HUMAN ANTIBODIES TO GFRα3 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 human glial cell-line derived neurotrophic factor (GDNF) family receptor alpha 3 (GFRα3), and therapeutic methods of using those antibodies.

STATEMENT OF RELATED ART

[0002] The glial cell line-derived neurotrophic factor related family is composed of glial cell line-derived neurotrophic factor (GDNF), neurturin (NRTN), artemin (ARTN) and persephin (PSPN). Each member of the GDNF family binds to a glycosylphosphatidylinositol (GPI)-anchored receptor associated with the plasma membrane. This family of receptors is referred to as the GDNF-family receptor alphas (GFRαs). This receptor family is composed of four different GFRα receptors, GFRα1–4. GDNF binds preferentially to GFRα1, NRTN binds preferentially to GFRα2, ARTN binds preferentially to GFRα3, and PSPN binds preferentially to GFRα4. Each GDNF family ligand signals through the RET ("rearranged during transfection") receptor tyrosine kinase, which was first discovered as a proto-oncogene. RET is activated by GDNF family members only if the ligand is first bound to its GFRα receptor (Airaksinen, M.S., *et al.* Nature Reviews Neuroscience (2002), 3:383-394).

[0003] Both ARTN and GFRα3 are highly expressed during development and are involved in sympathetic nervous system development. In adult, GFRα3 expression is largely restricted to the sensory neurons of the dorsal root ganglia (DRG) (Orozco, O.E., *et al*., European J. Neuroscience, (2001), 13:2177-2182). In adult mouse, artemin is expressed in testis, uterus, thyroid, prostate, and epididymis, as well as in olfactory bulbs and arterioles in the intestine and mesentery (Airaksinen, M.S., *et al.* Nature Reviews Neuroscience (2002), 3:383-394; Airaksinen, M.S. *et al*., Brain, Behavior and Evolution, (2006), 68:181-190).

[0004] A possible role for GFRα3 and artemin in hyperalgesia has been shown in several studies. For example, it has been demonstrated that an injection of the artemin protein into the hindpaw of a rodent caused thermal hyperalgesia and this nociception was enhanced when artemin was co-injected with NGF (Malin, S.A., *et al*., J. Neuroscience, (2006), 26(33): 8588-8599). Other studies showed that artemin mRNA expression was upregulated in a murine inflammatory model (Elitt, C.M., *et al*., J. Neuroscience, (2006), 26(33): 8578-8587). Furthermore, other studies showed that artemin transgenic mice have elevated expression of TRPV1 and TRPA1 and have increased behavioral sensitivity to heat and cold (Elitt, C.M., *et al*., J. Neuroscience, (2006), 26(33): 8578-8587). In addition, a possible role for GFRα3 in visceral hypersensitivity has been shown by studies in GFRα3 knockout mice, whereby these mice showed attenuation of visceral hypersensitivity after intracolonic treatment with TNBS

(2,4,6-trinitrobenzene sulfonic acid) relative to wild type C57BL/6 mice (Tanaka, T., *et al*., Am. J. Physiol. Gastrointest. Liver Physiol. (2011), 300:G418-G424). A possible role for artemin and its receptor GFRα3 in pain associated with pancreatitis has also been shown by a study done in patients undergoing pancreatic head resection (Ceyhan, G.O., *et al.,* Gut, (2007), 56:534-544). Based on the foregoing, further studies are warranted to determine whether patients suffering from pain/hyperalgesia and/or hypersensitivity could benefit by treatment with an inhibitor of GFRα3 activity.

[0005] Antibodies that bind GFRα3 are described in US 6,861,509. In addition, US 6,677,135 discloses a full length GFRα3 sequence, whereas splice variants of the GFRα3 molecule are described in US 7,026,138; US2007/0232535 and US2006/0216289. US 7,138,251 discloses sequences that have 99% identity to full length GFRα3 and the preparation of humanized monoclonal antibodies to this molecule is described in this patent.

BRIEF SUMMARY OF THE INVENTION

[0006] In a first aspect, the invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that bind to human GFRα3 and inhibit or block its activity, for example, block the binding of GFRα3 to the glial cell line-derived neurotrophic factor, artemin, and possibly blocking the subsequent activation of the RET receptor tyrosine kinase and/or blocking signaling through RET and/or blocking signaling through a mediator other than RET. The antibodies or antigen binding fragments thereof may be useful for treating hyperalgesia, allodynia and/or hypersensitivity to any sensory stimulus, including, but not limited to pressure, heat and/or cold. The antibodies may also be used to treat pain/hypersensitivity associated with a wide range of conditions and disorders in which blocking the interaction of GFRα3 with artemin is desired. The antibodies may also be used to inhibit tumor cell growth, proliferation and/or metastasis.

[0007] In one embodiment, the invention provides an isolated antibody or an antigen-binding fragment thereof that specifically binds to human GFRα3 and has one or more of the following characteristics:

(i) exhibits a K_D ranging from about 10^{-8} M to about 10^{-13} M as measured by surface plasmon resonance;

(ii) demonstrates the ability to block about 50-100% of the binding of GFRα3 to its ligand, artemin, with an IC_{50} value ranging from about 40 pM to about 15 nM;

(iii) demonstrates the ability to block about 20% to about 100% of the binding of GFRα3 to a solid support coated with a mixture of artemin and RET;

(iv) blocks or inhibits artemin-dependent activation of RET with an IC_{50} ranging from about 200 pM to about 50 nM;

(v) inhibits or reduces one or more nociceptive responses in an *in vivo* model of bone cancer pain;

(vi) inhibits or reduces artemin-sensitized thermal hyperalgesia *in vivo*;

(vii) inhibits or reduces allodynia in an *in vivo* model of osteoarthritis;

(viii) does not cross-react with other GFR co-receptors for RET;

(ix) 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, 290, 306, 322, 338, 354, 381 and 397; or

(x) 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, 298, 314, 330, 346, 362, 389 and 405.

[0008] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof is selected from the group consisting of a murine, chimeric, humanized and a human antibody.

[0009] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof does not cross-react with human GFRα1 or human GFRα2.

[0010] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof comprises (a) 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, 290, 306, 322, 338, 354, 381 and 397 and (b) 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, 298, 314, 330, 346, 362, 389 and 405.

[0011] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof demonstrates the ability to block about 50-95% of the binding of human GFRα3 to its ligand, artemin, with an IC_{50} value ranging from about 40 pM to about 750 pM.

[0012] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof blocks about 75-100% of the binding of human GFRα3 to its ligand, artemin, with an IC_{50} value ranging from about 400 pM to about 15 nM.

[0013] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof blocks or inhibits artemin-dependent activation of human RET with an IC_{50} ranging from about 300 pM to about 5 nM.

[0014] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof blocks or inhibits artemin-dependent activation of cynomolgus RET with an IC_{50} ranging from about 0.7 nM to about 2.5 nM.

[0015] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof comprises 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, 290, 306, 322, 338, 354, 381 and 397; and 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, 298, 314, 330, 346, 362, 389 and 405.

[0016] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof 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, 290, 306, 322, 338, 354, 381 and 397.

[0017] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof 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, 298, 314, 330, 346, 362, 389 and 405.

[0018] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof 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, 290/298, 306/314, 322/330, 338/346, 354/362, 381/389 and 397/405.

[0019] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NO: 50/58, 146/154, 210/218 and 290/298.

[0020] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof 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, 292, 308, 324, 340, 356, 383 and 399**;**

(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, 294, 310, 326, 342, 358, 385 and 401;

(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, 296, 312, 328, 344, 360, 387 and 403;

(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, 300, 316, 332, 348, 364, 391 and 407;

(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, 302, 318, 334, 350, 366, 393 and 409; 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, 304, 320, 336, 352, 368, 395 and 411.

[0021] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof competes for specific binding to human GFRα3 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, 290/298, 306/314, 322/330, 338/346 and 354/362, 381/389 and 397/405.

[0022] In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof binds the same epitope on human GFRα3 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, 290/298, 306/314, 322/330, 338/346 and 354/362, 381/389 and 397/405.

[0023] 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).

[0024] In one embodiment, the isolated antibody or antigen-binding fragment thereof that binds specifically to human GFRα3, comprises a HCVR comprising the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within the HCVR amino acid sequences 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, 290, 306, 322, 338, 354, 381 and 397; and/or a LCVR comprising the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within the 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, 298, 314, 330, 346, 362, 389 and 405. 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.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0025] In one embodiment, the isolated antibody or antigen-binding fragment that specifically binds human GFRα3 comprises:

(a) 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, 296, 312, 328, 344, 360, 387 and 403; and

(b) 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, 304, 320, 336, 352, 368, 395 and 411.

[0026] In one embodiment, the isolated antibody or antigen-binding fragment that specifically binds human GFRα3, as described in (a) and (b) above, further comprises:

(c) 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, 292, 308, 324, 340, 356, 383 and 399**;**

(d) 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, 294, 310, 326, 342, 358, 385 and 401;

(e) 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, 300, 316, 332, 348, 364, 391 and 407; and

(f) 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, 302, 318, 334, 350, 366, 393 and 409.

[0027] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to human GFRα3, 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, 290, 306, 322, 338, 354, 381 and 397; (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, 298, 314, 330, 346, 362, 389 and 405; (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, 296, 312, 328, 344, 360, 387 and 403, 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 NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 395 and 411 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, 292, 308, 324, 340, 356, 383 and 399 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 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, 294, 310, 326, 342, 358, 385 and 401 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 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, 300, 316, 332, 348, 364, 391 and 407 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 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, 302, 318, 334, 350, 366, 393 and 409 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) exhibits a K_D ranging from about 10^{-8} M to about 10^{-13} M as measured by surface plasmon resonance; (vi) demonstrates the ability to block about 50-100% of the binding of GFRα3 to its ligand, artemin, with an IC_{50} value ranging from about 40 pM to about 15 nM; (vii) demonstrates the ability to block about 20% to about 100% of the binding of GFRα3 to a solid support coated with a mixture of artemin and RET; (viii) blocks or inhibits artemin-dependent activation of RET with an IC_{50} ranging from about 200 pM to about 50 nM; (ix) inhibits or reduces one or more nociceptive responses in an *in vivo* model of bone cancer pain; (x) inhibits or reduces artemin-sensitized thermal hyperalgesia *in vivo*; (xi) inhibits or reduces allodynia in an *in vivo* model of osteoarthritis; (xii) does not cross-react with other GFR co-receptors for RET**.**

[0028] In one embodiment, the present invention provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain having an amino acid sequence selected from any of those shown in Table 1, 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 any of those shown in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0029] In one embodiment, the invention provides an antibody or fragment thereof further comprising a HCDR1 domain having an amino acid sequence of any of those shown in Table 1, 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 having an amino acid sequence of any of those shown on Table 1, 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 having an amino acid sequence of any of those shown in Table 1, 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 having an amino acid sequence of any of those shown in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0030] In certain embodiments, the antibody or antigen-binding portion of an antibody that specifically binds to human GFRα3 comprises a HCDR3/LCDR3 amino acid sequence pair selected from any of the HCDR3/LCDR3 amino acid sequences shown in Table 1. According to certain embodiments, the antibody or antigen-binding portion of an antibody comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOs: 8/16, 24/32, 40/48, 56/64, 72/80, 88/96, 104/112, 120/128, 136/144, 152/160, 168/176, 184/192, 200/208, 216/224, 232/240, 248/256, 264/272, 280/288, 296/304, 312/320, 328/336, 344/352, 360/368, 387/395 and 403/411.

[0031] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 4, 6 and 8, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 12, 14 and 16, respectively.

[0032] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 20, 22 and 24, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 28, 30 and 32, respectively.

[0033] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 36, 38 and 40, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 44, 46 and 48, respectively.

[0034] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 52, 54 and 56, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 60, 62 and 64, respectively.

[0035] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 68, 70 and 72, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 76, 78 and 80, respectively.

[0036] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 84, 86 and 88, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 92, 94 and 96, respectively.

[0037] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 100, 102 and 104, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 108, 110 and 112, respectively.

[0038] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 116, 118 and 120, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 124, 126 and 128, respectively.

[0039] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 132, 134 and 136, respectively and

LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 140, 142 and 144, respectively.

[0040] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 148, 150 and 152, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 156, 158 and 160, respectively.

[0041] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 164, 166 and 168, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 172, 174 and 176, respectively.

[0042] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 180, 182 and 184, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 188, 190 and 192, respectively.

[0043] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 196, 198 and 200, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 204, 206 and 208, respectively.

[0044] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 212, 214 and 216, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 220, 222 and 224, respectively.

[0045] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 228, 230 and 232, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 236, 238 and 240, respectively.

[0046] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 244, 246 and 248, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 252, 254 and 256, respectively.

[0047] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 260, 262 and 264, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 268, 270 and 272, respectively.

[0048] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 276, 278 and 280, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 284, 286 and 288, respectively.

[0049] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 292, 294 and 296, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 300, 302 and 304, respectively.

[0050] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 308, 310 and 312, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 316, 318 and 320, respectively.

[0051] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 324, 326 and 328, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 332, 334 and 336, respectively.

[0052] In one embodiment, the antibody or antigen binding fragment thereof comprises the

HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 340, 342 and 344, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 348, 350 and 352, respectively.

[0053] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 356, 358 and 360, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 364, 366 and 368, respectively.

[0054] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 383, 385 and 387, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 391, 393 and 395, respectively.

[0055] In one embodiment, the antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NOs: 399, 401 and 403, respectively and LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 407, 409 and 411, respectively.

[0056] Certain non-limiting, exemplary antibodies and antigen-binding fragments of the invention comprise HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 domains, respectively, selected from any of the amino acid sequences shown in Table 1.

[0057] In a second aspect, the invention provides nucleic acid molecules encoding anti-GFRα3 antibodies or fragments thereof. 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.

[0058] 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 NOs: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 380 and 396 or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In one embodiment, the HCVR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 49, 145, 209 and 289.

[0059] 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 NOs: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361, 388 and 404 or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In one embodiment, the LCVR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 57, 153, 217 and 297.

[0060] 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 located within the variable regions from any of the antibodies shown in Table 1, 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 any of those shown in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0061] In one embodiment, the invention provides an antibody or fragment thereof further comprising a HCDR1 domain encoded by a nucleotide sequence of any of those shown in Table 1, 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 of any of those shown in Table 1, 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 of any of those shown in Table 1, 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 shown in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0062] In a third aspect, the invention features a human anti-hGFRα3 antibody or antigen-binding fragment of an antibody 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, with combinations as shown in Table 2.

[0063] The invention encompasses anti-hGFRα3 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).

[0064] In a fourth aspect, the invention features a pharmaceutical composition comprising a recombinant human antibody or fragment thereof, which specifically binds hGFRα3 and a pharmaceutically acceptable carrier. 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. The second therapeutic agent may be any agent that is advantageously combined with the antibody or fragment thereof of the invention, for example, an agent capable of reducing pain, such as, but not limited to, opioids, morphine, a COX-2 inhibitor, aspirin, or other non-steroidal anti-inflammatories, acetaminophen, duloxetine, local anesthetics, NMDA modulators, cannabinoid receptor agonists, P2X family modulators, VR1 antagonists, and substance P antagonists**.** The second therapeutic agent may be an interleukin-1 (IL-1) inhibitor, for example, a fusion protein (US 6,927,044); or an antiepileptic/anticonvulsant drug, such as gabapentin, pregabalin, topiramate; or a tricyclic antidepressant, such as amitriptyline; a cytokine inhibitor or antagonist, such as an antagonist to IL-6, IL-6R, IL-18 or IL-18R, or an inhibitor of a voltage-gated sodium channel, such as a

$Na_v1.7$ inhibitor, or a $Na_v1.8$ inhibitor, or a $Na_v1.9$ inhibitor; an inhibitor of a potassium channel or calcium channel; or a NGF inhibitor (a small molecule inhibitor or an anti-NGF antibody), or a second inhibitor or antagonist to GFRα3, a tumor necrosis factor (TNF) or TNF receptor inhibitor, an inhibitor of TWEAK (TNF-related WEAK inducer of apoptosis), a RET inhibitor, an inhibitor of a GDNF family ligand, an inhibitor of GFRα1, GFRα2 or GFRα4, an inhibitor of an acid sensing ion channel (*e.g.* ASIC1 or ASIC3), or a selective serotonin reuptake inhibitor (SSRI), or a serotonin norepinephrine reuptake inhibitor (SNRI), or an inhibitor of a prekineticin receptor (*e.g.* PROK1 and PROK2), or a caspase inhibitor, a p38 inhibitor, an IKK1/2 inhibitor, CTLA-4Ig, or a corticosteroid. The second therapeutic agent may be a small molecule drug or a protein/polypeptide inhibitor. The second therapeutic agent may be synthetic or naturally derived. The second therapeutic agent may be a second antibody specific for GFRα3, a polypeptide antagonist, a siRNA or an antisense molecule specific for GFRα3. 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.

[0065] In a fifth aspect, the invention features methods for inhibiting hGFRα3 activity using an anti-hGFRα3 antibody or antigen-binding portion of an antibody of the invention, wherein the methods comprise administering a therapeutically effective amount of one or more antibodies of the invention, or antigen binding fragments thereof, or a pharmaceutical composition comprising one or more antibodies of the invention or antigen-binding fragments thereof.

[0066] In a sixth aspect, the invention features a method for treating a GFRα3-related condition or disease, or the pain associated with a GFRα3-related condition or disease, the method comprising administering an anti-GFRα3 antibody or antigen-binding portion of an antibody of the invention, or a composition comprising an anti-GFRα3 antibody or a fragment thereof, to a patient in need thereof, wherein the GFRα3-related condition or disease is prevented, ameliorated, or reduced in severity or frequency of occurrence, or the pain associated with the condition or disease is prevented, ameliorated, or reduced in severity or frequency of occurrence.

[0067] In one embodiment, the invention provides for the isolated antibody or antigen-binding

fragment thereof, or a pharmaceutical composition comprising at least one antibody or antigen-binding fragment thereof of the invention for use in treating a GFRα3-related condition or disease, or the pain associated with the GFRα3-related condition or disease, wherein the GFRα3-related condition or disease is prevented, ameliorated, or reduced in severity or frequency of occurrence, or the pain associated with the condition or disease is prevented, ameliorated, or reduced in severity or frequency of occurrence**.**

[0068] In one embodiment, the invention provides for use of an isolated antibody or antigen-binding fragment thereof of the invention, or a pharmaceutical composition comprising at least one antibody of the invention in the manufacture of a medicament for treating a GFRα3-related condition or disease, or the pain associated with the GFRα3-related condition or disease, wherein the GFRα3-related condition or disease is prevented, ameliorated, or reduced in severity or frequency of occurrence, or the pain associated with the condition or disease is prevented, ameliorated, or reduced in severity or frequency of occurrence**.**

[0069] In one embodiment, the GFRα3-related condition or disease is selected from the group consisting of acute pain, chronic pain, neuropathic pain, inflammatory pain, a functional pain syndrome, arthritis, pancreatitis, osteoarthritis, cluster headaches, trigeminal neuralgia, herpetic neuralgia, general neuralgias, neurodegenerative disorders, movement disorders, neuroendocrine disorders, ataxia, 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, hereditary erythromelalgia, dental pain, rhinitis, cancer pain, complex regional pain syndrome (CRPS), inflammatory bowel disease (*e.g.* Crohn's disease or ulcerative colitis) and bladder disorders**.**

[0070] In one embodiment, the functional pain syndrome is selected from the group consisting of chronic low back pain, irritable bowel syndrome (IBS), fibromyalgia (FM), chronic fatigue syndrome, abdominal pain, temporomandibular joint disorder (TMJD), painful bladder syndrome (interstitial cystitis), functional gastrointestinal disorders/syndromes, functional chest pain syndrome, migraines and tension type headaches, chronic pelvic pain syndrome, painful prostate syndrome (chronic prostatitis), multiple chemical sensitivity syndrome and Gulf War syndrome.

[0071] In one embodiment, the cancer pain is associated with a cancer selected from the group consisting of endometrial cancer, prostate cancer, breast cancer, cervical cancer, liver cancer, pancreatic cancer, colon cancer, stomach cancer, uterine cancer, ovarian cancer, kidney cancer, non-small cell lung cancer, brain cancer, a leukemia, a lymphoma, bone cancer and pain associated with metastasis of a cancer.

[0072] In one embodiment, the antibody or antigen-binding fragment is administered to the patient in combination with a second therapeutic agent.

[0073] In one embodiment, the second therapeutic agent is selected from the group consisting of an opioid, a COX-2 inhibitor, a local anesthetic, an NMDA modulator, a cannabinoid receptor

agonist, a P2X family modulator, a VR1 antagonist, a substance P antagonist, a second GFRα3 antagonist, a cytokine or cytokine receptor antagonist, a nerve growth factor (NGF) inhibitor (a small molecular inhibitor or an anti-NGF antibody), aspirin, a NSAID, a steroid, morphine, a selective serotonin reuptake inhibitor (SSRI), a serotonin norepinephrine reuptake inhibitor (SNRI), a tricyclic, an inhibitor of a voltage-gated sodium channel (Na_v), a calcium channel inhibitor, a potassium channel inhibitor, a tumor necrosis factor (TNF) or TNF receptor inhibitor, an inhibitor of TWEAK (TNF-related WEAK inducer of apoptosis), a RET inhibitor, an inhibitor of a GDNF family ligand, an inhibitor of an acid sensing ion channel (ASIC1 or ASIC3), an anti-convulsant (gabapentin or pregabalin), an inhibitor of a prekineticin receptor (PROK1 and PROK2), a caspase inhibitor, a p38 inhibitor, an IKK1/2 inhibitor, CTLA-4Ig and a corticosteroid.

[0074] In one embodiment, the second GFRα3 antagonist is a small organic molecule, a second antibody specific for GFRα3, a polypeptide antagonist, a siRNA or an antisense molecule specific for GFRα3.

[0075] In one embodiment, the cytokine or cytokine receptor antagonist is an interleukin-1 (IL-1) antagonist, an IL-6 antagonist, or an IL-18 antagonist.

[0076] The disorder treated is any disease or condition, which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of hGFRα3 activity. Specific populations treatable by the therapeutic methods of the invention include a disease, disorder, or condition selected from acute, chronic, ischemic, neuropathic**,** or inflammatory pain, hypersensitivity, such as visceral, thermal, or mechanical hypersensitivity, chronic pancreatitis, arthritis, migraine, cluster headaches, trigeminal neuralgia, herpetic neuralgia, general neuralgias, epilepsy or epileptic conditions, myotonia, arrhythmia, movement disorders, neuroendocrine disorders, ataxia, inflammatory bowel disease, spleen inflammation, stomach pain, trigonitis, fibroids, peritonitis, faecal urgency, incontinence, rectal hypersensitivity, visceral pain, osteoarthritis pain, post-herpetic neuralgia, diabetic neuropathy, radicular pain, sciatica, back pain, head or neck pain, breakthrough pain, post-surgical pain, cancer pain, or chemotherapy-induced pain. Other conditions treatable by the therapeutic methods of the invention include Hirschsprung disease, hereditary erythromelalgia, bladder disorders, rhinitis, prostate cancer, breast cancer, cervical cancer, liver cancer, pancreatic cancer, colon cancer, stomach cancer, uterine cancer, ovarian cancer, kidney cancer, a hematologic (blood-borne) cancer, such as a leukemia or a lymphoma, bone cancer, or pain associated with metastasis of a cancer, for example, pain associated with metastasis of a cancer to the bone. 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; pancreatic; chronic headache pain; tension headache; diabetic neuropathy; 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 (CRPS, also known as Reflex Sympathetic Dystrophy); phantom pain; intractable pain; acute musculoskeletal pain; joint pain; acute gout pain; mechanical low back pain; neck pain; tendonitis; injury/exercise pain; abdominal pain; pyelonephritis; appendicitis; cholecystitis; intestinal obstruction; hernias; etc; 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; breakthrough pain; 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; and hyperactivity bladder. In one embodiment the antibodies of the invention may be used to treat a functional pain syndrome, wherein the functional pain syndrome is selected from the group consisting of chronic low back pain, irritable bowel syndrome (IBS), fibromyalgia (FM), chronic fatigue syndrome, abdominal pain, temporomandibular joint disorder (TMJD), painful bladder syndrome (interstitial cystitis), functional gastrointestinal disorders/syndromes, functional chest pain syndrome, migraines and tension type headaches, chronic pelvic pain syndrome, painful prostate syndrome (chronic prostatitis), multiple chemical sensitivity syndrome and Gulf War syndrome.

[0077] The antibodies of the invention or antigen-binding fragments thereof may also be used to inhibit tumor cell growth/proliferation, or metastasis of tumor cells. In certain embodiments, the antibodies of the invention or antigen-binding fragments thereof, may be used to treat a cancer, or the "pain associated with a cancer" or "cancer-associated pain", including, for example, but not limited to, endometrial cancer, prostate cancer, breast cancer, cervical cancer, liver cancer, pancreatic cancer, colon cancer, stomach cancer, uterine cancer, ovarian cancer, kidney cancer, small cell lung cancer, non-small cell lung cancer, brain cancer, a hematologic (blood-borne) cancer, such as a leukemia or a lymphoma, bone cancer, or pain associated with metastasis of a cancer, for example, pain associated with metastasis of a cancer to the bone. "Cancer-associated pain" also includes pain more generally associated with cancerous conditions such as, *e.g.*, renal cell carcinoma, pancreatic carcinoma, head and neck cancer, malignant gliomas, osteosarcoma, colorectal cancer, gastric cancer, malignant mesothelioma, multiple myeloma, synovial sarcoma, thyroid cancer, or melanoma. The antibodies of the present invention are also useful for treating or preventing pain caused by or associated with cancer therapy or anti-cancer medical treatments, *e.g.*, chemotherapy-induced neuropathic pain such as pain caused by or associated with treatment with paclitaxel (Taxol™), docetaxel (Taxotere®); nitrosourea, cyclophosphamide, doxorubicin, epirubicin, 5-fluorouracil, topotecan, irinotecan, carmustine, estramustine, and platinum-based chemotherapeutic compounds, such as cisplatin, carboplatin, and iproplatin.

[0078] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0079] Figure 1. Inhibition of artemin-sensitized capsaicin thermal hyperalgesia in animals injected with mouse GFRα3 antibodies (indirect blocker M1M6977N or direct blocker M1M6986N, n=8 each) or isotype (negative) control antibody (M2M180N, n=8) at 30mg/kg s.c. 2 days before receiving capsaicin (1 day before receiving 0.5µg artemin).

[0080] Figure 2A and 2B. Tactile allodynia measured by von Frey Hairs in animals from two experiments (A & B) injected with fibrosarcoma and treated with isotype (negative) control antibody (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=8-11 per group). *p<.05, **p<.01, or ***p<.001 compared to isotype control at the same time point.

[0081] Figure 3A and 3B. Percent ipsilateral weight bearing in animals from two experiments (A & B) injected with fibrosarcoma and treated with isotype (negative) control (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=8-11 per group).

[0082] Figure 4A and 4B. Guarding scores in animals from two experiments (A & B) injected with fibrosarcoma cells and treated with isotype (negative) control (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=8-11 per group). **p<.01 compared to isotype control at the same time point.

[0083] Figure 5. Tactile allodynia measured by von Frey Hairs in animals injected with carcinoma and treated with isotype (negative) control (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=9-10 per group). *p<.05, **p<.01, or ***p<.001 compared to isotype (negative) control at the same time point.

[0084] Figure 6A and 6B. Percent ipsilateral weight bearing at two time points (A=11 days & B=18 days) injected with carcinoma and treated with isotype (negative) control (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=9-10 per group). *p<.05 compared to isotype control antibody by post hoc Dunnett's analysis.

[0085] Figure 7. Guarding scores in animals injected with carcinoma and treated with isotype (negative) control (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=9-10 per group). *p<.05, ***p<.001 compared to isotype control at the same time point.

[0086] Figure 8. Tactile allodynia measured by von Frey Hairs in animals with DMM treated with isotype (negative) control (M2M180N) or M1M6977N or M1M6986N anti-mouse GFRα3 antibodies (n=10 per group). **p<.01 or ***p<.001 compared to isotype control at the same time point.

[0087] Figure 9. Cross-Competition Analysis of anti-GFRα3 Antibodies for Binding to Biotin-hGFRα3-mmH.

DETAILED DESCRIPTION

[0088] 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.

[0089] 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. 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.

Definitions

[0090] "GFRα3," or "hGFRα3", as used herein, refers to the glycosylphosphatidylinositol (GPI)-anchored protein receptor for artemin, which belongs to the family of glial cell line derived neurotrophic factors (GDNF). It is one of the GDNF family receptor alpha proteins that, once bound to its ligand, artemin, mediates activation of the receptor tyrosine kinase RET ("rearranged during transfection"). Four members of the GFRα family have been recognized to date, GFRα-1-4 (Lindsay RM *et al*., Neuron, (1996), 17:571-574; Airaksinen, MS, *et al.*, Mol. Cell Neurosci., (1999), 13:313-325). GFRα3 is also known in the art as GDNF family receptor alpha 3 GPI-linked receptor, or glial cell line-derived neurotrophic factor receptor alpha-3. The expression "GFRα3," or "hGFRα3", or fragments thereof, as used herein, refers to the human GFRα3 protein or fragment thereof, unless specified as being from a non-human species, *e.g*. "mouse GFRα3", "rat GFRα3", or "monkey GFRα3". Moreover, "GFRα3," or "hGFRα3", as used herein, refers to human GFRα3 encoded by the nucleic acid sequence shown in SEQ ID NO: 374 (Genbank accession number NM_001496) and has the amino acid sequence as shown in SEQ ID NO: 375 (Genbank accession number NP_001487.2), or a biologically active fragment thereof. The signal sequence spans amino acid residues 1-31 of SEQ ID NO: 375, the mature protein spans amino acid residues 32-382 of SEQ ID NO: 375, whereas the C-terminal Pro region spans amino acid residues 383-400 of SEQ ID NO: 375. The GPI cleavage site is found at amino acid residue 374 of SEQ ID NO: 375 (asparagine). The amino acid sequence of human artemin is found in Genbank as accession number Q5T4W7 and the amino acid sequence of human artemin (from amino acids A108-G220 of accession number Q5T4W7) with a myc-myc-hexahistidine tag is shown as SEQ ID NO: 369 (with amino acid residues 114-141 of SEQ ID NO: 369 being the myc-myc hexahistidine tag).

[0091] Although GFRα3 is structurally and functionally similar to the other members of the GFRα family, GFRα3 is the most distantly related of the four family members. GFRα1 and GFRα2 share about 50% identity (Sanicola, M. et al., PNAS, USA, (1997), 94:6238-43; Klein, RD, et al., (1997), Nature, 387:717-21; Buj-Bello, A. et al., Nature (1997), 387:721-4; Baloh, RH, et al., Neuron, (1997), 18:793-802), while GFRα3 has only 32 and 37% identity,

respectively, with these proteins (Masure, S. et al., Eur. J. Biochem., (1998), 251:622-30; Nomoto, S. et al. , BBRC, (1998), 244:849-53). The amino acid sequence of mouse GFRα3 has the following Genbank Accession Number: NP_034410.3. The amino acid sequence of human GFRα1 has the following Genbank Accession Number: NP_005255.1 and is also found as SEQ ID NO: 376. The amino acid sequence of cynomolgus GFRα3 is shown in SEQ ID NO: 377 and the amino acid sequence of cynomolgus RET is shown in SEQ ID NO: 378.

[0092] 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 anti-GFRα3 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.

[0093] 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).

[0094] 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.

[0095] The fully-human anti-hGFRα3 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 back-mutated to the corresponding germline residue(s) or to a conservative amino acid substitution (natural or non-natural) of the corresponding germline residue(s) (such sequence changes are referred to herein as "germline back-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 back-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 germline sequence. In other embodiments, only certain residues are mutated back to the 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 to germline back-mutations within all framework regions FR1, FR2, FR3, FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. Furthermore, the antibodies of the present invention may contain any combination of two or more germline back-mutations within the framework and/or CDR regions, *i.e.*, wherein certain individual residues are mutated back to the germline sequence while certain other residues that differ from the germline sequence are maintained. Once obtained, antibodies and antigen-binding fragments that contain one or more germline back-mutations can be easily tested for one or more desired properties 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.

[0096] 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. The anti-human GFRα3 antibodies of the invention may be designated as “anti-hGFRα3” or “anti-GFRα3”.

[0097] The term "specifically binds," 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. An isolated antibody that specifically binds hGFRα3 may, however, exhibit cross-reactivity to other antigens such as GFRα3 molecules from other species. Moreover, multi-specific antibodies that bind to hGFRα3 and one or more additional antigens or a bi-specific that binds to two different regions of hGFRα3 are nonetheless considered antibodies that "specifically bind" hGFRα3, as used herein.

[0098] As used herein, the term "does not bind" to a specified target molecule (e.g. a particular GFRα3 peptide) means that the antibody, when tested for binding to the target molecule at 25°C in a Plasmon resonance assay, exhibits a K_D of greater than 500 nM, or if tested for binding to the target molecule at 25°C in an enzyme linked immunosorbent assay (ELISA) exhibits an EC_{50} of greater than 50 nM, or fails to exhibit any binding in either type of assay or equivalent thereof.

[0099] The term "high affinity" antibody refers to those mAbs having a binding affinity to hGFRα3 of at least 10^{-9} M; preferably 10^{-10} M; more preferably 10^{-11} M, even more preferably 10^{-12} M, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[0100] By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from hGFRα3 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™.

[0101] 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 hGFRα3.

[0102] The specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as an opioid, a COX-2 inhibitor, a local anesthetic, a cytokine antagonist, such as an IL-1 or IL-6 inhibitor, a second GFRα3 inhibitor, an NMDA modulator, a cannabinoid receptor agonist, a P2X family modulator, a VR1 antagonist, a substance P antagonist, a chemotherapeutic agent, or a radioisotope.

[0103] 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 hGFRα3, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than hGFRα3).

[0104] A "neutralizing antibody", as used herein (or an "antibody that neutralizes GFRα3 activity"), is intended to refer to an antibody whose binding to hGFRα3 results in inhibition of at least one biological activity of GFRα3. This inhibition of the biological activity of GFRα3 can be assessed by measuring one or more indicators of GFRα3 biological activity by one or more of

several standard *in vitro* or *in vivo* assays known in the art (see examples below).

[0105] 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.).

[0106] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0107] 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.

[0108] 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.

[0109] 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). 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.* (See Gonnet *et al.,* Science, (1992), 256:1443 45). A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0110] 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 402).

[0111] 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.

[0112] 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).

[0113] The term "functional pain syndrome(s)", refers to chronic symptom-based syndromes that affect up to 15% of the population worldwide. They are characterized by chronic pain and discomfort referred to in different regions of the body. No generally agreed-upon structural, inflammatory, or biochemical abnormalities have been identified that could fully explain the symptoms. Patients show a greatly reduced quality of life, yet treatment options are limited, and the development of novel therapeutic approaches has been disappointing. Some of the common disorders, which fall into this category, include chronic low back pain, irritable bowel syndrome (IBS), fibromyalgia (FM), chronic fatigue syndrome, functional abdominal pain syndrome, temporomandibular joint disorder (TMJD), painful bladder syndrome (interstitial cystitis), functional gastrointestinal disorders/syndromes, functional rectal pain syndrome, functional chest pain syndrome, migraines and tension type headaches, chronic pelvic pain syndrome, painful prostate syndrome (chronic prostatitis), multiple chemical sensitivity syndrome, and Gulf War syndrome.

General Description

[0114] The glial cell line-derived neurotrophic factor related family includes glial cell line-derived neurotrophic factor (GDNF), neurturin (NRTN), persephin (PSPN), and artemin (ARTN). GDNF family proteins are differentially involved in the development and maintenance of sensory, enteric, sympathetic and parasympathetic neurons and a variety of non-neural tissues (Henderson, C.E., *et al.,* (1994), Science 266:1062-1064; Kotzbauer, P.T. *et al.,* (1996), Nature 384:467-470; Springer, J.E., *et al*. (1994), Exp. Neurol. 127:167-170; Schaar. D.G., *et al*., (1993), Exp. Neurol. 124:368-371). GDNF is an especially potent survival factor for

dopaminergic, noradrenergic and spinal motor neurons (Yan, Q. *et al.* (1995), Nature, 373:341-344; Henderson, C.E., *et al.,* (1994), Science, 266:1062-1064; Buj-Bello, A. *et al*., (1995), Neuron, 15:821-828). Other GDNF family growth members have functions outside the nervous system (Trupp, M. et al., (1995), J. Cell Biol. 130:137-148; Kotzbauer, P.T. *et al.,* (1996), Nature 384:467-470; Springer, J.E., *et al*. (1994), Exp. Neurol. 127:167-170; Schaar. D.G., *et al*., (1993), Exp. Neurol. 124:368-371). For example, NRTN, ARTN, and PSPN are also expressed in the developing kidney. GDNF also has critical roles outside the nervous system in the regulation of kidney morphogenesis and spermatogenesis (Airaksinen, M.S. *et al*., (2002), Nature Reviews 3:383-392).

[0115] Each member of the GDNF family binds preferentially to (*ie*, is a ligand for) a glycosylphosphatidylinositol (GPI)-anchored protein receptor dynamically associated with the plasma membrane. The GDNF-family receptor alpha family is composed of four different receptors: GFRalpha1 (GFRα1, GDNFR-alpha); GFRalpha2 (GFRα2/TrnR2/GDNFR-beta/NTNR-alpha/RETL2); GFRalpha3 (GFRα3); and GFRalpha4 (GFRα4). GDNF binds preferentially to GFRα1, NRTN binds preferentially to GFRα2, ARTN binds preferentially to GFRα3 and PSPN binds preferentially to GFRα4 (Airaksinen, M.S., *et al.* Nature Reviews Neuroscience (2002), 3:383-394).

[0116] GFRα2 is highly expressed in cortex, basal forebrain, and specific layers of the olfactory bulb, and poorly expressed in substantia nigra, cerebellum, and motor nuclei. GFRα3 is expressed in fetal and adult mouse nerves, sympathetic and sensory ganglia, intestine, heart, brain, lung and kidney. GFRα4 is expressed at low levels in different brain areas in the adult as well as in some peripheral tissues including testis and heart. While the GDNF family member binding preferences are shown above to be GDNF to GFRα1; neurturin to GFRα2; artemin to GFRα3; and persephin to GFRα4, the ligand receptor pairing is not stringent (Airaksinen, M.S., *et al.* Nature Reviews Neuroscience (2002), 3:383-394). For example, GDNF binds to GFRα2 and GFRα3 with lower efficiencies than it binds to GFRα1.

[0117] The GDNF family ligands, typically but not exclusively, transmit their signals through multi-component complexes composed of a ligand, its GFR alpha receptor and the receptor tyrosine kinase, c-Ret. Ret is a common element of these ligand signaling complexes. Ret is a proto-oncogene that strongly activates anti-apoptotic signals through the activation of the phosphoinositol-3 kinases (PI3-K)/PDK/AKT(PKB) and the Ras/Raf/MEK/ERK pathways. Ret is also able to activate phospholipase C gamma (PLCgamma) which elevates intracellular calcium and facilitates activation of members of the conventional and novel protein kinase C (PKC) family. GDNF family ligand receptor complexes are not restricted to signaling through Ret. GDNF:GFRalpha1 can bind to NCAM in cells lacking RET and activate Fyn and FAK. Under some conditions GDNF:GFRalpha complexes directly activate src kinase.

[0118] In certain embodiments of the present invention, any one or more of the three globular cysteine-rich domains (1, 2, or 3) of GFRα3, or a fragment thereof, may be used to prepare

antibodies that bind GFRα3 and inhibit its function, or inhibit its ability to bind its ligand, such as, artemin. In certain embodiments, an antibody of the invention specific for GFRα3 may bind to a ligand-binding domain on GFRα3, and as such, may block the binding of the ligand (artemin)-GFRα3 complex to RET. The full-length amino acid sequence of human GFRα3 is shown as SEQ ID NO: 375. The nucleic acid encoding human GFRα3 is shown in SEQ ID NO: 374. Domain 1 spans residues 44-124 of SEQ ID NO: 375; domain 2 spans residues 162-239 of SEQ ID NO: 375; domain 3 spans residues 248-340 of SEQ ID NO: 375. (See either SEQ ID NO. 375 or Genbank NP_001487.2).

[0119] Any of these domains, 1, 2, or 3, or fragments derived therefrom, may be used to prepare antibodies that bind specifically to GFRα3 and inhibit its activity, or at least one function associated with GFRα3. In certain embodiments, the antibodies of the invention bind specifically to GFRα3 and may prevent signaling mediated by GFRα3. In certain embodiments, the antibodies that bind specifically to GFRα3 may prevent binding of GFRα3 to its ligand, such as artemin (Wang, X. *et al.* Structure, (2006), 14:1083-1092). In certain embodiments, the antibodies that bind specifically to GFRα3 may prevent activation of the RET receptor tyrosine kinase. In certain embodiments, the antibodies of the invention may bind specifically to GFRα3 without preventing activation of the RET receptor tyrosine kinase. In certain embodiments, the antibodies of the invention may bind specifically to GFRα3 and prevent signaling through RET, or through a mediator other than RET. In certain embodiments, the antibodies of the invention may be used to inhibit tumor cell growth/proliferation and as such, may be useful for treating certain cancers/malignancies, or the pain associated with such cancers/malignancies, or the pain associated with metastasis of such cancers/malignancies (See Tang, J-Z, *et al*. Mol Cancer Ther (2010), 9(6): 1697-1708; Kang, J. *et al.* Oncogene, (2009), 28:2034-2045; Ceyhan, G.O. et al. Annals of Surgery, (2006), 244(2):274-281; Banerjee, A., *et al.* Breast Cancer Res (2011), 13:R112; Pandey, V. *et al.,* Endocrinology, (2010), 151(3):909-920; Kang, J. *et al*., Oncogene, (2010), 29:3228-3240; Li, S. *et al.* J Biomed Sci (2011), 18:24). In certain embodiments, antibodies that bind specifically to GFRα3 may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 10 to about 50 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 GFRα3 specific antibodies. As noted above, the length, or the number of amino acid residues encompassing the three domains of hGFRα3 may vary by about ten to fifty amino acid residues extending from either, or both, the N terminal or C terminal end of the full length domain, or a fragment thereof, for preparation of anti-hGFRα3 specific antibodies.

Antigen-Binding Fragments of Antibodies

[0120] 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 hGFRα3. 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.

[0121] 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)). Other engineered molecules, such as diabodies, triabodies, tetrabodies and minibodies, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0122] 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.

[0123] 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)).

[0124] 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

[0125] 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 human GFRα3.

[0126] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to GFRα3 are initially 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.

[0127] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-13} through about 10^{-8} M, or from about 10^{-12} through about 10^{-9} 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

[0128] The anti-GFRα3 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 human GFRα3. 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 anti-GFRα3 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 anti-GFRα3 antibody or antibody fragment that is essentially bioequivalent to an anti-GFRα3 antibody or antibody fragment of the invention.

[0129] 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 as a single dose or as multiple doses. 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.

[0130] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] Bioequivalent variants of anti-GFRα3 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 anti-GFRα3 antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Anti-GFRα3 Antibodies Comprising Fc Variants

[0135] According to certain embodiments of the present invention, anti-GFRα3 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-GFRα3 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).

[0136] For example, the present invention includes anti-GFRα3 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

[0137] In general, the antibodies of the present invention may function by binding to any one or more of the three globular cysteine-rich domains (1, 2, or 3) of hGFRα3. In certain embodiments, the antibodies of the present invention may bind to an epitope located on at least one of the cysteine-rich domains of hGFRα3. In certain embodiments, an antibody of the invention may bind to amino acid residues of domain 1 of GFRα3, ranging from about residue 44 to about residue 124 of SEQ ID NO: 375. In certain embodiments, an antibody of the invention may bind to amino acid residues of domain 2 of GFRα3, ranging from about residue 162 to about residue 239 of SEQ ID NO: 375. In certain embodiments, an antibody of the invention may bind to amino acid residues of domain 3 of GFRα3, ranging from about residue 248 to about residue 340 of SEQ ID NO: 375. In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting GFRα3 activity by binding to a region in any one of the domains that acts as the ligand binding domain, thus preventing binding of the ligand, such as, artemin, to that site. In certain embodiments, an antibody of the invention may bind to the ligand binding site on one of the domains of GFRα3 and prevent subsequent binding of the artemin-GFRα3 complex to RET. In one embodiment, an antibody of the invention may bind to any one or more of the epitopes in the artemin-GFRα3 complex that may determine or play a role in the specificity between ligand and GFRα3, such as in the region ranging from residues 167-184 of SEQ ID NO: 375. In certain embodiments, an antibody of the invention may bind to one or more of the residues of domain 2 that are responsible for the specificity between artemin and GFRα3, for example, the amino acid residues at positions 167 (met), 176 (asp) and/or position 184 (glu), of SEQ ID NO: 375 and in so binding, may prevent ligand binding to its receptor, and subsequently may prevent signaling through the RET receptor tyrosine kinase, or through a signaling mediator or modulator other than RET. In certain embodiments, the antibodies of the invention may bind to the membrane bound form of GFRα3 or to the soluble form of GFRα3. In certain embodiments, the antibodies of the invention may bind GFRα3, but do not cross react with GFRα1, GFRα2, or GFRα4. In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in one cysteine rich region of one domain and may also bind one cysteine-rich region in a second domain of hGFRα3. In certain embodiments, the bi-specific antibodies of the invention may bind to two different regions within the same domain. In certain embodiments, one arm of a bi-specific antibody of the invention may bind to one cysteine rich region of one domain of hGFRα3 and the other arm may bind to RET, or to a modulator other than RET. In certain embodiments, the bispecific antibodies may bind one domain in GFRα3 and one domain in GFRα1 or GFRα2.

[0138] More specifically, the anti-GFRα3 antibodies of the invention may exhibit one or more of the following characteristics:

(i) exhibits a K_D ranging from about 10^{-8} M to about 10^{-13} M as measured by surface plasmon resonance;

(ii) demonstrates the ability to block about 50-100% of the binding of GFRα3 to its ligand, artemin, with an IC_{50} value ranging from about 40 pM to about 15 nM;

(iii) demonstrates the ability to block about 20% to about 100% of the binding of GFRα3 to a solid support coated with a mixture of artemin and RET;

(iv) blocks or inhibits artemin-dependent activation of RET with an IC_{50} ranging from about 200 pM to about 50 nM;

(v) inhibits or reduces one or more nociceptive responses in an *in vivo* model of bone cancer pain;

(vi) inhibits or reduces artemin-sensitized thermal hyperalgesia *in vivo*;

(vii) inhibits or reduces allodynia in an *in vivo* model of osteoarthritis;

(viii) does not cross-react with other GFR co-receptors for RET;

(ix) 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, 290, 306, 322, 338, 354, 381 and 397; or

(x) 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, 298, 314, 330, 346, 362, 389 and 405.

[0139] Certain anti-GFRα3 antibodies of the present invention are able to inhibit or attenuate GFRα3 activity in an *in vitro* assay. The ability of the antibodies of the invention to bind to and inhibit the binding of GFRα3 to its ligand artemin alone or in the presence of RET may be measured using any standard method known to those skilled in the art, including binding assays, or assays to determine if the antibodies block the activation of RET by inhibiting the binding of GFRα3 to its receptor artemin, such as those described herein. Non-limiting, exemplary *in vitro* assays for measuring GFRα3 activity are illustrated in Examples 4 and 5, below.

[0140] The present invention includes anti-GFRα3 antibodies and antigen binding fragments thereof which bind to one or more of the cysteine rich globular domains of GFRα3, as shown in SEQ ID NO: 375, or to a fragment thereof. The antibodies specific for GFRα3 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

[0141] In one embodiment, the invention provides a fully human monoclonal antibody or

antigen-binding fragment thereof that specifically binds hGFRα3 and neutralizes hGFRα3 activity, 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 NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 381 and 397; (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, 298, 314, 330, 346, 362, 389 and 405; (iii) comprises any one or more of the heavy or light chain CDR1, CDR2, and CDR3 sequences depicted in Table 1 and combinations thereof; (iv) is specific for binding to and/or blocking GFRα3 activity without binding to and/or blocking other GFR alpha receptors, including GFRα1, GFRα2 and GFRα4; (v) demonstrates binding specificity for any one or more of the cysteine-rich globular domains of GFRα3; (vi) blocks activation of and signaling through the RET receptor tyrosine kinase; (vii) inhibits or reduces artemin-sensitized thermal hyperalgesia *in vivo*; (viii) inhibits or reduces allodynia in an *in vivo* model of osteoarthritis; or inhibits or reduces one or more nociceptive responses in an *in vivo* model of bone cancer pain.

Epitope Mapping and Related Technologies

[0142] 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.

[0143] 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.

[0144] 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 (See, *e.g*., US 2004/0101920). 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.

[0145] In certain embodiments, the anti-GFRα3 antibody or antigen-binding fragment of an antibody binds an epitope within at least one of the GFRα3 cysteine rich domains 1, 2, or 3, or a fragment thereof, wherein domain 1 ranges from about residue number 44 to about residue number 124 of SEQ ID NO: 375; domain 2 ranges from about residue number 162 to about residue number 239 of SEQ ID NO: 375; domain 3 ranges from about residue number 248 to about residue number 340 of SEQ ID NO: 375.

[0146] In certain embodiments, the anti-GFRα3 antibody or antigen-binding fragment of an antibody binds an epitope within domain 1, or a fragment thereof, of human GFRα3.

[0147] In certain embodiments, the anti-GFRα3 antibody or antigen-binding fragment of an antibody binds an epitope within domain 2, or a fragment thereof, of human GFRα3.

[0148] In certain embodiments, the anti-GFRα3 antibody or antigen-binding fragment of an antibody binds an epitope within domain 3, or a fragment thereof, of human GFRα3.

[0149] In certain embodiments, the antibody or antibody fragment binds an epitope, which includes more than one of the enumerated epitopes of GFRα3 within domain 1, 2, or 3, and/or within two different domains (for example, epitopes within the 1 and 2 domains, or within the 2 and 3 domains, or within the 1 and 3 domains).

[0150] In certain embodiments, the antibody is a bi-specific antibody that binds one epitope within one domain of GFRα3 and another epitope within a different domain of GFRα3. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in domain 1 of GFRα3 and another epitope in domain 2 of GFRα3. In one embodiment, the antibody is a bi-specific

antibody that binds one epitope in domain 1 of GFRα3 and another epitope within domain 3 of GFRα3. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in domain 2 of GFRα3 and another epitope within domain 3 of GFRα3.

[0151] The present invention includes anti-GFRα3 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.,* H4H2207N, H4H2212N, H4H2236N3, H4H2243N2, H4H2210N, H4H2234N, H4H2291S, H4H2292S, H4H2293P, H4H2294S, H4H2295S, H4H2296S, H4H2341S, H4H2342P, H4H2344S, H4H2345S, H4H2346S, H4H2350P, H4H2352S, H4H2354S, H4H2355S, H4H2357S, H4H2364S, H1M2243N and H1M2236N). Likewise, the present invention also includes anti-GFRα3 antibodies that compete for binding to GFRα3 or a GFRα3 fragment with any of the specific exemplary antibodies described herein.

[0152] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-GFRα3 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 anti-GFRα3 antibody of the invention, the reference antibody is allowed to bind to a GFRα3 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the GFRα3 molecule is assessed. If the test antibody is able to bind to GFRα3 following saturation binding with the reference anti-GFRα3 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-GFRα3 antibody. On the other hand, if the test antibody is not able to bind to the GFRα3 molecule following saturation binding with the reference anti-GFRα3 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-GFRα3 antibody of the invention.

[0153] To determine if an antibody competes for binding with a reference anti-GFRα3 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a GFRα3 molecule under saturating conditions followed by assessment of binding of the test antibody to the GFRα3 molecule. In a second orientation, the test antibody is allowed to bind to a GFRα3 molecule under saturating conditions followed by assessment of binding of the reference antibody to the GFRα3 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the GFRα3 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to GFRα3. 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.

[0154] 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.

[0155] 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.

Species Selectivity and Species Cross-Reactivity

[0156] According to certain embodiments of the invention, the anti-GFRα3 antibodies bind to human GFRα3 but not to GFRα3 from other species. Alternatively, the anti-GFRα3 antibodies of the invention, in certain embodiments, bind to human GFRα3 and to GFRα3 from one or more non-human species. For example, the anti-GFRα3 antibodies of the invention may bind to human GFRα3 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomolgus, marmoset, rhesus or chimpanzee GFRα3.

Immunoconjugates

[0157] The invention encompasses a human anti-GFRα3 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing pain and/or inflammation, a chemotherapeutic drug, or a radioisotope. The type of therapeutic moiety that may be conjugated to the anti-GFRα3 antibody will take into account the condition to be treated and the desired therapeutic effect to be achieved. For example, for treating acute or chronic pain, an agent such as an NSAID, an opioid, or a Cox-2 inhibitor, or a local anesthetic agent, or a second GFRα3 inhibitor may be conjugated to the GFRα3 antibody. Alternatively, if the desired therapeutic effect is to treat the inflammation associated with a painful condition, it may be advantageous to conjugate an anti-inflammatory agent to the anti-GFRα3 antibody, such as, but not limited to, celecoxib, or a cytokine antagonist, such as an IL-1 or an IL-6 inhibitor. If the condition to be treated is a cancerous condition, it may be beneficial to conjugate a chemotherapeutic drug, or a radioisotope to the GFRα3 antibody. Examples of suitable agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

Multi-specific Antibodies

[0158] 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 anti- GFRα3 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. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for human GFRα3 or a fragment thereof, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety. In certain embodiments of the invention, one arm of an immunoglobulin is specific for an epitope on one domain of hGFRα3 or a fragment thereof, and the other arm of the immunoglobulin is specific for an epitope on a second domain of hGFRα3. In certain embodiments, one arm of an immunoglobulin is specific for one epitope on one domain of hGFRα3 and the other arm is specific for a second epitope on the same domain of hGFRα3.

[0159] 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

[0160] The invention provides therapeutic compositions comprising the anti-GFRα3 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.

[0161] The dose of antibody 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 antibody of the present invention is used for treating pain associated with GFRα3 activity in various conditions and diseases, wherein the condition or disease results in acute or chronic pain, inflammatory pain, neuropathic pain, and the like, in an adult patient, it is advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted.

[0162] 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, 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, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0163] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0164] 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.

[0165] 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.

[0166] 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.

[0167] 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.

[0168] 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.

Therapeutic Uses of the Antibodies

[0169] The antibodies of the invention are useful for the treatment, prevention and/or amelioration of any disease, disorder, or condition associated with GFRα3 activity, or for amelioration of 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-GFRα3 antibodies of the present invention include acute, chronic, neuropathic, or inflammatory pain, arthritis, interstitial cystitis, pancreatitis, migraine, cluster headaches, trigeminal neuralgia, herpetic neuralgia, general neuralgias, epilepsy or epileptic conditions, myotonia, arrhythmia, movement disorders, neuroendocrine disorders, ataxia, irritable bowel syndrome, inflammatory bowel syndrome, fecal urgency, incontinence, rectal hypersensitivity, visceral pain, osteoarthritis pain, gout, post-herpetic neuralgia, diabetic neuropathy, radicular pain, sciatica, back pain, head or neck pain, breakthrough pain, post-surgical pain, cancer pain, including pain associated with bone cancer or pancreatic cancer.

[0170] Other conditions treatable by the therapeutic methods of the invention included hereditary erythromelalgia, rhinitis, prostate cancer, breast cancer, bone cancer, cervical cancer, or bladder disorders. 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; visceral pain, including, abdominal; pancreatic; chronic headache pain; tension headache, including, cluster headaches; diabetic neuropathy; 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 (CRPS); phantom pain; intractable pain; musculoskeletal pain; joint pain; acute gout pain; mechanical low back pain; neck pain; tendonitis; injury/exercise pain; abdominal pain; pyelonephritis; appendicitis; cholecystitis; intestinal obstruction; hernias; etc; 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; breakthrough pain; 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.

[0171] In one embodiment the antibodies of the invention may be used to treat a functional pain syndrome, wherein the functional pain syndrome is selected from the group consisting of chronic low back pain, irritable bowel syndrome (IBS), fibromyalgia (FM), chronic fatigue syndrome (CFS), abdominal pain, temporomandibular joint disorder (TMJD), painful bladder syndrome (interstitial cystitis), functional gastrointestinal disorders/syndromes, functional chest pain syndrome, migraines and tension type headaches, chronic pelvic pain syndrome, painful prostate syndrome (chronic prostatitis), multiple chemical sensitivity syndrome and Gulf War syndrome.

[0172] The antibodies of the invention or antigen-binding fragments thereof may also be used to inhibit tumor cell growth/proliferation or metastasis of tumor cells. Accordingly, in certain embodiments, the antibodies of the invention or antigen-binding fragments thereof, may be used to treat a cancer, including, but not limited to, endometrial cancer, prostate cancer, breast cancer, cervical cancer, liver cancer, pancreatic cancer, colon cancer, stomach cancer, uterine cancer, ovarian cancer, kidney cancer, non-small cell lung cancer, brain cancer, a leukemia, a lymphoma, bone cancer, or pain associated with metastasis of a cancer, for example, metastasis of a cancer to the bone. (See Tang, J-Z, *et al*. Mol Cancer Ther (2010), 9(6): 1697-1708; Kang, J. *et al.* Oncogene, (2009), 28:2034-2045; Ceyhan, G.O. et al. Annals of Surgery, (2006), 244(2):274-281; Banerjee, A., *et al.* Breast Cancer Res (2011), 13:R112; Pandey, V. *et al.,* Endocrinology, (2010), 151(3):909-920; Kang, J. *et al*., Oncogene, (2010), 29:3228-3240; Li, S. *et al.* J Biomed Sci (2011), 18:24).

[0173] The antibodies of the present invention are also useful for treating or preventing cancer-associated pain. "Cancer-associated pain" includes, *e.g.*, bone cancer pain, including pain from cancer that has metastasized to bone (*e.g.*, breast cancer, prostate cancer, lung cancer, sarcoma, kidney cancer, multiple myeloma, etc.). "Cancer-associated pain" also includes pain more generally associated with cancerous conditions such as, *e.g*., renal cell carcinoma, pancreatic carcinoma, breast cancer, head and neck cancer, prostate cancer, malignant gliomas, osteosarcoma, colorectal cancer, gastric cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, small cell lung cancer, non-small cell lung cancer, synovial sarcoma, thyroid cancer, or melanoma. The antibodies of the present invention are also useful for treating or preventing pain caused by or associated with cancer therapy or anti-cancer medical treatments, *e.g*., chemotherapy-induced neuropathic pain such as pain caused by or associated with treatment with paclitaxel (Taxol™), docetaxel (Taxotere®); nitrosourea,

cyclophosphamide, doxorubicin, epirubicin, 5-fluorouracil, topotecan, irinotecan, carmustine, estramustine, and platinum-based chemotherapeutic compounds, such as cisplatin, carboplatin, and iproplatin.

Combination Therapies

[0174] Combination therapies may include an anti-hGFRα3 antibody of the invention and, for example, another GFRα3 antagonist (*e.g.*, anti-GFRα3 antibody or small molecule inhibitor of GFRα3); a COX-2 inhibitor; a local anesthetic; an NMDA modulator; a cannabinoid receptor agonist; a P2X family modulator; a VR1 antagonist; a substance P antagonist; an inhibitor of a voltage-gated sodium channel (Na_v), for example, a $Na_v1.7$ antagonist, or a $Na_v1.8$ antagonist (*e.g.*, anti-$Na_v1.7$ or anti-$Na_v1.8$ antibody or small molecule inhibitor), a $Na_v1.9$ antagonist (*e.g.*, anti-$Na_v1.9$ antibody or small molecule inhibitor of $Na_v1.9$); a calcium channel inhibitor; a potassium channel inhibitor; a cytokine inhibitor or cytokine receptor antagonist (*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); an antiepileptic/anti-convulsant drug (*e.g.*, gabapentin, pregabalin); a nerve growth factor (NGF) inhibitor (*e.g.*, a small molecule NGF antagonist or an anti-NGF antibody); an inhibitor of BDNF, TrkA, TrkB or p75; an opioid; morphine; low dose cochicine; aspirin or another NSAID; steroids (*e.g.*, prednisone, methotrexate, etc.); low dose cyclosporine A; a selective serotonin reuptake inhibitor (SSRI); a serotonin norepinephrine reuptake inhibitor (SNRI); a tricyclic; a tumor necrosis factor (TNF) or TNF receptor inhibitor (*e.g.*, a small molecule TNF or TNFR antagonist or an anti-TNF or TNFR antibody); an inhibitor of TWEAK (TNF-related WEAK inducer of apoptosis); a RET inhibitor; an inhibitor of a GDNF family ligand; an inhibitor of GFRα1, GFRα2 or GFRα4; an inhibitor of an acid sensing ion channel (*e.g.* ASIC1 or ASIC3; uric acid synthesis inhibitors (*e.g.*, allopurinol); uric acid excretion promoters (*e.g.*, probenecid, sulfinpyrazone, benzbromarone, etc.); an inhibitor of a prekineticin receptor (PROK1 and PROK2); other inflammatory inhibitors (*e.g.*, inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, etc.); and/or corticosteroids.

Administration Regimens

[0175] According to certain embodiments of the present invention, multiple doses of an anti-GFRα3 antibody 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 anti-GFRα3 antibody. As used herein, "sequentially administering" means that each dose of anti-GFRα3 antibody 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 anti-GFRα3 antibody, followed by one or more secondary doses of the anti-GFRα3 antibody, and optionally followed by one or more tertiary doses of the anti-GFRα3 antibody.

[0176] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-GFRα3 antibody. 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 anti-GFRα3 antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-GFRα3 antibody contained in the initial, secondary and/or tertiary doses varies 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").

[0177] 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 anti- GFRα3 antibody, which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0178] 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 anti-GFRα3 antibody. 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.

[0179] 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.

Diagnostic Uses of the Antibodies

[0180] The anti-GFRα3 antibodies of the present invention may also be used to detect and/or measure GFRα3 in a sample, *e.g.*, for diagnostic purposes. For example, an anti-GFRα3 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of GFRα3. Exemplary diagnostic assays for GFRα3 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-GFRα3 antibody of the invention, wherein the anti-GFRα3 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-GFRα3 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 GFRα3 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0181] Samples that can be used in GFRα3 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of GFRα3 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of GFRα3 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal GFRα3 levels or activity) will be measured to initially establish a baseline, or standard, level of GFRα3. This baseline level of GFRα3 can then be compared against the levels of GFRα3 measured in samples obtained from individuals suspected of having a GFRα3 related disease or condition, or pain associated with such disease or condition.

EXAMPLES

[0182] 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 Human GFRα3

[0183] An immunogen comprising any one of the GFRα3 peptides having amino acid sequences shown as SEQ ID NOS: 370, 371, 372 and 373, or fragments thereof, may be utilized to generate antibodies to human GFRα3. These peptides are conjugated to a carrier, for example, KLH, then administered 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 is monitored by a GFRα3-specific immunoassay. When a desired immune response is achieved, splenocytes are harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines are screened and selected to identify cell lines that produce GFRα3-specific antibodies. Using this technique several anti-GFRα3 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained. The anti-GFRα3 antibodies generated using this method were designated H1M2207N, H1M2212N, H1M2236N, H1M2236N3, H1M2243N, H1M2243N2, H1M2210N and H1M2234N.

[0184] Anti-GFRα3 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1. Using this method, several fully human anti-GFRα3 antibodies (*i.e*., antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H2207N, H4H2212N, H4H2236N, H4H2243N, H4H2210N, H4H2234N, H4H2291S, H4H2292S, H4H2293P, H4H2294S, H4H2295S, H4H2296S, H4H2341S, H4H2342P, H4H2344S, H4H2345S, H4H2346S, H4H2350P, H4H2352S, H4H2354S, H4H2355S, H4H2357S and H4H2364S.

[0185] The biological properties of the exemplary anti-GFRα3 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 anti-GFRα3 antibodies 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. “2207” as shown in Table 1), followed by a “P”, “S”, or “N” suffix. Thus, according to this nomenclature, an antibody may be referred to as, e.g.

"H4H2207N". 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, S 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, S 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

	AMINO ACID SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H2207N	2	4	6	8	10	12	14	16
H4H2212N	18	20	22	24	26	28	30	32
H4H2236N3	34	36	38	40	42	44	46	48
H4H2243N2	50	52	54	56	58	60	62	64
H4H2210N	66	68	70	72	74	76	78	80
H4H2234N	82	84	86	88	90	92	94	96
H4H2291S	98	100	102	104	106	108	110	112
H4H2292S	114	116	118	120	122	124	126	128
H4H2293P	130	132	134	136	138	140	142	144
H4H2294S	146	148	150	152	154	156	158	160
H4H2295S	162	164	166	168	170	172	174	176
H4H2296S	178	180	182	184	186	188	190	192
H4H2341S	194	196	198	200	202	204	206	208
H4H2342P	210	212	214	216	218	220	222	224
H4H2344S	226	228	230	232	234	236	238	240
H4H2345S	242	244	246	248	250	252	254	256
H4H2346S	258	260	262	264	266	268	270	272
H4H2350P	274	276	278	280	282	284	286	288
H4H2352S	290	292	294	296	298	300	302	304
H4H2354S	306	308	310	312	314	316	318	320

H4H2355S	322	324	326	328	330	332	334	336
H4H2357S	338	340	342	344	346	348	350	352
H4H2364S	354	356	358	360	362	364	366	368
H1M2243N	381	383	385	387	389	391	393	395
H1M2236N	397	399	401	403	405	407	409	411

Example 2. Variable Gene Utilization Analysis

[0187] To analyze the structure of antibodies produced, the nucleic acids encoding antibody variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR). Table 2 sets forth the gene usage for selected antibodies in accordance with the invention.

Table 2

	HCVR			LCVR	
AbPID	V_H	D_H	J_H	V_K	J_K
H1M2207N	3-9	6-6	4	1-5	2
H1M2212N	3-23	1-26	4	4-1	1
H1M2236N	3-23	3-3	6	1-16	4
H4H2236N3	3-23	3-3	6	1-16	4
H1M2243N	1-18	6-6	6	1-16	3
H4H2243N2	1-18	6-6	6	1-16	3
H2M2210N	3-23	1-20	3	3-20	4
H2M2234N	3-23	5-18	4	4-1	1
H4H2291S	3-23	6-6	6	1D-12	4
H4H2292S	3-33	1-7	3	1-39	3
H4H2293P	3-33	2-15	3	1-39	2
H4H2294S	3-23	6-6	6	1D-12	3
H4H2295S	3-23	6-6	6	1D-12	3
H4H2296S	3-23	6-6	6	1D-12	3
H4H2341S	1-69	3-10	5	1-39	5
H4H2342P	3-23	1-26	4	1-27	3
H4H2344S	3-33	2-15	3	1-39	2
H4H2345S	3-9	1-26	4	1-27	4
H4H2346S	3-33	2-15	3	1-39	2
H4H2350P	4-59	2-21	4	1-9	1
H4H2352S	1-18	3-3	3	3-20	2
H4H2354S	3-33	2-15	3	1-39	2
H4H2355S	3-23	6-6	4	1-5	4
H4H2357S	3-23	3-10	6	1-12	4
H4H2364S	3-23	6-6	6	1D-12	3

Example 3. Binding Affinities of GFRα3 Antibodies

[0188] Binding associative and dissociative rate constants (k_a and k_d, respectively) and calculated equilibrium dissociation constants and dissociative half-lives (K_D and $t_{1/2}$, respectively) for antigen binding to anti-GFRα3 antibodies were determined using a real-time surface plasmon resonance biosensor (Biacore T200) assay at 25°C and 37°C. Antibodies were tested for binding to human GFRα3 expressed with either a C-terminal myc-myc-hexahistidine tag (hGFRα3-mmh; SEQ ID: 370, a C-terminal hFc tag (hGFRα3-hFc; SEQ ID:371), or a C-terminal mFc tag (hGFRα3-mFc; SEQ ID:372), as well as monkey GFRa3 expressed with a C-terminal myc-myc-hexahistidine tag (MfGFRα3-mmh; SEQ ID:373). Anti-GFRα3 antibodies were captured on either a goat anti-mouse IgG polyclonal antibody (GE Healthcare, # BR-1008-38) or a mouse anti-human IgG monoclonal antibody (GE Healthcare, #BR-1008-39) surface created through direct amine coupling to a Biacore CM5 sensor chip. Kinetic experiments were carried out using HBS-EP (10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05% Surfactant P20, at pH 7.4) or PBS buffer containing 0.05% v/v surfactant P20 as both the running buffer and the sample buffer. Binding to human GFRα3-mmh or monkey GFRα3-mmh was evaluated by injecting several concentrations ranging from 200 to 7.4 nM (3-fold dilutions) across the captured antibody surface. Binding to human GFRα3-mFc or human GFRα3-hFc was evaluated by injecting several concentrations ranging from 100 to 3.7 nM (3-fold dilutions) across the captured antibody surface. Antibody-antigen association was monitored for up to 4 minutes, while dissociation in buffer was monitored for up to 20 minutes. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium 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 $t_{½}$ (min) = [ln2/(60*k_d)].

[0189] As shown in Table 3, at 25ºC, all 25 anti-GFRα3 antibodies bound to hGFRα3-mmh with K_D values ranging from 82.0pM to 29.7nM. At 37ºC, all 25 anti-GFRα3 antibodies bound to hGFRα3-mmh with K_D values ranging from 118pM to 47.3nM. As shown in Table 4, at 25ºC, all 23 anti-GFRα3 antibodies bound to MfGFRα3-mmh with K_D values ranging from 2.90pM to 97.2nM. At 37ºC, all 23 anti-GFRα3 antibodies bound to MfGFRα3-mmh with K_D values ranging from 11.7pM to 145nM. As shown in Table 5, at 25ºC and 37ºC, 6 of the 23 anti-GFRα3 antibodies were tested for binding to hGFRα3-hFc, while the other 17 antibodies were tested for binding to hGFRα3-mFc. At 25ºC, the 6 anti-GFRα3 antibodies tested for binding to hGFRα3-hFc bound with K_D values ranging from 7.50pM to 220pM. At 37ºC, the 6 anti-GFRα3 antibodies tested for binding to hGFRα3-hFc bound with K_D values ranging from 41.3pM to 531pM. At 25ºC, the 17 anti-GFRα3 antibodies tested for binding to hGFRα3-mFc bound with K_D values

ranging from 0.467pM to 58.4pM. At 37ºC, the 17 anti-GFRα3 antibodies tested for binding to hGFRα3-mFc bound with K_D values ranging from 13.2pM to 106pM.

Table 3: Kinetics of hGFRα3-mmH binding to different anti-GFRα3 antibodies at 25ºC and at 37ºC

AbPID	25°C				37°C			
	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H2291S	4.18E+05	1.64E-04	3.93E-10	70	5.58E+05	3.23E-04	5.80E-10	36
H4H2292S	1.08E+05	5.19E-05	4.83E-10	223	1.28E+05	2.49E-04	1.94E-09	46
H4H2293P	5.81E+05	1.27E-04	2.19E-10	91	7.81E+05	2.44E-04	3.13E-10	47
H4H2294S	5.63E+05	7.99E-05	1.42E-10	145	7.73E+05	2.61E-04	3.38E-10	44
H4H2295S	4.75E+05	2.56E-04	5.40E-10	45	6.69E+05	1.19E-03	1.77E-09	10
H4H2296S	5.63E+05	1.87E-04	3.32E-10	62	7.65E+05	6.14E-04	8.02E-10	19
H4H2341S	1.59E+05	2.67E-04	1.68E-09	43	2.48E+05	7.37E-04	2.98E-09	16
H4H2342P	1.86E+05	2.04E-04	1.10E-09	57	3.30E+05	6.71E-04	2.03E-09	17
H4H2344S	1.83E+05	2.40E-04	1.31E-09	48	2.80E+05	7.24E-04	2.58E-09	16
H4H2345S	1.09E+05	3.23E-03	2.97E-08	4	1.84E+05	8.70E-03	4.73E-08	1
H4H2346S	1.86E+05	7.99E-05	4.30E-10	145	4.19E+05	5.89E-04	1.41E-09	20
H4H2350P	1.03E+05	2.07E-04	2.01E-09	56	1.34E+05	1.28E-03	9.61E-09	9
H4H2352S	7.09E+05	5.81E-05	8.20E-11	199	1.18E+06	1.39E-04	1.18E-10	83
H4H2354S	2.00E+05	1.10E-04	5.48E-10	105	2.81E+05	6.49E-04	2.31E-09	18
H4H2355S	1.86E+05	1.52E-04	8.21E-10	76	2.81E+05	1.23E-03	4.37E-09	9
H4H2357S	2.30E+05	5.57E-04	2.42E-09	21	2.95E+05	2.10E-03	7.12E-09	6
H4H2364S	3.53E+05	3.67E-05	1.04E-10	315	3.38E+05	2.73E-04	8.09E-10	42
H1M2207N	5.61E+04	9.00E-04	1.60E-08	13	1.33E+05	1.44E-03	1.08E-08	8
H2aM2210N	6.73E+04	8.63E-04	1.28E-08	13	2.12E+05	3.35E-03	1.58E-08	3
H1M2212N	8.00E+05	1.58E-04	1.97E-10	73	1.02E+06	3.03E-04	2.97E-10	38
H2aM2234N	6.57E+05	7.11E-04	1.08E-09	16	7.93E+05	2.72E-03	3.43E-09	4
H1M2236N	7.60E+05	2.08E-04	2.75E-10	56	1.03E+06	5.50E-04	5.32E-10	21
H4H2236N3	9.22E+05	2.33E-04	2.53E-10	50	1.96E+06	7.48E-04	3.82E-10	15
H4H2243N2	2.67E+05	7.80E-04	2.92E-09	15	4.69E+05	2.44E-03	5.20E-09	5
H1M2243N	1.28E+05	2.61E-04	2.04E-09	44	1.37E+05	5.95E-04	4.35E-09	19

Table 4: Kinetics of MfGFRα3-mmH binding to different anti-GFRα3 antibodies at 25ºC and at 37ºC

AbPID	25°C				37°C			
	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H2291S	1.51E+05	2.79E-04	1.85E-09	41	3.57E+05	6.93E-04	1.94E-09	17
H4H2292S	7.76E+04	1.22E-04	1.57E-09	95	8.95E+04	1.95E-04	2.18E-09	59
H4H2293P	2.90E+05	4.17E-05	1.44E-10	277	4.41E+05	1.58E-04	3.58E-10	73
H4H2294S	3.25E+05	2.04E-04	6.27E-10	57	4.33E+05	9.74E-04	2.25E-09	12
H4H2295S	1.83E+05	9.73E-04	5.32E-09	12	2.76E+05	5.61E-03	2.04E-08	2
H4H2296S	1.93E+05	6.51E-04	3.37E-09	18	2.77E+05	2.85E-03	1.03E-08	4
H4H2341S	9.38E+04	1.30E-04	1.39E-09	89	1.44E+05	5.08E-04	3.53E-09	23

H4H2342P	9.37E+04	5.50E-04	5.87E-09	21	1.50E+05	1.66E-03	1.11E-08	7
H4H2344S	1.04E+05	1.15E-04	1.10E-09	101	1.61E+05	5.75E-04	3.57E-09	20
H4H2345S	9.75E+04	2.79E-03	2.87E-08	4	1.34E+05	3.53E-03	2.63E-08	3
H4H2346S	1.08E+05	5.98E-05	5.56E-10	193	1.53E+05	5.11E-04	3.34E-09	23
H4H2350P	6.47E+04	1.11E-04	1.71E-09	104	7.48E+04	8.96E-04	1.20E-08	13
H4H2352S	3.45E+05	1.00E-06	2.90E-12	11550	5.57E+05	6.53E-05	1.17E-10	177
H4H2354S	1.09E+05	6.23E-05	5.74E-10	185	1.57E+05	4.35E-04	2.78E-09	27
H4H2355S	1.05E+05	6.78E-05	6.49E-10	170	1.73E+05	9.13E-04	5.29E-09	13
H4H2357S	1.40E+05	3.15E-04	2.26E-09	37	1.72E+05	1.35E-03	7.86E-09	9
H4H2364S	1.22E+05	1.30E-04	1.06E-09	89	1.68E+05	8.61E-04	5.14E-09	13
H1M2207N	3.99E+04	3.88E-03	9.72E-08	3	4.58E+04	6.63E-03	1.45E-07	2
H2aM2210N	4.16E+04	1.20E-03	2.89E-08	10	8.09E+04	6.18E-03	7.64E-08	2
H1M2212N	3.84E+05	1.12E-04	2.93E-10	103	8.84E+05	2.60E-04	2.94E-10	44
H2aM2234N	4.27E+05	5.71E-04	1.34E-09	20	4.29E+05	2.11E-03	4.91E-09	5
H1M2236N	2.96E+05	2.86E-04	9.70E-10	40	4.34E+05	1.12E-03	2.58E-09	10
H1M2243N	6.46E+04	6.58E-04	1.02E-08	18	5.02E+04	2.75E-03	5.47E-08	4

Table 5: Kinetics of hGFRα3-hFc or hGFRα3-mFc binding to different anti-GFRα3 antibodies at 25ºC and at 37ºC

AbPID	25°C				37°C			
	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H2291S	1.03E+06	4.78E-06	4.63E-12	2417	1.33E+06	3.84E-05	2.89E-11	301
H4H2292S	5.34E+05	1.00E-06	1.87E-12	11550	5.96E+05	2.57E-05	4.32E-11	449
H4H2293P	1.22E+06	1.93E-06	1.59E-12	5988	1.71E+06	5.13E-05	2.99E-11	225
H4H2294S	1.22E+06	5.63E-06	4.63E-12	2052	1.66E+06	3.46E-05	2.09E-11	334
H4H2295S	1.08E+06	1.69E-06	1.57E-12	6822	1.62E+06	4.88E-05	3.01E-11	237
H4H2296S	1.24E+06	4.17E-06	3.37E-12	2770	1.61E+06	4.74E-05	2.94E-11	244
H4H2341S	5.96E+05	1.00E-06	1.68E-12	11550	9.61E+05	7.58E-05	7.89E-11	152
H4H2342P	6.61E+05	1.00E-06	1.51E-12	11550	1.21E+06	4.28E-05	3.54E-11	270
H4H2344S	5.79E+05	1.56E-06	2.69E-12	7409	1.01E+06	8.96E-05	8.91E-11	129
H4H2345S	5.16E+05	3.01E-05	5.84E-11	384	7.00E+05	7.44E-05	1.06E-10	155
H4H2346S	6.47E+05	1.44E-05	2.22E-11	803	1.75E+06	1.37E-04	7.81E-11	84
H4H2350P	5.09E+05	2.12E-05	4.16E-11	545	2.56E+06	1.26E-04	4.92E-11	92
H4H2352S	2.14E+06	1.00E-06	4.67E-13	11550	2.28E+06	3.00E-05	1.32E-11	385
H4H2354S	5.80E+05	1.09E-05	1.89E-11	1056	1.02E+06	9.11E-05	8.95E-11	127
H4H2355S	6.11E+05	1.00E-06	1.64E-12	11550	1.10E+06	3.39E-05	3.10E-11	341
H4H2357S	7.79E+05	2.52E-05	3.24E-11	458	1.19E+06	9.40E-05	7.93E-11	123
H4H2364S	7.71E+05	1.00E-06	1.30E-12	11550	1.26E+06	4.14E-05	3.28E-11	279
H1M2207N*	1.59E+05	2.60E-05	1.63E-10	444	2.03E+05	1.08E-04	5.31E-10	107
H2aM2210N*	1.68E+05	3.69E-05	2.20E-10	313	3.16E+05	7.66E-05	2.42E-10	151
H1M2212N*	1.12E+06	1.44E-05	1.28E-11	800	1.49E+06	6.17E-05	4.13E-11	187
H2aM2234N*	9.50E+05	9.60E-05	1.01E-10	120	1.26E+06	1.46E-04	1.16E-10	79
H1M2236N*	1.28E+06	1.76E-05	1.37E-11	658	1.58E+06	9.65E-05	6.10E-11	120
H1M2243N*	1.34E+05	1.00E-06	7.50E-12	11550	1.86E+05	2.18E-05	1.17E-10	529

*Tested for binding to hGFRα3-hFc, all other antibodies tested for binding to hGFRα3-mFc

Example 4. Blocking of Human GFRα3 Binding to Human ARTEMIN by anti-GFRα3 Antibodies

[0190] The ability of anti-GFRα3 antibodies to block human GFRα3 binding to human ARTEMIN in the presence or absence of co-receptor human RET was determined using two different blocking ELISA formats.

[0191] In the first format, recombinant human ARTEMIN protein with a C-terminal myc-myc-hexahistidine tag (hARTEMIN-mmH; SEQ ID:369) was coated at 2 ug/ml in 96-well microtiter plates in PBS buffer overnight at 4°C and then blocked with a solution of 0.5% (w/v) BSA. A constant amount of human GFRα3 fused with a C-terminal human Fc tag (hGFRα3-hFc; SEQ ID:371) at 120 pM was pre-mixed with varying amounts of antibodies, ranging from 0 to ~10 nM in serial dilutions, followed by an 1 hour incubation at room temperature (RT) to allow antibody-hGFRα3-hFc binding to reach equilibrium. The equilibrated sample solutions were then transferred to the hARTEMIN-mmH-coated plate. After 1 hour of binding, the plate was washed, then the bound hGFRα3-hFc was detected using HRP-conjugated anti-human IgG Fc specific antibody (Jackson Immunochemical, #109-035-098), and colorimetric signals were developed using a TMB HRP substrate (BD Biosciences, #51-2606KC and #51-2607KC). Absorbance was recorded at 450nm on a Victor X5 plate reader (Perkin Elmer) to determine the amount of free hGFRα3-hFc in the pre-equilibrated hGFRα3-hFc-antibody solutions that was able to bind to the plate coated with hARTEMIN-mmH. IC_{50} values, defined as the concentration of antibody required to reduce the signal from a constant concentration of hGFRα3-hFc by 50%, were calculated from the data using Prism software from GraphPad. The absorbance measured at the constant amount of 120pM hGFRα3-hFc in the absence of anti-GFRα3 antibody is defined as 0% blocking and the absorbance with no added hGFRα3-hFc is defined as 100% blocking. The observed absorbance in the wells containing the highest antibody concentration was used to calculate the maximum blocking percent shown in the table. The results are summarized in Table 6.

[0192] In the second ELISA format, the plates, samples and data were processed similarly as for the first format except both hARTEMIN-mmH and human RET with a C-terminal 10 histidine tag (hRET-10His; R&D Systems, # 1168-CR/CF) were coated for the blocking ELISA experiment. The 96-well microtiter plates were coated with a mixture of 1.2 ug/ml hARTEMIN-mmH and 6.9 ug/ml hRET-10His proteins in PBS overnight at 4°C and then blocked with a solution of 0.5% (w/v) BSA. A constant amount of biotinylated human GFRα3 with a C-terminal myc-myc-hexahistidine tag (biotin-hGFRα3-mmH; SEQ ID:370) at 1nM was pre-mixed with varied amounts of anti-GFRα3 antibodies, ranging from 0 to ~100 nM in serial dilutions, followed by a 1 hour incubation at RT to allow antibody-biotin-

hGFRα3-mmH binding to reach equilibrium. The equilibrated samples were then transferred to the coated plate. After 1 hour of binding, the plate was washed, then the bound biotin-hGFRα3-mmH was detected using HRP conjugated streptavidin (Pierce, #N200), and colorimetric signals were developed using TMB HRP substrates. IC_{50} values and the maximal blocking by each antibody are shown in the Table 6.

[0193] As shown in Table 6, 9 of the 23 anti-GFRα3 antibodies blocked 51-94% of the hGFRα3-hFc binding to coated hARTEMIN-mmH with IC_{50} values ranging from 43.8pM to 723pM in the first ELISA format. Eight of the 23 anti-GFRα3 antibodies caused the hGFRα3-hFc binding signal to increase ("enhancer" in Table 6) at many of the higher tested antibody concentrations in the first ELISA format. Six of the 23 antibodies tested in the first ELISA format did not block or enhance the hGFRα3-hFc binding signal to coated hARTEMIN-mmH. As shown in Table 6, for the second ELISA format, 17 of the 23 anti-GFRα3 antibodies blocked 75-100% of the biotin-hGFRα3-mmH binding to dual-coated hARTEMIN-mmH and hRET-10His with IC_{50} values ranging from 403pM to 14.6nM. Also in the second ELISA format, five of the 23 anti-GFRα3 antibodies caused the biotin-hGFRα3-mmH binding signal to increase at lower antibody concentrations but blocked 28-95% of the biotin-hGFRα3-mmH binding to hARTEMIN-mmH and hRET-10His at antibody concentrations 1nM and above ("enhancer" in Table 6). One anti-GFRα3 antibody caused the biotin-hGFRα3-mmH binding signal to increase ("enhancer" in Table 6) at the higher tested antibody concentrations, with no blocking at any concentration, in the second ELISA format.

Table 6: ELISA Blocking of human GFRα3 to human ARTEMIN alone or human ARTEMIN and human RET

	ELISA format 1: Ab blocking 120pM hGFRα3-hFc binding to coated hARTEMIN-mmH		**ELISA format 2: Ab blocking 1nM biotin-hGFRα3-mmH binding to coated hARTEMIN-mmH and hRET-10His**	
AbPID	IC_{50} (M)	% max blocking	IC_{50} (M)	% max blocking
H4H2207N	enhancer	NB	7.30E-09	85
H4H2210N	enhancer	NB	enhancer	NB
H4H2212N	4.38E-11	81	4.03E-10	99
H4H2234N	8.29E-11	70	3.42E-09	97
H4H2236N3	enhancer	NB	enhancer	68
H4H2243N2	enhancer	NB	1.12E-09	95
H4H2291S	NB	NB	enhancer	28
H4H2292S	7.23E-10	89	1.76E-09	100
H4H2293P	2.60E-10	93	7.38E-09	95
H4H2294S	NB	NB	9.52E-10	95

H4H2295S	enhancer	NB	enhancer	44
H4H2296S	NB	NB	enhancer	91
H4H2341S	enhancer	NB	1.62E-09	98
H4H2342P	enhancer	NB	1.08E-09	97
H4H2344S	1.33E-10	94	4.59E-10	100
H4H2345S	enhancer	NB	1.46E-08	75
H4H2346S	9.78E-11	92	7.971E-10	99
H4H2350P	4.96E-10	93	1.29E-09	91
H4H2352S	6.58E-11	51	7.61E-10	100
H4H2354S	1.16E-10	92	1.32E-09	97
H4H2355S	NB	NB	enhancer	95
H4H2357S	NB	NB	3.28E-09	86
H4H2364S	NB	NB	1.45E-09	100

NB = non-blocker

Example 5. Measuring the Ability of Anti-GFRα3 Antibodies to Block Activation of GFRα3 and RET by the Ligand ARTEMIN *in vitro*

[0194] The ability of anti-GFRα3 antibodies to block activation of GFRα3 and RET by its ligand ARTEMIN *in vitro* was determined using a cell-based assay. HEK293 cells modified to stably express both human GFRα3 (amino acids 1-400 of accession number NP_001487.2) and human RET (amino acids 1-1072 of accession number NP_065681) were generated and then transduced with a SRE responsive luciferase reporter (SRE-luc; Sabiosciences, CCS-010L) (HEK293/hGFRα3/hRET cells).

[0195] Twenty thousand HEK293/hGFRα3/hRET/SRE-luc cells were seeded into Poly D-Lysine coated 96 well plates (Greiner, #35-4620) in Optimem (GIBCO, #31985) containing 0.5% FBS and then grown overnight in 5% CO_2 at 37°C. The cells were then incubated for 1 hour at room temperature with serial dilutions of anti-GFRα3 antibodies ranging from 5pM to 300nM. A constant dose (100pM) of human ARTEMIN expressed with a C-terminal myc myc hexahistidine tag (SEQ ID:369) was then added to the cells and incubated for an additional 6 hours. Luciferase activity was measured as relative light units (RLU) on a Victor luminometer (Perkin Elmer) after the addition of OneGlo reagent (Promega, #E6051). EC_{50} and IC_{50} values were calculated from a four-parameter logistic equation over a 12-point response curve using GraphPad Prism data analysis software.

[0196] Twenty-three anti-GFRα3 antibodies were tested for their ability to inhibit ARTEMIN-dependent activation of the HEK293/hGFRα3/hRET/SRE-luc cells. As shown in Table 7, all 23 antibodies tested blocked luciferase activity with IC_{50} values ranging from 0.2nM to 48.3nM, and 19 of 23 antibodies blocked to the baseline at a concentration of 300nM. Four of the 23 antibodies (H4H2344S, H4H2345S, H4H2346S, and H4H2354S-1) did not block to baseline at any of the antibody concentrations tested.

Table 7: Inhibition of ARTEMIN-dependent stimulation of HEK293/hGFRα3/hRET/ SRE-luc cells by anti-GFRα3 antibodies

AbPID	IC_{50} (nM)
H4H2294S	0.27
H4H2342P	1.0
H4H2212N	0.80
H4H2292S	4.6
H4H2243N2	0.30
H4H2352S	0.92
H4H2207N	2.4
H4H2210N	2.9
H4H2234N	2.3
H4H2236N3	1.5
H4H2291S	1.3
H4H2293P	20
H4H2294S	1.7
H4H2295S	1.5
H4H2296S	1.4
H4H2341S	7.1
H4H2344S	48
H4H2345S	26
H4H2346S	26
H4H2354S	22
H4H2355S	2.3
H4H2357S	4.9
H4H2364S	2.3

Example 6. Inhibition of ARTEMIN-sensitized capsaicin thermal hyperalgesia

[0197] To induce ARTEMIN-sensitized thermal hyperalgesia in mice, each mouse was pre-treated with an intra-plantar injection of 0.5 micrograms mouse recombinant ARTEMIN (R&D Systems, #1085-AR) 24 hours before administering an intra-plantar injection of 0.5 micrograms capsaicin (a sub-optimal dose) from a 100 mM solution in DMSO (Sigma-Aldrich, #M-2028). Thermal hyperalgesia was evaluated using the Hargreaves' Test, in

which a beam of light is directed at the injected paw until the animal withdraws its paw. The latency to withdraw is recorded as a behavioral measure of nociception. Thermal hyperalgesia is consistently ARTEMIN-sensitized at 3 days after capsaicin administration based on significantly decreased paw withdrawal latencies. For these studies, a baseline value for withdrawal latency was measured before dosing with either ARTEMIN or capsaicin followed by a second measurement three days after capsaicin treatment. The experimenter conducting these assays was blind to the treatment group of the animals.

[0198] For all experiments evaluating the efficacy of human anti-GFRα3 antibodies in ARTEMIN-sensitized hyperalgesia, adult mice homozygous for the expression of human GFRα3 in place of mouse GFRα3 ("humanized GFRα3") were used. Both male and female mice were used in each assay, with sex balanced across treatment groups (a total of 8 mice per treatment or control group). Humanized GFRα3 mice were previously determined to have ARTEMIN-induced capsaicin thermal hyperalgesia latency responses similar to those observed in wild-type mice.

[0199] Six anti-GFRα3 antibodies (H4H2212N, H4H2243N2, H4H2292S, H4H2294S, H4H2342P, and H4H2352S-1) were tested in the model. All antibodies were diluted in phosphate-buffered saline (PBS) and were administered subcutaneously at 25mg/kg in a 1ml/100g body weight volume 24 hours prior to ARTEMIN injection into the hindpaw. In each experiment, one group of animals received an isotype control antibody.

[0200] Pain sensitivity for each treatment or control group was defined as percentage of baseline withdrawal latency (%BWL), calculated as the fractional change for each animal's time-based withdrawal latency (WL) three days after capsaicin treatment compared to their baseline withdrawal latency without capsaicin treatment:

$$\%BWL = [(WL_{(capsaicin)} - WL_{(no\ capsaicin)})/WL_{(no\ capsaicin)}] \times 100$$

[0201] Using %BWL, larger negative values indicate greater thermal hyperalgesia.

[0202] Table 8 shows the summary of group means (in boldface type) and standard error of the means (in italics) for percentage of baseline withdrawal latency (%BWL) in the ARTEMIN-sensitized capsaicin thermal hyperalgesia model assessed at three days after capsaicin injection.

[0203] As shown in Table 8, mice treated with anti-hGFRα3 antibodies exhibited increases in %BWL (smaller negative or positive values) compared to mice treated with the isotype control antibody. Four antibodies, H4H2352S, H4H2243N2, H4H2294S, and H4H2342P promoted the greatest resistance to thermal hyperalgesia across all experiments performed.

Table 8: Data Summary in the ARTEMIN-sensitized capsaicin thermal hyperalgesia model assessed at three days after capsaicin injection.

AbPID	%BWL			
	Exp. 323	**Exp. 361**	**Exp. 367**	**Exp. 380**
Isotype control	**-17** ± *7.6*	**-32** ± *6.8*	**-40** ± *4.8*	**-35** ± *3.5*
H4H2292S	**0.72** ± *19*	***nd***	***nd***	**-26** ± *5.9*
H4H2352S	**27** ± *15*	***nd***	**-5.6** ± *9.4***	***nd***
H4H2243N2	**20** ± *18*	***nd***	***nd***	**21** ± *6.4****
H4H2294S	***nd***	**-9.0** ± *12*	**2.3** ± *12***	***nd***
H4H2342P	***nd***	**-12** ± *9.3*	**18** ± *8.3****	***nd***
H4H2212N	***nd***	**-22** ± *6.9*	***nd***	***nd***

significantly different than isotype control, **p<.01; ***p<.001
(nd=not tested in this experiment)

Example 7. Testing of anti-GFRα3 Antibodies for Cross-Reactivity with GFRα1 and GFRα2

[0204] The ability of anti-GFRα3 antibodies to bind to GDNF-family receptors was assessed using an Octet Red biosensor (Fortebio, Inc.). Antibodies were tested for binding to either human GFRα1 expressed with a C-terminal human Fc tag and a hexahistidine tag (hGFRα1-hFc-6His, R&D Systems # 714-GR), human GFRα1 expressed with only a C-terminal human Fc tag (hGFRα1-hFc; SEQ ID: 376), human GFRα2 expressed with a C-terminal human Fc tag and a hexahistidine tag (hGFRα2-hFc-6His, R&D Systems #613-FR), human GFRα3 expressed with a C-terminal human Fc tag (hGFRα3-hFc, SEQ ID:371), or an irrelevant human Fc tagged protein. Antigens were captured onto anti-human Fc sensor tips from 10ug/mL solutions for 5 minutes. The coated sensor tips were then blocked with a 100ug/mL solution of irrelevant human Fc antibodies for 5 minutes. Blocked sensor tips were then submerged into wells containing 667uM of each anti-GFRα3 antibody or buffer alone for 10 minutes. The experiment was performed at 25°C with a flow rate of 1000 rpm using HBST+BSA buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% w/v Surfactant P20, 0.1 mg/mL BSA, pH 7.4). The binding response (measured in units of nm) at each step of the experiment was monitored and recorded.

[0205] All six of the tested anti-GFRα3 antibodies showed binding above 1.0 nm to the hGFRα3-hFc protein, but did not demonstrate any measurable binding to the other GDNF-family receptors or to the irrelevant human Fc tagged protein, as shown in Table 9.

Table 9. Reactivity of Anti-GFRα3 Antibodies with GFRα1, GFRα2 and GFRα3

Antigen	Capture Level (nm) +/- Std dev	100 ug/mL H4H2294S	100 ug/mL H4H2342P Bound	100 ug/mL H4H2243N2	100 ug/mL H4H2212N	100 ug/mL H4H2352S	100 ug/mL H4H2292S	Buffer

		Bound (nm)	(nm)	Bound (nm)	Bound (nm)	Bound (nm)	Bound (nm)	
hGFR□1-hFc	1.89 ± 0.14	0.04	0.06	0.03	0.00	0.02	0.05	-0.07
hGFR□1-hFc-6his	1.66 ± 0.10	0.03	0.08	0.03	-0.01	0.03	0.03	-0.07
hGFR□2-hFc-6his	1.49 ± 0.09	0.06	0.12	0.06	0.02	0.04	0.05	-0.06
hGFR□3-hFc	1.68 ± 0.10	1.17	1.59	1.11	1.18	1.25	1.04	-0.09
Irrelevant hFc tagged protein	1.00 ± 0.06	0.03	0.07	0.03	0.00	0.03	0.02	-0.08

Example 8. Measuring the Ability of Anti-GFRa3 antibodies to block ARTEMIN stimulation in a HEK293/MfGFRα3/MfRet/SRE-Luc bioassay

[0206] The ability of anti-GFRα3 antibodies to block activation of cynomolgus GFRα3 and cynomolgus RET by its ligand ARTEMIN *in vitro* was determined using a cell-based assay. HEK293 cells modified to stably express both cynomolgus GFRα3 (MfGFRα3; SEQ ID: 377) and cynomolgus RET (MfRET; SEQ ID: 378) were generated and then transduced with a Cignal Lenti SRE Reporter (SA Biosciences, #CLS-010L) expressing the firefly luciferase gene under the control of a minimal CMV promoter and tandem repeats of the serum response element to generate the HEK293/MfGFRα3/MfRet/SRE-Luc cell line.

[0207] For the bioassay, 20,000 HEK293/MfGFRα3/MfRet/SRE-Luc cells were seeded onto Poly D-Lysine coated 96 well plates (Greiner, #35-4620) in Optimem (GIBCO, #31985) containing 0.5% FBS and then grown overnight at 5% CO_2 at 37°C. The cells were then incubated for 1 hour with serial dilutions of anti-GFRα3 antibodies ranging from 5pM to 300nM. A constant dose (500pM) of human ARTEMIN expressed with a C-terminal myc-myc-hexahistidine tag (Human ARTEMIN-MMH; SEQ ID: 369) was then added to the cells and incubated for an additional 6 hours. To determine the EC_{50} value of human ARTEMIN-MMH from dose response curves, serial dilutions of human ARTEMIN-MMH ranging from 0.5 pM to 10nM was added to the cells without antibodies and incubated for 6 hours at 37°C. Luciferase activity was measured as relative light units (RLU) on a Victor luminometer (Perkin Elmer) after the addition of OneGlo reagent (Promega, #E6051). EC_{50} and IC_{50} values were calculated from a four-parameter logistic equation over a 12-point response curve using GraphPad Prism data analysis software.

[0208] Six anti-GFRα3 antibodies were tested for their ability to inhibit ARTEMIN-dependent activation of the HEK293/MfGFRα3/MfRET/SRE-luc cells. As shown in Table 10, all six antibodies tested completely blocked luciferase activity with IC_{50} values ranging from 0.7nM to

2.5nM. Human ARTEMIN-MMH stimulated SRE-dependent luciferase activity in the HEK293/mfGFRα3/mfRet/SRE-LUC cell line with an EC_{50} value of 70pM.

Table 10: Inhibition of ARTEMIN-dependent stimulation of HEK293/MfGFRα3/MfRET/ SRE-luc cells by anti-GFRα3 antibodies

Antibody	IC_{50} (nM)
H4H2294S	0.7
H4H2342P	2.5
H4H2212N	1.8
H4H2292S	1.5
H4H2243N2	0.8
H4H2352S	1.3

Example 9. Generation of a Bi-specific Anti-GFRα3 Antibody

[0209] Various bi-specific antibodies are generated for use in practicing the methods of the invention. For example, GFRα3-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct epitopes on GFRα3 are linked together to confer dual-epitope specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall GFRα3 blocking efficacy through increasing both GFRα3 specificity and binding avidity. Variable regions with specificity for individual different epitopes within any of the three cysteine repeats or that can bind to different regions within one epitope of any of the three cysteine repeats are paired on a structural scaffold that allows each variable region to bind simultaneously to the separate epitopes, or to different regions within one epitope. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one epitope within one cysteine repeat are recombined with light chain variable regions (V_L) from a series of binders having specificity for a second epitope within any of the other two cysteine repeats 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).

[0210] Alternatively, antibodies that bind both GFRα3 and a second target, such as, but not limited to, for example, RET 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 GFRα3 regions that are extracellularly exposed are linked together with variable regions that bind to relevant sites on, for example, the ligand, artemin, other GFRα receptors, or to RET, to confer dual-antigen specificity within a single binding molecule. Variable regions with specificity for individual epitopes of GFRα3, are combined with a variable region with specificity for, for example, artemin and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[0211] The bi-specific binders are tested for binding and functional blocking of the target antigens, for example, GFRα3 and/or artemin, other GFRα receptors, or 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 with its antigen(s), such as Biacore, ELISA, size exclusion chromatography, multi-angle laser light scattering, direct scanning calorimetry, and other methods. Binding of bi-specific antibodies to cells expressing GFRα3 is determined through flow cytometry using a fluorescently labeled secondary antibody recognizing the target antigen on the cells. Binding experiments with peptides 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 the GFRα3 receptor by a bi-specific is determined using any bioassay such as that described herein, or by *in vivo* determination of reaction to pain in appropriate animal models, such as those described herein. Functional *in vitro* blocking of GFRα3 or its ligand, artemin, by a bi-specific is determined using any bioassay such as that described in WO2010/077854, or in US2010/0166768, or by *in vivo* determination of hypersensitivity to thermal stimuli in appropriate animal models, such as those described herein.

Example 10. Surface plasmon resonance derived binding affinities and kinetic constants of monoclonal anti-mouse GFRα3 antibodies

[0212] Binding associative and dissociative rate constants (k_a and k_d, respectively) and calculated equilibrium dissociation constants and dissociative half-lives (K_D and $t_{1/2}$, respectively) for antigen binding to anti-mouse GFRα3 antibodies were determined using a real-time surface plasmon resonance biosensor (Biacore 3000) assay at 25°C. Antibodies were tested for binding to mouse GFRα3 expressed with myc-myc-hexahistidine tag (mGFRα3-MMH; SEQ ID: 379,). Anti-mouse GFRα3 antibodies were captured on a goat anti-mouse IgG polyclonal antibody (GE Healthcare, # BR-1008-38) surface created through direct amine coupling to a Biacore CM5 sensor chip. Kinetic experiments were carried out using HBS-EP

(10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05% Surfactant P20, at pH 7.4) as both the running buffer and the sample buffer. Binding to mouse GFRα3-MMH was evaluated by injecting several concentrations ranging from 100nM to 6.25 nM (2-fold dilutions) across the captured antibody surface. Antibody-antigen association was monitored for up to 5 minutes, while dissociation in buffer was monitored for up to 10 minutes. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium 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 $t_{½}$ (min) = [ln2/(60*k_d)].

[0213] As shown in Table 11, the two anti-mouse GFRα3 antibodies tested, M1M6986N and M1M6977N, bound to mGFRα3-MMH at 25ºC with K_D values of 23.1pM and 107pM, respectively.

Table 11: Kinetics of mGFRα3-MMH binding to different anti-mouse GFRα3 antibodies at 25ºC

AbPID	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
M1M6986N	1.02E+06	2.35E-05	2.31E-11	491
M1M6977N	3.84E+05	4.1E-05	1.07E-10	282

Example 11. Mouse GFRα3 Blocking ELISA

[0214] The ability of anti-mouse GFRα3 antibodies to block mouse GFRα3 binding to mouse ARTEMIN in the presence or absence of co-receptor mouse RET was determined using two different blocking ELISA formats. In the first format, recombinant mouse ARTEMIN (R&D cat# 1085-AR/CF) protein was coated at 2ug/mL (166nM) in 96-well microtiter plates in PBS buffer overnight at 4°C and then blocked with a solution of 0.5% (w/v) BSA. A constant amount (3.5nM) of biotinylated mouse GFRα3 with a C-terminal myc-myc-hexahistidine tag (Biotin-mGFR□3-MMH, SEQ ID:379) was pre-mixed with varying amounts of antibodies, ranging from 100nM to 1.6 pM in serial dilutions, followed by 1 hour incubation at room temperature (RT) to allow antibody-biotin-mGFRα3-MMH binding to reach equilibrium. The equilibrated sample solutions were then transferred to the mARTEMIN-coated plate. After 1 hour of binding, the plate was washed, then the bound biotin-mGFRα3-MMH was detected using Streptavidin-HRP (Pierce, #N200) and colorimetric signals were developed using a TMB HRP substrate (BD Biosciences, #51-2606KC and #51-2607KC). Absorbance was recorded at 450nm on a Victor X5 plate reader (Perkin Elmer) to determine the amount of free biotin-mGFRα3-MMH in the pre-equilibrated biotin-mGFRα3-MMH-antibody solutions that was able to bind to the plate-coated mARTEMIN. IC_{50} values, defined as the concentration of antibody required to reduce the signal from a constant concentration of biotin-mGFRα3-MMH by 50%, were calculated from the data using Prism software from GraphPad. The absorbance measured at the constant amount of biotin-mGFRα3-MMH in the absence of anti-mouse GFRα3 antibody is defined as 0%

blocking and the absorbance with no added biotin-mGFRα3-MMH is defined as 100% blocking. The observed absorbance in the wells containing the highest antibody concentration was used to calculate the maximum blocking percent shown in the table. The results are summarized in Table 12.

[0215] In the second ELISA format, the plates, samples and data were processed similarly as for the first format except both mouse RET expressed with C-terminal hFc and hexahistidine tags (mRET-hFc-6His; R&D cat# 482-RT/CF) and mARTEMIN (R&D cat# 1085-AR/CF) were coated for the blocking ELISA experiment. The 96-well microtiter plates were coated with a mixture of 1.2ug/mL (100nM) mARTEMIN and 9.5ug/mL (100nM) mRET-hFc-6His proteins in PBS overnight at 4°C and then blocked with a solution of 0.5% (w/v) BSA. A constant amount (350pM) of biotin-mGFRα3-MMH was pre-mixed with varied amounts of anti-mouse GFRα3 antibodies, ranging from 100nM to 1.6pM in serial dilutions, followed by a 1 hour incubation at RT to allow antibody- biotin-mGFRα3-MMH binding to reach equilibrium. The equilibrated samples were then transferred to the coated plate. After 1 hour of binding, the plate was washed, then the bound biotin-mGFRα3-MMH was detected using HRP conjugated streptavidin and colorimetric signals were developed using TMB HRP substrates. IC_{50} values and the maximal blocking by each antibody are shown in the Table 12.

[0216] As shown in Table 12, only one anti-mouse GFRα3 antibody tested, M1M6986N, demonstrated the ability to block biotin-mGFRα3-MMH from binding to the coated mARTEMIN plate with an IC_{50} value of 69.1pM. The other anti-mouse GFRα3 antibody tested, M1M6977N, did not demonstrate any measurable blockade in this ELISA format. Both anti-mouse GFRα3 antibodies tested, M1M6986N and M1M6977N, demonstrated the ability to completely block biotin-mGFRα3-MMH from binding to the plates coated with both mARTEMIN and mRET-hFc-6His, with IC_{50} values of 47.2pM and 366pM, respectively.

Table 12: ELISA Blocking of mouse GFRα3 to mouse ARTEMIN alone or mouse ARTEMIN and mouse RET

	ELISA format 1: Antibody blocking 3.5nM biotin-mGFRα3-MMH binding to coated mARTEMIN		**ELISA format 2: Antibody blocking 350pM biotin-mGFRα3-MMH binding to coated mARTEMIN and mRET-hFc-6His**	
AbPID	IC_{50} (M)	% max blocking	IC_{50} (M)	% max blocking
M1M6986N	6.91E-11	100%	4.72E-11	100%
M1M6977N	NB		3.66E-10	100%
NB = non-blocker				

Example 12. Cell based Luciferase bioassay

[0217] The ability of anti-mouse GFRα3 antibodies to block activation of mouse GFRα3 and mouse RET by its ligand mouse ARTEMIN *in vitro* was determined using a cell-based assay. HEK293 cells modified to stably express both mouse GFRα3 (amino acids 1-397 of accession number AAH66202.1) and mouse RET (amino acids 1-1115 of accession number NP_033076.2) were generated and then transduced with a SRE responsive luciferase reporter (SRE-luc; Sabiosciences, CCS-010L) (293/mGFRα3/mRET/SRE-luc cells).

[0218] Twenty thousand 293/mGFRα3/mRET/SRE-luc cells were seeded into Poly D-Lysine coated 96 well plates (Greiner, #35-4620) in Optimem (GIBCO, #31985) containing 0.5% FBS and then grown overnight in 5% CO_2 at 37°C. The cells were then incubated for 1 hour at room temperature with serial dilutions of anti-mouse GFRα3 antibodies ranging from 3 nM to 44 nM. A constant dose (100 pM) of mouse ARTEMIN (R&D, # 1085-AR/CF) was then added to the cells and incubated for an additional 6 hours. Luciferase activity was measured as relative light units (RLU) on a Victor luminometer (Perkin Elmer) after the addition of OneGlo reagent (Promega, #E6051). EC_{50} and IC_{50} values were calculated from a four-parameter logistic equation over a 12-point response curve using GraphPad Prism data analysis software.

[0219] As shown in Table 13, both anti-mouse GFRα3 antibodies tested, M1M6986N and M1M6977N demonstrated the ability to inhibit mouse ARTEMIN-dependent stimulation of 293/mGFRα3/mRET/SRE-luc cells with IC_{50} values of approximately 44nM and 3nM, respectively.

Table 13: Inhibition of ARTEMIN-dependent stimulation of 293/mGFRα3/mRET/SRE-luc cells by anti-mouse GFRα3 antibodies

Antibody	IC_{50} (nM)
M1M6986N	44 ± 3 (n=2)
M1M6977N	3 ± 1 (n=3)

Examples 13, 14 and 15: The effect of anti-mouse GFRα3 antibodies in animal models of bone cancer pain and osteoarthritic pain

[0220] The antibodies described herein are high affinity human antibodies to the GPI-linked alpha receptor for artemin, GFRα3. Since these antibodies to human GFRα3 do not cross-react with mouse GFRα3, *in vivo* assays with these antibodies can only be conducted in mice genetically altered to replace the mouse GFRα3 sequence with that of human GFRα3. Initial *in vivo* experiments in these $GFR\alpha3^{hu/hu}$ mice using pharmacological inhibition of artemin-sensitized capsaicin revealed efficacy of four human antibodies in this *in vivo* assay. In order to expedite the generation of efficacy data, mouse GFRα3 antibodies were generated to serve as

surrogates to the human antibodies. Mice of a mixed C57BL6/129Sv strain that were homozygous for deletion of the endogenous GFRα3 gene were immunized with recombinant mouse GFRα3 extracellular domain expressed in Chinese hamster ovary cells. A specific immune response to GFRα3 was confirmed by immunoassays of serum from the immunized mice. Spleens were collected from mice exhibiting a high specific immune response, and antibody-producing hybridoma cells were generated by fusion of the isolated splenocytes with mouse myeloma cells following standard hybridoma procedures. Hybridoma supernatants were further screened in immunoassays for binding to GFRα3 and for their ability to block binding of GFRα3 to either artemin or artemin/Ret coated on a solid surface in an immunoassay format. Supernatants were also screened for their ability to block artemin stimulation of the GFRα3/Ret co-receptor pathway in a cell-based bioassay. Variable-region antibody sequences were obtained by PCR amplification of selected hybridoma clones whose antibody proteins exhibited potent blocking in the cell-based assay, and these sequences were used to produce full-length recombinant anti-mouse GFRα3 antibodies with a mouse IgG1 isotype. Two antibodies were selected for *in vivo* testing that potently inhibited artemin signaling in the cell-based assay. In the *in vitro* binding immunoassay, antibody M1M6986N blocked binding of GFRα3 to both artemin or artemin/Ret coated on a solid surface and is referred to here as a direct blocker. Antibody M1M6977N blocked binding of GFRα3 to coated artemin/Ret but not to artemin alone in the *in vitro* immunoassay and is referred to here as an indirect blocker. These two mouse antibodies, M1M6986N and M1M6977N, were selected for testing in the artemin-sensitized capsaicin thermal analgesia model (described in Example 6), since these two antibodies demonstrated similar binding and blocking profiles to the efficacious human antibodies. These two antibodies were screened for their ability to block artemin-induced sensitization of hyperalgesia *in vivo* in wild type mice. Like their human antibody counterparts, both antibodies significantly inhibited artemin's sensitizing effect on capsaicin thermal hyperalgesia three days after capsaicin injection (Figure 1).

Example 13: Fibrosarcoma Model of Bone Cancer Pain

Methodology

Subjects

[0221] Adult male mice on a C57Bl6 background strain were used for two fibrosarcoma experiments at approximately 12 weeks of age. The experimenters measuring outcome data for this experiment were blind to treatment group of the animals throughout data collection, compilation, and analysis.

Bone Cancer Model

[0222] To induce bone cancer pain, the mice were anesthetized and then injected

intrafemorally with $1.0x10^6$ MC57G fibrosarcoma cells. These cells are derived from a C57Bl/6 mouse fibrosarcoma tumor line. Tumors typically grow aggressively in this model, such that bone destruction is evident by 14 days after tumor implantation. Radiographs were taken at days 7, and 10, and 14 after implantation to verify tumor growth and bone destruction. Bone destruction was scored on a three-point scale such that 0 represented no destruction and 3 represented complete destruction of the femur in the region of the tumor.

Antibody Treatment

[0223] Each animal received 30mg/kg s.c. antibody injections administered the day before cancer cell implantation and again on day 7. Animals were pseudo-randomly assigned to one of two or three treatment groups: 1) M2M180N isotype (negative) control antibody in two separate experiments, 2) M1M6977N anti-mouse GFRα3 antibody in two separate experiments or 3) M1M6986N anti-mouse GFRα3 antibody in the second experiment only. M1M6986 blocks artemin's interaction with GFRα3, and is thereby considered a "direct" blocker of artemin's action. In contrast, M1M6977N inhibits artemin's action through the GFRα3/RET complex, and is therefore considered an "indirect" inhibitor.

Measures of Nociception

[0224] Nociceptive responses to the bone tumor were measured using the von Frey Hair test for evoked mechanical (tactile) allodynia, the dynamic weight bearing (DWB) test for willingness to bear weight on a limb, and guarding behavior. Von Frey test results are expressed as grams of pressure required for paw withdrawal. Weight bearing results are expressed as percent body weight placed on the ipsilateral limb. Guarding behavior is expressed as time spent guarding the limb over a two-minute period.

Results

Bone Destruction

[0225] There was no significant effect of antibody treatment on bone destruction score in either experiment suggesting that the antibody treatment had no impact on the severity of the bone cancer itself (data not shown).

Nociceptive Behavior

[0226] There was a statistically significant decrease in tactile allodynia with GFRα3 antibody treatment after fibrosarcoma injection in the first experiment ($F(1,20)=9.189$, $p=.007$, Figure 2A) and a statistical trend toward efficacy overall in the second experiment ($F(2,29)=3.069$, $p<.062$, Figure 2B), with individual comparisons sometimes achieving significance in the second study (Figure 2B).

[0227] There was no statistically significant effect of GFRα3 antibodies on dynamic weight bearing on the ipsilateral limb measured 14 days after implantation of bone with fibrosarcoma cells in either experiment, although the first experiment revealed a statistical trend toward efficacy with M1M6977N treatment ($t(10)=2.047$, $p=.068$, Figure 3A; $F(2,28)=1.598$, $p=.220$, Figure 3B).

[0228] GFRα3 antibodies significantly reduced limb guarding after bone cancer implantation in both fibrosarcoma experiments ($F(1,20)=12.270$, $p=.002$, Figure 4A; $F(2,29)=3.576$, $p=.041$, Figure 4B).

Conclusion

[0229] Treatment with anti-mouse GFRα3 antibodies significantly reduced nociceptive behaviors in this bone cancer pain model as measured by evaluation of guarding and the von Frey Test of tactile allodynia. In addition, there was a statistical trend toward efficacy of the M1M6977N antibody in weight bearing differential in one experiment. Bone destruction scores were not different in groups receiving anti-mouse GFRα3 antibodies, suggesting that differences in pain-related measures could not be accounted for by differences in cancer severity. Therefore, our data suggest that neutralizing antibodies against GFRα3 could be efficacious against bone cancer pain. Because sarcoma cells are more often primary tumors than metastases in bone, and because most bone cancers derive from metastases of primary tumors from other sites, these antibodies were also tested in a model of breast (mammary) carcinoma-induced bone cancer pain. Breast and prostate tumors are among the most common tumors found to metastasize to bone.

Example 14: Breast Carcinoma Model of Bone Cancer Pain

Methodology

Subjects

[0230] Adult male mice on a Balb/c background strain were used for a mammary carcinoma bone cancer experiment at approximately 12 weeks of age. The experimenters measuring outcome data for this experiment were blind to treatment group of the animals throughout data collection, compilation, and analysis.

Bone Cancer Model

[0231] To induce bone cancer pain, the mice were anesthetized and then injected intrafemorally with 10,000 4T-1 mammary carcinoma cells. These cells are derived from a Balb/c mammary carcinoma tumor line. Tumors typically grow aggressively in this model, such that tumors are severe by 18 days after implantation. Radiographs were taken at days 10, 14, and 19 after implantation to verify tumor growth and bone destruction. Bone destruction was

scored on a three-point scale such that 0 represented no destruction and 3 represented complete destruction of the femur in the region of the tumor.

Antibody Treatment

[0232] Each animal received 30mg/kg s.c. antibody injections administered the day before cancer cell implantation and two times per week thereafter. Animals were pseudo-randomly assigned to one of three treatment groups: 1) M2M180N isotype (negative) control antibody, 2) M1M6977N anti-mouse GFRα3 antibody, or 3) M1M6986N anti-mouse GFRα3 antibody. M1M6986N blocks artemin's interaction with GFRα3, and is thereby considered a "direct" blocker of artemin's action. In contrast, M1M6977N inhibits artemin's action through the GFRα3/RET complex, and is therefore considered an "indirect" inhibitor.

Measures of Nociception

[0233] Nociceptive responses to the bone tumor were measured using the von Frey Hair test for evoked mechanical (tactile) allodynia, the dynamic weight bearing (DWB) test for willingness to bear weight on a limb, and guarding behavior. Von Frey test results are expressed as grams of pressure required for paw withdrawal. Weight bearing results are expressed as percent body weight placed on the ipsilateral limb. Guarding behavior is expressed as time spent guarding the limb over a two-minute period.

Results

Bone Destruction

[0234] There was no significant effect of antibody treatment on bone destruction score in this model, suggesting that the antibody treatment had no impact on the severity of the bone cancer itself.

Nociceptive Behavior

[0235] There was a statistically significant decrease in tactile allodynia with GFRα3 antibody treatment after carcinoma ($F(2, 25)=8.626$, $p=.001$, Figure 5).

[0236] There were no statistically significant overall effects of GFRα3 antibodies on dynamic weight bearing on the ipsilateral limb, although the overall effect of treatment achieved a statistical trend and M1M6977N achieved significant efficacy on post hoc comparison at 11 days (A), but not 18 days (B), after implantation of bone with carcinoma cells (11 day $F(2,25)=2.939$, $p=.071$, Figure 6A; 18 day $F(2,25)=0.149$, $p=.862$, Figure 6B).

[0237] GFRα3 antibodies significantly reduced limb guarding after bone cancer implantation in this model ($F(2,25)=4.222$, $p=.026$, Figure 7).

Conclusion

[0238] Treatment with anti-mouse GFRα3 antibodies significantly reduced nociceptive behaviors in this bone cancer pain model as measured by evaluation of guarding and the von Frey Test of tactile allodynia. In addition, there was evidence of efficacy of the REGN1967 antibody in weight bearing differential at one time point. Bone destruction scores were not different in groups receiving anti-mouse GFRα3 antibodies, suggesting that differences in pain-related measures could not be accounted for by differences in cancer severity. Therefore, our data suggest that neutralizing antibodies against GFRα3 could be efficacious against bone cancer pain in this model of metastatic bone cancer pain.

Example 15. Destabilization of the Medial Meniscus (DMM) Model of Osteoarthritic Pain

Methodology

Subjects

[0239] Adult male mice on a C57Bl6 background strain were used for the DMM experiment starting at approximately 12 weeks of age. The experimenters measuring outcome data for this experiment were blind to treatment group of the animals throughout data collection, compilation, and analysis.

DMM Model

[0240] In the DMM model, the medial meniscus of one knee is destabilized and the animal is allowed to develop disease for 16 weeks. During the 16 week period, animals develop tactile allodynia and increases in bone volume and bone mineral content in the injured knee resembling early human osteoarthritis. Tactile allodynia was verified in animals by von Frey Test at 16 weeks before the initiation of antibody treatment.

Antibody Treatment

[0241] Each animal received 30mg/kg s.c. antibody injections administered weekly starting 16 weeks after DMM surgery. Animals were pseudo-randomly assigned to one of three treatment groups: 1) M2M180N isotype (negative) control antibody, 2) M1M6977N anti-mouse GFRα3 antibody, or 3) M1M6986N anti-mouse GFRα3 antibody. M1M6986N blocks artemin's interaction with GFRα3, and is thereby considered a "direct" blocker of artemin's action. In contrast, M1M6977N inhibits artemin's action through the GFRα3/RET complex, and is therefore considered an "indirect" inhibitor.

Measures of Nociception

[0242] Nociceptive responses to the knee pathology were measured using the von Frey Hair test for evoked mechanical (tactile) allodynia.

Results

Nociceptive Behavior

[0243] There was a statistically significant decrease in tactile allodynia with GFRα3 antibody treatment after DMM ($F(2, 27)=21.68$, $p=.0001$, Figure 8).

Conclusion

[0244] Treatment with mouse GFRα3 antibodies had a statistically significant effect on tactile allodynia such that the groups treated with the two GFRα3 antibodies consistently showed less allodynia than the isotype control starting 14 days after the initiation of weekly treatment. These data suggest the possibility that GFRα3 antibodies will be efficacious against chronic human osteoarthritic pain.

Example 16. Cross-Competition Analysis of anti-GFRα3 Antibodies

[0245] A cross-competition assay was conducted to assess the ability of select antibodies to compete with one another for binding to human GFRα3 using an Octet RED384 biosensor (Fortebio Inc.). The entire experiment was performed at 25°C with the flow rate of 1000rpm in Octet HBST buffer (0.01 M HEPES pH7.4, 0.15M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20, 0.1mg/mL BSA). To assess whether 2 antibodies were able to compete with one another for binding to their respective epitopes on biotinylated recombinant human GFRα3 expressed with a C-terminal myc-myc-hexahistidine tag (biotin-hGFRα3-mmH; SEQ ID:370), around ~1.2nm of biotin-hGFRα3-mmH was first captured onto streptavidin-coated Octet sensor tips (Fortebio Inc, # 18-5019) by submerging the tips for 1 minute into a 10µg/mL solution of biotin-hGFRα3-mmH. The antigen coated sensor tips were then placed into wells containing 25µg/mL solution of a first anti-GFRα3 monoclonal antibody for 4 minutes to saturate the biotin-hGFRα3-mmH surface. The sensor tips were then subsequently dipped into wells containing 25µg/mL solution of a second anti-GFRα3 monoclonal antibody. The sensor tips were washed in Octet HBST buffer in between every step of the experiment. The real-time binding response was monitored during the course of the experiment and the binding response at the end of every step was recorded as shown in Figure 9. The response of mAb-2 binding to biotin-hGFRα3-mmH pre-complexed with the first antibody was compared and competitive/non-competitive behavior of different anti-GFRα3 monoclonal antibodies was determined.

[0246] As shown in Figure 9, dark grey boxes with black font represent binding response for self-competition. Antibodies competing in both directions, independent of the order of binding are represented with black boxes and white font. No competition between antibodies that suggest distinct binding epitope is represented as white box with black font.

[0247] Nine antibodies (H4H2236N3, H4H2342P, H4H2295S, H4H2294S, H4H2291S, H4H2357S, H4H2355S, H4H2296S, and H4H2243N2) bi-directionally compete with each other for binding to biotin-hGFRα3-mmH. Eight of the 9 (H4H2236N3, H4H2342P, H4H2295S, H4H2294S, H4H2291S, H4H2357S, H4H2355S, and H4H2296S) do not compete with any other anti-GFRα3 antibody tested, while H4H2243N2 also bi-directionally competes with two additional anti-GFRα3 antibodies tested (H4H2212N and H4H2352S). H4H2212N and H4H2352S bi-directionally compete with each other and H4H2243N2 for binding to biotin-hGFRα3-mmH, but while H4H2212N does not compete with any other anti-GFRα3 antibodies tested, H4H2352S also bi-directionally competes with an additional anti-GFRα3 antibody tested (H4H2292S). One anti-GFRα3 antibody tested, H4H2350P, does not compete with any of the anti-GFRα3 antibodies tested for binding to biotin-hGFRα3-mmH.

What is claimed is:

1. An isolated monoclonal antibody or an antigen-binding fragment thereof that specifically binds to glial cell-line derived neurotrophic factor (GDNF) family receptor alpha 3 (GFRα3), wherein the antibody comprises:
   (a) a heavy chain complementarity determining region (HCDR) 1 domain of SEQ ID NO: 148;
   (b) a HCDR2 domain of SEQ ID NO: 150;
   (c) a HCDR3 domain of SEQ ID NO: 152;
   (d) a light chain complementarity determining region (LCDR) 1 domain of SEQ ID NO: 156;
   (e) a LCDR2 domain of SEQ ID NO: 158; and
   (f) a LCDR3 domain of SEQ ID NO: 160.

2. The isolated monoclonal antibody or an antigen-binding fragment thereof of claim 1, wherein the antibody does not cross-react with human GFRα1 or human GFRα2.

3. The isolated monoclonal antibody or an antigen-binding fragment thereof of claim 1, wherein the antibody comprises (a) a heavy chain variable region (HCVR) of SEQ ID NO: 146 and (b) a light chain variable region (LCVR) of SEQ ID NO: 154.

4. The isolated antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment comprises a HCVR of SEQ ID NO: 146.

5. The isolated antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment comprises a LCVR of SEQ ID NO: 154.

6. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of claim 1 and a pharmaceutically acceptable carrier or diluent.