ANTI-JAGGED1 ANTIBODIES AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to anti-Jagged antibodies and methods of using the same.

BACKGROUND

The Notch signaling pathway regulates a diverse array of cell functions (Kopan et al., *Cell* 137, 216–233 (2009)). Four Notch receptors have been identified in mammals, i.e., Notch 1-4, that share basic structural elements that include an extracellular domain, a transmembrane domain, and an intracellular domain. Similarly, the canonical ligands of Notch share certain structural similarities but a number of non-canonical ligands of Notch have also been identified (Kopan et al., *Cell* 137, 216–233 (2009)). The five canonical ligands in mammals are Delta-like 1, Delta-like 3, Delta-like 4, Jagged1 and Jagged2. Binding of a Notch ligand to the extracellular domain of a Notch receptor sets a signaling cascade in motion that begins with proteolytic cleavage at the extracellular S2 site by an alpha secretase of the ADAM (a disintegrin and metalloprotease) family. Cleavage at S2 is followed by proteolytic cleavage by a gamma secretase at the intracellular S3 site, which results in release of the intracellular domain and downstream events that ultimately activate Notch-dependent transcription factors such as Hes1 and Hey.

Aberrant Notch expression and signaling has been implicated in a number of diseases, including cancer (Koch et al., *Cell. Mol. Life Sci.* 64, 2746–2762 (2007)). It is clear that there continues to be a need for agents that have clinical attributes that are optimal for development as therapeutic agents. The invention described herein meets this need and provides other benefits.

SUMMARY

The disclosure provides anti-Jagged1 antibodies and methods of using the same.

The present inventors unexpectedly found that anti-Jagged1 antibody A-1 (*see* Figure 4 and PCT Publication No. 2014/028446) is cleaved in the heavy chain following heat treatment and/or freeze-thaw conditions. The poor stability of the antibody potentially reduces its value as a therapeutic. Analysis of the cleavage site revealed no known protease cleavage motifs. It was therefore not known whether changes to the antibody sequence could reduce the observed

cleavage. Further, because the cleavage site is in a heavy chain HVR, even if amino acid change(s) would reduce cleavage, it was not known whether such changes could be made without significantly reducing the antibody's affinity for Jagged1. The inventors found, surprisingly, that mutation of the amino acid at position 101 in HVR-H3 reduced cleavage with only a slight loss of affinity.

Disclosed is an isolated antibody that binds to human Jagged1 is provided, wherein the antibody comprises an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S. In one aspect, there is provided an isolated antibody that binds to human Jagged1, wherein the antibody comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S or H; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In some embodiments, the antibody comprises at least one, at least two, at least three, at least four, or five HVRs selected from HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In some embodiments, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55; or (b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59; or (c) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; and HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59. In any of the embodiments described herein, the antibody may comprise (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40; or (b) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; or (c) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

In some embodiments, an isolated antibody that binds to Jagged1 comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40; or (b) HVR-H1 comprising the amino acid sequence of SEQ

ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; or (c) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

In some embodiments, an isolated antibody that binds to Jagged1 comprises a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 54, 58, or 62. In some embodiments, an isolated antibody that binds to Jagged1 comprises a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 10, 26, or 34. In some embodiments, the antibody comprises (a) a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 54 and a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 34; or (b) a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 58 and a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 62 and a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 26.

In one aspect, an isolated antibody that binds to human Jagged1 is provided, comprising (a) a VH sequence of SEQ ID NO: 54, wherein X is any amino acid other than S or H, and a VL sequence of SEQ ID NO: 34; or (b) a VH sequence of SEQ ID NO: 58, wherein X is any amino acid other than S or H, and a VL sequence of SEQ ID NO: 10; or (c) a VH sequence of SEQ ID NO: 62, wherein X is any amino acid other than S or H, and a VL sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 54 and a VL sequence of SEQ ID NO: 34. In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 56 and the light chain comprises the amino acid sequence of SEQ ID NO: 53. In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 57 and the light chain comprises the amino acid sequence of SEQ ID NO: 53.

In any of the embodiments described herein, X may be any amino acid other than S or H. In any of the embodiments described herein, X may be selected from A, D, E, G, I, K, L, N, Q, R T, and V. In any of the embodiments described herein, X may be T.

In one aspect, an isolated antibody that binds human Jagged1 is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40; or (b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; or (c) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

In some embodiments, an isolated antibody that binds human Jagged1 is provided, comprising (a) a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 34; or (b) a VH sequence of SEQ ID NO: 65 and a VL sequence of SEQ ID NO: 10; or (c) a VH sequence of SEQ ID NO: 66 and a VL sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 34.

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

Any of the above embodiments may be a full-length IgG1 antibody. In some embodiments, the antibody is an IgG1 antibody lacking effector function. In some embodiments, the antibody is an IgG1 antibody comprising an N297G or N297A mutation. In some embodiments, the antibody is an IgG1 antibody comprising an N297G mutation.

In some embodiments, an isolated antibody that binds to Jagged1 is provided, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 51 and the light chain comprises the amino acid sequence of SEQ ID NO: 53. In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 52 and the light chain comprises the amino acid sequence of SEQ ID NO: 53. In some embodiments, the antibody comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 75; or (b) a heavy chain comprising the amino acid

sequence of SEQ ID NO: 70 and a light chain comprising the amino acid sequence of SEQ ID NO: 76; or (c) a heavy chain comprising the amino acid sequence of SEQ ID NO: 79 and a light chain comprising the amino acid sequence of SEQ ID NO: 75; or (d) a heavy chain comprising the amino acid sequence of SEQ ID NO: 80 and a light chain comprising the amino acid sequence of SEQ ID NO: 76.

In any of the embodiments described herein, the antibody may be an antagonist of Jagged1-mediated signaling. In some embodiments, the antibody binds human and murine Jagged1. In some embodiments, the antibody binds human, murine, rat, and cynomolgus monkey Jagged1. In some embodiments, the antibody binds Jagged1 but does not bind Jagged2. In some embodiments, the antibody binds human Jagged1 does not bind human Jagged2. In some embodiments, the antibody binds human and murine Jagged1 but does not bind human or murine Jagged2. In some embodiments, the antibody binds human, murine, rat, and cynomolgus monkey Jagged1 but does not bind human, cynomolgus monkey, or murine Jagged2. In some embodiments, the antibody binds Jagged1 but does not bind Jagged2 or DLL1. In some embodiments, the antibody binds human Jagged1 but does not bind human Jagged2 or human DLL1. In some embodiments, the antibody the antibody binds human and murine Jagged1 but does not bind human or murine Jagged2 or human or murine DLL1. In some embodiments, the antibody the antibody binds human, murine, and cynomolgus monkey Jagged1 but does not bind human, murine, or cynomolgus monkey Jagged2 or human, murine, or cynomolgus monkey DLL1. In some embodiments, the antibody binds Jagged1 but does not bind Jagged2, DLL1, or DLL4. In some embodiments, the antibody binds human Jagged1 but does not bind human Jagged2, human DLL1, or human DLL4. In some embodiments, the antibody binds human and murine Jagged1 but does not bind human or murine Jagged2, human or murine DLL1, or human or murine DLL4. In some embodiments, the antibody binds human, murine, rat, and cynomolgus monkey Jagged1 but does not bind human, murine, or cynomolgus monkey Jagged2, human, murine, or cynomolgus monkey DLL1, or human, murine, or cynomolgus monkey DLL4.

In some embodiments, the antibody binds human Jagged1 with an affinity (Kd) of 2 nM or stronger (i.e., less than 2 nM). In some embodiments, the antibody binds human Jagged1 with an affinity (Kd) of 1.5 nM or stronger, or 1 nM or stronger, or 0.9 nM or stronger, 0.8 nM or stronger, or 0.7 nM or stronger (i.e., less than 1.5 nM, less than 1 nM, less than 0.9 nM, less than 0.8 nM, or less than 0.7 nM). In some embodiments, the antibody binds murine Jagged1 with an affinity (Kd) of 2 nM or stronger (i.e., less than 2 nM). In some embodiments, the antibody binds murine Jagged1 with an affinity (Kd) of 1.5 nM or stronger, or 1 nM or

stronger, or 0.9 nM or stronger, 0.8 nM or stronger, 0.7 nM or stronger, or 0.6 nM or stronger, or 0.5 nM or stronger (i.e., less than 1.5 nM, less than 1 nM, less than 0.9 nM, less than 0.8 nM, less than 0.7 nM, less than 0.6 nM, or less than 0.5 nM). In some embodiments, affinity (Kd) is measured using surface plasmon resonance.

In some embodiments, the antibody binds human Jagged1 with an association constant (k_{on}) of at least 1.0E+04/Ms, or at least 1.5E+04/Ms, or at least 2.0E+04/Ms. In some embodiments, the antibody binds human Jagged1 with a dissociation constant (k_{off}) of less than 10.0E-04/s, or less than 9.0E-04/s, or less than 8.0E-04/s, or less than 7.0E-04/s. In some embodiments, the antibody binds murine Jagged1 with an association constant (k_{on}) of at least 1.0E+04/Ms, or at least 1.5E+04/Ms, or at least 2.0E+04/Ms, or at least 3.0E+04/Ms, or at least 4.0E+04/Ms, or at least 5.0E+04/Ms, or at least 6.0E+04/Ms, or at least 7.0E+04/Ms. In some embodiments, the antibody binds murine Jagged1 with a dissociation constant (k_{off}) of less than 10.0E-04/s, or less than 9.0E-04/s, or less than 8.0E-04/s, or less than 7.0E-04/s. In some embodiments, association and dissociation constants are measured using surface plasmon resonance.

In some embodiments, the antibody binds native, folded Jagged1, but does not bind denatured Jagged1. In some embodiments, the antibody binds Jagged1 in an enzyme-linked immunosorbent assay (ELISA) but does not bind Jagged1 on a Western blot. In some embodiments, the antibody binds folded Jagged1 in an enzyme-linked immunosorbent assay (ELISA) but does not bind denatured Jagged1 on a Western blot. In some embodiments, the antibody binds folded Jagged1 under physiological conditions but does not bind denatured Jagged1.

In some embodiments, the antibody reduces tumor growth in a mouse xenograft model without causing weight loss. In some embodiments, the mouse xenograft model is a liver cancer xenograft model. In some embodiments, tumor growth is reduced by at least 50, at least 60, at least 70, at least 80, or at least 90 AUC/day TGI%.

In some embodiments, use of the Jagged1 antibody reduces goblet cell metaplasia in lungs in a mouse model of airway hyperresponsiveness. In some embodiments, administration of the antibody reduces the number of goblet cells in the lungs by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%.In another embodiment, the disclosure provides an isolated antibody that competes with any of the above embodiments for specific binding to Jagged1. In some embodiments, an antibody is that competes for binding to human Jagged1 with an antibody comprising a heavy chain variable region comprising the

sequence of SEQ ID NO: 33 and a light chain variable region comprising the sequence of SEQ ID NO: 34, wherein the antibody is not antibody A, antibody A-1, or antibody A-2.

In another aspect, an isolated nucleic acid encoding an isolated antibody described above is provided. In a further aspect, a host cell comprising the isolated nucleic acid described above is provided. In a further aspect, a method of producing an antibody is provided, comprising culturing the host cell described above so that the antibody is produced.

In another aspect, an immunoconjugate is provided, comprising any of the antibodies described above and a cytotoxic agent.

In another aspect, a pharmaceutical formulation is provided, comprising any of the antibodies described above and a pharmaceutically acceptable carrier. In another aspect, a pharmaceutical formulation is provided, comprising an immunoconjugate described above and a pharmaceutically acceptable carrier.

In another aspect, any of the antibodies or immunoconjugates described above is provided for use as a medicament. In another aspect, any of the antibodies or immunoconjugates described above is provided for use in treating a cancer. In another aspect, any of the antibodies or immunoconjugates described above is provided for use in reducing cancer cell growth.

In one embodiment, a method of inhibiting Jagged1-mediated signaling is provided. In one embodiment, a method of inhibiting Jagged1-mediated signaling *in vitro* is provided. In one embodiment, a method of inhibiting Jagged1-mediated signaling *in vivo* is provided.

Disclosed is a use of any of the antibodies or immunoconjugates described above in the manufacture of a medicament. In another aspect, there is provided a use of the antibody described above or the immunoconjugate described above in the manufacture of a medicament for treating cancer. In another aspect, there is provided a use of the antibody described above or the immunoconjugate described above in the manufacture of a medicament for treating diseases associated with goblet cell metaplasia (e.g., in lung) and/or excess mucus. In one embodiment, a method of treating an individual having a cancer comprising administering to the individual an effective amount of an antibody of any of the above embodiments is provided. In one embodiment, the cancer is selected from the group consisting of: breast cancer, lung cancer, brain cancer, cervical cancer, colon cancer, liver cancer, bile duct cancer, pancreatic cancer, skin cancer, B-cell malignancies, and T-cell malignancies.

The present inventors discovered that treatment with Jagged1-antibody biases cell fate in the airways away from a secretory cell (including goblet cell) fate and toward a ciliated cell fate. Jagged1 signaling is important for maintaining the secretory cell fate, and inhibition of Jagged1 signaling prevented goblet cell metaplasia. The present inventors also showed that the club cell-to-ciliated cell conversion is direct and did not involve cell division (data not shown). This transdifferentiation of one cell type to another occurred in the adult lung and is distinct from cell fate choices that involve progenitor cell division, such as after damage or during development. Goblet cell metaplasia or excess mucus is a hallmark of several airway diseases, such as asthma, cystic fibrosis, COPD and Barrett's esophagus. These Jagged inhibition results provide the basis for therapeutic applications involving use of Jagged1 or Jagged2 inhibitors for prevention or reversal of goblet cell metaplasia and for treatment of conditions

characterized by excess mucus, as in airway diseases (e.g., asthma, COPD, cystic fibrosis) and Barrett's esophagus.

In some embodiments, provided are methods of converting a club cell to a ciliated cell, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, provided are methods of increasing conversion of club cells into ciliated cells, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, the club cell is present in the adult human airway (e.g., lung). In some embodiments, the conversion occurs in the absence of club cell division. In some embodiments, the individual has a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus. In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, provided are methods of decreasing the number of goblet cells, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, provided are methods of reducing conversion of club cells into goblet cells, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, the goblet cell(s) is present in the adult human airway (e.g., lung). In some embodiments, the individual has a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus. In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, provided are methods of reducing the formation of goblet cells in a subject, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, formation of goblet cells in the adult human airway (e.g., lung) is reduced. In some embodiments, the individual has a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus. In some embodiments, the disease is associated with

goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, provided are methods of decreasing mucus, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, the mucus is airway mucus. In some embodiments, the mucus is present in adult human airway (e.g., lung). In some embodiments, the individual has a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus. In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, provided are methods of increasing ciliated cell number, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, provided are methods of increasing formation of ciliated cells, comprising administering an antagonist antibody that binds to human Jagged1 (including, but not limited to, any of the anti-Jagged1 antibodies described herein) to an individual. In some embodiments, the ciliated cell is present in the adult human airway (e.g., lung). In some embodiments, the individual has a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus. In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein. In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell

metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein, wherein the antibody that binds to human Jagged1 increases conversion of club cells to ciliated cells in the adult human airway (e.g., lung). In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein, wherein the antibody that binds to human Jagged1 decreases the number of goblet cells in the adult human airway (e.g., lung). In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein, wherein the antibody that binds to human Jagged1 reduces the formation of goblet cells in the adult human airway (e.g., lung). In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein, wherein the antibody that binds to human Jagged1 decreases mucus in the adult human airway (e.g., lung). In some embodiments, the disease is associated

with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein, wherein the antibody that binds to human Jagged1 increases ciliated cell number in the adult human airway (e.g., lung). In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In some embodiments, an antibody that binds to human Jagged1 (including, for example, any of the anti-Jagged1 antibodies described herein) is provided for use in treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of an antibody of any of the embodiments described herein, wherein the antibody that binds to human Jagged1 increases formation of ciliated cells in the adult human airway (e.g., lung). In some embodiments, the disease is associated with goblet cell metaplasia. In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

Also provided are methods of (a) converting a ciliated cell to a club cell (e.g., wherein the ciliated cell is found in the adult human airway, e.g., lung), (b) increasing mucus (e.g., airway mucus), (c) decreasing ciliated cell number (e.g., human airway ciliated cell), comprising administering an agonist of Jagged signaling to an individual.

In some embodiments, a method of treating a disease selected from allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and diseases associated with excess mucus is provided, comprising administering to an individual with cancer an effective amount of antibody that binds to human Jagged1 (including, for example, any of the antibodies that bind to human Jagged1 described herein). In some embodiments, the disease is associated with goblet cell metaplasia (e.g., in the lung). In some embodiments, the disease is selected from asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and Barrett's esophagus.

In any of the embodiments described herein, wherein the Jagged1 antibody reduces tumor growth in a mouse xenograft model without causing weight loss.

In one aspect, an anti-Jagged1 antibody as described above conjugated to a label is provided. In some embodiments, the label is a positron emitter. In some embodiments, the positron emitter is ^{89}Zr. In one aspect, a method of detecting human Jagged1 in a biological sample is provided, comprising contacting the biological sample with an antibody described herein under conditions permissive for binding of the antibody to a naturally occurring human Jagged1, and detecting whether a complex is formed between the antibody and a naturally occurring human Jagged1 in the biological sample. In some embodiments, the biological sample is selected from breast cancer, lung cancer, brain cancer, cervical cancer, colon cancer, liver cancer, bile duct cancer, pancreatic cancer, skin cancer, B-cell malignancies, and T-cell malignancies.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows exemplary amino acid sequences of human and murine Jagged1 protein.

FIG. 2 shows exemplary amino acid sequences of human and murine Jagged2 protein.

FIGS. 3A-D 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. (A) 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: 82). The bold font at the C-terminus represents a short linker sequence (EFG), a thrombin cleavage site (LVPRGS) (SEQ ID NO: 83), a G spacer and the 6-His tag. (B) 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 at the C-terminus. (C) Amino acid sequence of expressed protein murine Jag2-DSL-EGF1-4 (M27-E388). The bold font at the N-terminus represents a short linker sequence (ADLGS) (SEQ ID NO: 82). The bold font at the C-terminus represents a short linker sequence (EFG), a thrombin cleavage site (LVPRGS) (SEQ ID NO: 83), a G spacer and the 6-His tag. (D) Amino acid sequence of expressed protein human Jag2-DSL-EGF1-4 (R2-E388). The bold font at the C-terminus represents a short linker sequence (EFG), a thrombin cleavage site (LVPRGS) (SEQ ID NO: 83), a G spacer and the 6-His tag.

FIGS. 4A-1-B-2 show an alignment of the amino acid sequences for the heavy (FIG. 4A-1 and FIG. 4A-2) and light (FIG. 4B-1 and FIG. 4B-2) chain variable domains of anti-

Jagged1 (A, A-1, A-2), anti-Jagged2 (B, B-1, B-2, B-3), and anti-Jagged1/2 antibodies (C, C-1, D, D-1, D-2, D-3, D-4, D-5). Amino acid positions of the complementarity determining regions (CDRs) are indicated.

FIGS. 5A-B show the H1, H2, and H3 heavy chain hypervariable region (HVR) sequences of anti-Jagged1 antibodies, as described in the Examples. Amino acid positions are numbered according to the Kabat numbering system as described herein and elsewhere.

FIG. 6 shows the L1, L2, and L3 light chain HVR sequences of anti-Jagged1 antibodies described in the Examples. Amino acid positions are numbered according to the Kabat numbering system as described below.

FIG. 7 shows light and heavy chain framework sequences of anti-Jagged1 antibodies described in the Examples. Numbers in superscript indicate amino acid positions according to Kabat.

FIGS. 8 shows binding specificity of antibodies obtained from the screening. Results of ELISA assays measuring binding specificity of Antibodies A and B, identified during screening using human Jag1-DSL-EGF1-4 (FIG. 3B) for antibody A and murine and human Jag2-DSL-EGF1-4 (FIG. 3C and D) for antibody B. Black columns = binding to human Jagged1; gray columns = binding to human Jagged2. C-1 is an antibody that binds to both Jagged1 and Jagged2.

FIGS. 9A-B show inhibition of Notch signaling by affinity matured anti-Jagged antibodies. Co-culture assays were performed as described in Example 3. Phage antibodies at the indicated concentration are indicated on the x-axis. DAPT at the indicated concentrations served as positive control for inhibition of Notch signaling; DMSO served as vehicle control. Signaling was induced by Jagged1 (dark gray columns) or by Jagged2 (light gray columns). Untreated = cultures that were not stimulated with ligand and not treated with antibody; No Stimulation = cultures not stimulated with ligand; agD = isotype control antibody; Stim/no AB = cultures stimulated with ligand but not treated with antibody; gamma-secretase inhibitor DAPT or the DAPT vehicle control of DMSO.

FIGS. 10A-B show that combined inhibition of Jagged1 and Jagged2 causes rapid weight loss. (A) Mice were dosed twice per week with the anti-Jagged1/2 antibody C-1 (anti-J1/2; 5-10 mpk), the anti-Jagged1 antibody A-2 (anti-J1; 5-20mpk), the anti-Jagged2 antibody B-3 (anti-J2; 5-20mpk), the antibody A-2 and B-3 together (anti-J1 & -2; 5mpk each) or an isotype control antibody (20mpk). Total antibody concentration of each dosing was brought up to 20mpk with the isotype control antibody, where necessary. The average body weight changes (y-axis) are graphed as a percentage of starting body weight over time (x-axis). (B)

Balb/c mice (ten per group, individually housed) were injected IP twice per week with either 30 mpk of anti-gD isotype control antibody or with a combination of 15 mpk antibody A-2 plus 15 mpk antibody B-3 for eight days. Food intake was assessed by daily weighing of the food delivered and remaining in each cage. Error bars represent standard deviations (n = 10).

FIGS. 11A-1-B-2 show inhibition of human lung cancer cell growth by an anti-Jagged1 antagonist antibody *in vivo*. Mice bearing human lung cancer xenografts were injected twice per week intraperitoneally (IP) with 20mpk anti-gD isotype control antibody (Isotype control Ab) or with anti-Jagged1 antibody A-2 (Anti-Jag1), with the injections starting after average tumor volumes (measured with calipers) reached approximately 180 mm^3. Tumor volumes (y-axis) were subsequently measured for 19 days. FIG. 11A-1 and FIG. 11A-2: The average tumor volumes for each group (n=10) were plotted over time (x-axis) using a linear mixed effects model (FIG. 11A-1). Tumor volumes for each mouse in each group are depicted in the two panels in FIG. 11A-2. FIG. 11B-1 and FIG. 11B-2: Total body weight of each mouse was measured and graphed as the percentage change averaged for each group (FIG. 11B-1) or for each mouse in each group (FIG. 11B-2).

FIGS. 12A-B show inhibition of human breast cancer cell growth by anti-Jagged1 and anti-Jagged2 antagonist antibodies *in vivo*. C.B-17 SCID.bg mice with human breast cancer xenografts were injected on days 0, 4, 7, 12, 15, 18, 22, 25, 29, 32, 36, 43, 50, and 57 with anti-gD isotype control antibody (Anti-gD), anti-ragweed isotype control antibody (anti-ragweed), anti-Jagged1 antibody A-2 in the human IgG1 backbone (anti-Jag1 A-2 (hIgG1)), anti-Jagged1 antibody A-2 in the murine IgG2a backbone (anti-Jag1 A-2 (mIgG2a)), or anti-Jagged2 antibody B-3 in the human IgG1 backbone (anti-Jag2 B-3 (hIgG1)). Tumor volumes (y-axis) of treatment groups (A) or individual animals (B) were plotted using a linear mixed effects model over time (x-axis).

FIGS. 13A-C show cleavage of the anti-Jagged1 antibodies in the heavy chain. (A) Antibodies A, A-1 and A-2 were analyzed by standard SDS-PAGE and protein staining methods in the absence (-) or presence (+) of the reducing agent DTT, as indicated. Molecular mass standards (in kD) are also shown. Panel (B) displays a representative scan of a mass spectrometry (MS) analysis (LC-MS/TOF, reducing conditions) of A-1. The positions of the relevant fragments (peaks) are annotated, and the molecular masses are shown above the peaks. This analysis indicated that the cleavage site was between HC amino acids G100 and S101 in CDR3, as diagramed in the HC amino acid sequence in panel (C), with the arrow marking the cleavage position.

FIGS. 14A-C shows N-terminal peptide sequencing of the anti-Jagged1 antibody heavy chain cleavage site. Following the standard methods for SDS-PAGE under reducing conditions and protein staining, the full-length and cleaved fragments of the indicated antibody preparations were separated and identified (A). The C-terminal (B) and N-terminal (C) cleaved peptide fragments were cut out of the gel and sequenced using standard methods for peptide N-terminal sequencing to precisely identify the site of cleavage. The sequences highlighted in red font in panels (B) and (C) note the N-terminal sequences of each of the peptides sequenced, with the sequence in panel (B) marking the cleavage site and the sequence in panel (C) marking the N-terminus of the HC.

FIG. 15 shows the effect of amino acid changes at heavy chain S101 of the anti-Jagged1 antibodies on heavy chain cleavage.

FIGS. 16A-B show (A) SDS-PAGE analysis of cleavage of anti-Jagged1 antibodies incubated at 70°C; and (B) a summary of the percent heavy chain cleavage observed for each antibody preparation at 70°C and at 95°C.

FIGS. 17A-B show (A) SDS-PAGE analysis of cleavage of anti-Jagged1 antibody A-1 after varying numbers of freeze-thaw cycles, and (B) the percent heavy chain cleavage observed under each condition.

FIGS. 18A-C show (A) inhibition of Jagged1-induced activation by anti-Jagged1 antibodies A-1 (left bars) and A-1(S101T) (right bars) at various concentrations. Panels (B) and (C) shows average firefly luciferase values and average Renilla luciferase values, respectively, which were used to calculate the data in (A), as described in Example 10.

FIG. 19 shows immunofluorescent staining of ciliated cells (as marked by immunofluorescent detection of alpha-tubulin in red) and club cells (as marked by immunofluorescent detection of CC10 in green) in the bronchiolar epithelium of mice administered anti-Jagged2 antibodies in combination with anti-Jagged1 antibody A-1 or A-1(S101T), or an isotype control, as described in Example 11.

FIGS. 20A-B show (A) a LME (linear mixed effects) graph of tumor volume in a liver cancer xenograft model mice treated with anti-Jagged1 antibody A-1 or A-1(S101T), and (B) the treatment groups shown in (A) and the dose, tumor volume on the last day of the study (day 44), AUC/Day %TGI (area under the curve per day percentage tumor growth inhibition (TGI) relative to control, in which lower and upper refer to the lowest and uppermost, respectively, %TGI values for individual animals in each group), tumor doubling time in days (TTP 2X), and the number of mice showing a partial response during the experiment (PR).

FIGS. 21A-B show (A) a LME (linear mixed effects) graph of mouse body weight over time for the mice shown in FIG. 20, and (B) the treatment groups shown in (A) and the dose, % change in body weight on the last day of the study (% BW Last Day), the maximum % change in body weight (Max %BW), and the day on which the maximum change in bodyweight occurred (Max %BW Day), and the (AUC/Day (lower,upper)).

FIGS. 22A-B show the amino acid sequences for the (A) heavy chain and (B) light chain variable domains of anti-Jagged1 antibody A-1(S101T). Amino acid positions of the complementarity determining regions (CDRs) are indicated.

FIGS. 23A-C show (A) Periodic Acid-Schiff staining of lung airways of control, anti-Jagged1, anti-Jagged2 or the combination of anti-Jagged1 + anti-Jagged2 treated mice, (B) quantification of the number of goblet cells in the airways of the different treatment groups, and (C) inflammation index as assessed be H&E staining.

FIG. 24 shows binding of (left) anti-Jagged1 antibody A-2 and (right) anti-Jagged1/2 antibody C-1, to human Jagged1, murine Jagged1, human Jagged 2, murine Jagged2, human DLL1, murine DLL1, human DLL4 and murine DLL4.

FIG. 25 shows clearance of anti-Jagged1 A-1-S101T antibody following a single intravenous administration of three different doses of the antibody in mice.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

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-Jagged antibody" and "an antibody that binds to Jagged" refer to an antibody that is capable of binding Jagged1, Jagged2, or Jagged1 and 2 (Jagged1/2) with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Jagged. In one embodiment, the extent of binding of an anti-Jagged antibody to an unrelated, non-Jagged protein is less than about 10% of the binding of the antibody to Jagged as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to Jagged has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 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-Jagged antibody binds to an epitope of Jagged that is conserved among Jagged from different species. The terms "anti-Jagged1 antibody" and "an antibody that binds to Jagged1" refer to an antibody that is capable of binding Jagged1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting Jagged1.

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.

As used herein, "asthma" refers to a complex disorder characterized by variable and recurring symptoms, reversible airflow obstruction (e.g., by bronchodilator) and bronchial hyperresponsiveness which may or may not be associated with underlying inflammation. Examples of asthma include aspirin sensitive/exacerbated asthma, atopic asthma, severe asthma, mild asthma, moderate to severe asthma, corticosteroid naïve asthma, chronic asthma, corticosteroid resistant asthma, corticosteroid refractory asthma, newly diagnosed and untreated asthma, asthma due to smoking, asthma uncontrolled on corticosteroids and other asthmas as mentioned in J Allergy Clin Immunol (2010) 126(5):926-938.

A "blocking" antibody or an "antagonist" antibody is one which significantly inhibits (either partially or completely) a biological activity of the antigen it binds.

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

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 "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.

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 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 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.

"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 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 therapeutic or prophylactic result.

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 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.

"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).

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 "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-Jagged 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 "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 "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 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.

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 Jagged 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 amino acid sequence of an exemplary human and murine Jagged1 and Jagged2 is shown in FIG. 1 and 2 (SEQ ID NOS:1-4), respectively.

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 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 the identification of anti-Jagged antibodies and fragments thereof. In certain embodiments, antibodies that bind to at least one Jagged are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment

of cancer. Accordingly, the invention provides methods, compositions, kits, and articles of manufacture related to anti-Jagged antibodies.

A. Exemplary Anti-Jagged1 Antibodies

In one aspect, the invention provides isolated antibodies that bind to Jagged1.

In some embodiments, the antibody is an antagonist of Jagged1-mediated signaling. In some embodiments, the antibody binds human and murine Jagged1. In some embodiments, the antibody binds human, murine, and cynomolgus monkey Jagged1. In some embodiments, the antibody binds Jagged1 but does not bind Jagged2. In some embodiments, the antibody binds human Jagged1 does not bind human Jagged2. In some embodiments, the antibody binds human and murine Jagged1 but does not bind human or murine Jagged2. In some embodiments, the antibody binds human, murine, and cynomolgus monkey Jagged1 but does not bind human, cynomolgus monkey, or murine Jagged2. In some embodiments, the antibody binds Jagged1 but does not bind Jagged2 or DLL1. In some embodiments, the antibody binds human Jagged1 but does not bind human Jagged2 or human DLL1. In some embodiments, the antibody the antibody binds human and murine Jagged1 but does not bind human or murine Jagged2 or human or murine DLL1. In some embodiments, the antibody the antibody binds human, murine, and cynomolgus monkey Jagged1 but does not bind human, murine, or cynomolgus monkey Jagged2 or human, murine, or cynomolgus monkey DLL1. In some embodiments, the antibody binds Jagged1 but does not bind Jagged2, DLL1, or DLL4. In some embodiments, the antibody binds human Jagged1 but does not bind human Jagged2, human DLL1, or human DLL4. In some embodiments, the antibody binds human and murine Jagged1 but does not bind human or murine Jagged2, human or murine DLL1, or human or murine DLL4. In some embodiments, the antibody binds human, murine, and cynomolgus monkey Jagged1 but does not bind human, murine, or cynomolgus monkey Jagged2, human, murine, or cynomolgus monkey DLL1, or human, murine, or cynomolgus monkey DLL4.

In some embodiments, the antibody binds human Jagged1 with an affinity (Kd) of 2 nM or stronger (i.e., less than 2 nM). In some embodiments, the antibody binds human Jagged1 with an affinity (Kd) of 1.5 nM or stronger, or 1 nM or stronger, or 0.9 nM or stronger, 0.8 nM or stronger, or 0.7 nM or stronger (i.e., less than 1.5 nM, less than 1 nM, less than 0.9 nM, less than 0.8 nM, or less than 0.7 nM). In some embodiments, the antibody binds murine Jagged1 with an affinity (Kd) of 2 nM or stronger (i.e., less than 2 nM). In some embodiments, the antibody binds murine Jagged1 with an affinity (Kd) of 1.5 nM or stronger, or 1 nM or stronger, or 0.9 nM or stronger, 0.8 nM or stronger, 0.7 nM or stronger, or 0.6 nM or stronger,

or 0.5 nM or stronger (i.e., less than 1.5 nM, less than 1 nM, less than 0.9 nM, less than 0.8 nM, less than 0.7 nM, less than 0.6 nM, or less than 0.5 nM).

In some embodiments, the antibody binds native, folded Jagged1, but does not bind denatured Jagged1. In some embodiments, the antibody binds Jagged1 in an enzyme-linked immunosorbent assay (ELISA) but does not bind Jagged1 on a Western blot. In some embodiments, the antibody binds folded Jagged1 in an enzyme-linked immunosorbent assay (ELISA) but does not bind denatured Jagged1 on a Western blot. In some embodiments, the antibody binds folded Jagged1 under physiological conditions but does not bind denatured Jagged1. "Native, folded" Jagged1 refers to Jagged1 that has undergone protein folding under physiological conditions and has been maintained in a folded state. In some embodiments, Jagged1 has been maintained in a folded state in solution.

In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55, wherein X is any amino acid other than S; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the

amino acid sequence of SEQ ID NO: 36; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 . In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T.

In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the invention provides an anti-Jagged1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S

and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55, wherein X is any amino acid other than S. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55, wherein X is any amino acid other than S. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55, wherein X is any amino acid other than S and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55, wherein X is any amino acid other than S, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55, wherein X is any amino acid other than S. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid

sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other than S, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59, wherein X is any amino acid other

than S. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 55 or 59, wherein X is any amino acid other than S; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 55, wherein X is any amino acid other than S; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-

L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 59, wherein X is any amino acid other than S; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 59, wherein X is any amino acid other than S; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 55 or 59, wherein X is any amino acid other than S; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 55, wherein X is any amino acid other than S; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In another aspect, an antibody of the invention

comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 59, wherein X is any amino acid other than S; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 59, wherein X is any amino acid other than S; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 37; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 64; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence

selected from SEQ ID NO: 64; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 37; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40. In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 64; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In another aspect, an antibody of the invention comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 64; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

In some embodiments, an anti-Jagged1 antibody comprises (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 71, wherein X1 is selected from P and G, X2 is selected from D and N, and X3 is selected from T and S, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 72, wherein X1 is any amino acid other than S, and X2 is W or L; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74, wherein X1 is S or Y, X2 is P or A, and X3 is P or T. In some embodiments, the X1 in SEQ ID NO: 72 is any amino acid other than S or H. In some embodiments, the X1 in SEQ ID NO:

72 is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, the X1 in SEQ ID NO: 72 is T.

In one embodiment, an anti-Jagged1 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-Jagged1 antibody comprises HVRs as in any of the above embodiments, and further comprises a VH comprising at least one, two, three, or four FRs selected from an FR1 comprising the amino acid sequence of SEQ ID NO: 47; an FR2 comprising the amino acid sequence of SEQ ID NO: 48; an FR3 comprising the amino acid sequence of SEQ ID NO: 49; and an FR4 comprising the amino acid sequence of SEQ ID NO: 50. In another embodiment, an anti-Jagged1 antibody comprises HVRs as in any of the above embodiments, and further comprises a VL comprising at least one, two, three, or four FRs selected from an FR1 comprising the amino acid sequence of SEQ ID NO: 43; an FR2 comprising the amino acid sequence of SEQ ID NO: 44; an FR3 comprising the amino acid sequence of SEQ ID NO: 45; and an FR4 comprising the amino acid sequence of SEQ ID NO: 46.

In another aspect, an anti-Jagged1 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 NO: 54, 58, or 62, wherein X is any amino acid other than S. In some such embodiments, the VH sequence comprises an HVR-H3 of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S. In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37 or 64. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T. In another aspect, an anti-Jagged1 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 NO: 33, 65, or 66. In some such embodiments, the VH sequence comprises an HVR-H3 of SEQ ID NO: 37 or 64. In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37 or 64. In certain embodiments, a VH 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-Jagged1 antibody comprising that sequence retains the ability to bind to at least one Jagged1. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In certain embodiments, an anti-Jagged1 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 NO: 33. In some such embodiments, the VH sequence comprises an HVR-H3 of SEQ ID NO: 37. In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the anti-Jagged1 antibody comprises the VH sequence in SEQ ID NO: 33, 65, or 66, including post-translational modifications of that sequence. In some embodiments, the anti-Jagged1 antibody comprises the VH sequence in SEQ ID NO: 33, including post-translational modifications of that sequence. In some embodiments, the anti-Jagged1 antibody comprises the VH sequence in SEQ ID NO: 65, including post-translational modifications of that sequence. In some embodiments, the anti-Jagged1 antibody comprises the VH sequence in SEQ ID NO: 66, including post-translational modifications of that sequence.

In another aspect, an anti-Jagged1 antibody 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 the amino acid sequence of SEQ ID NO: 10, 26, or 34. 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-Jagged1 antibody comprising that sequence retains the ability to bind to Jagged1. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 10, 26, or 34. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40. In some embodiments, the anti-Jagged1 antibody comprises the VL sequence in SEQ ID NO: 10, 26, or 34, including post-translational modifications of that sequence. In some embodiments, the anti-Jagged1 antibody comprises

the VL sequence in SEQ ID NO: 34, including post-translational modifications of that sequence. In some embodiments, the anti-Jagged1 antibody comprises the VL sequence in SEQ ID NO: 10, including post-translational modifications of that sequence. In some embodiments, the anti-Jagged1 antibody comprises the VL sequence in SEQ ID NO: 26, including post-translational modifications of that sequence.

In another aspect, an anti-Jagged1 antibody 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 the VH and VL sequences in SEQ ID NO: 54, wherein X is any amino acid other than S; and SEQ ID NO: 34, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 58, wherein X is any amino acid other than S; and SEQ ID NO: 10, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 62, wherein X is any amino acid other than S; and SEQ ID NO: 26, respectively, including post-translational modifications of those sequences. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T.

In another aspect, an anti-Jagged1 antibody 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 the VH and VL sequences in SEQ ID NO: 33 and SEQ ID NO: 34, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 65 and SEQ ID NO: 10, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 66 and SEQ ID NO: 26, respectively, including post-translational modifications of those sequences.

In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 57, wherein X is any amino acid other than S, and a light chain comprising the sequence of SEQ ID NO: 53. In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 67, wherein X is any amino acid other than S, and a light chain comprising the sequence of SEQ ID NO: 75. In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 68, wherein X is any amino acid other than S, and a light chain comprising the

sequence of SEQ ID NO: 76. In some embodiments, X is any amino acid other than S. In some embodiments, X is any amino acid other than S or H. In some embodiments, X is selected from A, D, E, G, I, K, L, N, Q, R T, and V. In some embodiments, X is T. In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 51, and a light chain comprising the sequence of SEQ ID NO: 53. In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 52, and a light chain comprising the sequence of SEQ ID NO: 53. In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 69, and a light chain comprising the sequence of SEQ ID NO: 75. In some embodiments, an anti-Jagged1 antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 70, and a light chain comprising the sequence of SEQ ID NO: 76.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-Jagged1 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-Jagged1 antibody comprising a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 34.

In a further aspect, the invention provides an antibody that competes for binding with any of the antibodies provided herein.

In a further aspect of the invention, an anti-Jagged1 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-Jagged1 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact human IgG1 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-Jagged1 antibody according to any of the above embodiments may incorporate 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.

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 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.

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, *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 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 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) 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 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. 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.

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 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 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. 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 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. 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.*, 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 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, 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 Jagged1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Jagged1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Jagged1. 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 Jagged1 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
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser

Original Residue	Exemplary Substitutions	Preferred Substitutions
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;

(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 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 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 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, 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 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 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.

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 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 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 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) Glycosylation variants

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). In some embodiments, an IgG1 constant region comprising an N297G or N297A mutation substantially lacks effector function. 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).

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 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**

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.

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. 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 Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. 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-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. 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 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)).

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).

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 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).

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-Jagged1 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-Jagged1 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-Jagged1 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

Anti-Jagged1 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, A-2, or A-1(S101T) for binding to human or murine Jagged1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by A, A-1, A-2, or A-1(S101T).

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 Jagged1 is incubated in a solution comprising a first labeled antibody that binds to Jagged1 (e.g., A, A-1, A-2, or A-1(S101T)) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to Jagged1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized Jagged1 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 Jagged1, excess unbound antibody is removed, and the amount of label associated with immobilized Jagged1 is measured. If the amount of label associated with immobilized Jagged1 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 Jagged1. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

In one aspect, assays are provided for identifying anti-Jagged1 antibodies thereof having biological activity. Biological activity may include, e.g., inhibition of Jagged1-induced signaling through Notch1. 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 Jagged1-induced Notch signaling. Nonlimiting exemplary assays are provided in the Examples. In certain embodiments, an antibody of the invention is tested for such biological activity. Antibodies having such biological activity in vivo and/or in vitro are also provided.

D. Immunoconjugates

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

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 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-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-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 anti-Jagged1 antibodies provided herein is useful for detecting the presence of Jagged1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as cancerous tissues.

In one embodiment, an anti-Jagged1 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of Jagged1 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-Jagged1 antibody as described herein under conditions permissive for binding of the anti-Jagged1 antibody to Jagged1, and detecting whether a complex is formed between the anti-Jagged1antibody and Jagged1. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-Jagged1 antibody is used to select subjects eligible for therapy with an anti-Jagged1 antibody, e.g. where Jagged1 is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention include cancer, e.g., breast cancer, lung cancer, brain cancer, cervical cancer, colon cancer, liver cancer, bile duct cancer, pancreatic cancer, skin cancer, B-cell malignancies, and T-cell malignancies.

In certain embodiments, labeled anti-Jagged1 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-Jagged1 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®, 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. 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 cytotoxic agent, e.g., a chemotherapeutic agent. 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 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).

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 may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

Any of the anti-Jagged1 antibodies provided herein may be used in therapeutic methods.

In one aspect, an anti-Jagged1 antibody for use as a medicament is provided. In further aspects, an anti-Jagged1 antibody for use in treating a disease or disorder associated with aberrant Notch signaling, e.g. a cancer, is provided. In certain embodiments, an anti-Jagged1 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-Jagged1 antibody for use in a method of treating an individual having a cancer comprising administering to the individual an effective amount of the anti-Jagged1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

In further embodiments, the invention provides an anti-Jagged1 antibody for use in inhibiting lung cancer growth. In certain embodiments, the invention provides an anti-Jagged1 antibody for use in a method of reducing lung cancer growth in an individual comprising administering to the individual an effective of the anti-Jagged1 antibody to reducing lung cancer growth. In certain embodiments, the invention provides an anti-Jagged1 antibody for use in a method of reducing breast cancer growth in an individual comprising administering to the individual an effective of the anti-Jagged1 antibody to reducing breast cancer growth. An "individual" according to any of the above embodiments is preferably a human.

In some embodiments, an anti-Jagged1 antibody is provided for treating allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and/or excess mucus. Other allergic diseases that may be treated with the anti-Jagged1 antibodies provided herein include, but are not limited to, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria; immune-mediated skin diseases include bullous skin diseases, erythema multiform and contact dermatitis; autoimmune disease include psoriasis, rheumatoid arthritis, juvenile chronic arthritis; inflammatory bowel disease (i.e., ulcerative

colitis, Crohn's disease); idiopathic interstitial pneumonia, diseases associated with goblet cell metaplasia (such as asthma, COPD, cystic fibrosis and Barrett's esophagus), lung diseases such as cystic fibrosis, gluten-sensitive enteropathy, and Whipple's disease; immunologic diseases of the lung such as eosinophilic pneumonia, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis; chronic obstructive pulmonary disease, RSV infection, uvelitis, scleroderma, osteoporosis, and Hodgkin's lymphoma.

In a further aspect, the invention provides for the use of an anti-Jagged1 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of a disease or disorder associated with aberrant Notch signaling. In one embodiment, the medicament is for treatment of a cancer. In a further embodiment, the medicament is for use in a method of treating a cancer comprising administering to an individual having a cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating a disease or disorder associated with aberrant Notch signaling. In one embodiment, the method comprises administering to an individual having such disease or disorder an effective amount of an anti-Jagged1 antibody. In one embodiment, the method comprises administering to an individual having a cancer an effective amount of an anti-Jagged1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for inhibiting cancer cell growth in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-Jagged1 antibody to inhibit cancer cell growth. In one embodiment, an "individual" is a human.

In some embodiments, the invention provides methods for treating allergy, asthma, autoimmune disease, diseases associated with goblet cell metaplasia (e.g., in lung) and/or excess mucus in an individual. In some embodiments, the invention provides methods for treating allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria; immune-mediated skin diseases include bullous skin diseases, erythema multiform and contact

dermatitis; autoimmune disease include psoriasis, rheumatoid arthritis, juvenile chronic arthritis; inflammatory bowel disease (i.e., ulcerative colitis, Crohn's disease); idiopathic interstitial pneumonia, diseases associated with goblet cell metaplasia (such as asthma, COPD, cystic fibrosis and Barrett's esophagus), lung diseases such as cystic fibrosis, gluten-sensitive enteropathy, and Whipple's disease; immunologic diseases of the lung such as eosinophilic pneumonia, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis; chronic obstructive pulmonary disease, RSV infection, uvelitis, scleroderma, osteoporosis, and/or Hodgkin's lymphoma in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-Jagged1 antibody provided herein. In some embodiments, an "individual" is a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-Jagged1 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-Jagged1 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-Jagged1 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

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 cytotoxic agent. In certain embodiments, an additional therapeutic agent is an antibody.

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 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-Jagged1 antibody and administration of an additional therapeutic agent occur within about one month, or within 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, 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.

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 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. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. 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. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose

of about 2 mg/kg of the antibody. 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-Jagged1 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. 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 an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. 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, 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 particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such 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-Jagged1 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 provided above.

Example 1. Generation of anti-Jagged antibodies.

a. Library Sorting and Screening to Identify anti-Jagged1 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 (SEQ ID NO:6) or human Jag2-DSL-EGF1-4 (SEQ ID NO:8) 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 Jagged1 or Jagged2. 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 Jagged1 (and not Jagged2), Jagged2 (and not Jagged1), or to both Jagged1 and Jagged2 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_HV_L libraries

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 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 70-10-10-10 mixtures of bases favoring the wild type nucleotides (Gallop et al., *Journal of Medicinal Chemistry* 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.

For clones originated from V_HV_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_HV_L library, as diversity of CDR-L3 was built into the naïve library. To achieve the soft 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

For affinity improvement selection, Jag1 or Jag2 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 150μl/well 50mM HCl, 500mM KCl for 30 minutes, and

subsequently neutralized by 50µl/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 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 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 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 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 to 75µl of in ELISA (enzyme linked 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 (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 either Jag1 or Jag2 by comparison to respective parental clones. Then the most affinity-improved clones were reformatted into human IgG1 for antibody production and further BIAcore binding kinetic analysis and other *in vitro* or *in vivo* assay.

Further screening rounds identified antibodies specific for only one of the Jagged family members, as determined by ELISA. Antibody A bound human and murine Jagged1, but not Jagged2 (FIG. 8; heavy chain variable region sequence shown in SEQ ID NO: 9, light chain variable region sequence shown in SEQ ID NO: 10). Conversely, antibody B (the parental antibody of antibody B-3) bound human and murine Jagged2, but not Jagged1 (FIG. 8). C-1 binds to both Jagged1 and Jagged2, and served as a control. The heavy chain and light chain variable region sequences for antibody C-1 are shown in FIG. 4.

Example 2. Antibody binding affinities and epitope mapping.

Binding affinities of anti-Jagged1 phage antibodies were measured by Surface Plasmon Resonance (SRP) using a BIAcore™-3000 instrument. Anti-Jagged1/2 phage human IgGs were captured by mouse anti-human IgG coated on the CM5 sensor chip to achieve approximately 150 response units (RU). For kinetics measurements, two-fold serial dilutions of human or mouse Jag1/2 DSL_EGF1-4 (1.95nM to 250nM) were injected in PBT buffer (PBS with 0.05% Tween 20) at 25°C with a flow rate of 30ml/min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (k_d) was calculated as the ratio k_{off}/k_{on}.

Table 2 summarizes the binding constants for antibodies A, A-1, A-2, B, and B-3 binding to purified human Jagged1, human Jagged2, and mouse Jagged2. Parent antibody A specifically bound to human and murine Jagged1. The affinity matured antibodies A-1 and A-2 bound both human and murine Jagged1 with high affinity. Antibodies A, A-1 and A-2 did not bind human or murine Jagged2. Conversely, antibodies B and B-3 did not bind human or murine Jagged1. B-3 specifically bound to human and mouse Jagged2.

Table 2: Biacore summary table

Ab	Human Jag1			Human Jag2			Mouse Jag2		
	kon (1/Ms)	koff (1/s)	Kd (M)	kon (1/Ms)	koff (1/s)	Kd (M)	kon (1/Ms)	koff (1/s)	Kd (M)
A	2.3E+04	2.1E-03	9.4E-08	No binding up to 0.5 μM					
A-1	8.3 E+04	5.9E-05	7.1E-10						
A-2	2.3 E+05	7.1E-05	3.0E-10						
B	No binding up to 0.5 μM			2.5E+06	2.6E-03	1.0E-09	2.5E+06	2.6E-03	1.0E-09
B-3							5.8E+05	1.75E-04	3.0E-10

The heavy chain and light chain variable region sequences for antibody A are shown in SEQ ID NOs: 9 and 10, respectively. The heavy chain and light chain variable region sequences for antibody A-1 are shown in SEQ ID NOs: 17 and 18, respectively. The heavy chain and light chain variable region sequences for antibody A-2 are shown in SEQ ID NOs: 25 and 26, respectively. The heavy chain and light chain variable region sequences for antibody B-3 are shown in SEQ ID NOs: 41 and 42, respectively. The heavy chain and light chain variable region sequences for antibody B are shown in FIG. 4.

Example 3. Anti-Jagged antagonist antibodies inhibit Jagged1-induced signaling *in vitro.*

To determine whether anti-Jagged antibodies can act as antagonists of Jagged-induced Notch signaling, co-culture experiments were performed essentially as described by Wu et al., *Nature* 464, 1052-1057 (15 April 2010). NIH-3T3 cells engineered to express Jagged1, as the Notch ligand, were co-cultured with NIH 3T3 cells that stably express Notch1 and that were transiently transfected to express a Notch-responsive TP-1 (12X CSL) firefly luciferase reporter and a constitutively expressed *Renilla* luciferase reporter (pRL-CMV, Promega). Strong Notch reporter signal (Firefly luciferase) was observed in the co-culture (FIG. 12, J1 induced-Positive Control). Reporter expression was reduced to background levels when a γ-secretase inhibitor was added to the co-culture, demonstrating Notch-dependent expression of the reporter construct. *Data not shown.*

Addition of increasing amounts (0.016-50 μg/ml) of anti-Jagged antibody A-2 (heavy chain and light chain variable region sequences of SEQ ID NOs: 25 and 26, respectively) or B-3 (heavy chain and light chain variable region sequences of SEQ ID NOs: 41 and 42, respectively) resulted in dose-dependent inhibition of reporter expression (FIG. 9). Signaling was induced by Jagged1 (FIG. 9A, dark gray columns) or by Jagged2 (FIG. 9B, light gray columns) and inhibition was determined as described above. Controls included cultures that were not stimulated with ligand and not treated with antibody (FIG. 9A and B, Untreated), not stimulated with ligand (FIG. 9A and B, No Stimulation), treated with 5-10 μg/ml isotype control antibody (FIG. 9A and B, agD), stimulated with ligand but not treated with antibody (FIG. 9A and B, Stim/no AB), treated with 5 μM of the gamma-secretase inhibitor DAPT or the DAPT vehicle control of DMSO.

Antibody A-2 inhibited Jagged1-induced signaling, but not Jagged2-induced signaling, in a dose-dependent manner (FIG. 9A). The IC_{50} for A-2 was between 2 and 10 μg/ml for Jagged1 inhibition whereas little or no Jagged2 inhibition was observed even at the highest concentration of 50 μg/ml. The results demonstrate that antibody A-2 is a Jagged1-selective

antagonist, i.e., antibody A-2 inhibits Jagged1-mediated signaling, but not Jagged2-mediated signaling. In contrast, antibody B-3 potently inhibited Jagged2-induced signaling at the lowest concentration tested but did not inhibit Jagged1-induced signaling at the highest concentration tested, thus establishing B-3 as a Jagged1-selective antagonist (FIG. 9B).

Example 4. Effect of anti-Jagged antibody treatment on body weight.

As described above, gamma-secretase inhibitors, and other inhibitors of multiple Notch receptors, cause weight loss and intestinal goblet cell metaplasia, which is undesirable for clinical administration. To determine how the antibodies described herein affect body weight and intestinal health, mice were dosed twice per week with the anti-Jagged1 antibody A-2 (5-20mpk; heavy chain and light chain variable region sequences of SEQ ID NOs: 25 and 26, respectively), the anti-Jagged2 antibody B-3 (5-20mpk; heavy chain and light chain variable region sequences of SEQ ID NOs: 41 and 42, respectively), the antibody A-2 and B-3 together (5mpk each), an anti-Jagged1/2 antibody that binds to both Jagged 1 and Jagged 2 (C-1; 5-10 mg antibody per kg mouse body weight (mpk); heavy and light chain variable regions sequence shown in FIG. 4), or the isotype control anti-gD antibody (20mpk). The isotype control antibody was also used to bring the total antibody concentration of each dosing to 20 mpk. Total body weight of each mouse was determined prior to first administration of antibodies and monitored until day 12 of the study. The average body weight changes are depicted in FIG. 10, graphed as a percentage of starting body weight. Dual inhibition of Jagged1 and Jagged2, using either the anti-Jagged1/2 antibody C-1 or a combination of the Jagged1-specific antibody A-2 and the Jagged2-specific antibody B-3 together, caused rapid and substantial weight loss (FIG. 10A). By day 4, some mice that received the anti-Jagged1/2 antibody C-1 had lost over 5% of their bodyweight, which progressed to nearly 8-10% loss in body weight by day 7 (FIG. 10A). Mice that received both A-2 and B-3 also lost weight rapidly, in some cases up to 17% by day 11 (FIG. 10A). In contrast, none of the Jagged1-specific or Jagged2-specific antibodies alone caused weight loss over the course of the study at either 5 or 20mpk (FIG. 10A). Treatment with the combination of anti-Jagged1 plus anti-Jagged2 antibodies resulted in decreased food intake (FIG. 10B), which correlated with the observed decrease in body weight (FIG. 10A) and suggested that decreased food intake could partly or entirely account for the correlated body weight decreases.

Example 5. Anti-Jagged1 antagonist antibodies inhibit human lung cancer cell growth *in vivo*.

Harlan athymic nude mice were inoculated subcutaneously with Calu-6 cells, a human non-small cell lung cancer line. After tumor volume reached approximately 200 cubic mm,

mice were injected intraperitoneally (IP) twice per week (days 0, 4, 7, 11, 14 and 18) with 20 mpk of either anti-gD isotype control antibody (n=10) or with anti-Jagged1 antibody A-2 (n=10; heavy chain and light chain variable region sequences of SEQ ID NOs: 25 and 26, respectively). Tumor volume in each mouse was measured with calipers for another 19 days. Total body weight of each mouse was monitored over the course of the study.

Tumors in mice treated with anti-Jagged1 showed a significant decrease in tumor volume relative to tumors in the control group (FIG. 11A). The effect of the anti-Jagged1 antibody treatment could be detected as early as day seven after treatment (FIG. 11A). At day 18, the average tumor volume in mice that received the anti-Jagged1 antibody reached approximately 500mm^3, while average tumor volume in control animals reached approximately 750 mm^3 at day 18. No significant change in body weight between the treatment and control group could be observed (FIG. 11B).

Example 6. Anti-Jagged1 and anti-Jagged2 antibodies inhibit human breast cancer cell growth *in vivo*.

C.B-17 SCID.bg mice were inoculated in the mammary fat pad with MDA-MD-468 cells, a human basal breast cancer line. After tumor volume reached approximately 200 cubic mm, mice were dosed IP with 30 mpk of either anti-gD isotype control antibody (human IgG1 isotype), anti-ragweed isotype control antibody (murine IgG2a isotype), anti-Jagged1 antibody A-2 (heavy chain and light chain variable region sequences of SEQ ID NOs: 25 and 26, respectively) in the human IgG1 backbone, anti-Jagged1 antibody A-2 in the murine IgG2a backbone or anti-Jagged2 antibody B-3 (heavy chain and light chain variable region sequences of SEQ ID NOs: 41 and 42, respectively) in the human IgG1 backbone on days 0, 4, 7, 12, 15, 18, 22, 25, 29, 32, 36, 43, 50, and 57. Tumor volume (y-axis) was measured with calipers for 60 days after the first injection. The tumor volumes for each group (n=9 per group) were plotted using a linear mixed effects model (FIG. 12A). Tumor volumes for each mouse in each group are depicted in FIG. 12B.

Example 7: Anti-Jagged1 antibodies are cleaved in the heavy chain

Antibodies A (heavy chain and light chain variable region sequences of SEQ ID NOs: 9 and 10, respectively), A-1 (heavy chain and light chain variable region sequences of SEQ ID NOs: 17 and 18, respectively), and A-2 (heavy chain and light chain variable region sequences of SEQ ID NOs: 25 and 26, respectively) were analyzed by SDS-PAGE and mass spectrometry for integrity of the heavy and light chains. For SDS-PAGE analysis, each antibody sample was mixed in a 1:1 v/v ratio with 2x Tris-Glycine SDS Sample Buffer (Novex LC2676), in the absence and presence of 10mM DTT. Samples were heated at 95°C for 5 minutes, and 2μg of

each sample was loaded onto a Novex 4-20% SDS-PAGE, 1.0mm gel (Novex EC6025). 10μL of Mark 12 molecular weight standard (Invitrogen 100006637) was also loaded onto the gel. Electrophoresis was run in 1x Tris-Glycine SDS Running Buffer (Invitrogen LC2675-5) at a constant 250V until the tracking dye reached the bottom of the gel. The gel was then stained with a Coommassie-based stain (Expedeon InstantBlue #ISB1L).

For mass spectrometry analysis, each antibody was diluted to a final concentration of 1mg/mL in PBS. The pH of the antibody was increased to 8.0 with addition of 1:10 v/v of 1.0M Tris, pH 8.0. DTT to a final concentration of 10mM in solution was added to reduce the antibody. The sample was then heated at 37°C for 15 minutes. Samples were then injected onto a PLRP-S 1000Å, 8μm, 2.1 x 50mm column (Agilent, PL1912-1802) heated to 80°C using an Agilent 1200 HPLC system, followed by electrospray ionization on an Agilent 6210 TOF LC/MS system.

The results of those analyses are shown in FIG. 13. The SDS-PAGE analysis (FIG. 13A) revealed that a fraction of the heavy chain (HC) is cleaved in each of the antibodies. The bands corresponding to the intact HC and light chain (LC), as well as the carboxy (C)-terminal and amino (N)-terminal cleaved fragments of the HC, are marked to the right of the gel. A representative mass spectrometry analysis, for antibody A-1, is shown in FIG. 13B. This analysis indicated that the cleavage site was between HC amino acids G100 and S101 (sequential numbering, corresponding to G96 and S97 according to Kabat numbering) in CDR3, as diagramed in the HC amino acid sequence in FIG. 13C, with the arrow marking the cleavage position. Similar analyses revealed that the HC cleavage occurred in all tested preparations of these antibodies (including following expression in two different cell types, CHO and 293) and that cleavage occurred independent of the type of antibody Fc region (human IgG1 or murine IgG2a).

An SDS-PAGE gel of the anti-Jagged1 antibodies was run substantially as described above. Rather than staining with Coomassie-based stain, antibody was transferred onto PVDF membrane (Invitrogen LC2002) using XCell Blot Module (Invitrogen EI9051) at constant 0.35A. The membrane was stained with Coomassie Blue R-250. The samples on membrane were subjected to N-terminal sequence analysis using the Applied Biosystems Procise Sequencer 494 according to the sequencing principle described in Niall, 1973, Meth. Enzymol. 27: 942–1010.

The results of the sequencing analysis are shown in FIG. 14. This method confirmed that the cleavage site was the same one predicted from the mass spectrometry results, which is

between G100 and S101 (sequential numbering, corresponding to G96 and S97 according to Kabat numbering) of the HC sequence.

The heavy chain cleavage of anti-Jagged1 antibodies A, A-1, And A-2 was unexpected. An analysis of the amino acid sequence surrounding the cleavage site identified no known protease cleavage sites. The mechanism of the cleavage is unclear, and is not readily apparent from the sequences of the antibodies.

Example 8: Mutation of heavy chain S101 reduces cleavage of anti-Jagged1 antibody heavy chain

Since the observed cleavage of the anti-Jagged1 antibodies was unexpected and the mechanism unclear, it was not known whether changes to the antibody sequence could prevent the cleavage, while retaining the affinity and efficacy of the antibody. In addition, it was not known what position(s) in the antibody sequence should be changed to prevent cleavage. To determine whether antibody cleavage could be prevented by changing the heavy chain sequence, a series of amino acid changes were made at heavy chain position S101 (sequential numbering of the heavy chain variable region sequence of SEQ ID NO: 17, corresponding to S97 according to Kabat numbering). The antibodies were expressed in mammalian cells and purified according to standard procedures. Cleavage was analyzed by SDS-PAGE as described in Example 7. The results are shown in FIG. 15. The amino acid changes at position S101 significantly reduced or eliminated HC cleavage, although some cleavage was detected with the S101H mutation (FIG. 15, lane 5). These results were confirmed by mass spectrometry, performed as described in Example 7.

To determine the effects of the changes on binding to a purified Jag1 extracellular domain protein fragment, mutant A-1 antibody binding affinities were measured using BIAcore. Table 3 shows a summary of the fragmentation and Jagged1 binding affinities for each of the mutant A-1 antibodies.

Table 3: Fragmentation and Jagged1 binding affinities of mutant A-1 antibodies

	HC Fragmentation Detected?		Jag 1 Binding (BIAcore)		
mAb Variants	Mass Spec	SDS-PAGE	k_a (1/Ms)	k_d (1/s)	KD (M)
A-1 G100, S101 (WT)	yes	yes	1.80E+05	1.44E-04	7.99E-10
A-1 G100A, S101	yes	yes			
A-1 G100, S101A	no	no	1.46E+05	6.75E-04	4.62E-09
A-1 G100, S101D	no	no	1.02E+05	5.58E-04	5.47E-09
A-1 G100, S101E	no	no	1.67E+05	1.15E-03	6.88E-09
A-1 G100, S101G	no	no	1.54E+05	8.72E-04	5.67E-09
A-1 G100, S101H	yes	yes	1.27E+05	5.62E-04	4.43E-09
A-1 G100, S101I	no	no	9.88E+04	5.90E-04	5.97E-09
A-1 G100, S101K	no	no	1.18E+05	8.43E-04	7.16E-09
A-1 G100, S101L	no	no	8.34E+04	6.49E-04	7.79E-09
A-1 G100, S101N	no	no	1.36E+05	7.97E-04	5.87E-09
A-1 G100, S101Q	no	no	1.03E+05	5.82E-04	5.64E-09
A-1 G100, S101R	no	no	1.02E+05	6.90E-04	6.75E-09
A-1 G100, S101T	no	no	1.31E+05	3.41E-04	2.61E-09
A-1 G100, S101V	no	no	1.20E+05	5.80E-04	4.84E-09

As shown in Table 3, the changes to the amino acid residue at position 101 reduced cleavage to undetectable levels, except for S101H. In addition, since the mechanism of cleavage was unknown, a change to position 100, G100A, was also tested. The G100A mutant was still cleaved. *See* Table 3. Surprisingly, given that the mutations were made in HVR-H3, the S101 mutant antibodies retained the ability to bind Jagged1.

Example 9: Effect of temperature and freeze-thaw cycling on cleavage of anti-Jagged1 antibody heavy chain

To assess whether anti-Jagged1 antibody cleavage is increased by incubation at increasing temperatures, various independent preparations of anti-Jagged1 antibodies A-1 (heavy chain and light chain variable region sequences of SEQ ID NOs: 17 and 18, respectively) and A-2 (heavy chain and light chain variable region sequences of SEQ ID NOs: 25 and 26, respectively), as well as an isotype control antibody (not anti-Jagged1), were incubated for 10 min at 70°C or 95°C. The extent of cleavage was assessed using standard SDS-PAGE and protein staining. The results are shown in FIG. 16. Each of the anti-Jagged1 antibody preparations was cleaved at 70°C, while the control antibody was not (FIG. 16A). As

summarized in the table (FIG. 16B), the extent of anti-Jagged1 cleavage was not significantly or consistently altered by incubation at 95°C versus 70°C.

To assess whether cleavage resulted from freeze-thaw cycling of anti-Jagged1 antibody preparations, antibody A-1 (heavy chain and light chain variable region sequences of SEQ ID NOs: 17 and 18, respectively) was subjected to multiple rounds of freezing at -80°C followed by thawing. 2 μg of each sample was then analyzed by SDS-PAGE and protein staining under non-reducing (-DTT) or reducing (+DTT) conditions, as indicated. The stained gel was imaged using a Biorad GelDoc Easy Imager instrument and densitometry analysis was performed using Biorad Image Lab software. The results of that experiment are shown in FIG. 17. F/T 1 refers to the original sample, with increasing numbers indicating the number of additional rounds of freeze-thaw cycles. For each cycle, the A-1 antibody was frozen at -80°C and then thawed at room temperature. An aliquot was removed for SDS-PAGE. The freeze/thaw was repeated twice more for a total of 3 freeze/thaw cycles, with aliquots being removed after each thaw step. The table (FIG. 17B) summarizes the percentage of cleavage under each condition. The experiment revealed that additional rounds of freeze-thaw had little impact on the percentage of cleavage.

Example 10: Jagged1 blocking activity of A-1 and A-1(S101T) *in vitro*

In vitro co-culture assays of Jag1-induced Notch reporter activity were performed to measure the Jag1 blocking activity of A-1 (heavy chain and light chain variable region sequences of SEQ ID NOs: 17 and 18, respectively) and A-1(S101T) (heavy chain and light chain variable region sequences of SEQ ID NOs: 33 and 34, respectively). U87MG cells, which express high levels of Notch2, were co-transfected with a Notch-responsive TP-1 (12X CSL) Firefly (FF) luciferase reporter and a constitutively expressed Renilla luciferase reporter (pRL-CMV, Promega) to control for transfection efficiency. *See* Wu et al., 2010, Nature 464: 1052-1057. Anti-Jagged1 antibody A-1 or A-1(S101T), isotype control antibody, 5 μM DAPT (Calbiochem), or DMSO vehicle control was added with ligand-expressing cells (NIH-3T3 cells stably transfected with human Jag1 or a no ligand control) 6 hours after transfection. Luciferase activities were measured after 20 hours of co-culture (Promega, Dual Glo Luciferase). Typically, four replicates were analyzed for each condition, and values were expressed as relative luciferase units (Firefly signal divided by the Renilla signal) and graphed as a percentage of Jag1-induced activity per anti-Ragweed control.

The results of that experiment are shown in FIG. 18. FF and Renilla luciferase measurements were taken (FIGS. 18B and C, respectively) according to standard methods, and

the FF to Renilla luciferase ratios were graphed as a normalized measurement of Jag1-induced Notch activity (FIG. 18A). The results show that A-1 and A-1(S101T) both inhibit Jag1-induced Notch signaling in a dose-dependent manner. *See* FIGS. 18A and B. Thus, A-1(S101T) retains the anti-Jagged1 blocking activity of parental antibody A-1.

Example 11: Jagged1 blocking activity of A-1 and A-1(S101T) *in vivo*

To assess Jagged1 blocking activity of anti-Jagged1 antibodies *in vivo*, the club and ciliated cell composition of mouse lung bronchiolar epithelium was measured after dosing mice with control or anti-Jagged1 antibodies. Wild-type, eight-week old BALB/c female mice (three mice per group) were dosed on day 0 as follows (all groups contained anti-Jagged2 antibody (B-3) to sensitize the bronchiolar epithelium to reveal clearly measurable effects of anti-Jagged1 activity):

1. Control 1X – isotype control (15 mg antibody per kg mouse body weight) + anti-Jag2 B-3 (15mg/kg; heavy chain and light chain variable region sequences of SEQ ID NOs: 41 and 42, respectively)
2. A-1 1X – anti-Jag1 A-1 (15 mg/kg; heavy chain and light chain variable region sequences of SEQ ID NOs: 17 and 18, respectively)+ anti-Jag2 B-3 (15mg/kg)
3. A-1 .5X – anti-Jag1 A-1 (7.5mg/kg) + anti-Jag2 B-3 (15mg/kg)
4. A-1 .25X – anti-Jag1 A-1 (3.75mg/kg) + anti-Jag2 B-3 (15mg/kg)
5. A-1-S101T 2X – anti-Jag1 A-1(S101T) (30mg/kg; heavy chain and light chain variable region sequences of SEQ ID NOs: 33 and 34, respectively) + anti-Jag2 B-3 (15mg/kg)
6. A-1-S101T 1X – anti-Jag1 A-1(S101T) (15mg/kg) + anti-Jag2 B-3 (15mg/kg)
7. A-1-S101T .5X – anti-Jag1 A-1(S101T) (7.5mg/kg) + anti-Jag2 B-3 (15mg/kg)
8. A-1-S101T .25X – anti-Jag1 A-1(S101T) (3.75mg/kg) + anti-Jag2 B-3 (15mg/kg)

On day 5, lungs were harvested, inflated, fixed and stained for immunofluorescence (IF) as follows. Lungs were inflated with 4% PFA in PBS. The entire lung was transferred to 10% neutral buffered formalin (NBF) and fixed over-night at room temperature. Fixed lungs were transferred to 70% ethanol for at least 24 hours. The lungs were paraffin embedded and sectioned at 5 μm. Immunofluorescence staining for ciliated and Clara cells was as follows. Slides were de-paraffinized and antigens were retrieved by boiling the slides in citrate buffer (Dako S1700) in a pressure cooker for 15 minutes at 125°C. Slides were briefly rinsed in 2X PBS, and then permeablized with 0.2% Triton-X100 in PBS for 45 minutes or 3 x 15 minutes. Sections were blocked with 5% FBS/2% BSA for 1 hour. Slides were then incubated with goat anti-CC10 (1:1000) and mouse anti-acetylated alpha tubulin (1:200) in blocking buffer for 2 to

3 hours or overnight. Slides were washed 3 times for 15 minutes with PBS. Slides were incubated with secondary antibodies for 1 hour (Invitrogen Alexa Fluor secondary antibodies diluted 1:1000), then rinsed twice for 15 minutes with PBS. Nuclei were stained with DAPI (0.5ug/ml) for 15 minutes. Slides were then rinsed twice for 15 minutes with PBS and coverslipped.

The results of that experiment are shown in FIG. 19. Blocking Jagged1 and Jagged2 signaling causes an increase in the number of ciliated cells (as marked by immunofluorescent detection of alpha-tubulin in red) and a decrease in the number of club cells (as marked by immunofluorescent detection of CC10 in green) in the mouse bronchiolar epithelium. Blocking both Jagged1 plus Jagged2 results in a near complete loss of club cells, such that the resulting epithelium consists primarily of ciliated cells (red; *see* "A-1, 1X" and "A-1-S101T, 2X" in FIG. 19). A-1 and A-1(S101T) both inhibited Jagged1-induced Notch signaling *in vivo* in a dose-dependent manner. Thus, A-1(S101T) retains the anti-Jagged1 blocking activity of parental antibody A-1 *in vivo*.

Example 12: Anti-Jagged1 antibodies A-1 and A-1(S101T) inhibit growth of liver cancer tumors *in vivo*

A human patient-derived liver cancer tumor, LIV#78 (Genendesign, China), was grown as a subcutaneous xenograft in BALB-c nude immunocompromised mice. When tumors grew to between 150 to 200 mm^3, the mice were grouped into seven treatment groups with ten mice per group and dosed once per week (except for group 5, which was dosed once every three weeks) at the indicated dose (in mg of antibody per kg of mouse body weight) of the indicated antibody (A-1(S101T) antibody has the heavy chain and light chain sequences of SEQ ID NOs: 51 and 53, respectively; "A-1-DANG(effectorless)" has the heavy and light chain sequences of SEQ ID NOs: 52 and 53, respectively; and A-1 antibody has the heavy chain and light chain sequences of SEQ ID NOs: 81 and 53, respectively). *See* FIG. 20B. Tumor volumes were assessed by caliper measurements (length x width x height/2).

FIG. 20A shows a LME (linear mixed effects) graph of tumor volume in each treatment group over the course of the study. FIG. 20B summarizes the growth statistics, group identities and dosing regimens. The anti-Jagged1 A-1 and A-1(S101T) antibodies significantly inhibited liver cancer growth *in vivo*; multiple PRs (partial responses) were observed with each of the anti-Jagged1 antibodies. Likewise, none of the anti-Jagged1 treated groups showed a doubling of tumor volume during the 44 days of the study, whereas the control group showed a time to progression of tumor doubling (TTP 2X) of 18.5 days. *See* FIG. 20B. The percentage of tumor growth inhibition (%TGI, where "lower" and "upper" refer to the lowest and

uppermost %TGI measurements, respectively, for individual animals in each group) as a function of area under the curve per day (AUC/day) compared to the control group depended on the dose of anti-Jagged1 A-1-S101T, consistent with tumor growth inhibition reflecting the extent of Jagged1 inhibition. Likewise, inhibition of tumor growth was similar using A-1 or A-1(S101T). *Compare, e.g.*, groups 3 and 7, FIG. 20B. Tumor growth inhibition did not depend on antibody effector function because a heavy chain N297G mutant form of A-1, which lacks effector function, inhibited tumor growth as effectively as A-1. *Compare* groups 6 and 7, FIG. 20B.

FIG. 21A shows a LME (linear mixed effects) graph of mouse body weight over time for the mice shown in FIG. 20. FIG. 20B shows various body weight parameters for the treatment groups, including the % change in body weight on the last day of the study (% BW Last Day), the maximum % change in body weight (Max %BW), and the day on which the maximum change in bodyweight occurred (Max %BW Day), and the (AUC/Day (lower,upper)). The body weight graphs for the treatment groups, including the control group, are statistically indistinguishable, indicating that anti-Jagged1 treatment was well-tolerated.

Example 13: Blocking Jagged1 inhibits goblet cell metaplasia *in vivo*

Following a 35 day period of sensitization to intraperitonealy injected ovalbumin, mice were challenged with aerosolized ovalbumin for 7 consecutive days, after which they were sacrificed and analyzed for the number of goblet cells. Mice were treated with control, anti-Jagged1 A-2 antibody (with murine IgG2a Fc), anti-Jagged2 B-3 antibody (with murine IgG2a Fc) or the combination of anti-Jagged1 + anti-Jagged2 antibodies 24 hours and 96 hours after the first aerosol challenge.

FIG. 23A shows periodic acid-Schiff staining of lung airways in the mice treated with anti-Jagged1, anti-Jagged2, anti-Jagged1 + anti-Jagged2, or control antibody. FIG. 23B shows quantification of the goblet cells in the airways of the different treatment groups. An abundance of goblet cells are evident in the control and anti-Jagged2 groups. Few goblet cells were present in the anti-Jagged1 group and practically no goblet cells were detected in the anti-Jagged1 + anti-Jagged2 group. FIG. 23C shows the inflammation index as assessed by Haematoxylin Eosin (H&E) staining. Treatment with either Jagged1 or -2 blocking antibody does not affect inflammation in the lungs.

Jagged1-induced Notch signaling biases cell fate in the airways towards a secretory cell (including goblet cell) fate and away from a ciliated cell fate. Jagged1 signaling is important for maintaining the secretory cell fate, and inhibition of Jagged1 signaling prevented goblet cell

metaplasia. We also showed that the club cell-to-ciliated cell conversion is direct and did not involve cell division (data not shown). Club cells give rise to goblet cells in the lung. This transdifferentiation of one cell type to another occurred in the adult lung and is distinct from cell fate choices that involve progenitor cell division, such as after damage or during development. Goblet cell metaplasia or excess mucus is a hallmark of several airway diseases, such as asthma, cystic fibrosis, COPD and Barrett's esophagus. These Jagged inhibition results provide the basis for therapeutic applications involving use of Jagged1 or Jagged2 inhibitors for prevention or reversal of goblet cell metaplasia and for treatment of conditions characterized by excess mucus, as in airway diseases (e.g., asthma, COPD, cystic fibrosis) and Barrett's esophagus.

Example 14: Specific binding of anti-Jagged1 and anti-Jagged2 antibodies

Antibodies A-2 (FIG. 24, left panel) and C-1 (FIG. 24, right panel) were tested for binding to recombinant purified Notch ligands human Jagged1 (hJag-1), human Jagged2 (hJag-2), murine Jagged2 (mJag-2), human Delta-like 1 (hDLL1), murine Delta-like 1 (mDLL1), and human Delta-like 4 (hDLL4) using a standard enzyme-linked immunosorbent assay (ELISA). 1μg/ml of Notch ligand protein (as indicated) in PBS, pH7.4, was coated onto ELISA plates (Nunc Maxisorp) at 40°C overnight. Plates were blocked with Casein blocker in PBS (Pierce) for one hour at room temperature. Serial 3-fold dilutions of antibody IgGs (as indicated) in PBST buffer (PBT buffer (PBS + 0.05% (v/v) Tween 20) with 0.5% (w/v) BSA) were added to the plates and incubated for one hour at room temperature. The plates were then washed with PBST and bound antibodies were detected with peroxidase-conjugated goat anti-human Fab specific IgG (Sigma). TMB substrate (3,3',5,5'-tetramethylbenzidine) was used and absorbance at 630nM was read using a standard ELISA plate reader. Absorbance was plotted against concentrations of IgGs using KaleidaGraph (Synergy Software). FIG. 24 shows the results, with A_{630} on the y-axis representing the extent of binding.

Antibody A2 bound human and murine Jagged1, but did not bind human Jagged 2, murine Jagged2, human DLL1, murine DLL1, human DLL4, or murine DLL4 (FIG. 24, left panel). Antibody C1 bound human and murine Jagged1, human and murine DLL1, human and murine Jagged2, but did not bind human or murine DLL4 (Figure 24, right panel).

Antibody binding affinities and rate constants were measured by Surface Plasmon Resonance (SPR) using a BIAcore™-T200 instrument. Human IgG1 antibodies were captured by mouse anti-human IgG coated on the CM5 sensor chip to achieve approximately 150 response units (RU). For kinetic or affinity measurements, four-fold serial dilutions of human

Jagged1, murine Jagged1, human Jagged2, murine Jagged2, human DLL1, murine DLL1, human DLL4, murine DLL4, and rat Jagged1were injected in HBS-T buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 0.05% v/v Surfactant P20, GE Healthcare) at 25°C with a flow rate of 30ml/min. The ligand fragments were the DSL-EGF1-4 fragments, except for rat Jagged1, which was purchased from R&D Systems. For kinetic analysis, association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore T200 Evaluation Software version 2.0). The equilibrium dissociation constant (k_d) was calculated as the ratio k_{off}/k_{on}. For affinity analysis, K_d was calculated using a Steady State Affinity model (BIAcore T200 Evaluation Software version 2.0).

Table 3 summarizes the binding constants for antibodies A-2, B-3, C-1, A-1, and A-1 S101T binding to purified human Jagged1, murine Jagged1, human Jagged2, murine Jagged2, human DLL1, murine DLL1, human DLL4, murine DLL4, and/or rat Jagged1. n.d. = not detected, n.t. = not tested. Antibodies A-2, A-1, and A-1-S101T.NG (antibody A-1S101T with N297G mutation) specifically bound to human and murine Jagged1 with high affinity. Antibodies A-1 and A-1-S101T.NG bound rat Jagged1 with high affinity also. Antibody B-3 specifically bound to human and murine Jagged2 with high affinity. Antibody C-1 specifically bound to human and murine Jagged1 and Jagged 2. Antibodies B-3 and C-1, but not antibody A-2, showed some binding to human and mouse DLL1. None of the antibodies tested bound human or mouse DLL4.

Table 3: Binding constants for anti-Jagged antibodies

	A-2			B-3			C-1		
	k_{on} (1/Ms)	k_{off} (1/s)	K_d (M)	k_{on} (1/Ms)	k_{off} (1/s)	K_d (M)	k_{on} (1/Ms)	k_{off} (1/s)	K_d (M)
hJag1	2.42E+05	9.23E-05	3.82E-10	n.d.	n.d.	> 1.00E-06*	2.90E+05	9.00E-05	3.10E-10
mJag1	3.85E+05	5.77E-05	1.50E-10	n.d.	n.d.	> 1.00E-06*	8.69E+05	1.54E-04	1.77E-10
hJag2	n.d.	n.d.	n.d.	4.58E+06	1.88E-04	4.10E-11	7.64E+06	5.56E-04	7.28E-11
mJag2	n.d.	n.d.	n.d.	7.71E+05	4.40E-05	5.71E-11	1.16E+06	8.03E-05	6.92E-11
hDLL1	n.d.	n.d.	n.d.	n.d.	n.d.	3.49E-07*	n.d.	n.d.	7.83E-08*
mDLL1	n.d.	n.d.	n.d.	n.d.	n.d.	7.23E-08*	n.d.	n.d.	5.55E-08*
hDLL4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
mDLL4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
rJag1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

Table 3 (cont.): Binding constants for anti-Jagged antibodies

	A-1			A-1.S101T.NG		
	k_{on} (1/Ms)	k_{off} (1/s)	K_d (M)	k_{on} (1/Ms)	k_{off} (1/s)	K_d (M)
hJag1	2.91E+04	2.25E-04	7.75E-09	2.09E+04	5.88E-04	2.82E-08
mJag1	1.59E+05	7.42E-05	4.57E-10	9.51E+04	6.22E-04	6.54E-09
hJag2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
mJag2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
hDLL1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
mDLL1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
hDLL4	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
mDLL4	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
rJag1	4.34E+04	9.80E-07	2.26E-11	4.55E+04	1.97E-04	4.32E-09

Example 15: Pharmacokinetics of anti-Jagged1 A-1-S101T antibody

The pharmacokinetic profile of anti-Jagged1 A-1-S101T antibody following a single intravenous injection at 1, 10, and 100 mg/kg were evaluated in female Balb/c nude mice (Charles River Laboratories, Hollister, CA). The mice were 5-8 weeks old and weighed approximately 17.3-21.8 g. Serum samples were collected and antibody concentrations were analyzed by specific enzyme linked immunosorbent assays (ELISA). The specific ELISA was coated with JAG1 extracellular domain-Histidine and detected with goat anti-human Fc. The assay sensitivity has a less than standard value of 6.25 ng/mL. Pharmacokinetic parameters were estimated using a non-compartmental model with Phoenix™ WinNonlin® (v.6.3; Pharsight Corporation; Mountain View, CA). All PK analysis was based on naïve pool of individual animal data.

A greater than dose proportional increase in exposure was observed following IV administration of anti-Jagged1 A-1-S101T antibody within the dose range of 1 and 100 mg/kg, suggesting a target-mediated clearance mechanism of the antibody (Figure 25 and Table 4). The clearance values ranged from approximately 13 to 75 mL/day/kg.

Table 4: Pharmacokinetic properties of anti-Jagged1 A-1-S101T antibody

Treatment	ELISA	C_{max} (µg/mL)	AUC_{last} (day*µg/mL)	AUC_{inf} (day*µg/mL)	CL (mL/day/kg)	V_{ss} (mL/kg)
1 mg/kg anti-JAG1	specific	14.5±0.497	13.3±0.552	13.3	75.0	80.7
10 mg/kg anti-JAG1	specific	160±2.00	458±19.3	458	21.8	87.6
100 mg/kg anti-JAG1	specific	1480±68.4	7220±443	7410	13.5	115

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.

Table of Sequences

SEQ ID NO	Description	Sequence
1	Human Jag1	MRSPRTRGRS GRPLSLLLAL LCALRAKVCG ASGQFELEIL SMQNVNGELQ NGNCCGGARN PGDRKCTRDE CDTYFKVCLK EYQSRVTAGG PCSFGSGSTP VIGGNTFNLK ASRGNDRNRI VLPFSFAWPR SYTLLVEAWD SSNDTVQPDS IIEKASHSGM INPSRQWQTL KQNTGVAHFE YQIRVTCDDY YYGFGCNKFC RPRDDFFGHY ACDQNGNKTC MEGWMGPECN RAICRQGCSP KHGSCKLPGD CRCQYGWQGL YCDKCIPHPG CVHGICNEPW QCLCETNWGG QLCDKDLNYC GTHQPCLNGG TCSNTGPDKY QCSCPEGYSG PNCEIAEHAC LSDPCHNRGS CKETSLGFEC ECSPGWTGPT CSTNIDDCSP NNCSHGGTCQ DLVNGFKCVC PPQWTGKTCQ LDANECEAKP CVNAKSCKNL IASYYCDCLP GWMGQNCDIN INDCLGQCQN DASCRDLVNG YRCICPPGYA GDHCERDIDE CASNPCLNGG HCQNEINRFQ CLCPTGFSGN LCQLDIDYCE PNPCQNGAQC YNRASDYFCK CPEDYEGKNC SHLKDHCRTT PCEVIDSCTV AMASNDTPEG VRYISSNVCG PHGKCKSQSG GKFTCDCNKG FTGTYCHENI NDCESNPCRN GGTCIDGVNS YKCICSDGWE GAYCETNIND CSQNPCHNGG TCRDLVNDFY CDCKNGWKGK TCHSRDSQCD EATCNNGGTC YDEGDAFKCM CPGGWEGTTC NIARNSSCLP NPCHNGGTCV VNGESFTCVC KEGWEGPICA QNTNDCSPHP CYNSGTCVDG DNWYRCECAP GFAGPDCRIN INECQSSPCA FGATCVDEIN GYRCVCPPGH SGAKCQEVSG RPCITMGSVI PDGAKWDDDC NTCQCLNGRI ACSKVWCGPR PCLLHKGHSE CPSGQSCIPI LDDQCFVHPC TGVGECRSSS LQPVKTKCTS DSYYQDNCAN ITFTFNKEMM SPGLTTEHIC SELRNLNILK NVSAEYSIYI ACEPSPSANN EIHVAISAED IRDDGNPIKE ITDKIIDLVS KRDGNSSLIA AVAEVRVQRR PLKNRTDFLV PLLSSVLTVA WICCLVTAFY WCLRKRRKPG SHTHSASEDN TTNNVREQLN QIKNPIEKHG ANTVPIKDYE NKNSKMSKIR THNSEVEEDD MDKHQQKARF AKQPAYTLVD REEKPPNGTP TKHPNWTNKQ DNRDLESAQS LNRMEYIV
2	Murine Jag1	MRSPRTRGRP GRPLSLLLAL LCALRAKVCG ASGQFELEIL SMQNVNGELQ NGNCCGGVRN PGDRKCTRDE CDTYFKVCLK EYQSRVTAGG PCSFGSGSTP VIGGNTFNLK ASRGNDRNRI VLPFSFAWPR SYTLLVEAWD SSNDTIQPDS IIEKASHSGM INPSRQWQTL KQNTGIAHFE YQIRVTCDDH YYGFGCNKFC RPRDDFFGHY ACDQNGNKTC MEGWMGPDCN KAICRQGCSP KHGSCKLPGD CRCQYGWQGL YCDKCIPHPG CVHGTCNEPW QCLCETNWGG QLCDKDLNYC GTHQPCLNRG TCSNTGPDKY QCSCPEGYSG PNCEIAEHAC LSDPCHNRGS CKETSSGFEC ECSPGWTGPT CSTNIDDCSP NNCSHGGTCQ DLVNGFKCVC PPQWTGKTCQ LDANECEAKP CVNARSCKNL IASYYCDCLP GWMGQNCDIN INDCLGQCQN DASCRDLVNG YRCICPPGYA GDHCERDIDE CASNPCLNGG HCQNEINRFQ CLCPTGFSGN LCQLDIDYCE PNPCQNGAQC YNRASDYFCK CPEDYEGKNC SHLKDHCRTT TCEVIDSCTV AMASNDTPEG VRYISSNVCG PHGKCKSQSG GKFTCDCNKG FTGTYCHENI NDCESNPCKN GGTCIDGVNS YKCICSDGWE GAHCENNIND CSQNPCHYGG TCRDLVNDFY CDCKNGWKGK TCHSRDSQCD EATCNNGGTC YDEVDTFKCM CPGGWEGTTC NIARNSSCLP NPCHNGGTCV VNGDSFTCVC KEGWEGPICT QNTNDCSPHP CYNSGTCVDG DNWYRCECAP GFAGPDCRIN INECQSSPCA FGATCVDEIN GYQCICPPGH SGAKCHEVSG RSCITMGRVI LDGAKWDDDC NTCQCLNGRV ACSKVWCGPR PCRLHKSHNE CPSGQSCIPV LDDQCFVRPC TGVGECRSSS LQPVKTKCTS DSYYQDNCAN ITFTFNKEMM SPGLTTEHIC SELRNLNILK NVSAEYSIYI ACEPSLSANN EIHVAISAED IRDDGNPVKE ITDKIIDLVS KRDGNSSLIA AVAEVRVQRR PLKNRTDFLV PLLSSVLTVA WVCCLVTAFY WCVRKRRKPS SHTHSAPEDN TTNNVREQLN QIKNPIEKHG ANTVPIKDYE NKNSKMSKIR THNSEVEEDD MDKHQQKVRF AKQPVYTLVD REEKAPSGTP TKHPNWTNKQ DNRDLESAQS LNRMEYIV
3	Human Jag2	MRAQGRGRLP RRLLLLLALW VQAARPMGYF ELQLSALRNV NGELLSGACC DGDGRTTRAG GCGHDECDTY VRVCLKEYQA KVTPTGPCSY GHGATPVLGG NSFYLPPAGA AGDRARARAR AGGDQDPGLV VIPFQFAWPR SFTLIVEAWD WDNDTTPNEE LLIERVSHAG MINPEDRWKS LHFSGHVAHL ELQIRVRCDE NYYSATCNKF CRPRNDFFGH YTCDQYGNKA CMDGWMGKEC KEAVCKQGCN LLHGGCTVPG ECRCSYGWQG RFCDECVPYP GCVHGSCVEP WQCNCETNWG GLLCDKDLNY CGSHHPCTNG GTCINAEPDQ YRCTCPDGYS GRNCEKAEHA CTSNPCANGG SCHEVPSGFE CHCPSGWSGP TCALDIDECA SNPCAAGGTC VDQVDGFECI CPEQWVGATC QLDANECEGK PCLNAFSCKN LIGGYYCDCI PGWKGINCHI NVNDCRGQCQ HGGTCKDLVN GYQCVCPRGF GGRHCELERD ECASSPCHSG GLCEDLADGF HCHCPQGFSG PLCEVDVDLC EPSPCRNGAR CYNLEGDYYC ACPDDFGGKN CSVPREPCPG GACRVIDGCG SDAGPGMPGT

		AASGVCGPHG RCVSQPGGNF SCICDSGFTG TYCHENIDDC LGQPCRNGGT CIDEVDAFRC FCPSGWEGEL CDTNPNDCLP DPCHSRGRCY DLVNDFYCAC DDGWKGKTCH SREFQCDAYT CSNGGTCYDS GDTFRCACPP GWKGSTCAVA KNSSCLPNPC VNGGTCVGSG ASFSCICRDG WEGRTCTHNT NDCNPLPCYN GGICVDGVNW FRCECAPGFA GPDCRINIDE CQSSPCAYGA TCVDEINGYR CSCPPGRAGP RCQEVIGFGR SCWSRGTPFP HGSSWVEDCN SCRCLDGRRD CSKVWCGWKP CLLAGQPEAL SAQCPLGQRC LEKAPGQCLR PPCEAWGECG AEEPPSTPCL PRSGHLDNNC ARLTLHFNRD HVPQGTTVGA ICSGIRSLPA TRAVARDRLL VLLCDRASSG ASAVEVAVSF SPARDLPDSS LIQGAAHAIV AAITQRGNSS LLLAVTEVKV ETVVTGGSST GLLVPVLCGA FSVLWLACVV LCVWWTRKRR KERERSRLPR EESANNQWAP LNPIRNPIER PGGHKDVLYQ CKNFTPPPRR ADEALPGPAG HAAVREDEED EDLGRGEEDS LEAEKFLSHK FTKDPGRSPG RPAHWASGPK VDNRAVRSIN EARYAGKE
4	Murine Jag2	MRARGWGRLP RRLLLLLVLC VQATRPMGYF ELQLSALRNV NGELLSGACC DGDGRTTRAG GCGRDECDTY VRVCLKEYQA KVTPTGPCSY GYGATPVLGG NSFYLPPAGA AGDRARARSR TGGHQDPGLV VIPFQFAWPR SFTLIVEAWD WDNDTTPDEE LLIERVSHAG MINPEDRWKS LHFSGHVAHL ELQIRVRCDE NYYSATCNKF CRPRNDFFGH YTCDQYGNKA CMDGWMGKEC KEAVCKQGCN LLHGGCTVPG ECRCSYGWQG KFCDECVPYP GCVHGSCVEP WHCDCETNWG GLLCDKDLNY CGSHHPCVNG GTCINAEPDQ YLCACPDGYL GKNCERAEHA CASNPCANGG SCHEVPSGFE CHCPSGWSGP TCALDIDECA SNPCAAGGTC VDQVDGFECI CPEQWVGATC QLDANECEGK PCLNAFSCKN LIGGYYCDCL PGWKGINCQI NINDCHGQCQ HGGTCKDLVN GYQCVCPRGF GGRHCELEYD KCASSPCRRG GICEDLVDGF RCHCPRGLSG LHCEVDMDLC EPSPCLNGAR CYNLEGDYYC ACPEDFGGKN CSVPRDTCPG GACRVIDGCG FEAGSRARGV APSGICGPHG HCVSLPGGNF SCICDSGFTG TYCHENIDDC MGQPCRNGGT CIDEVDSFRC FCPSGWEGEL CDINPNDCLP DPCHSRGRCY DLVNDFYCAC DDGWKGKTCH SREFQCDAYT CSNGGTCYDS GDTFRCACPP GWKGSTCTIA KNSSCVPNPC VNGGTCVGSG DSFSCICRDG WEGRTCTHNT NDCNPLPCYN GGICVDGVNW FRCECAPGFA GPDCRINIDE CQSSPCAYGA TCVDEINGYR CSCPPGRSGP RCQEVVIFTR PCWSRGMSFP HGSSWMEDCN SCRCLDGHRD CSKVWCGWKP CLLSGQPSDP SAQCPPGQQC QEKAVGQCLQ PPCENWGECT AEEPLPPSTP CQPRSSHLDN NCARLTLRFN RDQVPQGTTV GAICSGIRAL PATRAAAHDR LLLLLCDRAS SGASAVEVAM SFSPARDLPD SSLIQSTAHA IVAAITQRGN SSLLLAVTEV KVETVVMGGS STGLLVPVLC SVFSVLWLAC VVICVWWTRK RRKERERSRL PRDESTNNQW APLNPIRNPI ERPGGSGLGT GGHKDILYQC KNFTPPPRRA GEALPGPAGH GAGGEDEEDE ELSRGDGDSP EAEKFISHKF TKDPSCSLGR PACWAPGPKV DNRAVRSTKD VRRAGRE
5	Murine Jag1-DSL-EGF1-4 (mouse Jag1 antigen)	ADLGSQFELE ILSMQNVNGE LQNGNCCGGV RNPGDRKCTR DECDTYFKVC LKEYQSRVTA GGPCSFGSGS TPVIGGNTFN LKASRGNDRN RIVLPFSFAW PRSYTLLVEA WDSSNDTIQP DSIIEKASHS GMINPSRQWQ TLKQNTGIAH FEYQIRVTCD DHYYGFGCNK FCRPRDDFFG HYACDQNGNK TCMEGWMGPD CNKAICRQGC SPKHGSCKLP GDCRCQYGWQ GLYCDKCIPH PGCVHGTCNE PWQCLCETNW GGQLCDKDLN YCGTHQPCLN RGTCSNTGPD KYQCSCPEGY SGPNCEIAEH ACLSDPCHNR GSCKETSSGF ECECSPGWTG PTCSTNIDDE FGLVPRGSGH HHHHH
6	human Jag1-DSL-EGF1-4 (human Jag1 antigen)	QFELEILSMQ NVNGELQNGN CCGGARNPGD RKCTRDECDT YFKVCLKEYQ SRVTAGGPCS FGSGSTPVIG GNTFNLKASR GNDRNRIVLP FSFAWPRSYT LLVEAWDSSN DTVQPDSIIE KASHSGMINP SRQWQTLKQN TGVAHFEYQI RVTCDDYYYG FGCNKFCRPR DDFFGHYACD QNGNKTCMEG WMGPECNRAI CRQGCSPKHG SCKLGDCRCQ YGWQGLYCDK CIPHPGCVHG ICNEPWQCLC ETNWGGQLCD KDLNYCGTHQ PCLNGGTCSN TGPDKYQCSC PEGYSGPNCE IAEHACLSDP CHNRGSCKET SLGFECECSP GWTGPTCSTN IDD
7	murine Jag2-DSL-EGF1-4 (mouse Jag2 antigen)	ADLGSMGYFE LQLSALRNVN GELLSGACCD GDGRTTRAGG CGRDECDTYV RVCLKEYQAK VTPTGPCSYG YGATPVLGGN SFYLPPAGAA GDRARARSRT GGHQDPGLVV IPFQFAWPRS FTLIVEAWDW DNDTTPDEEL LIERVSHAGM INPEDRWKSL HFSGHVAHLE LQIRVRCDEN YYSATCNKFC RPRNDFFGHY TCDQYGNKAC MDGWMGKECK EAVCKQGCNL LHGGCTVPGE CRCSYGWQGK FCDECVPYPG CVHGSCVEPW HCDCETNWGG LLCDKDLNYC GSHHPCVNGG TCINAEPDQY LCACPDGYLG KNCERAEHAC ASNPCANGGS CHEVPSGFEC HCPSGWNGPT CALDIDEEFG LVPRGSGHHH HHH
8	human Jag2-DSL-EGF1-4 (human Jag2 antigen)	ARPMGYFELQ LSALRNVNGE LLSGACCDGD GRTTRAGGCG HDECDTYVRV CLKEYQAKVT PTGPCSYGHG ATPVLGGNSF YLPPAGAAGD RARARARAGG DQDPGLVVIP FQFAWPRSFT LIVEAWDWDN DTTPNEELLI ERVSHAGMIN PEDRWKSLHF SGHVAHLELQ IRVRCDENYY SATCNKFCRP RNDFFGHYTC DQYGNKACMD GWMGKECKEA VCKQGCNLLH GGCTVPGECR CSYGWQGRFC DECVPYPGCV HGSCVEPWQC NCETNWGGLL CDKDLNYCGS HHPCTNGGTC

		INAEPDQYRC TCPDGYSGRN CEKAEHACTS NPCANGGSCH EVPSGFECHC PSGWSGPTCA LDIDEEFGLV PRGSGHHHHH H
9	Antibody A heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG SWFAYWGQGT LVTVSS
10	Antibody A light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIK
11	Antibody A heavy chain hypervariable region 1 (HVR-H1)	GFTFSNYGIH
12	Antibody A HVR-H2	WITPDGGYTDYADSVKG
13	Antibody A HVR-H3	AGSWFAY
14	Antibody A light chain hypervariable region 1 (HVR-L1)	RASQDVSTAVA
15	Antibody A HVR-L2	SASFLYS
16	Antibody A HVR-L3	QQSYTTPPT
17	Antibody A-1 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG SLFAYWGQGT LVTVSS
18	Antibody A-1 light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYTTATTFGQ GTKVEIK
19	Antibody A-1 HVR-H1	GFTFSNYGIH
20	Antibody A-1 HVR-H2	WITPDGGYTDYADSVKG
21	Antibody A-1 HVR-H3	AGSLFAY
22	Antibody A-1 HVR-L1	RASQDVSTAVA
23	Antibody A-1 HVR-L2	SASFLYS
24	Antibody A-1 HVR-L3	QQYYTTATT
25	Antibody A-2 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITGNGGYSDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG SWFAYWGQGT LVTVSS
26	Antibody A-2 light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIK
27	Antibody A-2 HVR-H1	GFTFSNYGIH
28	Antibody A-2 HVR-H2	WITGNGGYSDYADSVKG
29	Antibody A-2	AGSWFAY

	HVR-H3	
30	Antibody A-2 HVR-L1	RASQDVSTAVA
31	Antibody A-2 HVR-L2	SASFLYS
32	Antibody A-2 HVR-L3	QQSYTTPPT
33	Antibody A-1(S101T) heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TLFAYWGQGT LVTVSS
34	Antibody A-1(S101T) light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYTTATTFGQ GTKVEIK
35	Antibody A-1(S101T) HVR-H1	GFTFSNYGIH
36	Antibody A-1(S101T) HVR-H2	WITPDGGYTDYADSVKG
37	Antibody A-1(S101T) HVR-H3	AGTLFAY
38	Antibody A-1(S101T) HVR-L1	RASQDVSTAVA
39	Antibody A-1(S101T) HVR-L2	SASFLYS
40	Antibody A-1(S101T) HVR-L3	QQYYTTATT
41	Antibody B-3 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFT SYDIHWVRQA PGKGLEWVGG ISPADGDTDY ANSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARND YDVRFVGSGM DYWGQGTLVT VSS
42	Antibody B-3 light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SFTAPPTFGQ GTKVEIK
43	Antibody A, A-1, A-2, A-1(S101T), B-3 light chain framework 1 (LC-FR1)	DIQMTQSPSS LSASVGDRVT ITC
44	Antibody A, A-1, A-2, A-1(S101T), B-3 LC-FR2	WYQQKP GKAPKLLIY
45	Antibody A, A-1, A-2, A-1(S101T), B-3 LC-FR3	GVPS RFSGSGSGTD FTLTISSLQP EDFATYYC
46	Antibody A, A-1, A-2, A-1(S101T), B-3 LC-FR4	FGQ GTKVEIK

47	Antibody A, A-1, A-2, A-1(S101T), B-3 heavy chain framework 1 (HC-FR1)	EVQLVESGGG LVQPGGSLRL SCAAS
48	Antibody A, A-1, A-2, A-1(S101T), B-3 HC-FR2	WVRQA PGKGLEWVG
49	Antibody A, A-1, A-2, A-1(S101T), B-3 HC-FR3	RFTI SADTSKNTAY LQMNSLRAED TAVYYCAR
50	Antibody A, A-1, A-2, A-1(S101T), B-3 HC-FR4	WGQGT LVTVSS
51	Antibody A-1(S101T) IgG1 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TLFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
52	Antibody A-1(S101T) IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TLFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
53	Antibody A-1(S101T) light chain; antibody A-1 light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYTTATTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
54	Antibody A-1(S101X) heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XLFAYWGQGT LVTVSS X is any amino acid other than S.
55	Antibody A-1(S101X) HVR-H3	AGXLFAY X is any amino acid other than S.
56	Antibody A-1(S101X) IgG1 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XLFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK X is any amino acid other than S.
57	Antibody A-1(S101X) IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XLFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL

		PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK X is any amino acid other than S.
58	Antibody A(S101X) heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XWFAYWGQGT LVTVSS X is any amino acid other than S.
59	Antibody A(S101X) HVR-H3; Antibody A-2(S101X) HVR-H3	AGXWFAY X is any amino acid other than S.
62	Antibody A-2(S101X) heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITGNGGYSDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XWFAYWGQGT LVTVSS X is any amino acid other than S.
63	Antibody A-1 IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG SLFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
64	Antibody A(S101T) HVR-H3; Antibody A-2(S101T) HVR-H3	AGTWFAY
65	Antibody A(S101T) heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TWFAYWGQGT LVTVSS
66	Antibody A-2(S101T) heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITGNGGYSDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TWFAYWGQGT LVTVSS
67	Antibody A(S101X) IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XWFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK X is any amino acid other than S.
68	Antibody A-2(S101X) IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITGNGGYSDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG XWFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK X is any amino acid other than S.

69	Antibody A(S101T) IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TWFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
70	Antibody A-2(S101T) IgG1 heavy chain N297G	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITGNGGYSDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TWFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYGSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
71	HVR-H2 consensus	WIT(X1)(X2)GGY(X3)DYADSVKG X1 is P or G; X2 is D or N; X3 is T or S
77	HVR-H3 consensus	AG(X1)(X2)FAY X1 is S or T; X2 is W or L
72	HVR-H3 consensus (101X)	AG(X1)(X2)FAY X1 is any amino acid other than S; X2 is W or L
73	HVR-H3 consensus (101T)	AGT(X1)FAY X1 is W or L
74	HVR-L3 consensus	QQ(X1)YTT(X2)(X3)T X1 is S or Y; X2 is P or A; X3 is P or T
75	Antibody A light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
76	Antibody A-2 light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
78	Antibody A, A-1, A-1(S101T), A-2 alternative HVR-H1	NYGIH
79	Antibody A(S101T) IgG1 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TWFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
80	Antibody A-2(S101T) IgG1 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITGNGGYSDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG TWFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL

		PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
81	Antibody A-1 IgG1 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYGIHWVRQA PGKGLEWVGW ITPDGGYTDY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARAG SLFAYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to human Jagged1, wherein the antibody comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 35 or 78; an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28 or 36; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55 or 59, wherein X is any amino acid other than S or H; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16 or 40.

2. The isolated antibody of claim 1, wherein the antibody comprises:

a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 55; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40; or

b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; or

c) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 59; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

3. The isolated antibody of any one of the preceding claims, wherein the antibody comprises a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 54, 58, or 62.

4. The isolated antibody of any one of the preceding claims, wherein the antibody comprises a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 10, 26, or 34.

5. The isolated antibody of any one of the preceding claims, wherein the antibody comprises:

a) a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 54 and a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 34; or

b) a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 58 and a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 10; or

c) a VH sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 62 and a VL sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 26.

6. An isolated antibody that binds human Jagged1, comprising:

a) a VH sequence of SEQ ID NO: 54, wherein X is any amino acid other than S or H, and a VL sequence of SEQ ID NO: 34; or

b) a VH sequence of SEQ ID NO: 58, wherein X is any amino acid other than S or H, and a VL sequence of SEQ ID NO: 10; or

c) a VH sequence of SEQ ID NO: 62, wherein X is any amino acid other than S or H, and a VL sequence of SEQ ID NO: 26.

7. The isolated antibody of claim 6, wherein the antibody comprises a VH sequence of SEQ ID NO: 54 and a VL sequence of SEQ ID NO: 34.

8. The isolated antibody of claim 7, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 56 and the light chain comprises the amino acid sequence of SEQ ID NO: 53.

9. The isolated antibody of claim 7, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 57 and the light chain comprises the amino acid sequence of SEQ ID NO: 53.

10. The isolated antibody of any one of the preceding claims, wherein X is selected from A, D, E, G, I, K, L, N, Q, R T, and V.

11. The isolated antibody of any one of the preceding claims, wherein X is T.

12. An isolated antibody that binds human Jagged1, comprising:

a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid

sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 40; or

b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; or

c) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 78; HVR-H2 comprising the amino acid sequence of SEQ ID NO: 28; HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38; HVR-L2 comprising the amino acid sequence of SEQ ID NO: 39; and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16.

13. The isolated antibody of claim 12, wherein the antibody comprises:

a) a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 34; or

b) a VH sequence of SEQ ID NO: 65 and a VL sequence of SEQ ID NO: 10; or

c) a VH sequence of SEQ ID NO: 66 and a VL sequence of SEQ ID NO: 26.

14. The isolated antibody of claim 13, wherein the antibody comprises a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 34.

15. The antibody of any one of the preceding claims, which is a full length IgG1 antibody.

16. The antibody of claim 15, wherein the antibody substantially lacks effector function.

17. The antibody of claim 15, wherein the heavy chain comprises a N297G or N297A mutation.

18. The isolated antibody of claim 14, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 51 and the light chain comprises the amino acid sequence of SEQ ID NO: 53.

19. The isolated antibody of claim 14, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 52 and the light chain comprises the amino acid sequence of SEQ ID NO: 53.

20. The antibody of claim 13, wherein the antibody comprises:

a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 75; or

b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 70 and a light chain comprising the amino acid sequence of SEQ ID NO: 76; or

c) a heavy chain comprising the amino acid sequence of SEQ ID NO: 79 and a light chain comprising the amino acid sequence of SEQ ID NO: 75; or

d) a heavy chain comprising the amino acid sequence of SEQ ID NO: 80 and a light chain comprising the amino acid sequence of SEQ ID NO: 76.

21. The antibody of any one of the preceding claims, wherein the antibody is an antagonist of Jagged1-mediated signaling.

22. The antibody of any one of the preceding claims, wherein the antibody does not bind human Jagged2.

23. The antibody of any one of the preceding claims, wherein the antibody does not bind human DLL4.

24. The antibody of any one of the preceding claims, wherein the antibody does not bind human DLL1.

25. The antibody of any one of the preceding claims, wherein the antibody does not bind murine Jagged2, murine DLL4, and/or murine DLL1.

26. The antibody of any one of the preceding claims, wherein the antibody reduces tumor growth in a mouse xenograft model without causing weight loss.

27. The antibody of claim 26, wherein the mouse xenograft model is a liver cancer xenograft model.

28. The antibody of claim 26 or claim 27, wherein tumor growth is reduced by at least 50, at least 60, at least 70, at least 80, or at least 90 AUC/day TGI%.

29. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

30. A host cell comprising the isolated nucleic acid of claim 29.

31. A method of producing an antibody comprising culturing the host cell of claim 30 so that the antibody is produced.

32. An immunoconjugate comprising the antibody of any one of claims 1 to 28 and a cytotoxic agent.

33. A pharmaceutical formulation comprising the antibody of any one of the claims 1 to 28 and a pharmaceutically acceptable carrier.

34. A pharmaceutical formulation comprising the immunoconjugate of claim 32 and a pharmaceutically acceptable carrier.

35. The antibody of any one of claims 1 to 28 or the immunoconjugate of claim 32 for use as a medicament.

36. The antibody of any one of claims 1 to 28 or the immunoconjugate of claim 32 for use in treating cancer.

37. The antibody of any one of claims 1 to 28 or the immunoconjugate of claim 32 for reducing cancer cell growth.

38. Use of the antibody of any one of claims 1 to 28 or the immunoconjugate of claim 33 in the manufacture of a medicament for treating cancer.

39. The use of claim 38, wherein the medicament is for reducing cancer cell growth.

40. Use of the antibody of any one of claims 1 to 28 or the immunoconjugate of claim 33 in the manufacture of a medicament for treating diseases associated with goblet cell metaplasia (e.g., in lung) and/or excess mucus.

41. The use of claim 40, wherein the medicament is for treating a disease associated with goblet cell metaplasia.

42. The use of claim 41, wherein the medicament is for treating asthma, cystic fibrosis, chronic obstructive pulmonary disease, or Barrett's esophagus.

43. The use of claim 38, wherein the cancer is selected from breast cancer, lung cancer, brain cancer, cervical cancer, colon cancer, liver cancer, bile duct cancer, pancreatic cancer, skin cancer, B-cell malignancies, and T-cell malignancies.

44. The isolated antibody of any one of claims 1 to 28 conjugated to a label.

45. The isolated antibody of claim 44, wherein the label is a positron emitter.

46. The isolated antibody of claim 45, wherein the positron emitter is ^{89}Zr.

47. A method of detecting human Jagged1 in a biological sample comprising contacting the biological sample with the antibody of any one of claims 1 to 28 and 44 to 46 under conditions permissive for binding of the antibody to a naturally occurring human Jagged1, and detecting whether a complex is formed between the antibody and a naturally occurring human Jagged1 in the biological sample.

48. The method of claim 47, wherein the biological sample is selected from breast cancer, lung cancer, brain cancer, cervical cancer, colon cancer, liver cancer, bile duct cancer, pancreatic cancer, skin cancer, B-cell malignancies, and T-cell malignancies.