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(54) Title: HUMAN ANTIBODIES THAT BIND RET AND METHODS OF USE THEREOF

(57) Abstract: The present invention provides fully human antibodies that bind to the RET receptor tyrosine kinase, compositions comprising the antibodies and methods of use. The antibodies of the invention are useful for treating diseases, disorders, or conditions associated with expression, activation, or signaling of the RET receptor tyrosine kinase gene, or a rearranged form thereof, including cancerous conditions and the pain associated with the cancer, or for alleviating the pain associated with other conditions attributed to, at least in part, by expression, activation or signaling of RET. The antibodies specific for RET may be useful for slowing tumor cell growth or tumor cell proliferation and also for alleviating the pain associated with the cancer and other conditions. The antibodies may also be useful for diagnosis of a disease, disorder or condition associated with RET activation or signaling.

HUMAN ANTIBODIES THAT BIND RET AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind to RET (rearranged during transfection) receptor tyrosine kinase and compositions comprising these antibodies and therapeutic methods of using these antibodies.

SEQUENCE LISTING

[0002] An official copy of the sequence listing is submitted concurrently with the specification electronically via EFS-Web as an ASCII formatted sequence listing with a file name of 10582WO01_SeqList_ST25.TXT, a creation date of April 09, 2020, and a size of about 168 kilobytes. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

STATEMENT OF RELATED ART

[0003] The RET (REarranged during Transfection) receptor tyrosine kinase is expressed during development in a variety of tissues, including the peripheral and central nervous systems and the kidney (Arighi, E. *et al*, (2005), Cytokine Growth Factor Rev 16:441-467; Borrello, MG, *et al,* (2013), Expert Opin. Ther. Targets, 17(4):403-419; Golden, JP, *et al.* (1998), J. Comp. Neurol. 398:139-150; Golden, JP, *et al.* (1999), Exp. Neurol. 158:504-528). It is also expressed in neural crest-derived cells and regulates cell proliferation, migration and survival (Coulpier, M. *et al,* (2002), J Biol Chem, 277:1991-1999; Golden, JP, *et al.* (1998), J. Comp. Neurol. 398:139-150; Golden, JP, *et al.* (1999), Exp. Neurol. 158:504-528). RET knockout mice exhibit renal agenesis and lack enteric neurons in the digestive tract. A very similar phenotype is observed in both GFRα1 and GDNF knockout mice, confirming a major role for GDNF/GFRα1 in the activation of RET signaling during development.

[0004] RET is the signaling receptor for ligands of the glial-derived neurotrophic factor (GDNF) family, which comprises GDNF, artemin, neurturin and persephin. GDNF family ligands interact with and activate RET only in the presence of one of four GPI-linked co-receptors, known as GDNF family □ receptors GFR□(1-4) (Baloh, RH, *et al.* (2000), Curr Opin Neurobiol 10:103-110; Borrello, MG, *et al* (2013), Expert Opin. Ther. Targets, 17(4):403-419). The primary ligands for the co-receptors GFRα1, GFRα2, GFRα3 and GFRα4 are GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN), respectively, although cross-talk between ligands and co-receptors have been observed *in vitro*.

[0005] The role of RET as a driver of tumorigenesis has been established by the activating mutations that are frequently observed in multiple endocrine neoplasia syndromes MEN2A and MEN2B and in familial medullary thyroid cancer (Mulligan, LM, *et al,* (1994), Nat. Genet.

6:70-74). Furthermore, a large percentage of sporadic medullary thyroid cancers contain somatic activating mutations in RET (Fusco, A. *et al,* (1987), Nature 328:170-172; Grieco, M. *et al*, (1990), Cell 60:557-563). These mutations can occur in the kinase domain or in the extracellular domain, where they result in unpaired cysteines that are thought to promote ligand-independent RET dimerization and activation. Thus, the tumorigenic potential of RET in humans has been clearly established via genetic studies.

[0006] In addition to its role in endocrine cancers, recent work has identified RET as a potential therapeutic target in breast cancer. RET and GFRα1 are expressed in breast cancer cell lines and in primary human breast cancer samples. Interestingly, the expression of RET and GFRα1 can be induced by estrogen *in vitro*. Consistent with this observation, RET and GFRα1 are preferentially expressed in the estrogen receptor-positive subset of breast cancers. Furthermore, GDNF-induced RET signaling promotes anchorage-independent growth of estrogen receptor-positive breast cancer cells and potentiates the effects of estrogen on the growth and survival of these cells, indicating a functional cooperation between these two pathways. Thus, RET signaling appears to be an important driver of the oncogenic phenotype in breast cancer cells (Wang, C. *et al* (2012), Breast Cancer Res Treat 133(2):487-500; Stine, ZE, *et al,* (2011), Human Molecular Genetics 20(19):3746-3756).

[0007] Activation of RET is initiated by binding of GDNF to GFRα1. The GDNF/GFRα1 complex then binds to RET, resulting in receptor dimerization and activation. There are several small molecules that have the ability to inhibit RET, including an agent (vandetanib) that has exhibited activity in medullary thyroid cancer patients (See Wells, SA *et al,* (2012), J Clin Oncol 30:134-141; Leboulleux, S. et al, (2012), Lancet Oncol 13:897-905). Other small molecules have been identified that bind to and inhibit RET signaling (Borrello, MG, *et al,* (2013), Expert Opin. Ther. Targets, 17(4):403-419). Unfortunately, due to their lack of specificity, certain of these compounds have shown adverse events in clinical trials that have precluded further development.

[0008] To date, there have been no reports of therapeutic anti-RET monoclonal antibodies for use in a clinical setting to treat tumors expressing RET. The studies reported herein describe the generation of fully human monoclonal antibodies that bind to RET and prevent the interaction of RET with one or more GDNF family members complexed with their corresponding co-receptors.

[0009] The domain structure of the RET extracellular region is shown in Figure 1 and consists of four cadherin-like domains followed by a cysteine-rich domain (See Borrello, MG, *et al* (2013), Expert Opin. Ther. Targets, 17(4):403-419). While the structure of the active RET signaling complex has not been solved, it appears that the GDNF/GFRα1 complex contacts the RET extracellular domain at multiple sites, including the fourth cadherin-like

domain and the cysteine-rich domain. Thus, antibodies directed against multiple domains of RET could potentially inhibit signaling. Antibodies to RET have been described and can be found in US6861509 and US2009/0136502.

[0010] However, given the role that RET plays in tumor cell growth and proliferation, and given the fact that there are few agents approved to target this molecule, a need still exists for inhibitors of RET, for example, human antibodies that specifically bind to RET, which are highly potent and which produce no adverse effects that would preclude approval for clinical use. It is an object of the invention to go at least some way to addressing this need and/or at least to provide the public with a useful choice.

BRIEF SUMMARY OF THE INVENTION

[0010a] In a first aspect, the invention provides an isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET (Rearranged during Transfection) receptor tyrosine kinase, wherein the antibody or antigen-binding fragment therefore comprises a HCVR comprising three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and a LCVR comprising three light chain CDRs (LCDR1, LCDR2 and LCDR3), and:

(a) HCDR1 comprises the amino acid sequence of SEQ ID NO:4, HCDR2 comprises the amino acid sequence of SEQ ID NO:6, HCDR3 comprises the amino acid sequence of SEQ ID NO:8, LCDR1 comprises the amino acid sequence of SEQ ID NO:12, LCDR2 comprises the amino acid sequence of SEQ ID NO:14, and LCDR3 comprises the amino acid sequence of SEQ ID NO:16; or

(b) HCDR1 comprises the amino acid sequence of SEQ ID NO:20, HCDR2 comprises the amino acid sequence of SEQ ID NO:22, HCDR3 comprises the amino acid sequence of SEQ ID NO:24, LCDR1 comprises the amino acid sequence of SEQ ID NO:28, LCDR2 comprises the amino acid sequence of SEQ ID NO:30, and LCDR3 comprises the amino acid sequence of SEQ ID NO:32; or

(c) HCDR1 comprises the amino acid sequence of SEQ ID NO:36, HCDR2 comprises the amino acid sequence of SEQ ID NO:38, HCDR3 comprises the amino acid sequence of SEQ ID NO:40, LCDR1 comprises the amino acid sequence of SEQ ID NO:44, LCDR2 comprises the amino acid sequence of SEQ ID NO:46, and LCDR3 comprises the amino acid sequence of SEQ ID NO:48; or

(d) HCDR1 comprises the amino acid sequence of SEQ ID NO:52, HCDR2 comprises the amino acid sequence of SEQ ID NO:54, HCDR3 comprises the amino acid sequence of SEQ ID NO:56, LCDR1 comprises the amino acid sequence of SEQ ID NO:60, LCDR2 comprises the amino acid sequence of SEQ ID NO:62, and LCDR3 comprises the amino acid sequence of SEQ ID NO:64; or

(e) HCDR1 comprises the amino acid sequence of SEQ ID NO:68, HCDR2 comprises the amino acid sequence of SEQ ID NO:70, HCDR3 comprises the amino acid sequence of SEQ ID NO:72, LCDR1 comprises the amino acid sequence of SEQ ID NO:76, LCDR2 comprises the amino acid sequence of SEQ ID NO:78, and LCDR3 comprises the amino acid sequence of SEQ ID NO:80; or

(f) HCDR1 comprises the amino acid sequence of SEQ ID NO:84, HCDR2 comprises the amino acid sequence of SEQ ID NO:86, HCDR3 comprises the amino acid sequence of SEQ ID NO:88, LCDR1 comprises the amino acid sequence of SEQ ID NO:92, LCDR2 comprises the amino acid sequence of SEQ ID NO:94, and LCDR3 comprises the amino acid sequence of SEQ ID NO:96; or

(g) HCDR1 comprises the amino acid sequence of SEQ ID NO:100, HCDR2 comprises the amino acid sequence of SEQ ID NO:102, HCDR3 comprises the amino acid sequence of SEQ ID NO:104, LCDR1 comprises the amino acid sequence of SEQ ID NO:108, LCDR2 comprises the amino acid sequence of SEQ ID NO:110, and LCDR3 comprises the amino acid sequence of SEQ ID NO:112; or

(h) HCDR1 comprises the amino acid sequence of SEQ ID NO:116, HCDR2 comprises the amino acid sequence of SEQ ID NO:118, HCDR3 comprises the amino acid sequence of SEQ ID NO:120, LCDR1 comprises the amino acid sequence of SEQ ID NO:124, LCDR2 comprises the amino acid sequence of SEQ ID NO:126, and LCDR3 comprises the amino acid sequence of SEQ ID NO:128; or

(i) HCDR1 comprises the amino acid sequence of SEQ ID NO:132, HCDR2 comprises the amino acid sequence of SEQ ID NO:134, HCDR3 comprises the amino acid sequence of SEQ ID NO:136, LCDR1 comprises the amino acid sequence of SEQ ID NO:140, LCDR2 comprises the amino acid sequence of SEQ ID NO:142, and LCDR3 comprises the amino acid sequence of SEQ ID NO:144; or

(j) HCDR1 comprises the amino acid sequence of SEQ ID NO:148, HCDR2 comprises the amino acid sequence of SEQ ID NO:150, HCDR3 comprises the amino acid sequence of SEQ ID NO:152, LCDR1 comprises the amino acid sequence of SEQ ID NO:156, LCDR2 comprises the amino acid sequence of SEQ ID NO:158, and LCDR3 comprises the amino acid sequence of SEQ ID NO:160; or

(k) HCDR1 comprises the amino acid sequence of SEQ ID NO:164, HCDR2 comprises the amino acid sequence of SEQ ID NO:166, HCDR3 comprises the amino acid sequence of SEQ ID NO:168, LCDR1 comprises the amino acid sequence of SEQ ID NO:172, LCDR2 comprises the amino acid sequence of SEQ ID NO:174, and LCDR3 comprises the amino acid sequence of SEQ ID NO:176; or

(l) HCDR1 comprises the amino acid sequence of SEQ ID NO:180, HCDR2 comprises the amino acid sequence of SEQ ID NO:182, HCDR3 comprises the amino

acid sequence of SEQ ID NO:184, LCDR1 comprises the amino acid sequence of SEQ ID NO:188, LCDR2 comprises the amino acid sequence of SEQ ID NO:190, and LCDR3 comprises the amino acid sequence of SEQ ID NO:192; or

(m) HCDR1 comprises the amino acid sequence of SEQ ID NO:196, HCDR2 comprises the amino acid sequence of SEQ ID NO:198, HCDR3 comprises the amino acid sequence of SEQ ID NO:200, LCDR1 comprises the amino acid sequence of SEQ ID NO:204, LCDR2 comprises the amino acid sequence of SEQ ID NO:206, and LCDR3 comprises the amino acid sequence of SEQ ID NO:208; or

(n) HCDR1 comprises the amino acid sequence of SEQ ID NO:212, HCDR2 comprises the amino acid sequence of SEQ ID NO:214, HCDR3 comprises the amino acid sequence of SEQ ID NO:216, LCDR1 comprises the amino acid sequence of SEQ ID NO:220, LCDR2 comprises the amino acid sequence of SEQ ID NO:222, and LCDR3 comprises the amino acid sequence of SEQ ID NO:224; or

(o) HCDR1 comprises the amino acid sequence of SEQ ID NO:228, HCDR2 comprises the amino acid sequence of SEQ ID NO:230, HCDR3 comprises the amino acid sequence of SEQ ID NO:232, LCDR1 comprises the amino acid sequence of SEQ ID NO:236, LCDR2 comprises the amino acid sequence of SEQ ID NO:238, and LCDR3 comprises the amino acid sequence of SEQ ID NO:240; or

(p) HCDR1 comprises the amino acid sequence of SEQ ID NO:244, HCDR2 comprises the amino acid sequence of SEQ ID NO:246, HCDR3 comprises the amino acid sequence of SEQ ID NO:248, LCDR1 comprises the amino acid sequence of SEQ ID NO:252, LCDR2 comprises the amino acid sequence of SEQ ID NO:254, and LCDR3 comprises the amino acid sequence of SEQ ID NO:256; or

(q) HCDR1 comprises the amino acid sequence of SEQ ID NO:260, HCDR2 comprises the amino acid sequence of SEQ ID NO:262, HCDR3 comprises the amino acid sequence of SEQ ID NO:264, LCDR1 comprises the amino acid sequence of SEQ ID NO:268, LCDR2 comprises the amino acid sequence of SEQ ID NO:270, and LCDR3 comprises the amino acid sequence of SEQ ID NO:272; or

(r) HCDR1 comprises the amino acid sequence of SEQ ID NO:276, HCDR2 comprises the amino acid sequence of SEQ ID NO:278, HCDR3 comprises the amino acid sequence of SEQ ID NO:280, LCDR1 comprises the amino acid sequence of SEQ ID NO:284, LCDR2 comprises the amino acid sequence of SEQ ID NO:286, and LCDR3 comprises the amino acid sequence of SEQ ID NO:288; or

(s) HCDR1 comprises the amino acid sequence of SEQ ID NO:292, HCDR2 comprises the amino acid sequence of SEQ ID NO:294, HCDR3 comprises the amino acid sequence of SEQ ID NO:296, LCDR1 comprises the amino acid sequence of SEQ ID

NO:300, LCDR2 comprises the amino acid sequence of SEQ ID NO:302, and LCDR3 comprises the amino acid sequence of SEQ ID NO:304.

[0010b] In a second aspect, the invention provides an isolated nucleic acid molecule encoding the antibody or antigen-binding fragment according to the first aspect of the invention.

[0010c] In a third aspect, the invention provides an expression vector comprising the nucleic acid molecule according to the second aspect of the invention.

[0010d] In a fourth aspect, the invention provides a pharmaceutical composition comprising any one or more of the antibodies that specifically bind RET, or an antigen-binding fragment thereof according to the first aspect of the invention, and a pharmaceutically acceptable carrier or diluent.

[0010e] In a fifth aspect, the invention relates to a method of treating a disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene or the pain associated with the disorder or condition, the method comprising administering one or more antibodies or antigen-binding fragments thereof according to the first aspect of the invention, or the pharmaceutical composition according to the fourth aspect of the invention to a patient in need thereof.

[0010f] In a sixth aspect, the invention relates to use of one or more antibodies or antigen-binding fragments thereof according to the first aspect of the invention, or the pharmaceutical composition according to the fourth aspect of the invention, in the manufacture of a medicament for treating a disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene or the pain associated with the disorder or condition, comprising administering the medicament to a patient in need thereof.

[0010g] In a seventh aspect, the invention relates to a method for inhibiting tumor growth or tumor cell proliferation, wherein the tumor or tumor cell expresses RET, the method comprising administering one or more antibodies or antigen-binding fragments thereof according to the first aspect of the invention, or the pharmaceutical composition according to the fourth aspect of the invention to a patient in need thereof.

[0010h] In an eighth aspect, the invention relates to use of one or more antibodies or antigen-binding fragments thereof according to the first aspect of the invention, or the pharmaceutical composition according to the fourth aspect of the invention, in the manufacture of a medicament for inhibiting tumor growth or tumor cell proliferation, wherein the tumor or tumor cell expresses RET, comprising administering the medicament to a patient in need thereof.

[0010i] In a ninth aspect, the invention relates to a method of down-modulating RET function, the method comprising administering one or more antibodies or antigen-binding fragments thereof according to the first aspect of the invention, or the pharmaceutical composition according to the fourth aspect of the invention, to a patient in need thereof.

[0010j] In a tenth aspect, the invention relates to use of one or more antibodies or antigen-binding fragments thereof according to the first aspect of the invention, or the pharmaceutical composition according to the fourth aspect of the invention, in the manufacture of a medicament for down-modulating RET function, comprising administering the medicament to a patient in need thereof.

[0010k] The invention is defined in the claims. However, the disclosure preceding the claims may refer to additional methods and other subject matter outside the scope of the present claims. This disclosure is retained for technical purposes.

[0011] The invention provides fully human monoclonal antibodies (mAbs) or antigen-binding fragments thereof that bind specifically to RET and inhibit the binding or interaction of RET with one or more GDNF family member ligands (GDNF, neurturin, artemin, and persephin) complexed with their corresponding co-receptors (GFRα1, GFRα2, GFRα3, and GFRα4, respectively). In one embodiment, the human anti-RET antibodies described herein prevent the interaction of RET with the GDNF/GFRα1 complex. In a related embodiment, the human anti-RET antibodies described herein prevent the interaction of RET with the artemin/GFRα3 complex. In a related embodiment, the human anti-RET antibodies described herein prevent the interaction of RET with the neurturin/GFRα2 complex, or the persephin/GFRα4 complex.

[0012] The studies described herein have demonstrated that these antibodies are capable of modulating ligand dependent RET signaling. In certain embodiments, antibodies have been identified that antagonize ligand dependent RET signaling.

[0013] Given the role that RET plays in the development of multiple endocrine neoplastic syndromes, as well as in other cancers, the antibodies of the invention that antagonize/inhibit the signaling activity of RET may be used in the treatment of these neoplastic syndromes and cancers to inhibit the growth/proliferation of a tumor cell. Examples of cancerous conditions that may be treated using the RET antagonistic antibodies of the invention include, but are not limited to, thyroid tumors, lung tumors, pancreatic tumors, skin cancers, breast cancer and leukemias. A thyroid tumor that may be treatable using an antagonistic anti-RET antibody of the invention may include a papillary thyroid carcinoma (PTC) or a medullary thyroid carcinoma (MTC). The medullary thyroid carcinoma that may be treatable using an antagonistic anti-RET antibody of the invention may include a hereditary MTC selected from the group consisting of MEN2A, MEN2B and familial medullary thyroid carcinoma (FMTC) syndrome, or the medullary thyroid carcinoma

may be a sporadic MTC. The antibodies of the invention may also be used to treat pain associated with these cancerous conditions, as well as pain associated with other diseases, disorders, or conditions in which RET activity or signaling may play a role.

[0014] The antibodies may be used as stand-alone therapy or may be used in conjunction with a second agent useful for treating a disease or disorder associated with RET expression. In certain embodiments, the antibodies may be given therapeutically in conjunction with a second agent to treat the disease or disorder, or to ameliorate at least one symptom associated with the disease or disorder. If the antibody inhibits RET activity or signaling and is under consideration for use in treating for example, a cancerous condition, the second agent may be a chemotherapeutic agent, or a bone marrow restorative agent, or may be radiation therapy to treat the tumor. If the antibody inhibits RET activity or signaling and is under consideration for treating pain associated with a certain condition, and if the treatment warrants the use of a second pain reducing agent, the second agent may be any agent that is also useful for alleviating pain associated with that condition, such as aspirin or another NSAID, morphine, steroids (*e.g.*, prednisone), a nerve growth factor (NGF) inhibitor (*e.g.*, a small molecule NGF antagonist or an anti-NGF antibody), an anti-$Na_v1.7$ antibody, or small molecule inhibitor of $Na_v1.7$, a $Na_v1.8$ antagonist (*e.g.*, anti-$Na_v1.8$ antibody or small molecule inhibitor of $Na_v1.8$), a $Na_v1.9$ antagonist (*e.g.*, anti-$Na_v1.9$ antibody or small molecule inhibitor of $Na_v1.9$), a cytokine inhibitor (*e.g.*, an interleukin-1 (IL-1) inhibitor (such as rilonacept ("IL-1 trap") or anakinra (KINERET®), a small molecule IL-1 antagonist, or an anti-IL-1 antibody; an IL-18 inhibitor (such as a small molecule IL-18 antagonist or an anti-IL-18 antibody); an IL-6 or IL-6R inhibitor (such as a small molecule IL-6 antagonist, an anti-IL-6 antibody or an anti-IL-6 receptor antibody), inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, or an opioid.

[0015] The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, *e.g.,* to eliminate residual effector functions (Reddy *et al.,* (2000), J. Immunol. 164:1925-1933).

[0016] Accordingly, in a first aspect, the invention provides an isolated human monoclonal antibody or an antigen-binding fragment thereof that specifically binds to RET (REarranged during Transfection) receptor tyrosine kinase, wherein the antibody has one or more of the following characteristics:

(a) is a fully human antibody;

(b) exhibits a K_D ranging from about 1.0 X 10^{-7} M to about 1.0 X 10^{-12} M as measured by Surface Plasmon Resonance;

(c) inhibits or blocks the binding, or interaction of RET with one or more GDNF family member ligands (GDNF, neurturin, artemin, and persephin) complexed with

their corresponding co-receptors (GFRα1, GFRα2, GFRα3, and GFRα4, respectively);

(d) inhibits RET signaling mediated by one or more GDNF family member ligands selected from GDNF, neurturin, artemin, and persephin;

(e) enhances RET internalization/degradation following binding of the antibody to the RET receptor;

(f) comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290; or

(g) comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298.

[0017] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET blocks the binding of human RET to the GDNF:GFRα1 co-complex with an IC_{50} value ranging from about 100 pM to about 7.0 nM.

[0018] In a related embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET blocks the binding of human RET to the GDNF:GFRα1 co-complex with an IC_{50} value ranging from about 250 pM to about 5.2 nM.

[0019] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET blocks the binding of human RET to the GDNF:GFRα1 co-complex by about 40% to about 100%.

[0020] In a related embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET blocks the binding of human RET to the GDNF:GFRα1 co-complex by about 57% to about 97%.

[0021] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits GDNF-mediated RET signaling with an IC_{50} value ranging from about 50 pM to greater than 100 nM.

[0022] In a related embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits GDNF-mediated RET signaling with an IC_{50} value ranging from about 143 pM to greater than 100 nM.

[0023] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits GDNF-mediated RET signaling by about 40% to about 100%.

[0024] In a related embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits GDNF-mediated RET signaling by about 60% to about 100%.

[0025] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits artemin-mediated RET signaling with an IC_{50} value ranging from about 100 pM to about 500 nM.

[0026] In a related embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits artemin-mediated RET signaling with an IC_{50} value ranging from about 250 pM to about 341 nM.

[0027] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET inhibits artemin-mediated RET signaling by about 57% to about 100%.

[0028] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET, comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290.

[0029] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET, comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs : 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298.

[0030] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET, comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290; and a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs : 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298.

[0031] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282 and 290/298.

[0032] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET comprises a HCVR comprising the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a HCVR amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290; and a LCVR comprising the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a LCVR amino acid sequence selected from the group consisting of SEQ ID NOs : 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298.

[0033] Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, (1997), *J. Mol. Biol. 273*:927-948; and Martin *et al.*, (1989), *Proc. Natl. Acad. Sci. USA 86*:9268-9272. Public databases are also available for identifying CDR sequences within an antibody.

[0034] In one embodiment, the isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276 and 292**;**

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278 and 294;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280 and 296;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284 and 300;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286 and 302; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288 and 304.

[0035] In one embodiment, the invention provides for an isolated antibody or antigen-binding fragment thereof that specifically binds to RET, which competes for specific binding to RET with an antibody or antigen-binding fragment comprising heavy and light chain sequence pairs selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282 and 290/298.

[0036] In one embodiment, the invention provides for an isolated antibody or antigen-binding fragment thereof that specifically binds to RET, which binds the same epitope on RET that is recognized by an antibody comprising heavy and light chain sequence pairs selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282 and 290/298.

[0037] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280 and 296, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288 and 304, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276 and 292, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278 and 294, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (vi) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284 and 300, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (vii) and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286 and 302, or a substantially similar sequence

thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (viii) exhibits a K_D ranging from about 1 X 10^{-7} M to about 1 X 10^{-12} M; (ix) is capable of blocking the binding of human RET to the GDNF:GFRα1 co-complex with an IC_{50} value of less than about 5.2 nM; or (x) demonstrates the ability to inhibit ligand dependent RET signaling by about 60 to 100% with an IC_{50} value ranging from about 143 pM to greater than 100 nM.

[0038] In a second aspect, the invention provides nucleic acid molecules encoding antibodies or fragments thereof that specifically bind to RET. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0039] In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273 and 289 or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0040] In one embodiment, the antibody or fragment thereof further comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281 and 297, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0041] In one embodiment, the invention also provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167, 183, 199, 215, 231, 247, 263, 279 and 295, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287 and 303, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0042] In one embodiment, the invention provides an antibody or fragment thereof further comprising a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275 and 291, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 5, 21, 37, 53, 69, 85,

101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277 and 293, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283 and 299, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285 and 301, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0043] In a third aspect, the invention features a human antibody or antigen-binding fragment specific for RET comprising a HCVR encoded by nucleotide sequence segments derived from V_H, D_H and J_H germline sequences, and a LCVR encoded by nucleotide sequence segments derived from V_K and J_K germline sequences.

[0044] The invention encompasses antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or e.g., removal of a fucose moiety to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0045] In a fourth aspect, the invention provides a pharmaceutical composition comprising at least one isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to RET and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising two fully human monoclonal antibodies or antigen-binding fragments thereof, which either bind to the same epitope or bind to two different epitopes on RET and a pharmaceutically acceptable carrier or diluent. It is to be understood that any combination of antibodies as described herein may be used in a pharmaceutical composition to achieve the desired results in the patient population in need of such therapy. For example, two antibodies that recognize and/or bind RET may be used in a composition.

[0046] In one embodiment, the composition comprises an antibody that binds RET and has a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282 and 290/298.

[0047] In one embodiment, the pharmaceutical composition comprises at least one antibody that binds RET, wherein the antibody comprises the three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) amino acid sequences selected from the group consisting of

SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290; and the three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) amino acid sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298.

[0048] In one embodiment, the antibodies of the invention, or compositions containing one or more antibodies of the invention may be used to inhibit at least one activity or function associated with RET expressed on a cell. In one embodiment, the cell may be a tumor cell. In one embodiment, the activity may be cell signaling.

[0049] In one embodiment, the invention features a composition, which is a combination of an antibody or antigen-binding fragment of an antibody of the invention, and a second therapeutic agent.

[0050] The second therapeutic agent may be a small molecule drug, a protein/polypeptide, an antibody, a nucleic acid molecule, such as an anti-sense molecule, or a siRNA. The second therapeutic agent may be synthetic or naturally derived.

[0051] The second therapeutic agent may be any agent that is advantageously combined with the antibody or fragment thereof of the invention, for example, if the anti-RET antibody is an inhibitor of RET to be used to treat a cancerous condition, the second agent may be selected from a chemotherapeutic agent, a radionuclide, an siRNA specific for RET, a second antibody specific for RET, a small molecule RET inhibitor, and a bone marrow restorative agent, such as G-CSF, GM-CSF, or M-CSF, or biological agents having colony stimulating, or bone marrow restorative activity. In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with the antibody or antigen-binding fragment of an antibody of the invention, if such side effect(s) should occur. In certain embodiments, the second therapeutic agent may be an agent useful for diminishing pain associated with certain conditions characterized by pain and/or inflammation. Such second agent may include a nerve growth factor (NGF) inhibitor (*e.g.*, a small molecule NGF antagonist or an anti-NGF antibody), aspirin or another NSAID, morphine, steroids (*e.g.*, prednisone), an anti-$Na_v1.7$ antibody, or small molecule inhibitor of $Na_v1.7$, a $Na_v1.8$ antagonist (*e.g.*, anti-$Na_v1.8$ antibody or small molecule inhibitor of $Na_v1.8$), a $Na_v1.9$ antagonist (*e.g.*, anti-$Na_v1.9$ antibody or small molecule inhibitor of $Na_v1.9$), a cytokine inhibitor (*e.g.*, an interleukin-1 (IL-1) inhibitor (such as rilonacept ("IL-1 trap"); Regeneron) or anakinra (KINERET®, Amgen), a small molecule IL-1 antagonist, or an anti-IL-1 antibody; an IL-18 inhibitor (such as a small molecule IL-18 antagonist or an anti-IL-18 antibody); an IL-6 or IL-6R inhibitor (such as a small molecule IL-6 antagonist, an anti-IL-6 antibody or an anti-IL-6 receptor antibody), inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, or an opioid.

[0052] It will also be appreciated that the antibodies and pharmaceutically acceptable compositions of the present invention can be employed in combination therapies, that is, the antibodies and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an antibody may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (*e.g.,* control of any adverse effects). As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are appropriate for the disease, or condition, being treated.

[0053] If it is contemplated that a small molecule RET inhibitor is to be combined with an antibody of the invention, the small molecule RET inhibitor may be selected from the group consisting of vandetanib, sorafenib, sunitinib, cabozantinib, motesanib, RPI-1, PP-1 and NVP-AST478, cediranib, (AZD2171), gefitinib, erlotinib, SU14813, vatalanib, (BAY43-9006), XL-647, XL-999, AG-013736, BIBF1120, TSU68, GW786034, AEE788, CP-547632, KRN951, CHIR258, CEP-7055, OSI-930, ABT-869, E7080, ZK-304709, BAY57-9352, L-21649, BMS582664, XL-880, XL-184, XL-820, RPI-1, PP-1 and NVP-AST478.

[0054] When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art.

[0055] A fifth aspect of the invention provides a method for treating a disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene, or a rearranged form thereof, or the pain associated with the disorder or condition, the method comprising administering an antibody or antigen-binding fragment of any of the anti-RET antibodies described herein together with a pharmaceutically acceptable carrier or diluent to a patient in need thereof.

[0056] In one embodiment, the disorder or condition is a cancer selected from the group consisting of thyroid cancer, lung cancer, pancreatic cancer, skin cancer, breast cancer and a blood-borne cancer. In one embodiment, the disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene, or a rearranged form thereof is selected from the group consisting of acute pain, chronic pain, neuropathic pain, inflammatory pain, arthritis, osteoarthritis, migraine, cluster headaches, trigeminal neuralgia, herpetic neuralgia, general neuralgias, neurodegenerative disorders, neuroendocrine disorders, visceral pain, acute gout, post-herpetic neuralgia, diabetic neuropathy, sciatica, back pain, head or neck pain, severe or intractable pain, breakthrough

pain, post-surgical pain, dental pain, rhinitis, cancer pain, or bladder disorders.

[0057] In a related aspect, the invention provides a method for inhibiting tumor growth or tumor cell proliferation, wherein the tumor or tumor cell expresses RET, or a rearranged form thereof, the method comprising administering an antibody or an antigen-binding fragment thereof of the invention to a patient in need thereof.

[0058] In one embodiment, the tumor is a solid tumor or a blood-borne tumor.

[0059] In one embodiment, the solid tumor is selected from the group consisting of a thyroid tumor, a lung tumor, a pancreatic tumor, a skin tumor and a breast tumor.

[0060] In one embodiment, the thyroid tumor is a papillary thyroid carcinoma (PTC) or a medullary thyroid carcinoma (MTC).

[0061] In one embodiment, the medullary thyroid carcinoma is a hereditary MTC selected from the group consisting of MEN2A, MEN2B and familial medullary thyroid carcinoma (FMTC) syndrome, or wherein the medullary thyroid carcinoma is a sporadic MTC.

[0062] In one embodiment, the lung tumor is a lung adenocarcinoma.

[0063] In one embodiment, the lung tumor is a non-small cell lung cancer (NSCLC).

[0064] In one embodiment, the skin tumor is a melanoma.

[0065] In one embodiment, the blood-borne tumor is a leukemia.

[0066] In one embodiment, the leukemia is chronic myelomonocytic leukemia.

[0067] In a related aspect, the invention provides a method of down-modulating RET expression and/or function, the method comprising administering an antibody or an antigen-binding fragment thereof of the invention.

[0068] In one embodiment, the down-modulating of RET expression and/or function results in down-regulation of a downstream signaling pathway selected from the group consisting of the RAS/RAF/ERK and the PI3K pathways. In certain embodiments, the down-modulating of RET expression and/or function results in down-regulation of a signaling pathway selected from the group consisting of the PKC, SRC and STAT3 pathways.

[0069] In one embodiment, an anti-RET antibody of the invention may interfere with, or prevent, the interaction between RET and one or more GDNF family member ligands (GDNF, neurturin, artemin, and persephin) complexed with their corresponding co-receptors (GFRα1, GFRα2, GFRα3, and GFRα4, respectively). In one embodiment, the human anti-RET antibodies described herein may interfere with, or prevent the interaction of RET with the GDNF/GFRα1 complex. In a related embodiment, the human anti-RET antibodies described herein may interfere with, or prevent the interaction of RET with the artemin/GFRα3 complex. In other related embodiments, the human anti-RET antibodies described herein may interfere with, or prevent the interaction of RET with the neurturin/GFRα2, or the persephin/GFRα4 complexes.

[0070] Once activated, RET recruits a variety of signaling molecules that mediate biological

responses. RET can activate various signaling pathways, such as RAS/RAF/ERK (extracellular signal-regulated kinase), phosphatidylinositol 3-kinase (PI3K)/AKT, PKC and SRC. These signaling pathways are activated via binding of adaptor proteins to intracellular tyrosine residues of RET phosphorylated by its own kinase activity.

[0071] Accordingly, in certain aspects of the invention, an anti-RET antibody of the invention may block the biological responses attributed at least in part to activation of other signaling pathways by RET. In certain embodiments, an anti-RET antibody of the invention may interfere with the signal transduction through a pathway comprising RET and RAS. In certain embodiments, an anti-RET antibody may interfere with cell proliferation, migration, or invasion, or the phosphorylation of ERK1/2 (Extracellular Signal-Regulated Kinase 1/2). In some embodiments, an anti-RET antibody may interfere with the signal transduction through a pathway comprising RET and PI3K (phosphatidylinositol-3-Kinase). In certain embodiments, an anti-RET antibody may interfere with cell proliferation, migration, or invasion, or the phosphorylation of Akt (protein kinase B).

[0072] The antibody or antigen-binding fragment may be administered to the patient in combination with a second therapeutic agent suitable for treating the disease, disorder or condition. If the disease or condition to be treated by an anti-RET antibody is a cancerous condition, the second therapeutic agent may be selected from the group consisting of a chemotherapeutic agent, a radionuclide (alone, or as part of a drug targeting regimen), an antibody-drug conjugate, a small molecule RET inhibitor, an anti-tumor agent, an siRNA specific for RET, and a second antibody specific for RET. If an anti-RET antibody is envisioned for treating pain associated with the cancerous condition, or for treating pain associated with other conditions that may be attributed at least in part to RET activation or signaling, the second therapeutic agent may be selected from any one or more of the following: a nerve growth factor (NGF) inhibitor (*e.g.*, a small molecule NGF antagonist or an anti-NGF antibody), aspirin or another NSAID, morphine, steroids (*e.g.*, prednisone), an anti-$Na_v1.7$ antibody, or small molecule inhibitor of $Na_v1.7$, a $Na_v1.8$ antagonist (*e.g.*, anti-$Na_v1.8$ antibody or small molecule inhibitor of $Na_v1.8$), a $Na_v1.9$ antagonist (*e.g.*, anti-$Na_v1.9$ antibody or small molecule inhibitor of $Na_v1.9$), a cytokine inhibitor (*e.g.*, an interleukin-1 (IL-1) inhibitor (such as rilonacept ("IL-1 trap"); Regeneron) or anakinra (KINERET®, Amgen), a small molecule IL-1 antagonist, or an anti-IL-1 antibody; an IL-18 inhibitor (such as a small molecule IL-18 antagonist or an anti-IL-18 antibody); an IL-6 or IL-6R inhibitor (such as a small molecule IL-6 antagonist, an anti-IL-6 antibody or an anti-IL-6 receptor antibody), inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, or an opioid.

[0073] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0074] **Figure 1.** A schematic diagram of the human RET receptor.

DETAILED DESCRIPTION

[0075] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0076] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0077] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[0078] "REarranged during Transfection", also referred to as "RET" is a receptor tyrosine kinase that is expressed during development in a variety of tissues, including the peripheral and central nervous systems and the kidney. The *RET* oncogene was identified in 1985 by Takahashi *et al.,* who reported on a novel gene rearrangement with transforming activity in NIH/3T3 cells that were transfected with human lymphoma DNA (See Takahashi, M., *et al.,* (1985) Cell, 42:581-588). *RET* was later confirmed to be an oncogene, being somatically rearranged in DNA of papillary thyroid carcinoma (PTC) patients and later designated as RET/PTCs. (Fusco, A. et al., (1987), Nature, 328:170-172; Grieco, M. et al. , (1990), Cell, 60:557-63). RET protein is composed of three domains: an extracellular ligand-binding domain, a hydrophobic transmembrane domain and a cytoplasmic portion with the tyrosine kinase domain split by an insertion of 27 amino acids (See Figure 1). There are two main isoforms of RET generated by alternative splicing. The short and long RET isoforms, which differ by 9 and 51 unrelated C-terminal amino acids, are referred to as RET9 and RET51, respectively. They are highly conserved over a broad range of species (Carter, MT, *et al.* (2001), Cytogenet Cell Genet, 95:169-76). Both isoforms display transforming activity by focus formation assay (Rossel, M. *et al.* (1997), Oncogene, 14:265-75).

[0079] The cDNA sequence and the amino acid sequence of isoform A of RET (also known

as RET51) is provided in GenBank as accession numbers NM_020975.4 and NP_066124.1, respectively, and are provided herein as SEQ ID NOs: 309 and 310, respectively.

[0080] The cDNA sequence and the amino acid sequence of isoform C of RET (also known as RET9) is provided in GenBank as accession numbers NM_020630.4 and NP_065681.1, respectively, and are provided herein as SEQ ID NOs: 311 and 312, respectively. RET, or immunogenic fragments thereof, may be used in preparing human monoclonal antibodies specific for RET. The RET protein, or fragments thereof may be recombinantly produced using standard methods known in the art. Exemplary fusion proteins containing the ecto-domain of RET are shown as SEQ ID NOs: 305, 306, 307 and 309. These fusion proteins may be used as immunogens, or they may be used to target therapeutic agents to cells or tissues expressing RET.

[0081] RET is the signaling receptor for ligands of the "glial-derived neurotrophic factor (GDNF) family", which comprises GDNF (See GenBank accession No.NP_000505.1), artemin (See GenBank accession No. Q5T4W7), neurturin (See GenBank accession No. NM_004558), and persephin (See GenBank accession No. AF040962). GDNF family ligands interact with and activate RET only in the presence of, or complexed with, one of four GPI-linked “co-receptors”, known as GDNF family □ receptors GFRα1 (See GenBank accession No.NP_005255.1), GFRα2 (See GenBank accession No. NM_001495.4), GFRα3 (See GenBank accession No.NP_001487.2), and GFRα4 (See GenBank accession No. NM_022139 for GFRα4a and NM_145762.2 for GFRα4b), (Baloh, RH, *et al.* (2000), Curr Opin Neurobiol 10:103-110; Borrello, MG, *et al* (2013), Expert Opin. Ther. Targets, 17(4):403-419). The primary ligands for the co-receptors GFRα1, GFRα2, GFRα3 and GFRα4 are GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN), respectively.

[0082] The term “IC_{50}” refers to the “half maximal inhibitory concentration”, which value measures the effectiveness of compound (e.g. anti-RET antibody) inhibition towards a biological or biochemical utility. This quantitative measure indicates the quantity required for a particular inhibitor to inhibit a given biological process by half.

[0083] As used herein, the terms "treat," "treatment" and "treating" refer to either the reduction in progression of a disease, disorder, or condition attributed to in part, or associated with RET expression in a cell or tissue in a subject, for example, slowing the rate of tumor cell proliferation in patients bearing a tumor that expresses RET, when administered an antagonistic/inhibitory antibody of the invention, or reduction in the pain associated with a cancerous condition, or pain associated with any other disease or condition which is caused at least in part by RET expression.

[0084] As used herein, the terms "prevent," "preventing," and "prevention" refer to the inhibition of the development or onset of a disease, disorder, or condition attributed to in part, or associated with, RET expression in a cell or tissue in a subject, for example, certain

cancers, or the inhibition of tissue damage that occurs in a patient after an injury, or the inhibition or amelioration of pain associated with a disease or condition attributed to in part RET expression.

[0084a] The term “comprising” as used in this specification and claims means “consisting at least in part of”. When interpreting statements in this specification and claims which include the term “comprising”, other features besides the features prefaced by this term in each statement can also be present. Related terms such as “comprise” and “comprised” are to be interpreted in similar manner.

[0085] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region (“HCVR” or “V_H”) and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region (“LCVR or “V_L”) and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the invention, the FRs of the antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0086] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[0087] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative

substitutions.

[0088] The fully human monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0089] The present invention also includes fully monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one

or more conservative substitutions. For example, the present invention includes antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0090] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (e.g., mouse), have been grafted onto human FR sequences.

[0091] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-6}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, which bind specifically to RET. Moreover, multi-specific antibodies that bind to RET protein and one or more additional antigens or a bi-specific that binds to two different regions of RET are nonetheless considered antibodies that "specifically bind", as used herein.

[0092] The term "high affinity" antibody refers to those mAbs having a binding affinity to RET, expressed as K_D, of at least 10^{-7} M, of at least 10^{-8} M; preferably 10^{-9} M; more preferably 10^{-10}M, more preferably 10^{-11}M, more preferably 10^{-12}M as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[0093] By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from RET, with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[0094] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding portion" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retains the ability to bind to RET.

[0095] The specific embodiments, antibody or antibody fragments of the invention may be

conjugated to a therapeutic moiety ("immunoconjugate" or "antibody-drug conjugate"), such as a small molecule RET inhibitor, an anti-tumor agent, a radionuclide, a growth factor, a bone marrow restorative agent or colony stimulating factor, or any other therapeutic moiety useful for treating a disease, disorder, or condition associated with RET expression, such as a cancer, or a damaged tissue.

[0096] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds RET, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than RET.

[0097] A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes RET activity"), is intended to refer to an antibody whose binding to RET results in inhibition of at least one biological activity of RET, such as cell signaling. For example, an antibody of the invention may aid in blocking the binding of RET to its ligand, or to one of the GFRα co-receptors, or prevent or treat a disease associated with RET expression. Alternatively, an antibody of the invention may demonstrate the ability to ameliorate at least one symptom of the disease or condition associated with RET expression. This inhibition of the biological activity of RET can be assessed by measuring one or more indicators of RET biological activity by one or more of several standard *in vitro* assays (such as any of the assays as described herein) or *in vivo* assays known in the art (for example, animal models to look at inhibition of tumor cell growth *in vivo*) following administration of one or more of the antibodies described herein.

[0098] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[0099] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[00100] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen-binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of

molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[00101] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[00102] As applied to polypeptides, the term "substantial similarity" or “substantially similar” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00103] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25:3389-3402, each of which is herein incorporated by reference.

[00104] In specific embodiments, the antibody or antibody fragment for use in the method of the invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide. An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise an Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 mAbs; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 mAbs; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 mAbs. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[00105] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose

of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

General Description

[00106] "REarranged during Transfection", also referred to as "RET" is a receptor tyrosine kinase that is expressed during development in a variety of tissues, including the peripheral and central nervous systems and the kidney (Arighi, E. *et al.* (2005), Cytokine Growth Factor Rev 16:441-67; Borrello, MG, *et al.,* (2013), Expert Opin. Ther. Targets, 17(4): 403-419). The *RET* oncogene was identified in 1985 by Takahashi *et al.,* who reported on a novel gene rearrangement with transforming activity in NIH/3T3 cells that were transfected with human lymphoma DNA (See Takahashi, M., *et al.,* (1985) Cell, 42:581-588). *RET* was later confirmed to be an oncogene, being somatically rearranged in DNA of papillary thyroid carcinoma (PTC) patients and later designated as RET/PTCs. (Fusco, A. *et al.*, (1987), Nature, 328:170-172; Grieco, M. *et al.* , (1990), Cell, 60:557-63). RET protein is composed of three domains: an extracellular ligand-binding domain, a hydrophobic transmembrane domain and a cytoplasmic portion with the tyrosine kinase domain split by an insertion of 27 amino acids (See Figure 1). There are two main isoforms of RET generated by alternative splicing. The short and long RET isoforms, which differ by 9 and 51 unrelated C-terminal amino acids, are referred to as RET9 and RET51, respectively. They are highly conserved over a broad range of species (Carter, MT, *et al.* (2001), Cytogenet Cell Genet, 95:169-76). Both isoforms display transforming activity by focus formation assay (Rossel, M. *et al.* (1997), Oncogene, 14:265-75).

[00107] Genetic alterations of RET have been shown to be involved in the etiology of thyroid cancers and more recent data suggests that RET is also involved in lung adenocarcinomas (Viglietto, G. *et al.* (1995), Oncogene, 11:1207-10; Fischer, AH, *et al.,* (1998), Am J Pathol. 153:1443-50). Other studies suggest that RET may be involved in additional tumors, including breast, pancreas, leukemias and melanoma (Ballerini, P. *et al,* (2012), Leukemia, 26:2384-9; Sawai, H. *et al.* (2005), 65(24):11536-44; Narita, N. *et al.,* (2009), Oncogene, 28:3058-68).

[00108] Vandetanib (ZD6474, CAPRELSA®, Astra Zeneca), is an orally available aminoquinazoline compound, which was initially developed as a VEGFR2 inhibitor, but was later found to be active against RET, VEGFR3, EGFR and PDGFR. Vandetanib is currently approved by the FDA and EMA for advanced and metastatic medullary thyroid carcinoma (MTC) (Wells, SA, *et al.* ,(2012), J. Clin. Oncol. 30:134-41).

[00109] Sorafenib (BAY43-9006, NEXAVAR®, Bayer Pharmaceuticals) is a bisarylurea compound initially developed to target the serine/threonine kinase BRAF, but was

subsequently found to be a potent agent against Flt-3, VEGFR1-3, PDGFR, c-kit and RET (Wilhelm, S. *et al.,* (2006), Nat Rev Drug Discov, 5:835-44). Sorafenib was approved by the FDA for advanced liver and kidney cancers.

[00110] Sunitinib (SU11248, SUTENT®, Pfizer) is an indolinone compound that targets mainly VEGFR2, PDGFR, c-kit, FLT3 and RET kinases ((Chow, LQ, *et al.*, (2007), J. Clin. Oncol. 25:884-96). Sunitinib was approved by the FDA for imatinib-resistant GIST patients, as well as for advanced pancreatic neuroendocrine tumors and renal cell cancer.

[00111] Cabozantinib (Cometriq, formerly known as XL-184, Exelixis) is a small-molecule, multikinase inhibitor that targets MET, VEGFR2 and RET. It is currently in clinical trials in a large number of tumor types, including medullary thyroid cancer, prostate cancer, ovarian cancer, non-small cell lung cancer (NSCLC), hepatocellular, renal cell and breast cancer, as well as melanoma and glioblastoma ((Zhang, Y. *et al.*, (2010), IDrugs 13:112-21).

[00112] Another RET-targeting agent in clinical development is motesanib (AMG-706, Amgen), which is a multikinase inhibitor that targets VEGFR1-3, Flt3, Kit, PDGFR and RET.

[00113] Other RET inhibitors in pre-clinical development are RPI-1, which is an indoline compound; PP-1, which is a pyrazolopyrimidine compound active on RET and Src; and NVP-AST478, which is a biphenyl-urea compound that has potent anti-RET kinase activity *in vitro* and *in vivo* (Cuccuru, G. *et al.* (2004), J Natl Cancer Inst 96:1006-14).

[00114] However, one problematic aspect of the above-noted RET inhibitors is that they are not specific for RET, that is, they appear to act through multiple mechanisms and as such, could potentially exert other untoward effects *in vivo*. For example, certain of the inhibitors noted above induce adverse events such as hypertension and QTc prolongation. Thus, the non-selective profile of these agents may limit the therapeutic window. It would be of benefit to identify agents, such as an anti-RET antibody that selectively binds to RET, which could result in superior clinical efficacy with a more favorable safety profile.

[00115] Accordingly, there is still a need for effective therapies against RET-driven tumors, and furthermore, there is a need to identify an agent specific for RET for preventing and treating other diseases, disorders, or conditions associated with RET expression without the adverse side effects associated with the agents described above. Such specificity and efficacy may be achieved through use of an anti-RET antibody, such as those described herein.

[00116] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a whole human RET protein, or with a recombinant form of the protein, or a fragment thereof, or with a fusion protein that contains the extracellular/ecto-domain of human RET. (See GenBank accession number NP_066124.1 (SEQ ID NO: 310) or GenBank accession number NP_065681.1 (SEQ ID NO: 312), or a recombinantly produced RET fusion protein (See SEQ ID NOs: 305, 306, 307 and

313), followed by immunization with a secondary immunogen (purified human RET protein), or with an immunogenically active fragment of the RET protein, such as the ecto-domain of RET.

[00117] The immunogen may be DNA encoding the human RET protein (See GenBank accession number NM_020975.4 and SEQ ID NO: 309 for isoform A; or GenBank accession number NM_020630.4 and SEQ ID NO: 311 for isoform C) or an active fragment thereof.

[00118] The immunogen may be derived from the extracellular domain of the RET protein, which spans amino acid residues 1-635 of any of SEQ ID NOs: 305, 307, 310, 312, and 313 (including the signal sequence); from amino acid residue 1-636 of SEQ ID NO: 306 (including the signal sequence). An immunogen may be derived from a fragment of any of the above-noted regions of the RET protein.

[00119] The full-length amino acid sequence of RET51 is shown as SEQ ID NO: 310 and is also shown in GenBank accession number NP_066124.1. The full-length amino acid sequence of RET9 is shown as SEQ ID NO: 312 and is also shown in GenBank accession number NP_065681.1. Exemplary immunogens may be the recombinant constructs shown in SEQ ID NOs: 307 or 313.

[00120] In certain embodiments, antibodies that bind specifically to RET may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of RET specific antibodies. In certain embodiments, any one or more of the above-noted regions of RET, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

Antigen-Binding Fragments of Antibodies

[00121] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding portion" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to RET. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. Antigen-binding fragments of an antibody

may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[00122] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[00123] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H - V_H, V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[00124] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H -C_H1; (ii) V_H -C_H2; (iii) V_H -C_H3; (iv) V_H -C_H1-C_H2; (v) V_H -C_H1-C_H2-C_H3; (vi) V_H -C_H2-C_H3; (vii) V_H -C_L; (viii) V_L -C_H1; (ix) V_L -C_H2; (x) V_L -C_H3; (xi) V_L -C_H1-C_H2; (xii) V_L -C_H1-C_H2-C_H3; (xiii) V_L -C_H2-C_H3; and (xiv) V_L -C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A

hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[00125] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

Preparation of Human Antibodies

[00126] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to RET.

[00127] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to RET are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[00128] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be

produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[00129] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[00130] In certain embodiments, the antibodies of the instant invention possess affinities (K_D) ranging from about 1.0 x 10^{-7} M to about 1.0 x 10^{-12}M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[00131] The anti-RET antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind RET. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment of the invention.

[00132] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug

concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[00133] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[00134] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[00135] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[00136] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[00137] Bioequivalent variants of the antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-RET Antibodies Comprising Fc Variants

[00138] According to certain embodiments of the present invention, anti-RET antibodies are provided comprising an Fc domain comprising one or more mutations, which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-RET antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges

from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P).

[00139] For example, the present invention includes anti-RET antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

Biological Characteristics of the Antibodies

[00140] In general, the antibodies of the present invention may function by binding to RET and in so doing act to block, or prevent RET activation and/or signaling. The antibodies of the present invention may also function by binding to RET and in so doing interfere with, or prevent the interaction, or binding of RET with one or more GDNF family members complexed with their corresponding co-receptors, e.g GDNF/GFRα1, neurturin/GFRα2, artemin/GFRα3, or persephin/GFRα4. Based on the fact that the tumorigenic potential of RET has been established in humans, an antagonistic antibody that specifically binds to RET may prove to have a beneficial effect in inhibiting tumor cell growth in patients suffering from a cancerous condition.

[00141] In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting RET activity by binding to any region or fragment of the full length protein, the amino acid sequence of which is shown in SEQ ID NO: 310 (RET51), also shown as GenBank accession number NP_066124.1 and in SEQ ID NO: 312 (RET9), also shown as GenBank accession number NP_065681.1. The antibodies may also bind to any region which is found in SEQ ID NO: 310 or 312, or to a fragment found within SEQ ID NO: 310 or 312.

[00142] In one embodiment, the invention provides a fully human monoclonal antibody or

antigen-binding fragment thereof that binds to the RET protein, wherein the antibody or fragment thereof exhibits one or more of the following characteristics:

(a) is a fully human antibody;

(b) exhibits a K_D ranging from about 1.0 X 10^{-7} M to about 1.0 X 10^{-12} M as measured by Surface Plasmon Resonance;

(c) inhibits or blocks the binding, or interaction of RET with one or more GDNF family member ligands (GDNF, neurturin, artemin, and persephin) complexed with their corresponding co-receptors (GFRα1, GFRα2, GFRα3, and GFRα4, respectively);

(d) inhibits RET signaling mediated by one or more GDNF family member ligands selected from GDNF, neurturin, artemin, and persephin;

(e) enhances RET internalization/degradation following binding of the antibody to the RET receptor;

(f) comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274 and 290; or

(g) comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282 and 298.

[0142] Certain anti-RET antibodies of the present invention are able to bind to the RET protein and inhibit the activation and/or signaling associated with RET. In so doing, the antibodies may function to inhibit the growth of tumors that depend on activation of RET signaling for growth. Such antagonistic anti-RET antibodies may be used alone to treat a cancerous condition, or may be used as adjunct therapy with any other anti-cancer agent, such as a chemotherapeutic small molecule, or radiation therapy, or with a bone marrow restorative agent.

[0143] In certain embodiments, the anti-RET antibodies may be capable of inhibiting multiple signaling pathways, including RAS/RAF pathway, which leads to activation of the mitogen activated protein kinases (MAPK) ERK1 and ERK2 (Trupp, M. *et al.*, (1999), J Biol. Chem. 274:20885-94; Santoro, M. *et al.*, (1994), Mol. Cell Biol. 14:663-75; van Weering, DHJ, *et al.* (1995), 11:2207-14; Worby, CA, *et al.,* (1996), J Biol Chem, 271:23619-22), phosphatidylinositol 3-kinase (PI3K), resulting in activation of the serine/threonine kinase Akt (Trupp, M. *et al.,* (1999), J Biol. Chem. 274:20885-94; van Weering, DHJ, (1997), J Biol Chem 272:249-54; Segouffin-Cariou, C., *et al,* (2000), 275:3568-76; Maeda, K. et al, (2004), 323: 345-54).

[0144] Non-limiting, exemplary *in vitro* assays for measuring the ability of the anti-RET antibodies of the invention to block the binding of RET to the GFRα1/GDNF co-complex and

in vitro assays to measure the effect of the antibodies on RET signaling, activation, or internalization are illustrated in Examples 4 and 5, respectively. In Example 3, the binding affinities and kinetic constants of human anti-RET antibodies were determined by surface plasmon resonance and the measurements were conducted on a Biacore 4000 or T200 instrument. In Example 4, the ability of the antibodies to block the binding of RET to the GFRα1/GDNF co-complex was tested using a competition sandwich ELISA assay. Example 5 demonstrates the ability of the antibodies of the invention to inhibit ligand dependent RET signaling in a serum-response factor (SRE)-luciferase reporter assay. More particularly, the data presented in Example 5 show that the anti-RET antibodies of the invention display a range of inhibitory activity on RET signaling in the presence of the glial family ligands, GDNF and artemin.

Epitope Mapping and Related Technologies

[0145] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, a routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol Biol 248:443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues that correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A.

[0146] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino

acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0147] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

[0148] The present invention includes anti-RET antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein in Table 1. Likewise, the present invention also includes anti-RET antibodies that compete for binding to RET or a fragment thereof with any of the specific exemplary antibodies described herein in Table 1.

[0149] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-RET antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference RET antibody of the invention, the reference antibody is allowed to bind to a RET protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the RET molecule is assessed. If the test antibody is able to bind to RET following saturation binding with the reference anti-RET antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-RET antibody. On the other hand, if the test antibody is not able to bind to the RET molecule following saturation binding with the reference anti-RET antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-RET antibody of the invention.

[0150] To determine if an antibody competes for binding with a reference anti-RET antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a RET molecule under saturating conditions followed by assessment of binding of the test antibody to the RET molecule. In a second orientation, the test antibody is allowed to bind to a RET molecule under saturating conditions followed by assessment of binding of the reference antibody to the RET molecule.

If, in both orientations, only the first (saturating) antibody is capable of binding to the RET molecule, then it is concluded that the test antibody and the reference antibody compete for binding to RET. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0151] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.,* Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0152] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0153] The invention encompasses a human RET monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of inhibiting the proliferation of tumor cells, or to ameliorate at least one symptom associated with a RET associated condition, such as a cancerous condition. Such an agent may be a second different antibody to RET, or an anti-tumor chemotherapeutic agent, or may be a radionuclide that when targeted to a tumor cell expressing RET acts to kill the tumor cell. The type of therapeutic moiety that may be conjugated to the anti- RET antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. Alternatively, if the desired therapeutic effect is to treat the sequelae or symptoms associated with expression of RET by certain tissues, or any other condition resulting from RET expression, such as, but not limited to, cancer, it may be advantageous to conjugate an agent appropriate to treat the sequelae or symptoms of the condition, or to alleviate any side effects of the antibodies of the invention. Examples of suitable agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

Multi-specific Antibodies

[0154] The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.,* Tutt *et al.,* 1991, J. Immunol. 147:60-69; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The antibodies of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.,* by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multi-specific antibody with a second binding specificity.

[0155] An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_{H3} domain and a second Ig C_{H3} domain, wherein the first and second Ig C_{H3} domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_{H3} domain binds Protein A and the second Ig C_{H3} domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_{H3} may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_{H3} include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

Therapeutic Administration and Formulations

[0156] The invention provides therapeutic compositions comprising the anti-RET antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations

include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0157] The dose of each of the antibodies of the invention may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibodies of the present invention are used for treating a RET associated disease, or condition in a patient, or for treating one or more symptoms associated with a condition that depends on RET activation or signaling, such as certain tumors expressing RET in a patient, or for lessening the severity of the disease, it is advantageous to administer each of the antibodies of the present invention intravenously or subcutaneously normally at a single dose of about 0.01 to about 30 mg/kg body weight, more preferably about 0.1 to about 20 mg/kg body weight, or about 0.1 to about 15 mg/kg body weight, or about 0.02 to about 7 mg/kg body weight, about 0.03 to about 5 mg/kg body weight, or about 0.05 to about 3 mg/kg body weight, , or about 1 mg/kg body weight, or about 3.0 mg/kg body weight, or about 10 mg/kg body weight, or about 20 mg/kg body weight. Multiple doses may be administered as necessary. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibodies or antigen-binding fragments thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 600 mg, about 5 to about 300 mg, or about 10 to about 150 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibodies or antigen-binding fragments thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0158] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.* (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by

infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, nasal mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. It may be delivered as an aerosolized formulation (See US2011/0311515 and US2012/0128669). The delivery of agents useful for treating respiratory diseases by inhalation is becoming more widely accepted (See A. J. Bitonti and J. A. Dumont, (2006), Adv. Drug Deliv. Rev, 58:1106-1118). In addition to being effective at treating local pulmonary disease, such a delivery mechanism may also be useful for systemic delivery of antibodies (See Maillet et al. (2008), Pharmaceutical Research, Vol. 25, No. 6, 2008).

[0159] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0160] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0161] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0162] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the

empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0163] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK ™ Autoinjector (Amgen, Thousands Oaks, CA), the PENLET ™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA ™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

[0164] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Administration Regimens

[0165] According to certain embodiments of the present invention, multiple doses of an antibody to RET may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an antibody to RET. As used herein, "sequentially administering" means that each dose of antibody to RET is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially

administering to the patient a single initial dose of an antibody to RET, followed by one or more secondary doses of the antibody to RET and optionally followed by one or more tertiary doses of the antibody to RET.

[0166] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the antibody to RET. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of antibody to RET, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of antibody to RET contained in the initial, secondary and/or tertiary doses vary from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0167] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of antibody to RET which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0168] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an antibody to RET. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0169] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately

preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Therapeutic Uses of the Antibodies

[0170] Due to their binding to/interaction with the RET protein expressed on certain cells and tissues, the present antibodies are useful for preventing the interaction of the RET protein with one or more ligand/co-receptor complexes *e.g.* GDNF/GFRα1, artemin/GFRα3, neurturin/GFRα2, or persephin/GFRα4. Given the ability of the anti-RET antibodies of the invention to prevent or inhibit this interaction, the antagonistic antibodies of the present invention may prove useful for inhibition of tumor cell growth when the tumor cell depends on RET signaling for growth, or they may prove useful for inhibiting pain associated with the cancerous condition, as well as pain associated with other diseases or disorders in which RET activation or signaling plays a role. The antibodies of the present invention may be used to slow the growth, and/or metastasis of tumors in a subject with a RET expressing tumor, or to treat the pain associated with the cancerous condition, when administered alone or in conjunction with another anti-tumor agent or therapeutic regimen, or with one or more agents used to further ameliorate the pain associated with the condition. Alternatively, the antibodies of the present invention may be useful for ameliorating at least one symptom associated with the cancerous condition.

[0171] It is contemplated that the antibodies of the invention may be used alone, or in conjunction with a second agent, or third agent for treating a RET associated disease or condition, or for alleviating at least one symptom or complication associated with the RET associated disease or condition. A "RET associated disease or condition" is any disease or condition whereby RET is known to be expressed in cells or tissues affected by the disease or condition, and which responds favorably to treatment with either a small molecule therapeutic known to inhibit activation and/or signaling of RET, or which responds favorably to treatment with an anti-RET antibody of the present invention. The second or third agents may be delivered concurrently with the antibodies of the invention, or they may be administered separately, either before or after the antibodies of the invention. The second or third agent may be a small organic molecule, or a biological agent, such as a protein or polypeptide. The second or third agent may be synthetic, or naturally derived. The second or third agent may be an anti-tumor agent, such as a chemotherapeutic drug, or radiation therapy, or a bone-marrow restorative agent, or other agents to reduce fever or pain, another second but different antibody that specifically binds RET, an agent (*e.g.* an antibody) that binds to a RET ligand, *e.g.* GDNF, neurturin, artemin, or persephin, or that binds to the co-

receptor for RET, such as GFRα1, GFRα2, GFRα3, or GFRα4, or an siRNA specific for the RET molecule.

[0172] In yet a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for treating patients suffering from a RET associated disease or condition. In yet another embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for reducing the proliferation of tumor cells, or reducing the tumor burden in a patient that has a tumor that depends for growth on RET signaling. In a further embodiment of the invention the present antibodies are used as adjunct therapy with any other agent useful for treating a RET associated disease or condition, including a chemotherapeutic agent, radiation therapy, a bone marrow restorative agent, a second RET antibody, or any other antibody specific for a RET antigen, or an antibody specific for GDNF or GFRα1, or any other palliative therapy known to those skilled in the art.

[0173] The antibodies of the invention are useful for the treatment, prevention and/or amelioration of any disease, disorder, or condition associated with RET activity, or for ameliorating at least one symptom associated with the disease, disorder, or condition, or for alleviating the pain associated with such disease, disorder, or condition. Exemplary conditions, diseases and/or disorders, and/or the pain associated with such conditions, diseases, or disorders, that can be treated with the anti-RET antibodies of the present invention include acute or chronic pain, including, but not limited to, neuropathic pain, inflammatory pain, arthritis, migraine, cluster headaches, trigeminal neuralgia, herpetic neuralgia, general neuralgias, irritable bowel syndrome, inflammatory bowel syndrome, visceral pain including abdominal pain, osteoarthritis pain, gout, post-herpetic neuralgia, diabetic neuropathy, radicular pain, sciatica, back pain, head or neck pain, breakthrough pain, post-surgical pain, bone pain, cancer pain. Other conditions treatable by the antibodies and therapeutic methods of the invention included thyroid cancers, familial medullary thyroid carcinoma (FMTC) syndrome, sporadic medullary carcinoma (MTC), multiple endocrine neoplasia syndromes MEN2A and MEN2B, prostate cancer, breast cancer, cervical cancer, colon cancer, or bladder cancer and the pain associated with these conditions. The cancers treatable by the antibodies of the invention may be solid tumors or they may be blood-borne tumors, such as a leukemia. The antibodies of the invention or antigen-binding fragments thereof may also be used to treat the following conditions: non-malignant acute, chronic, or fracture bone pain; rheumatoid arthritis, spinal stenosis; neuropathic low back pain; myofascial pain syndrome; fibromyalgia; temporomandibular joint pain; pancreatic pain; chronic headache pain; tension headache; HIV-associated neuropathy; Charcot-Marie Tooth neuropathy; hereditary sensory neuropathies; peripheral nerve injury; painful neuromas; ectopic proximal and distal discharges; radiculopathy;

chemotherapy induced neuropathic pain; radiotherapy-induced neuropathic pain; post-mastectomy pain; central pain; spinal cord injury pain; post-stroke pain; thalamic pain; complex regional pain syndrome; phantom pain; intractable pain; musculoskeletal pain; joint pain; acute gout pain; mechanical low back pain; neck pain; tendonitis; injury/exercise pain; pyelonephritis; appendicitis; cholecystitis; intestinal obstruction; hernias; chest pain, including, cardiac pain; pelvic pain, renal colic pain, acute obstetric pain, including, labor pain; cesarean section pain; burn and trauma pain; endometriosis; herpes zoster pain; sickle cell anemia; acute pancreatitis; orofacial pain including sinusitis pain, dental pain; multiple sclerosis pain; leprosy pain; Behcet's disease pain; adiposis dolorosa; phlebitic pain; Guillain-Barre pain; painful legs and moving toes; Haglund syndrome; Fabry's disease pain; bladder and urogenital disease; hyperactivity bladder; painful bladder syndrome; interstitial cystitis; or prostatitis.

Combination Therapies

[0174] As noted above, the methods of the present invention, according to certain embodiments, comprise administering to the subject one or more additional therapeutic agents in combination with an antibody to RET. As used herein, the expression "in combination with" means that the additional therapeutic agents are administered before, after, or concurrent with the pharmaceutical composition comprising the anti-RET antibody. The term “in combination with” also includes sequential or concomitant administration of the anti-RET antibody and a second therapeutic agent.

[0175] For example, when administered "before" the pharmaceutical composition comprising the anti-RET antibody, the additional therapeutic agent may be administered about 72 hours, about 60 hours, about 48 hours, about 36 hours, about 24 hours, about 12 hours, about 10 hours, about 8 hours, about 6 hours, about 4 hours, about 2 hours, about 1 hour, about 30 minutes, about 15 minutes or about 10 minutes prior to the administration of the pharmaceutical composition comprising the anti-RET antibody. When administered "after" the pharmaceutical composition comprising the anti-RET antibody, the additional therapeutic agent may be administered about 10 minutes, about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours or about 72 hours after the administration of the pharmaceutical composition comprising the anti-RET antibodies. Administration "concurrent" or with the pharmaceutical composition comprising the anti-RET antibody means that the additional therapeutic agent is administered to the subject in a separate dosage form within less than 5 minutes (before, after, or at the same time) of administration of the pharmaceutical composition comprising the anti-RET antibody, or administered to the subject as a single combined dosage

formulation comprising both the additional therapeutic agent and the anti-RET antibody.

[0176] Combination therapies may include an anti-RET antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

For example, a second or third therapeutic agent may be employed to aid in reducing the tumor burden in the patient, such as a chemotherapeutic agent, or radiation therapy useful in inhibiting the proliferation of tumor cells in a subject. Alternatively, the antibodies may be used as adjunct therapy after surgical removal of the tumor and may be used alone, or in conjunction with a chemotherapeutic agent, with radiation therapy or with a bone marrow restorative agent. The antibodies may also be used in conjunction with other therapies, as noted above, including a second antibody specific for RET, or an antibody specific for a RET ligand, or with an antibody, or fusion molecule that binds GFRα1 (See SEQ ID NO: 308).

Diagnostic Uses of the Antibodies

[0177] The anti-RET antibodies of the present invention may also be used to detect and/or measure RET in a sample, *e.g.*, for diagnostic purposes. It is envisioned that confirmation of a disease or condition thought to be associated with RET may be made by measuring the presence of RET in, for example, a biopsy sample from a tumor (*i.e.* tumor cells) that depends on growth through RET signaling. Exemplary diagnostic assays for RET may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-RET antibody of the invention, wherein the anti-RET antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate a cell expressing the RET protein from patient samples. Alternatively, an unlabeled anti-RET antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure RET containing the F protein in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0178] Samples that can be used in RET diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of RET protein, or fragments thereof, under normal or pathological conditions. Generally, levels of RET in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with the presence of RET) will be measured to initially establish a baseline, or standard, level of the RET protein. This baseline level of RET can then be compared against the levels of RET measured in samples

obtained from individuals suspected of having a disease or condition associated with RET, or symptoms associated with such conditions.

[0178a] In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

EXAMPLES

[0179] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to RET Protein

[0180] An immunogen comprising any one of the following can be used to generate antibodies to RET. In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a full length RET protein (See for example, SEQ ID NO: 310 for the human RET51 isoform, also found in ATCC accession number NP_066124.1; and SEQ ID NO: 312 for the human RET9 isoform, also found in ATCC accession number NP_065681.1, both having signal sequences from residue numbers 1-28). The mice may be given one or more booster shots containing either the same molecule, or they may be boosted with immunogenic fragments thereof, such as with the human RET extracellular domain, which ranges from amino acids 1-635 of SEQ ID NO: 313, also found in ATCC accession number NP_066124.1 with signal sequence ranging from amino acid residues 1-28). In certain embodiments, the mice are injected with the full length RET protein, followed by boosting with any of the constructs shown as SEQ ID NOs: 305, 306, 307 and 313 or with a recombinantly prepared molecule.

[0181] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a biologically active RET molecule, or an immunogenic fragment of the RET protein, or DNA encoding the full length protein or the active fragment thereof. The immunogen may be delivered to the animal via any route including but not limited to intramuscularly, subcutaneously, intravenously or intranasally.

[0182] In certain embodiments, the full length RET protein or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

[0183] The full length protein, or a fragment thereof, that were used as immunogens, as noted above, were administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by a RET immunoassay. When a desired immune response was achieved, splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce RET-specific antibodies. Using this technique, and the various immunogens described above, several chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; certain exemplary antibodies generated in this manner were designated as, for example, H2M7086N.

[0184] Anti-RET antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-RET antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H8044P, H4H8045P, H4H8046P, H4H8048P, H4H8056P, H4H8058P, H4H8060P, H4H8062P, H4H8066P, H4H8067P, H4H8071P, H4H8076P, H4H8079P, H4H8080P, H4H8083P, H4H8084P, H4H8085P and H4H8087P.

[0185] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0186] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected antibodies specific for RET protein and their corresponding antibody identifiers. Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (e.g. "H4H", "H1M, "H2M"), followed by a numerical identifier (e.g. "7086" as shown in Table 1), followed by a "P" or "N" suffix. Thus, according to this nomenclature, an antibody may be referred to as, e.g. "H2M7086N". The H4H, H1M, and H2M prefixes on the antibody designations used herein indicate the particular Fc region of the antibody. For example, an "H2M" antibody has a mouse IgG2 Fc, whereas an "H4H" antibody has a human IgG4 Fc. As will be appreciated by a person of ordinary skill in the art, an H1M or H2M antibody can be converted to an H4H antibody, and vice versa, but in any event, the variable domains (including the CDRs), which are indicated by the numerical identifiers shown in Table 1, will remain the same. Antibodies having the same numerical

antibody designation, but differing by a letter suffix of N, B or P refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (*i.e.,* in the framework regions). Thus, N, B and P variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but differ from one another within their framework regions.

Table 1

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H2M7086N	2	4	6	8	10	12	14	16
H4H8044P	18	20	22	24	26	28	30	32
H4H8045P	34	36	38	40	42	44	46	48
H4H8046P	50	52	54	56	58	60	62	64
H4H8048P	66	68	70	72	74	76	78	80
H4H8056P	82	84	86	88	90	92	94	96
H4H8058P	98	100	102	104	106	108	110	112
H4H8060P	114	116	118	120	122	124	126	128
H4H8062P	130	132	134	136	138	140	142	144
H4H8066P	146	148	150	152	154	156	158	160
H4H8067P	162	164	166	168	170	172	174	176
H4H8071P	178	180	182	184	186	188	190	192
H4H8076P	194	196	198	200	202	204	206	208
H4H8079P	210	212	214	216	218	220	222	224
H4H8080P	226	228	230	232	234	236	238	240
H4H8083P	242	244	246	248	250	252	254	256
H4H8084P	258	260	262	264	266	268	270	272
H4H8085P	274	276	278	280	282	284	286	288
H4H8087P	290	292	294	296	298	300	302	304

Example 3. Surface Plasmon Resonance Derived Binding Affinities & Kinetic Constants of Human Monoclonal anti-RET Antibodies

[0187] Binding affinities and kinetic constants of human anti-RET antibodies were determined by surface plasmon resonance (Biacore T200) at 25°C & 37°C (Tables 2-3). Antibodies, expressed as human IgG4 Fc (*i.e.*, "H4H" designations), were captured onto an anti-human Fc sensor surface (mAb-capture format), and soluble monomeric (hRET.mmh; SEQ ID NO: 305, *macaca fascicularis* (mf) RET.mmh; SEQ ID NO: 306) or dimeric

(hRET.mFc ;SEQ ID NO: 307) RET protein was injected over the sensor surface. Binding equilibrium dissociation constants (K_D) and dissociative half-lives ($t_{1/2}$) were calculated from the kinetic rate constants as: K_D [M] = k_d / k_a; and $t_{1/2}$ (min) = (ln2/(60*k_d). Calculations were performed using BiacoreT200 evaluation software v1.0.

[0188] Several antibodies of the invention displayed sub-nanomolar affinities to human and monkey RET protein (Tables 2-3).

Table 2: Biacore binding affinities of human Fc mAbs at 25°C

	Binding at 25°C/ Mab Capture Format				
AbPID	**Analyte**	**ka (Ms^{-1})**	**Kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H4H7086N	hRET.mmh	TBD	TBD	TBD	TBD
	hRET.mFc	TBD	TBD	TBD	TBD
	mfRET.mmh	TBD	TBD	TBD	TBD
H4H8066P	hRET.mmh	1.27E+05	2.57E-04	2.02E-09	45
	hRET.mFc	2.07E+05	1.20E-04	5.80E-10	96
	mfRET.mmh	6.43E+04	8.82E-04	1.37E-08	13
H4H8067P	hRET.mmh	1.11E+05	2.35E-04	2.12E-09	49
	hRET.mFc	1.68E+05	1.58E-04	9.40E-10	73
	mfRET.mmh	1.08E+05	4.15E-02	3.85E-07	0.3
H4H8071P	hRET.mmh	1.56E+05	2.19E-04	1.41E-09	53
	hRET.mFc	2.00E+05	1.79E-04	8.90E-10	65
	mfRET.mmh	1.17E+05	2.72E-04	2.32E-09	43
H4H8076P	hRET.mmh	2.24E+05	3.38E-04	1.51E-09	34
	hRET.mFc	2.50E+05	1.71E-04	6.86E-10	68
	mfRET.mmh	2.12E+05	3.27E-04	1.54E-09	35
H4H8079P	hRET.mmh	1.43E+05	3.84E-04	2.68E-09	30
	hRET.mFc	2.09E+05	1.51E-04	7.20E-10	77
	mfRET.mmh	1.17E+05	4.35E-04	3.73E-09	27
H4H8044P	hRET.mmh	8.88E+04	4.88E-04	5.49E-09	24
	hRET.mFc	1.07E+05	2.11E-04	1.97E-09	55
	mfRET.mmh	9.20E+04	1.35E-03	1.47E-08	9
H4H8045P	hRET.mmh	2.79E+05	2.46E-04	8.83E-10	47
	hRET.mFc	3.81E+05	8.71E-05	2.29E-10	133
	mfRET.mmh	1.92E+05	2.80E-03	1.46E-08	4
H4H8046P	hRET.mmh	3.06E+05	3.01E-04	9.84E-10	38
	hRET.mFc	2.80E+05	1.81E-04	6.45E-10	64
	mfRET.mmh	2.78E+05	3.15E-04	1.13E-09	37
H4H8048P	hRET.mmh	2.54E+05	4.93E-04	1.94E-09	23
	hRET.mFc	2.31E+05	2.67E-04	1.16E-09	43
	mfRET.mmh	2.30E+05	5.35E-04	2.33E-09	22
H4H8080P	hRET.mmh	2.11E+05	5.02E-04	2.38E-09	23
	hRET.mFc	1.83E+05	2.42E-04	1.32E-09	48
	mfRET.mmh	NB	NB	NB	NB
H4H8083P	hRET.mmh	1.92E+05	4.84E-04	2.52E-09	24
	hRET.mFc	1.97E+05	2.24E-04	1.14E-09	52
	mfRET.mmh	2.07E+05	7.11E-04	3.44E-09	16

H4H8084P	hRET.mmh	7.23E+05	3.75E-04	5.18E-10	31
	hRET.mFc	7.20E+05	2.04E-04	2.84E-10	57
	mfRET.mmh	6.95E+05	3.93E-04	5.65E-10	29
H4H8085P	hRET.mmh	8.01E+04	1.87E-04	2.34E-09	62
	hRET.mFc	9.40E+04	1.25E-04	1.33E-09	92
	mfRET.mmh	6.60E+04	2.25E-04	3.40E-09	51
H4H8087P	hRET.mmh	8.96E+05	2.42E-04	2.70E-10	48
	hRET.mFc	1.22E+06	1.07E-04	8.70E-11	108
	mfRET.mmh	6.41E+05	2.72E-04	4.24E-10	42
H4H8056P	hRET.mmh	2.11E+05	5.39E-04	2.56E-09	21
	hRET.mFc	1.85E+05	2.72E-04	1.47E-09	42
	mfRET.mmh	2.16E+05	5.83E-04	2.69E-09	20
H4H8058P	hRET.mmh	2.48E+05	5.18E-04	2.09E-09	22
	hRET.mFc	2.36E+05	2.59E-04	1.10E-09	45
	mfRET.mmh	2.30E+05	5.40E-04	2.35E-09	21
H4H8060P	hRET.mmh	7.61E+05	3.11E-04	4.09E-10	37
	hRET.mFc	8.27E+05	1.49E-04	1.79E-10	78
	mfRET.mmh	7.51E+05	3.93E-04	5.23E-10	29
H4H8062P	hRET.mmh	5.05E+05	3.70E-04	7.33E-10	31
	hRET.mFc	4.00E+05	2.20E-04	4.90E-10	53
	mfRET.mmh	5.01E+05	7.33E-04	1.46E-09	16

NB: No binding observed under conditions used

Table 3: Biacore binding affinities of human Fc mAbs at 37°C

	Binding at 37°C/ Mab Capture Format				
AbPID	**Analyte**	**ka (Ms^{-1})**	**Kd (s^{-1})**	**K_D (Molar)**	**t½ (min)**
H4H7086N	hRET.mmh	TBD	TBD	TBD	TBD
	hRET.mFc	TBD	TBD	TBD	TBD
	mfRET.mmh	TBD	TBD	TBD	TBD
H4H8066P	hRET.mmh	1.95E+05	1.06E-03	5.43E-09	11
	hRET.mFc	2.62E+05	3.35E-04	1.28E-09	34
	mfRET.mmh	8.19E+04	3.23E-03	3.94E-08	4
H4H8067P	hRET.mmh	1.59E+05	1.16E-03	7.31E-09	10
	hRET.mFc	2.04E+05	4.58E-04	2.24E-09	25
	mfRET.mmh	3.07E+05	6.03E-02	1.96E-07	0.2
H4H8071P	hRET.mmh	2.36E+05	8.96E-04	3.79E-09	13
	hRET.mFc	3.00E+05	3.12E-04	1.04E-09	37
	mfRET.mmh	1.61E+05	1.14E-03	7.04E-09	10
H4H8076P	hRET.mmh	3.15E+05	1.36E-03	4.31E-09	9
	hRET.mFc	3.14E+05	6.75E-04	2.15E-09	17
	mfRET.mmh	2.89E+05	1.40E-03	4.84E-09	8
H4H8079P	hRET.mmh	2.07E+05	1.89E-03	9.10E-09	6
	hRET.mFc	2.61E+05	5.23E-04	2.00E-09	22
	mfRET.mmh	1.54E+05	2.05E-03	1.33E-08	6
H4H8044P	hRET.mmh	1.51E+05	2.81E-03	1.86E-08	4
	hRET.mFc	1.37E+05	8.50E-04	6.21E-09	14

	mfRET.mmh	1.08E+05	5.21E-03	4.85E-08	2
H4H8045P	hRET.mmh	3.95E+05	1.27E-03	3.22E-09	9
	hRET.mFc	4.59E+05	3.82E-04	8.34E-10	30
	mfRET.mmh	2.61E+05	1.31E-02	5.03E-08	1
H4H8046P	hRET.mmh	7.18E+05	1.30E-03	1.81E-09	9
	hRET.mFc	3.38E+05	6.94E-04	2.05E-09	17
	mfRET.mmh	7.04E+05	1.35E-03	1.92E-09	9
H4H8048P	hRET.mmh	3.39E+05	2.42E-03	7.14E-09	5
	hRET.mFc	2.80E+05	1.09E-03	3.89E-09	11
	mfRET.mmh	2.95E+05	2.54E-03	8.60E-09	5
H4H8080P	hRET.mmh	2.85E+05	2.67E-03	9.35E-09	4
	hRET.mFc	2.69E+05	1.07E-03	3.98E-09	11
	mfRET.mmh	NB	NB	NB	NB
H4H8083P	hRET.mmh	2.82E+05	2.55E-03	9.04E-09	5
	hRET.mFc	2.64E+05	8.00E-04	3.02E-09	14
	mfRET.mmh	2.83E+05	4.00E-03	1.42E-08	3
H4H8084P	hRET.mmh	1.06E+06	1.65E-03	1.56E-09	7
	hRET.mFc	1.05E+06	8.50E-04	8.09E-10	14
	mfRET.mmh	1.01E+06	1.78E-03	1.76E-09	6
H4H8085P	hRET.mmh	1.44E+05	8.97E-04	6.23E-09	13
	hRET.mFc	9.90E+04	5.50E-04	5.50E-09	21
	mfRET.mmh	1.52E+05	1.26E-03	8.30E-09	9
H4H8087P	hRET.mmh	1.27E+06	1.23E-03	9.68E-10	9
	hRET.mFc	1.26E+06	4.41E-04	3.49E-10	26
	mfRET.mmh	8.36E+05	1.31E-03	1.57E-09	9
H4H8056P	hRET.mmh	3.26E+05	2.89E-03	8.88E-09	4
	hRET.mFc	2.27E+05	1.28E-03	5.62E-09	9
	mfRET.mmh	3.03E+05	3.13E-03	1.03E-08	4
H4H8058P	hRET.mmh	5.28E+05	2.21E-03	4.18E-09	5
	hRET.mFc	5.52E+05	1.21E-03	2.18E-09	10
	mfRET.mmh	5.43E+05	2.26E-03	4.16E-09	5
H4H8060P	hRET.mmh	1.11E+06	1.57E-03	1.41E-09	7
	hRET.mFc	1.06E+06	5.92E-04	5.58E-10	19
	mfRET.mmh	1.12E+06	2.09E-03	1.88E-09	6
H4H8062P	hRET.mmh	6.82E+05	1.81E-03	2.65E-09	6
	hRET.mFc	3.66E+05	8.80E-04	2.40E-09	13
	mfRET.mmh	6.78E+05	4.28E-03	6.31E-09	3

NB: No binding observed under conditions used

Example 4. Anti-RET Antibodies Potently Block the Binding of Human RET to the GFRα1/GDNF Co-Complex

[0189] The ability of anti-RET antibodies to block the binding of human RET to pre-complexed plate bound GDNF:GFRα1 was assessed using a competition sandwich ELISA. The majority of RET antibodies potently blocked the binding of RET to plate bound GDNF/GFRα1 co-complex (Table 4). IC_{50} values ranged from 5.2nM to below the

theoretical bottom of the assay (250pM), with maximum blocking ranging from 72-96 %.

Detailed Methods

[0190] Recombinant human dimeric GDNF (R&D systems) and human GFRα1.mFc (SEQ ID: 308) were mixed at a 1:1 molar ratio in PBS to obtain a final co-complex concentration of ~ 2.0ug/ml. The GDNF-GFRα1 co-complex was incubated for 1 hr at room temp (RT) before coating 96-well microtiter plates overnight at 4°C. Nonspecific binding sites were blocked with BSA.

[0191] Separately, 1nM of biotinylated monomeric RET protein (biot-hRET.mmh; SEQ ID: 305) was titrated with varying amounts of serially diluted antibodies ranging between 0-120 nM. Antibody-RET mixtures were incubated for 1 hour at RT and then transferred to microtiter plates previously coated with the hGDNF/hGFRα1 co-complex. Binding was allowed to proceed for 1 hr at RT followed by extensive washing. Plate-bound biot-hRET.mmh was detected with HRP-conjugated streptavidin and developed with TMB. Plates were read at 450 nm and data analysis used a sigmoidal dose-response model within Prism™ software.

[0192] IC_{50} values, calculated as the concentration of antibody required to block 50% of hRET binding to hGDNF/hGFRα1, was used as an indicator of blocking potency. Maximum blocking values represent the ability of anti-RET antibodies to block hRET binding relative to baseline. Baseline values were calculated as the absorbance measured at the constant amount of hRET on the dose curve (0 % blocking) and the absorbance measured with no added hRET (100 % blocking). The absorbance values of the wells containing the highest concentration for each antibody were used to determine the blocking percent at maximum concentration antibody tested. A summary of IC_{50}'s and percent maximum blocking are shown in Table 4.

Table 4: IC_{50} values for anti-RET antibodies blocking plate coated pre-complexed GDNF/GFRα1

AbPID	Blocking 1nM Biot-hRET.mmh against plate coated Pre-complexed GDNF/GFRα1 IC50, [M]	% Max Blocking
H2M7086N	3.3E-10	96
H4H7086N	3.9E-10	97
H4H8044P	5.2E-09	91
H4H8045P	3.4E-10	75
H4H8046P	<2.5E-10 #	95
H4H8048P	<2.5E-10 #	96
H4H8056P	IC	73

H4H8058P	2.5E-10	93
H4H8060P	<2.5E-10 #	95
H4H8062P	<2.5E-10 #	97
H4H8066P	4.1E-10	57
H4H8067P	<2.5E-10 #	95
H4H8071P	3.6E-10	72
H4H8076P	<2.5E-10 #	95
H4H8079P	<2.5E-10 #	93
H4H8080P	<2.5E-10 #	82
H4H8083P	<2.5E-10 #	95
H4H8084P	<2.5E-10 #	96
H4H8085P	<2.5E-10 #	96
H4H8087P	<2.5E-10 #	92

Below assay theoretical bottom of <2.5E-10 M; IC-inconclusive

Example 5. Anti-RET Antibodies Inhibit Ligand Dependent RET Signaling in an SRE-Luciferase Reporter Assay & Display Strong Internalization

[0193] In this example, the effect of anti-RET antibodies on RET signaling and internalization was examined using MCF7 and hRET engineered reporter cell lines.

[0194] The glial family ligands GDNF and Artemin trigger the activation of RET through the formation of a high affinity co-complex with GFRα1 or 3, respectively, bringing together two RET molecules and initiating the phosphorylation of specific tyrosine residues. Trans-phosphorylation of RET activates several downstream intracellular cascades, and the upregulation of RET signaling has been implicated in several disease pathologies, including cancer (Borrello, MG, *et al.*, (2013), Expert. Opin. Ther. Targets 17(4): 403-419).

[0195] To test the ability of RET antibodies to block GDNF mediated signaling, the human breast adenocarcinoma cell line MCF7, which expresses RET and GFRα1, was transduced with a serum response factor (SRE)-regulated luciferase reporter gene to create a MCF7/SRE-Luc line. Antibodies of this invention displayed potent inhibition of GDNF stimulated RET signaling, with IC_{50} values ranging from 143 pM to >100 nM (Table 5). The percent of inhibition ranged from 60-100%. Several non-blocking antibodies were also identified; H4H8085P stimulated luciferase activity to 50% of the levels observed with GDNF, while H4H8044P, H4H8076P and H4H8046P were weaker activators of luciferase response (2-5% activation).

[0196] To determine if the antibodies that blocked GDNF mediated RET signaling would also be efficacious against artemin triggered activity engineered HEK293/hGFRa3/hRET SRELuc cell lines were constructed. Most GDNF-dependent blockers of RET-signaling were also

blockers of artemin dependent signaling activity in this cell line (Table 5; column 5-6). Interestingly H4H8048P was identified to be a more potent blocker against artemin-

dependent signaling compared to GDNF-dependent signaling, perhaps reflecting different epitopes bound by the GDNF-GFRα1 and artemin-GFRα3 co-complexes on the RET receptor.

[0197] Lastly, to understand if the blocking activity observed might also be due to degradation of the RET receptor upon antibody binding, several antibodies were tested in an internalization assay (Table 6). Among the seven antibodies tested, H4H8087P was identified as the strongest internalizer, with H4H8079P and H4H7086P also demonstrating potent internalization.

[0198] In conclusion, this example illustrates that the anti-RET antibodies of this invention display a range of activating and inhibitory activity on RET signaling in the presence of the glial family ligands, GDNF and artemin.

Table 5: IC_{50} and EC_{50} values of anti-RET antibodies in SRE-Luciferase ligand-dependent RET signaling assay

mAb	MCF7/SRE-luc Avg. IC_{50} (nM)	MCF7/SRE-Luc Avg % Blocking	MCF7/SRE-Luc % Activation	293/hGFRα3/hRET/SRE-Luc IC_{50} (nM)	293/hGFRα3/hRET SRE-luc %Blocking
H2M7086N	2.6	100	0	4	98
H4H8044P	2.1	85	2	1.6	77
H4H8045P	0.14	69	0	0.42	96
H4H8046P	44	78	5	11.8	91
H4H8048P	>100	82	0	8.3	99
H4H8056P	2	73	0	ND	ND
H4H8058P	5.4	85	0	ND	ND
H4H8060P	>100	87	0	ND	ND
H4H8062P	5.2	100	0	ND	ND
H4H8066P	0.39	60	0	1.5	93
H4H8067P	3.8	91	0	4.8	99
H4H8071P	0.45	84	0	ND	ND
H4H8076P	55.7	74	3	ND	ND
H4H8079P	0.15	90	0	0.8	96
H4H8080P	>100	41	0	341	57
H4H8083P	>100	68	0	57	81
H4H8084P	10.4	100	0	2.1	94
H4H8085P	NB	0	47	127	72
H4H8087P	0.25	100	0	0.25	100

Table 6: Percent internalization of anti-RET antibodies at 37°C relative to H4H8087P

PID	Internalization (% H4H8087P)
H4H7086N	59.03
H4H8058P	42.02

H4H8062P	44.94
H4H8067P	58.20
H4H8079P	62.78
H4H8048P	35.37
H4H8087P	100.00

Detailed Methods

Generation of the MCF7/SRE-Luciferase Stable Cell lines

[0199] MCF7 cells naturally express RET and GFRα1. Production of MCF7/SRELuc cells utilized a stably incorporated SRE-Luciferase generated via transduction of MCF7 with the Cignal Lenti SRE Reporter kit (SABiosciences) and a two-week selection in puromycin. The lentivirus expresses the firefly luciferase gene under the control of a minimal CMV promoter and tandem repeats of the serum response element (SRE).

Generation of HEk293/ hGFRa1 (or 3) / hRET/SRE-Luciferase Stable Cell Lines

[0200] Human GFRα (1 or 3) and hRET were stably introduced into HEK293 cells via sequential rounds of Lipofectamine2000-mediated transfection, and selected for at least two weeks in 500ug/ml G418 (hGFRa1 or 3) and 100ug/ml hygromycin B (hRET). The HEK293 double stable lines expressing hGFRa1/hRET or hGFRa3/hRET were then transduced with the Cignal Lenti SRE Reporter kit, as described above, to generate the HEK293/hGFRa1/hRET/SRE-Luc cell line and the artemin-responsive HEK293/GFRa3/hRET/SRE-Luc cell line.

Inhibition of GDNF-stimulated Luciferase activity in MCF7/SRE-Luciferase engineered cell lines

[0201] Twenty thousand MCF7-SRE-luc cells were seeded in PDL coated 96 well plates in Optimem + 0.5% FBS and grown overnight at 37°C, 5% CO_2. For inhibition curves, cells were incubated for 1hr with serially diluted anti hRET mAbs ranging from 1.6 pM to 1uM. A constant dose of human GDNF (4-10pM) was then added and cells were incubated for an additional 6 hr.

[0202] To assess activating properties of anti-RET mAbs, MCF7-SRE-Luc cells were incubated for 6hr with serially diluted anti hRET mAbs ranging from 1.6 pM to 1uM in the absence of ligand.

[0203] GDNF dose response curves were measured using serially diluted GDNF, ranging from 0.05 pM to 10 nM, added to wells without antibodies and incubated for 6 hr at 37°C. Luciferase activity was measured with ONE GLO™ reagent (Promega) and relative light units (RLUs) were measured on a Victor luminometer (Perkin Elmer).

Inhibition of Artemin-stimulated Luciferase activity in HEK293/hGFRa3/hRET engineered cell lines

[0204] Inhibition of Artemin-stimulated luciferase activity in HEK293/hGFRa3/ hRET/SRE-Luc cell lines was assessed using anti-RET antibodies via the method described for MCF7/SRE-Luc cells. To generate inhibition curves, cells were incubated for 1hr with serially diluted anti hRET antibodies ranging from 1.6 pM to 1uM. Cells were then stimulated with a constant dose of hArtemin (100pM) for 6 hr. Artemin dose response curves were generated by adding serially diluted hArtemin (0.17 pM – 10 nM) to cells for 6 hr at 37°C without the addition of antibody. Luciferase activity measurements and curve fitting was performed as described for GDNF stimulated luciferase activity.

Calculation of EC_{50}/IC_{50} values

[0205] EC_{50}/IC_{50} values were determined from a four-parameter logistic equation over a 12-point response curve using GraphPad Prism. Percent blocking is reported for the highest antibody dose and data is reported as average ± standard deviation (SD).

Quantitative analysis of internalization properties of anti-RET antibodies

[0206] To test anti hRET mAbs for internalization, HEK293/hGFRa1/hRET/SRE-Luc cells were incubated with antibodies (10 ug/ml) for 30 minutes on ice, followed by one wash. Cells were then incubated with alexa488 conjugated anti-hFc Fab secondary antibodies for 30 minutes followed by a second wash. Antibodies were allowed to internalize for 4hr at 37°C or remain at 4°C to prevent internalization. Cells were fixed in 4% formaldehyde and cell surface alexa488 was quenched by incubation with an anti alexa488-quenching antibody for 1hr at 4°C. Nuclei were stained with Hoechst stain and images were acquired on the ImageXpress micro XL (Molecular Devices).

[0207] Total alexa488 intensity in the intracellular vesicles at 37°C in the quenched samples was quantitated via Columbus image analysis software (Perkin Elmer). The total internalized mAb intensity is expressed as a percentage of the strongest internalizing mAb.

Example 6. Generation of a Bi-specific Antibody

[0208] Various bi-specific antibodies are generated for use in practicing the methods of the invention. For example, RET specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct domains of the RET protein are linked together to confer dual-domain specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall RET neutralization efficacy through increasing both specificity and binding avidity. Variable regions with specificity for individual domains are paired on a structural scaffold that allows each region to bind simultaneously to separate

epitopes, or to different regions within one domain. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one domain are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second domain to identify non-cognate V_L partners that can be paired with an original V_H without disrupting the original specificity for that V_H. In this way, a single V_L segment (*e.g.,* V_L1) can be combined with two different V_H domains (*e.g.,* V_H1 and V_H2) to generate a bi-specific comprised of two binding "arms" (V_H1- V_L1 and V_H2- V_L1). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

[0209] Alternatively, antibodies that bind RET and a second target, such as, but not limited to, for example, a a tumor antigen, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct regions may be linked together with variable regions that bind to relevant sites on, for example, a different antigen to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. For example, in the case of a bi-specific antibody that binds *ie.* RET and one of its ligands, one may be able to better inhibitor tumor cell growth, without the need for administration of a composition containing two separate antibodies. Variable regions with specificity for RET, are combined with a variable region with specificity for one of its ligands and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[0210] The bi-specific binders are tested for binding and functional blocking of the target antigen, for example, RET, in any of the assays described above for antibodies. For example, standard methods to measure soluble protein binding are used to assess the bispecific interaction, such as Biacore, ELISA, size exclusion chromatography, multi-angle laser light scattering, direct scanning calorimetry, and other methods. Binding of bi-specific antibodies to both RET and one of its ligands is determined through use of an ELISA binding assay in which synthetic peptides representing the different antigens are coated onto the wells of microtiter plates, and binding of a bi-specific is determined through use of a secondary detection antibody. Binding experiments can also be conducted using surface plasmon resonance experiments, in which real-time binding interaction of peptide to antibody is measured by flowing a peptide or bi-specific across a sensor surface on which bi-specific or peptide, respectively, is captured. Functional *in vitro* blocking of both RET and one of its ligands by a bi-specific is determined using any bioassay such as the assays described herein, or by *in vivo* protection studies in appropriate animal models, such as tumor bearing animal models.

WE CLAIM

1. An isolated human monoclonal antibody or antigen-binding fragment thereof that specifically binds to RET (Rearranged during Transfection) receptor tyrosine kinase, wherein the antibody or antigen-binding fragment therefore comprises a HCVR comprising three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and a LCVR comprising three light chain CDRs (LCDR1, LCDR2 and LCDR3), and:

(a) HCDR1 comprises the amino acid sequence of SEQ ID NO:4, HCDR2 comprises the amino acid sequence of SEQ ID NO:6, HCDR3 comprises the amino acid sequence of SEQ ID NO:8, LCDR1 comprises the amino acid sequence of SEQ ID NO:12, LCDR2 comprises the amino acid sequence of SEQ ID NO:14, and LCDR3 comprises the amino acid sequence of SEQ ID NO:16; or

(b) HCDR1 comprises the amino acid sequence of SEQ ID NO:20, HCDR2 comprises the amino acid sequence of SEQ ID NO:22, HCDR3 comprises the amino acid sequence of SEQ ID NO:24, LCDR1 comprises the amino acid sequence of SEQ ID NO:28, LCDR2 comprises the amino acid sequence of SEQ ID NO:30, and LCDR3 comprises the amino acid sequence of SEQ ID NO:32; or

(c) HCDR1 comprises the amino acid sequence of SEQ ID NO:36, HCDR2 comprises the amino acid sequence of SEQ ID NO:38, HCDR3 comprises the amino acid sequence of SEQ ID NO:40, LCDR1 comprises the amino acid sequence of SEQ ID NO:44, LCDR2 comprises the amino acid sequence of SEQ ID NO:46, and LCDR3 comprises the amino acid sequence of SEQ ID NO:48; or

(d) HCDR1 comprises the amino acid sequence of SEQ ID NO:52, HCDR2 comprises the amino acid sequence of SEQ ID NO:54, HCDR3 comprises the amino acid sequence of SEQ ID NO:56, LCDR1 comprises the amino acid sequence of SEQ ID NO:60, LCDR2 comprises the amino acid sequence of SEQ ID NO:62, and LCDR3 comprises the amino acid sequence of SEQ ID NO:64; or

(e) HCDR1 comprises the amino acid sequence of SEQ ID NO:68, HCDR2 comprises the amino acid sequence of SEQ ID NO:70, HCDR3 comprises the amino acid sequence of SEQ ID NO:72, LCDR1 comprises the amino acid sequence of SEQ ID NO:76, LCDR2 comprises the amino acid sequence of SEQ ID NO:78, and LCDR3 comprises the amino acid sequence of SEQ ID NO:80; or

(f) HCDR1 comprises the amino acid sequence of SEQ ID NO:84, HCDR2 comprises the amino acid sequence of SEQ ID NO:86, HCDR3 comprises the amino acid sequence of SEQ ID NO:88, LCDR1 comprises the amino acid sequence of SEQ ID NO:92,

LCDR2 comprises the amino acid sequence of SEQ ID NO:94, and LCDR3 comprises the amino acid sequence of SEQ ID NO:96; or

(g) HCDR1 comprises the amino acid sequence of SEQ ID NO:100, HCDR2 comprises the amino acid sequence of SEQ ID NO:102, HCDR3 comprises the amino acid sequence of SEQ ID NO:104, LCDR1 comprises the amino acid sequence of SEQ ID NO:108, LCDR2 comprises the amino acid sequence of SEQ ID NO:110, and LCDR3 comprises the amino acid sequence of SEQ ID NO:112; or

(h) HCDR1 comprises the amino acid sequence of SEQ ID NO:116, HCDR2 comprises the amino acid sequence of SEQ ID NO:118, HCDR3 comprises the amino acid sequence of SEQ ID NO:120, LCDR1 comprises the amino acid sequence of SEQ ID NO:124, LCDR2 comprises the amino acid sequence of SEQ ID NO:126, and LCDR3 comprises the amino acid sequence of SEQ ID NO:128; or

(i) HCDR1 comprises the amino acid sequence of SEQ ID NO:132, HCDR2 comprises the amino acid sequence of SEQ ID NO:134, HCDR3 comprises the amino acid sequence of SEQ ID NO:136, LCDR1 comprises the amino acid sequence of SEQ ID NO:140, LCDR2 comprises the amino acid sequence of SEQ ID NO:142, and LCDR3 comprises the amino acid sequence of SEQ ID NO:144; or

(j) HCDR1 comprises the amino acid sequence of SEQ ID NO:148, HCDR2 comprises the amino acid sequence of SEQ ID NO:150, HCDR3 comprises the amino acid sequence of SEQ ID NO:152, LCDR1 comprises the amino acid sequence of SEQ ID NO:156, LCDR2 comprises the amino acid sequence of SEQ ID NO:158, and LCDR3 comprises the amino acid sequence of SEQ ID NO:160; or

(k) HCDR1 comprises the amino acid sequence of SEQ ID NO:164, HCDR2 comprises the amino acid sequence of SEQ ID NO:166, HCDR3 comprises the amino acid sequence of SEQ ID NO:168, LCDR1 comprises the amino acid sequence of SEQ ID NO:172, LCDR2 comprises the amino acid sequence of SEQ ID NO:174, and LCDR3 comprises the amino acid sequence of SEQ ID NO:176; or

(l) HCDR1 comprises the amino acid sequence of SEQ ID NO:180, HCDR2 comprises the amino acid sequence of SEQ ID NO:182, HCDR3 comprises the amino acid sequence of SEQ ID NO:184, LCDR1 comprises the amino acid sequence of SEQ ID NO:188, LCDR2 comprises the amino acid sequence of SEQ ID NO:190, and LCDR3 comprises the amino acid sequence of SEQ ID NO:192; or

(m) HCDR1 comprises the amino acid sequence of SEQ ID NO:196, HCDR2 comprises the amino acid sequence of SEQ ID NO:198, HCDR3 comprises the amino acid sequence of SEQ ID NO:200, LCDR1 comprises the amino acid sequence of SEQ ID NO:204,

LCDR2 comprises the amino acid sequence of SEQ ID NO:206, and LCDR3 comprises the amino acid sequence of SEQ ID NO:208; or

(n) HCDR1 comprises the amino acid sequence of SEQ ID NO:212, HCDR2 comprises the amino acid sequence of SEQ ID NO:214, HCDR3 comprises the amino acid sequence of SEQ ID NO:216, LCDR1 comprises the amino acid sequence of SEQ ID NO:220, LCDR2 comprises the amino acid sequence of SEQ ID NO:222, and LCDR3 comprises the amino acid sequence of SEQ ID NO:224; or

(o) HCDR1 comprises the amino acid sequence of SEQ ID NO:228, HCDR2 comprises the amino acid sequence of SEQ ID NO:230, HCDR3 comprises the amino acid sequence of SEQ ID NO:232, LCDR1 comprises the amino acid sequence of SEQ ID NO:236, LCDR2 comprises the amino acid sequence of SEQ ID NO:238, and LCDR3 comprises the amino acid sequence of SEQ ID NO:240; or

(p) HCDR1 comprises the amino acid sequence of SEQ ID NO:244, HCDR2 comprises the amino acid sequence of SEQ ID NO:246, HCDR3 comprises the amino acid sequence of SEQ ID NO:248, LCDR1 comprises the amino acid sequence of SEQ ID NO:252, LCDR2 comprises the amino acid sequence of SEQ ID NO:254, and LCDR3 comprises the amino acid sequence of SEQ ID NO:256; or

(q) HCDR1 comprises the amino acid sequence of SEQ ID NO:260, HCDR2 comprises the amino acid sequence of SEQ ID NO:262, HCDR3 comprises the amino acid sequence of SEQ ID NO:264, LCDR1 comprises the amino acid sequence of SEQ ID NO:268, LCDR2 comprises the amino acid sequence of SEQ ID NO:270, and LCDR3 comprises the amino acid sequence of SEQ ID NO:272; or

(r) HCDR1 comprises the amino acid sequence of SEQ ID NO:276, HCDR2 comprises the amino acid sequence of SEQ ID NO:278, HCDR3 comprises the amino acid sequence of SEQ ID NO:280, LCDR1 comprises the amino acid sequence of SEQ ID NO:284, LCDR2 comprises the amino acid sequence of SEQ ID NO:286, and LCDR3 comprises the amino acid sequence of SEQ ID NO:288; or

(s) HCDR1 comprises the amino acid sequence of SEQ ID NO:292, HCDR2 comprises the amino acid sequence of SEQ ID NO:294, HCDR3 comprises the amino acid sequence of SEQ ID NO:296, LCDR1 comprises the amino acid sequence of SEQ ID NO:300, LCDR2 comprises the amino acid sequence of SEQ ID NO:302, and LCDR3 comprises the amino acid sequence of SEQ ID NO:304.

2. The isolated human monoclonal antibody or antigen-binding fragment of claim 1, wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 212, HCDR2 comprises the amino acid sequence of SEQ ID NO: 214, HCDR3 comprises the amino acid sequence of SEQ ID NO: 216, LCDR1 comprises the amino acid sequence of SEQ ID NO: 220, LCDR2 comprises the amino acid sequence of SEQ ID NO: 222, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 224.

3. The isolated human monoclonal antibody or antigen-binding fragment of claim 1, wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 36, HCDR2 comprises the amino acid sequence of SEQ ID NO: 38, HCDR3 comprises the amino acid sequence of SEQ ID NO: 40, LCDR1 comprises the amino acid sequence of SEQ ID NO: 44, LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 48.

4. The isolated human monoclonal antibody or antigen-binding fragment of claim 1, wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 148, HCDR2 comprises the amino acid sequence of SEQ ID NO: 150, HCDR3 comprises the amino acid sequence of SEQ ID NO: 152, LCDR1 comprises the amino acid sequence of SEQ ID NO: 156, LCDR2 comprises the amino acid sequence of SEQ ID NO: 158, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 160.

5. The isolated human monoclonal antibody or antigen-binding fragment of claim 1, wherein:

(a) the HCVR comprises the amino acid sequence of SEQ ID NO:2 and the LCVR comprises the amino acid sequence of SEQ ID NO:10; or

(b) the HCVR comprises the amino acid sequence of SEQ ID NO:18 and the LCVR comprises the amino acid sequence of SEQ ID NO:26; or

(c) the HCVR comprises the amino acid sequence of SEQ ID NO:34 and the LCVR comprises the amino acid sequence of SEQ ID NO:42; or

(d) the HCVR comprises the amino acid sequence of SEQ ID NO:50 and the LCVR comprises the amino acid sequence of SEQ ID NO:58; or

(e) the HCVR comprises the amino acid sequence of SEQ ID NO:66 and the LCVR comprises the amino acid sequence of SEQ ID NO:74; or

(f) the HCVR comprises the amino acid sequence of SEQ ID NO:82 and the LCVR comprises the amino acid sequence of SEQ ID NO:90; or

(g) the HCVR comprises the amino acid sequence of SEQ ID NO:98 and the LCVR comprises the amino acid sequence of SEQ ID NO:106; or

(h) the HCVR comprises the amino acid sequence of SEQ ID NO:114 and the LCVR comprises the amino acid sequence of SEQ ID NO:122; or

(i) the HCVR comprises the amino acid sequence of SEQ ID NO:130 and the LCVR comprises the amino acid sequence of SEQ ID NO:138; or

(j) the HCVR comprises the amino acid sequence of SEQ ID NO:146 and the LCVR comprises the amino acid sequence of SEQ ID NO:154; or

(k) the HCVR comprises the amino acid sequence of SEQ ID NO:162 and the LCVR comprises the amino acid sequence of SEQ ID NO:170; or

(l) the HCVR comprises the amino acid sequence of SEQ ID NO:178 and the LCVR comprises the amino acid sequence of SEQ ID NO:186; or

(m) the HCVR comprises the amino acid sequence of SEQ ID NO:194 and the LCVR comprises the amino acid sequence of SEQ ID NO:202; or

(n) the HCVR comprises the amino acid sequence of SEQ ID NO:210 and the LCVR comprises the amino acid sequence of SEQ ID NO:218; or

(o) the HCVR comprises the amino acid sequence of SEQ ID NO:226 and the LCVR comprises the amino acid sequence of SEQ ID NO:234; or

(p) the HCVR comprises the amino acid sequence of SEQ ID NO:242 and the LCVR comprises the amino acid sequence of SEQ ID NO:250; or

(q) the HCVR comprises the amino acid sequence of SEQ ID NO:258 and the LCVR comprises the amino acid sequence of SEQ ID NO:266; or

(r) the HCVR comprises the amino acid sequence of SEQ ID NO:274 and the LCVR comprises the amino acid sequence of SEQ ID NO:282; or

(s) the HCVR comprises the amino acid sequence of SEQ ID NO:290 and the LCVR comprises the amino acid sequence of SEQ ID NO:298.

6. The isolated human monoclonal antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment thereof comprises a HCVR having the amino acid sequence of SEQ ID NO: 210 and a LCVR having the amino acid sequence of SEQ ID NO: 218.

7. The isolated human monoclonal antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment thereof comprises a HCVR having the amino acid sequence of SEQ ID NO: 34 and a LCVR having the amino acid sequence of SEQ ID NO: 42.

8. The isolated human monoclonal antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment thereof comprises a HCVR having the amino acid sequence of SEQ ID NO: 146 and a LCVR having the amino acid sequence of SEQ ID NO: 154.

9. The isolated human monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-8, wherein the antibody is a fully human antibody

10. An isolated nucleic acid molecule encoding the antibody or antigen-binding fragment of any of claims 1-8.

11. An expression vector comprising the nucleic acid molecule of claim 10.

12. A pharmaceutical composition comprising any one or more of the antibodies that specifically bind RET, or an antigen-binding fragment thereof of any of claims 1-9 and a pharmaceutically acceptable carrier or diluent.

13. A method of treating a disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene or the pain associated with the disorder or condition, the method comprising administering one or more antibodies or antigen-binding fragments thereof of any of claims 1-9, or the pharmaceutical composition of claim 12 to a patient in need thereof.

14. Use of one or more antibodies or antigen-binding fragments thereof of any of claims 1-9, or the pharmaceutical composition of claim 12, in the manufacture of a medicament for treating a disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene or the pain associated with the disorder or condition, comprising administering the medicament to a patient in need thereof.

15. The method of claim 13 or the use of claim 14, wherein the disorder or condition associated with expression, activation or signaling of the RET receptor tyrosine kinase gene is:

a cancer and wherein the cancer is selected form the group consisting of thyroid cancer, lung cancer, pancreatic cancer, skin cancer, breast cancer and a blood-borne cancer; or

selected from the group consisting of acute pain, chronic pain, neuropathic pain, inflammatory pain, arthritis, osteoarthritis, migraine, cluster headaches, trigeminal neuralgia, herpetic neuralgia, general neuralgias, neurodegenerative disorders, neuroendocrine disorders, visceral pain, acute gout, post-herpetic neuralgia, diabetic neuropathy, sciatica, back pain, head or neck pain, severe or intractable pain, breakthrough pain, post-surgical pain, dental pain, rhinitis, cancer pain, or bladder disorders.

16. A method for inhibiting tumor growth or tumor cell proliferation, wherein the tumor or tumor cell expresses RET, the method comprising administering one or more antibodies or antigen-binding fragments thereof of any of claims 1-9, or the pharmaceutical composition of claim 12 to a patient in need thereof.

17. Use of one or more antibodies or antigen-binding fragments thereof of any of claims 1-9, or the pharmaceutical composition of claim 12, in the manufacture of a medicament for inhibiting tumor growth or tumor cell proliferation, wherein the tumor or tumor cell expresses RET, comprising administering the medicament to a patient in need thereof.

18. The method of claim 16 or the use of claim 17, wherein:

the tumor is a solid tumor or a blood-borne tumor;

the tumor is a solid tumor and the solid tumor is selected from the group consisting of a thyroid tumor, a lung tumor, a pancreatic tumor, a skin tumor, and a breast tumor; or

the tumor is a thyroid tumor, and the thyroid tumor is a papillary thyroid carcinoma (PTC) or a medullary thyroid carcinoma (MTC);

the tumor is a thyroid tumor, and the thyroid tumor is a medullary thyroid carcinoma (MTC), and the medullary thyroid carcinoma is a hereditary MTC selected from the group consisting of multiple endocrine neoplasia type 2 or 3 (MEN2A, MEN2B) and familial medullary thyroid carcinoma (FMTC) syndrome, or wherein the medullary thyroid carcinoma is a sporadic MTC;

the tumor is a lung tumor, and the lung tumor is a lung adenocarcinoma or non-small cell lung cancer (NSCLC);

the tumor is a skin tumor, and the skin tumor is melanoma;

the tumor is a blood-borne tumor, and the blood-borne tumor is a leukemia; or

the tumor is a blood-borne tumor, and the blood-borne tumor is a leukemia, and the leukemia is chronic myelomonocytic leukemia.

19. A method of down-modulating RET function, the method comprising administering one or more antibodies or antigen-binding fragments thereof of any of claims 1-9, or the pharmaceutical composition of claim 12 to a patient in need thereof.

20. Use of one or more antibodies or antigen-binding fragments thereof of any of claims 1-9, or the pharmaceutical composition of claim 12, in the manufacture of a medicament for down-modulating RET function, comprising administering the medicament to a patient in need thereof.

21. The method of claim 19 or the use of claim 20, wherein the down-modulating of RET function results in down-regulation of a downstream signaling pathway selected from the group consisting of the RAS/RAF pathway and the PI3K pathway.

22. The method or use of any of claims 13-21, wherein:

the antibody or antigen-binding fragment is administered to the patient in combination with a second therapeutic agent; or

the antibody or antigen-binding fragment is administered to the patient in combination with a second therapeutic agent and the second therapeutic agent is selected from the group consisting of a small molecule tyrosine kinase inhibitor, an anti-tumor agent, an siRNA specific for RET, a second antibody specific for RET, and a pain-reducing agent.

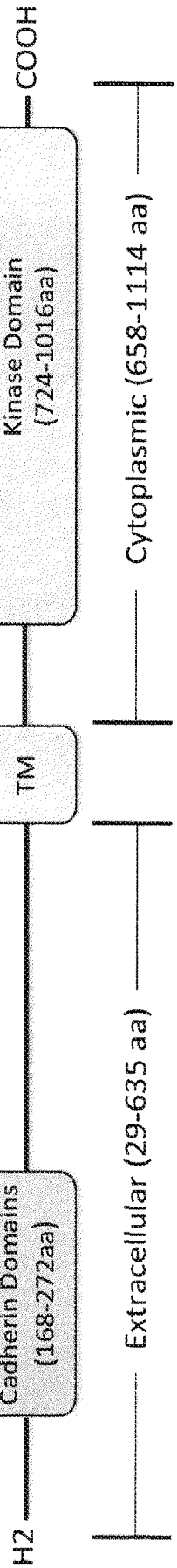
23. The method or use of claim 22, wherein:

the small molecule tyrosine kinase inhibitor is selected from the group consisting of vandetanib, cediranib, (AZD2171), gefitinib, erlotinib, SU14813, vatalanib, sorafenib, sorafenib (BAY43-9006), sunitinib, cabozantinib, motesanib, XL-647, XL-999, AG-013736, BIBF1120, TSU68, GW786034, AEE788, CP-547632, KRN951, CHIR258, CEP-7055, OSI-930, ABT-869, E7080, ZK-304709, BAY57-9352, L- 21649, BMS582664, XL-880, XL-184, XL-820, RPI-1, PP-1 and NVP-AST478;

the anti-tumor agent is selected from the group consisting of a chemotherapeutic agent, a radionuclide and an antibody-drug conjugate; or

the pain-reducing agent is selected from the group consisting of a nerve growth factor (NGF) inhibitor (*e.g.*, a small molecule NGF antagonist or an anti-NGF antibody), aspirin or another NSAID, morphine, steroids (*e.g.*, prednisone), an anti-Nav1.7 antibody, or small molecule inhibitor of Nav1.7, a Nav1.8 antagonist (*e.g.*, anti-Nav1.8 antibody or small molecule inhibitor of Nav1.8), a Nav1.9 antagonist (*e.g.*, anti-Nav1.9 antibody or small molecule inhibitor of Nav1.9), a cytokine inhibitor (*e.g.*, an interleukin-1 (IL-1) inhibitor (such as rilonacept ("IL-1 trap"); Regeneron) or anakinra (KINERET®, Amgen), a small molecule IL-1 antagonist, or an anti-IL-1 antibody; an IL-18 inhibitor (such as a small molecule IL-18 antagonist or an anti-IL-18 antibody); an IL-6 or IL-6R inhibitor (such as a small molecule IL-6 antagonist, an anti-IL-6 antibody or an anti- IL-6 receptor antibody), inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, or an opioid.

Figure 1



Schematic representation of the Human Ret receptor, annotated according to UniProt entry P07949.