arg Gly Arg Ser Gly Arg Pro Leu Ser Leu
1   5   10   15

Leu Leu Ala Leu Leu Cys Ala Leu Arg Ala Lys Val Cys Gly Ala Ser
            20   25   30

Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly Glu
        35   40   45

Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp Arg
    50   55   60

Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu Lys
65   70   75   80

Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly Ser
            85   90   95

Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala Ser
            100   105   110

Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala Trp
        115   120   125

Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn Asp
    130   135   140

Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly Met
                              Page 40

145   150   155   160

Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly Val
                165   170   175

Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr Tyr
                180   185   190

Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe Gly
                195   200   205

His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly Trp
    210   215   220

Met Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly Cys Ser Pro
225   230   235   240

Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr Gly
                245   250   255

Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys Val
                260   265   270

His Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn Trp
                275   280   285

Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His Gln
    290   295   300

Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys Tyr
305   310   315   320

Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile Ala
                325   330   335

Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys Lys
                340   345   350

Glu Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr Gly
                355   360   365

Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn Asn Cys Ser
    370   375   380

His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys Cys Val Cys
385   390   395   400

Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala Asn Glu Cys
                405   410   415

Glu Ala Lys Pro Cys Val Asn Ala Lys Ser Cys Lys Asn Leu Ile Ala

Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln Asn Cys Asp
        435   440   445

Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp Ala Ser Cys
    450   455   460

Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro Gly Tyr Ala
465   470   475   480

Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser Asn Pro Cys
            485   490   495

Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe Gln Cys Leu
            500   505   510

Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp Ile Asp Tyr
        515   520   525

Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr Asn Arg Ala
    530   535   540

Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly Lys Asn Cys
545   550   555   560

Ser His Leu Lys Asp His Cys Arg Thr Thr Pro Cys Glu Val Ile Asp
            565   570   575

Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu Gly Val Arg
        580   585   590

Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser Gln
        595   600   605

Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly Thr
    610   615   620

Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Arg Asn
625   630   635   640

Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys Ile Cys Ser
            645   650   655

Asp Gly Trp Glu Gly Ala Tyr Cys Glu Thr Asn Ile Asn Asp Cys Ser
        660   665   670

Gln Asn Pro Cys His Asn Gly Gly Thr Cys Arg Asp Leu Val Asn Asp
        675   680   685

Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr Cys His Ser

                690   695   700

Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly Gly Thr Cys
705   710   715   720

Tyr Asp Glu Gly Asp Ala Phe Lys Cys Met Cys Pro Gly Gly Trp Glu
                    725   730   735

Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu Pro Asn Pro
                740   745   750

Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Glu Ser Phe Thr Cys
            755   760   765

Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Ala Gln Asn Thr Asn
    770   775   780

Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys Val Asp Gly
785   790   795   800

Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro Asp
                    805   810   815

Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys Ala Phe Gly
                820   825   830

Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Arg Cys Val Cys Pro Pro
            835   840   845

Gly His Ser Gly Ala Lys Cys Gln Glu Val Ser Gly Arg Pro Cys Ile
    850   855   860

Thr Met Gly Ser Val Ile Pro Asp Gly Ala Lys Trp Asp Asp Asp Cys
865   870   875   880

Asn Thr Cys Gln Cys Leu Asn Gly Arg Ile Ala Cys Ser Lys Val Trp
                885   890   895

Cys Gly Pro Arg Pro Cys Leu Leu His Lys Gly His Ser Glu Cys Pro
                900   905   910

Ser Gly Gln Ser Cys Ile Pro Ile Leu Asp Asp Gln Cys Phe Val His
            915   920   925

Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu Gln Pro Val
    930   935   940

Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn Cys Ala Asn
945   950   955   960

Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly Leu Thr Thr

                    965   970   975

Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu Lys Asn Val
            980   985   990

Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser Pro Ser Ala
        995   1000   1005

Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile Arg Asp
    1010   1015   1020

Asp Gly Asn Pro Ile Lys Glu Ile Thr Asp Lys Ile Ile Asp Leu
    1025   1030   1035

Val Ser Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Ala
    1040   1045   1050

Glu Val Arg Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp Phe
    1055   1060   1065

Leu Val Pro Leu Leu Ser Ser Val Leu Thr Val Ala Trp Ile Cys
    1070   1075   1080

Cys Leu Val Thr Ala Phe Tyr Trp Cys Leu Arg Lys Arg Arg Lys
    1085   1090   1095

Pro Gly Ser His Thr His Ser Ala Ser Glu Asp Asn Thr Thr Asn
    1100   1105   1110

Asn Val Arg Glu Gln Leu Asn Gln Ile Lys Asn Pro Ile Glu Lys
    1115   1120   1125

His Gly Ala Asn Thr Val Pro Ile Lys Asp Tyr Glu Asn Lys Asn
    1130   1135   1140

Ser Lys Met Ser Lys Ile Arg Thr His Asn Ser Glu Val Glu Glu
    1145   1150   1155

Asp Asp Met Asp Lys His Gln Gln Lys Ala Arg Phe Ala Lys Gln
    1160   1165   1170

Pro Ala Tyr Thr Leu Val Asp Arg Glu Glu Lys Pro Pro Asn Gly
    1175   1180   1185

Thr Pro Thr Lys His Pro Asn Trp Thr Asn Lys Gln Asp Asn Arg
    1190   1195   1200

Asp Leu Glu Ser Ala Gln Ser Leu Asn Arg Met Glu Tyr Ile Val
    1205   1210   1215

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<211> 1218
<212> PRT
<213> Mus sp.

<400> 79
Met Arg Ser Pro Arg Thr Arg Gly Arg Pro Gly Arg Pro Leu Ser Leu
1   5   10   15

Leu Leu Ala Leu Leu Cys Ala Leu Arg Ala Lys Val Cys Gly Ala Ser
            20   25   30

Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly Glu
            35   40   45

Leu Gln Asn Gly Asn Cys Cys Gly Gly Val Arg Asn Pro Gly Asp Arg
        50   55   60

Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu Lys
65   70   75   80

Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly Ser
                85   90   95

Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala Ser
            100   105   110

Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala Trp
        115   120   125

Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn Asp
        130   135   140

Thr Ile Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly Met
145   150   155   160

Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly Ile
                165   170   175

Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp His Tyr Tyr
            180   185   190

Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe Gly
            195   200   205

His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly Trp
        210   215   220

Met Gly Pro Asp Cys Asn Lys Ala Ile Cys Arg Gln Gly Cys Ser Pro
225   230   235   240

Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr Gly
                245   250   255

Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys Val
            260   265   270

His Gly Thr Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn Trp
        275   280   285

Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His Gln
    290   295   300

Pro Cys Leu Asn Arg Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys Tyr
305   310   315   320

Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile Ala
            325   330   335

Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys Lys
            340   345   350

Glu Thr Ser Ser Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr Gly
        355   360   365

Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn Asn Cys Ser
    370   375   380

His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys Cys Val Cys
385   390   395   400

Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala Asn Glu Cys
            405   410   415

Glu Ala Lys Pro Cys Val Asn Ala Arg Ser Cys Lys Asn Leu Ile Ala
            420   425   430

Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln Asn Cys Asp
        435   440   445

Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp Ala Ser Cys
    450   455   460

Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro Gly Tyr Ala
465   470   475   480

Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser Asn Pro Cys
            485   490   495

Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe Gln Cys Leu
            500   505   510

Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp Ile Asp Tyr
    515   520   525

Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr Asn Arg Ala
    530   535   540

Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly Lys Asn Cys
545   550   555   560

Ser His Leu Lys Asp His Cys Arg Thr Thr Thr Cys Glu Val Ile Asp
            565   570   575

Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu Gly Val Arg
            580   585   590

Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser Gln
        595   600   605

Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly Thr
    610   615   620

Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Lys Asn
625   630   635   640

Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys Ile Cys Ser
            645   650   655

Asp Gly Trp Glu Gly Ala His Cys Glu Asn Asn Ile Asn Asp Cys Ser
            660   665   670

Gln Asn Pro Cys His Tyr Gly Gly Thr Cys Arg Asp Leu Val Asn Asp
    675   680   685

Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr Cys His Ser
    690   695   700

Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly Gly Thr Cys
705   710   715   720

Tyr Asp Glu Val Asp Thr Phe Lys Cys Met Cys Pro Gly Gly Trp Glu
            725   730   735

Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu Pro Asn Pro
            740   745   750

Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Asp Ser Phe Thr Cys
    755   760   765

Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Thr Gln Asn Thr Asn
770   775   780

Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys Val Asp Gly
785   790   795   800

Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro Asp
                805   810   815

Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys Ala Phe Gly
            820   825   830

Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Gln Cys Ile Cys Pro Pro
        835   840   845

Gly His Ser Gly Ala Lys Cys His Glu Val Ser Gly Arg Ser Cys Ile
    850   855   860

Thr Met Gly Arg Val Ile Leu Asp Gly Ala Lys Trp Asp Asp Asp Cys
865   870   875   880

Asn Thr Cys Gln Cys Leu Asn Gly Arg Val Ala Cys Ser Lys Val Trp
            885   890   895

Cys Gly Pro Arg Pro Cys Arg Leu His Lys Ser His Asn Glu Cys Pro
            900   905   910

Ser Gly Gln Ser Cys Ile Pro Val Leu Asp Asp Gln Cys Phe Val Arg
            915   920   925

Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu Gln Pro Val
    930   935   940

Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn Cys Ala Asn
945   950   955   960

Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly Leu Thr Thr
            965   970   975

Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu Lys Asn Val
            980   985   990

Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser Leu Ser Ala
    995   1000   1005

Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile Arg Asp
    1010   1015   1020

Asp Gly Asn Pro Val Lys Glu Ile Thr Asp Lys Ile Ile Asp Leu
    1025   1030   1035

Val Ser Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Ala
    1040   1045   1050

Glu Val Arg Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp Phe
    1055   1060   1065

```
Leu Val  Pro Leu Leu Ser Ser  Val Leu Thr Val Ala  Trp Val Cys
    1070                 1075                 1080


Cys Leu  Val Thr Ala Phe Tyr  Trp Cys Val Arg Lys  Arg Arg Lys
    1085                 1090                 1095


Pro Ser  Ser His Thr His Ser  Ala Pro Glu Asp Asn  Thr Thr Asn
    1100                 1105                 1110


Asn Val  Arg Glu Gln Leu Asn  Gln Ile Lys Asn Pro  Ile Glu Lys
    1115                 1120                 1125


His Gly  Ala Asn Thr Val Pro  Ile Lys Asp Tyr Glu  Asn Lys Asn
    1130                 1135                 1140


Ser Lys  Met Ser Lys Ile Arg  Thr His Asn Ser Glu  Val Glu Glu
    1145                 1150                 1155


Asp Asp  Met Asp Lys His Gln  Gln Lys Val Arg Phe  Ala Lys Gln
    1160                 1165                 1170


Pro Val  Tyr Thr Leu Val Asp  Arg Glu Glu Lys Ala  Pro Ser Gly
    1175                 1180                 1185


Thr Pro  Thr Lys His Pro Asn  Trp Thr Asn Lys Gln  Asp Asn Arg
    1190                 1195                 1200


Asp Leu  Glu Ser Ala Gln Ser  Leu Asn Arg Met Glu  Tyr Ile Val
    1205                 1210                 1215


<210> 80
<211> 365
<212> PRT
<213> Mus sp.

<400> 80
Ala Asp Leu Gly Ser Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn
1               5                   10                  15


Val Asn Gly Glu Leu Gln Asn Gly Asn Cys Cys Gly Gly Val Arg Asn
                20                  25                  30


Pro Gly Asp Arg Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys
            35                  40                  45


Val Cys Leu Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys
    50                  55                  60


Ser Phe Gly Ser Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn
65                  70                  75                  80


Leu Lys Ala Ser Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe
```

Ser Phe Ala Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp
            100   105   110

Ser Ser Asn Asp Thr Ile Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser
            115   120   125

His Ser Gly Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln
    130   135   140

Asn Thr Gly Ile Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp
145   150   155   160

Asp His Tyr Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp
            165   170   175

Asp Phe Phe Gly His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys
            180   185   190

Met Glu Gly Trp Met Gly Pro Asp Cys Asn Lys Ala Ile Cys Arg Gln
            195   200   205

Gly Cys Ser Pro Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg
    210   215   220

Cys Gln Tyr Gly Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His
225   230   235   240

Pro Gly Cys Val His Gly Thr Cys Asn Glu Pro Trp Gln Cys Leu Cys
            245   250   255

Glu Thr Asn Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys
            260   265   270

Gly Thr His Gln Pro Cys Leu Asn Arg Gly Thr Cys Ser Asn Thr Gly
    275   280   285

Pro Asp Lys Tyr Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn
    290   295   300

Cys Glu Ile Ala Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg
305   310   315   320

Gly Ser Cys Lys Glu Thr Ser Ser Gly Phe Glu Cys Glu Cys Ser Pro
            325   330   335

Gly Trp Thr Gly Pro Thr Cys Ser Thr Asn Ile Asp Asp Glu Phe Gly
            340   345   350

Leu Val Pro Arg Gly Ser Gly His His His His His His

<210> 81
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 81
Gly Phe Thr Phe Ser Asn Tyr Gly Ile His
1   5   10

<210> 82
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 82
Trp Ile Thr Pro Asp Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val Lys
1   5   10   15

Gly

<210> 83
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 83
Trp Ile Thr Gly Asn Gly Gly Tyr Ser Asp Tyr Ala Asp Ser Val Lys
1   5   10   15

Gly

<210> 84
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<220>

<221> VARIANT
<222> (4)..(4)
<223> /replace="Gly"

<220>
<221> VARIANT
<222> (5)..(5)
<223> /replace="Asn"

<220>
<221> VARIANT
<222> (9)..(9)
<223> /replace="Ser"

<220>
<221> misc_feature
<222> (1)..(17)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 84
Trp Ile Thr Pro Asp Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val Lys
1   5   10   15

Gly

<210> 85
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 85
Ala Gly Ser Trp Phe Ala Tyr
1   5

<210> 86
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 86
Ala Gly Ser Leu Phe Ala Tyr
1   5

<210> 87
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

peptide"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Leu"

<220>
<221> misc_feature
<222> (1)..(7)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 87
Ala Gly Ser Trp Phe Ala Tyr
1   5

<210> 88
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 88
Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1   5   10

<210> 89
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 89
Ser Ala Ser Phe Leu Tyr Ser
1   5

<210> 90
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 90
Gln Gln Ser Tyr Thr Thr Pro Pro Thr
1   5

<210> 91
<211> 9
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        peptide"

<400> 91
Gln Gln Tyr Tyr Thr Thr Ala Thr Thr
1   5

<210> 92
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        peptide"

<220>
<221> VARIANT
<222> (3)..(3)
<223> /replace="Tyr"

<220>
<221> VARIANT
<222> (7)..(7)
<223> /replace="Ala"

<220>
<221> VARIANT
<222> (8)..(8)
<223> /replace="Thr"

<220>
<221> misc_feature
<222> (1)..(9)
<223> /note="Variant residues given in the sequence have no
        preference with respect to those in the annotations
        for variant positions"

<400> 92
Gln Gln Ser Tyr Thr Thr Pro Pro Thr
1   5

<210> 93
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        polypeptide"

<400> 93
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20   25   30

Gly Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Gly Trp Ile Thr Pro Asp Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Ala Gly Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
            100   105   110

Thr Val Ser Ser
        115

<210> 94
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 94
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20   25   30

Gly Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Gly Trp Ile Thr Pro Asp Gly Gly Tyr Thr Asp Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Ala Gly Ser Leu Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
            100   105   110

Thr Val Ser Ser
        115

```
<210> 95
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 95
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30


Gly Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Gly Trp Ile Thr Gly Asn Gly Gly Tyr Ser Asp Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Ala Gly Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110


Thr Val Ser Ser
        115


<210> 96
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 96
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30


Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
```

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
                100   105

<210> 97
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        polypeptide"

<400> 97
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
                20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Ala Thr
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
                100   105

<210> 98
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
        polypeptide"

<400> 98
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30


Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro
            85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

```
<210> 99
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 99
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly
1               5                   10
```

```
<210> 100
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 100
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asn Ile Lys Arg Phe
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

```
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Arg Ser Pro His
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 101
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 101
```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Tyr Thr Thr Pro Ser
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 102
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 102
```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
            20                  25                  30
```

```
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
            35                  40                  45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Asn Ser Tyr Pro Phe
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
                100                 105
```

```
<210> 103
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 103
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
                20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
            35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
                100                 105
```

```
<210> 104
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"
```

<400> 104
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20   25   30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35   40   45

Ser Tyr Ile Tyr Pro Tyr Ser Gly Ala Thr Tyr Tyr Ala Asp Ser Val
        50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg His Ser Gly Tyr Tyr Arg Ile Ser Ser Ala Met Asp Val Trp
            100   105   110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            115   120

<210> 105
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 105
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20   25   30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35   40   45

Ala Arg Ile Asn Pro Pro Asn Arg Ser Asn Gln Tyr Ala Asp Ser Val
        50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Gly Ser Gly Phe Arg Trp Val Met Asp Tyr Trp Gly Gln Gly
            100   105   110

Thr Leu Val Thr Val Ser Ser
        115

<210> 106
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 106
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20   25   30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35   40   45

Gly Ala Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Gln Tyr Tyr Arg Asp Met Asp Val Trp Gly Gln Gly Thr Leu
            100   105   110

Val Thr Val Ser Ser
        115

<210> 107
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 107
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr

                20   25   30

Gly Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Gly Trp Ile Thr Gly Asn Gly Gly Tyr Ser Asp Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Ala Gly Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
            100   105   110

Thr Val Ser Ser
        115