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(54) Titre: ANTICORPS CONTRE NOTUM PECTINACETYLESTERASE (54) Title: ANTIBODIES THAT BIND NOTUM PECTINACETYLESTERASE

(57) Abrégé/Abstract:

Antibodies that neutralize Notum Pectinacetylesterase are described, as well as compositions comprising them, and methods of their use to treat diseases and disorders affecting the bone.



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ANTIBODIES THAT BIND NOTUM PECTINACETYLESTERASE

[001]

1. FIELD OF THE INVENTION

[002] This invention relates to antibody inhibitors of Notum Pectinacetylesterase, compositions comprising them, and methods of their use.

2. BACKGROUND OF THE INVENTION

[003] Bone health depends on the coordinated activities of bone forming osteoblasts and bone resorbing osteoclasts. "Bone turnover reflects a balance between these anabolic and catabolic cellular functions and ensures that the mature skeleton can repair itself when damaged and sustain its endocrine function by release of minerals such as calcium and phosphorous into the circulation." Allen, J.G. et al., J. Med. Chem., 53 (June 10, 2010), pp. 4332 – 4353, 4332. Many disease states alter this balance, resulting in increased or decreased bone mass or changes in bone quality. Gradual loss of bone mineral density is known as osteopenia; severe loss of bone is known as osteoporosis. *Id*.

[004] The current standard of care for the treatment and prevention of osteoporosis utilizes the bisphosphonate class of oral, small molecule antiresportives. *Id.* at 4333. Zoledronic acid, raloxifene, calcium, and vitamin D supplements are also typically used in the osteoporosis treatment. *Id.* While antiresorptive agents can help prevent bone loss, anabolic agents "are capable of increasing bone mass to a greater degree ... and also have the capacity to improve bone quality and increase bone strength." Guo, H., et al., J. Med. Chem., 53 (February 25, 2010), pp. 1819 – 1829, 1819. In the United States, human PTH is the only FDA-approved anabolic agent. *Id.*; Allen at 4333. "Because of the paucity of available anabolic agents for osteoporosis treatment, there is an urgent need to develop small molecular compounds to treat this disease that are nontoxic, cost-effective, and easy to administer." Guo, at 1819.

[005] "Although the development of pharmacological agents that stimulate bone formation is less advanced compared to antiresorptive therapies, several pathways are known to facilitate osteoblast function." Allen at 4338. These pathways include bone morphogenic proteins, transforming growth factor β , parathyroid hormone, insulin-like growth factor, fibroblast growth factor, and wingless-type MMTV integration site (WNT) signaling. *Id.* Guo and coworkers recently reported results concerning the first of these pathways. Guo, *supra*. In particular, they reported that certain substituted benzothiophene and benzofuran compounds enhance bone morphogenic protein 2 expression in mice and rats. Two of the compounds reportedly stimulate bone formation and trabecular connectivity restoration *in vivo*. *Id.* at 1819.

[006] Another of these pathways is the WNT pathway, which is implicated in a variety of developmental and regenerative processes. Allen at 4340. The pathway is complex, however, and much about it and about how its components affect bone remains unclear. For example, it has been suggested that LRP-5, mutations of which are associated with increased bone mass in humans, and β -catenin, through which canonical WNT signaling occurs, "may not be linked directly via WNT signaling to the control of bone mass." *Id.*

[007] Recent analysis of gene expression data has led to the identification of new targets of WNT signaling. *See, e.g.,* Torisu, Y., et al., <u>Cancer Sci.</u>, 99(6):1139-1146, 1143 (2008). One such target is Notum Pectinacetylesterase, also known as NOTUM and LOC174111.

3. SUMMARY OF THE INVENTION

[008] In some embodiments, a monoclonal antibody that binds human notum pectinacetylesterase (NOTUM) and neutralizes at least one activity of NOTUM is provided. In some embodiments, the antibody binds to a NOTUM selected from mouse NOTUM, guinea pig NOTUM, cynomolgus monkey NOTUM, and rhesus monkey NOTUM. In some embodiments, the antibody has at least one activity selected from reducing NOTUM activity in a trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) assay *in vitro*, and reducing NOTUM activity in a Wnt signaling assay *in vitro*. In some embodiments, the antibody has at least one activity selected from increasing serum PINP levels *in vivo*, increasing bone mineral density *in vivo*, increasing midshaft femur cortical thickness *in vivo*, increasing midshaft femur bone area *in vivo*, increasing midshaft humerus cortical thickness *in vivo*, increasing endocortical bone formation *in vivo*, increasing the proportion of cortical bone volume in the LV5 vertebral body *in vivo*, and increasing the proportion of femoral neck bone volume to femoral neck total volume *in vivo*. In some embodiments, an antibody that binds NOTUM binds to a polypeptide having the amino acid sequence of SEQ ID NO: 1 with K_D of less than 50 nM, less than 20 nM, or less than 10 nM.

[009] In some embodiments, the antibody has at least one binding characteristic selected from: a) binds to a polypeptide having the amino acid sequence of SEQ ID NO: 83 with a binding affinity that is at least 5-fold stronger than the binding affinity of the antibody for a polypeptide having the amino acid sequence of SEQ ID NO: 84; b) binds to a polypeptide having the amino acid sequence of SEQ ID NO: 85 with a binding affinity that is at least 5-fold stronger than the binding affinity of the antibody for a polypeptide having the amino acid sequence of SEQ ID NO: 86; c) binds to a polypeptide having the amino acid sequence of SEQ ID NO: 1 with a binding affinity that is at least 5-fold stronger than the binding affinity of the antibody for a polypeptide having the amino acid sequence of SEQ ID NO: 94; d) binds to a polypeptide having the amino acid sequence of SEQ ID NO: 1 with a binding affinity that is at least 5-fold stronger than the binding affinity of the antibody for a polypeptide having the amino acid sequence of SEQ ID NO: 99; e) binds to a polypeptide

having the amino acid sequence of SEQ ID NO: 95 with a binding affinity that is at least 5-fold stronger than the binding affinity of the antibody for a polypeptide having the amino acid sequence of SEQ ID NO: 2; f) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO: 8; g) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 15 and a light chain variable region having the amino acid sequence of SEQ ID NO: 16; h) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 23 and a light chain variable region having the amino acid sequence of SEQ ID NO: 24; i) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 31 and a light chain variable region having the amino acid sequence of SEQ ID NO: 32; j) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 39 and a light chain variable region having the amino acid sequence of SEQ ID NO: 40; k) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 47 and a light chain variable region having the amino acid sequence of SEQ ID NO: 48; and I) competes for binding to NOTUM with an antibody comprising a heavy chain variable region having an amino acid sequence of SEQ ID NO: 55 and a light chain variable region having the amino acid sequence of SEQ ID NO: 56.

[010] In some embodiments, the antibody is selected from a mouse antibody, a chimeric antibody, a humanized antibody, and a human antibody.

[011] In some embodiments, an antibody that binds NOTUM comprises a heavy chain and a light chain, wherein the heavy chain comprises at least one CDR selected from: a) a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 9, 17, 25, 33, 41, 49, and 90; b) a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 10, 18, 26, 34, 42, and 50; and c) a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 11, 19, 27, 35, 43, 51, and 91. In some embodiments, the heavy chain comprises a set comprising a CDR1, a CDR2, and a CDR3, wherein the set is selected from: a) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 9, a CDR2 having the amino acid sequence of SEQ ID NO: 10, and a CDR3 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 19; d) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27; e) a set comprising a CDR1 having the amino acid sequence

of SEQ ID NO: 25, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27; f) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 35; h) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 41, a CDR2 having the amino acid sequence of SEQ ID NO: 42, and a CDR3 having the amino acid sequence of SEQ ID NO: 43; i) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 50, and a CDR3 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 59. In some embodiments, the heavy chain comprises a heavy chain variable regions comprising an amino acid sequence selected from SEQ ID NO: 7, 15, 23, 31, 39, 47, 63, 67, 71, 75, and 79.

[012] In some embodiments, an antibody that binds NOTUM comprises a heavy chain and a light chain, wherein the light chain comprises at least one CDR selected from: a) a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 12, 20, 28, 36, 44, 52, and 92; b) a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 13, 21, 29, 37, 45, 53, 61, and 93; and c) a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 14, 22, 30, 38, 46, 54, and 62. In some embodiments, the light chain comprises a set comprising a CDR1, a CDR2, and a CDR3, wherein the set is selected from: a) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 12, a CDR2 having the amino acid sequence of SEQ ID NO: 13, and a CDR3 having the amino acid sequence of SEQ ID NO: 14; b) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; c) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; d) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; e) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 28, a CDR2 having the amino acid sequence of SEQ ID NO: 29, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; f) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; g) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 36, a CDR2 having the amino acid sequence of SEQ ID NO: 37, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; h) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 44, a CDR2 having the amino acid sequence of SEQ ID NO: 45, and a CDR3

having the amino acid sequence of SEQ ID NO: 46; i) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 52, a CDR2 having the amino acid sequence of SEQ ID NO: 53, and a CDR3 having the amino acid sequence of SEQ ID NO: 54; and j) a set comprising a CDR1 having the amino acid sequence of SEQ ID NO: 60, a CDR2 having the amino acid sequence of SEQ ID NO: 61, and a CDR3 having the amino acid sequence of SEQ ID NO: 62. In some embodiments, the light chain comprises a light chain variable regions comprising an amino acid sequence selected from SEQ ID NO: 8, 16, 24, 32, 40, 48, 56, 65, 69, 73, 77, and 81.

[013] In some embodiments, an antibody that binds NOTUM comprises a heavy chain variable region and a light chain variable region, wherein: a) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 9, a CDR2 having the amino acid sequence of SEQ ID NO: 10, and a CDR3 having the amino acid sequence of SEQ ID NO: 11, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 12, a CDR2 having the amino acid sequence of SEQ ID NO: 13, and a CDR3 having the amino acid sequence of SEQ ID NO: 14; or b) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 91, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; or c) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 19, and the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; or d) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; or e) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 25, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 28, a CDR2 having the amino acid sequence of SEQ ID NO: 29, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; or f) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 91, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ

ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; or g) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 35, and the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 36, a CDR2 having the amino acid sequence of SEQ ID NO: 37, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; or h) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 41, a CDR2 having the amino acid sequence of SEQ ID NO: 42, and a CDR3 having the amino acid sequence of SEQ ID NO: 43, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 44, a CDR2 having the amino acid sequence of SEQ ID NO: 45, and a CDR3 having the amino acid sequence of SEQ ID NO: 46; or i) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 49, a CDR2 having the amino acid sequence of SEQ ID NO: 50, and a CDR3 having the amino acid sequence of SEQ ID NO: 51, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 52, a CDR2 having the amino acid sequence of SEQ ID NO: 53, and a CDR3 having the amino acid sequence of SEQ ID NO: 54; or j) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 59, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 60, a CDR2 having the amino acid sequence of SEQ ID NO: 61, and a CDR3 having the amino acid sequence of SEQ ID NO: 62.

[014] In some embodiments, an antibody that binds NOTUM comprises a heavy chain variable region and a light chain variable region, wherein a) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 8; or b) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 15 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16; or c) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 71 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73; or d) the heavy chain comprises the amino acid sequence of SEQ ID NO: 72 and the light chain comprises the amino acid sequence of SEQ ID NO: 74; or e) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 23 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 24; or f) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 75 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 77; or g) the heavy chain comprises the amino acid sequence of SEQ ID NO: 76 and the light chain comprises the amino acid sequence of SEQ ID NO: 78; or h) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 31 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 32; or i) the heavy

chain variable region comprises the amino acid sequence of SEQ ID NO: 79 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 81; or j) the heavy chain comprises the amino acid sequence of SEQ ID NO: 80 and the light chain comprises the amino acid sequence of SEQ ID NO: 82; or k) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 39 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 40; or I) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 67 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 69; or m) the heavy chain comprises the amino acid sequence of SEQ ID NO: 68 and the light chain comprises the amino acid sequence of SEQ ID NO: 70; or n) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 47 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 48; or o) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 55 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 56; or p) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 63 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 65; or q) the heavy chain comprises the amino acid sequence of SEQ ID NO: 64 and the light chain comprises the amino acid sequence of SEQ ID NO: 66.

[015] In some embodiments, a nucleic acid molecule is provided that comprises a polynucleotide sequence that encodes a heavy chain or a light chain of an antibody that binds NOTUM and neutralizes at least one activity of NOTUM. In some embodiments, the nucleic acid molecule comprises a first polynucleotide sequence that encodes the heavy chain, and a second polynucleotide sequence that encodes the light chain. In some embodiments, the nucleic acid molecule is a vector. In some embodiments, a host cell comprising a nucleic acid molecule that comprises a polynucleotide sequence that encodes a heavy chain or a light chain of an antibody that binds NOTUM and neutralizes at least one activity of NOTUM is provided. In some embodiments, a host cell comprising a nucleic acid molecule that comprises a first polynucleotide sequence that encodes a heavy chain, and a second polynucleotide sequence that encodes a light chain, is provided. In some embodiments, a host cell comprises a first nucleic acid molecule comprising a polynucleotide sequence that encodes a heavy chain, and a second nucleic acid molecule comprising a polynucleotide sequence that encodes a light chain. In some embodiments, a method of producing an antibody that binds to NOTUM and neutralizes at least one activity of NOTUM is provided, comprising incubating a host cell under conditions sufficient to express the antibody.

[016] In some embodiments, a pharmaceutical composition comprising an antibody that binds NOTUM and neutralizes at least one activity of NOTUM is provided. In some embodiments, a method of stimulating endocortical bone formation in a patient, comprising administering an effective amount of the pharmaceutical composition is provided. In some embodiments, a method

of treating, managing, or preventing a disease or disorder characterized by bone loss in a patient, comprising administering an effective amount of the pharmaceutical composition is provided. In some embodiments, the disease or disorder is osteoporosis. In some embodiments, a single unit dosage form comprising the pharmaceutical composition is provided.

4. BRIEF DESCRIPTION OF THE FIGURES

- [017] Figure 1 provides a graphical representation of differences between the cortical thicknesses of various bone sites in NOTUM homozygous knockout mice ("HOM") and those in their wildtype littermates ("WT").
- [018] Figure 2 provides a graphical representation of an increase in cortical bone thicknesses observed in both NOTUM homozygous and heterozygous ("HET") knockout mice as compared to their wildtype littermates.
- [019] Figure 3 provides a graphical representation of results obtained from femur breaking strength and spine compression tests performed on the bones of male NOTUM homozygous and heterozygous knockout mice and their wildtype littermates.
- [020] Figure 4 provides a graphical representation of results obtained from femur breaking strength and spine compression tests performed on the bones of female NOTUM homozygous and heterozygous knockout mice and their wildtype littermates
- [021] Figure 5 provides a graphical representation of certain human/mouse chimeric proteins, and indicates a region that appears to be involved in binding of NOTUM neutralizing antibodies in Bin 1, as described in Example 6.7.
- [022] Figure 6 provides a graphical representation of midshaft femur cortical thickness measurements obtained in mice after eight weeks of administering MAb 2.1029 or MAb 2.78, as described in Example 6.9.1.
- [023] Figure 7 provides a graphical representation of midshaft femur cortical thickness measurements obtained in mice after four weeks of administering various dosages of MAb 2.1029, as described in Example 6.9.2.
- [024] Figure 8 provides a graphical representation of midshaft femur cortical thickness measurements obtained in mice after four weeks of administering various dosages of MAb 2.78b, as described in Example 6.9.3. Figure 8A shows 3 mg/kg, 10 mg/kg, and 30 mg/kg dosages of MAb 2.78b. Figure 8B shows 0.3 mg/kg, 1 mg/kg, and 3 mg/kg dosages of MAb 2.78b.
- [025] Figure 9 provides a graphical representation of midshaft femur cortical thickness measurements (A) and serum PINP levels (B) obtained in mice after 4 weeks of administering MAb 2.78b, with and without pretreatment with zoledronate, as described in Example 6.9.4.

[026] Figure 10 provides a graphical representation of midshaft femur cortical thickness measurements obtained in mice after 4 weeks of administering MAb 2.78a, as described in Example 6.9.5.

- [027] Figure 11 provides a graphical representation of midshaft femur cortical thickness measurements (A) and midshaft humerus cortical thickness measurements (B) obtained in mice after 12 weeks of administering MAb 2.78a, as described in Example 6.9.6.
- [028] Figure 12 provides a graphical representation of midshaft femur cortical thickness measurements (A), midshaft humerus cortical thickness measurements (B), and ninth rib cortical thickness (C) obtained in mice after 24 weeks of administering MAb 2.78a, as described in Example 6.9.6.
- [029] Figure 13 provides a graphical representation of midshaft femur cortical thickness (A) and midshaft femur mineralized bone area (B) in sham surgery and ovariectomized mice administered NOTUM neutralizing antibody 2.78b or control antibody, as described in Example 6.10.3.
- [030] Figure 14 provides a graphical representation of the proportion in the LV5 vertebral body of bone volume to total volume (A), the proportion in the LV5 vertebral body of cortical bone volume to total volume (B), and the proportion in the LV5 vertebral body of trabecular bone volume to total volume (C) in sham surgery and ovariectomized mice administered NOTUM neutralizing antibody 2.78b or control antibody, as described in Example 6.10.3.
- [031] Figure 15 provides a graphical representation of the proportion of femoral neck bone volume to total volume in sham surgery and ovariectomized mice administered NOTUM neutralizing antibody 2.78b or control antibody, as described in Example 6.10.3.
- [032] Figure 16 provides a graphical representation of the percentage of the endocortical surface of the midshaft femur cross-sections that were labeled with calcein, alizarin, and tetracycline in sham surgery and ovariectomized mice administered NOTUM neutralizing antibody 2.78b or control antibody, as described in Example 6.10.4.
- [033] Figure 17 provides a graphical representation of the mineral apositional rate (A) and the volume-referent bone formation rate (B) in sham surgery and ovariectomized mice administered NOTUM neutralizing antibody 2.78b or control antibody, as described in Example 6.10.4.

5. <u>DETAILED DESCRIPTION OF THE INVENTION</u>

[034] This invention is based, in part, on the discovery that inhibition of NOTUM can affect endocortical bone formation. Particular aspects of the invention are based on studies of mice lacking a functional NOTUM gene ("knockout mice"), on the development of antibodies that inhibit

NOTUM, and on the discovery that such antibodies can be used to stimulate cortical bone formation in mice and rats.

[035] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

In the event that one

or more of the incorporated literature and similar materials defines a term that contradicts that term's definition in this application, this application controls.

5.1. <u>Definitions</u>

- [036] The term "antibody," as used herein, refers to an intact antibody or a fragment of an antibody that competes with the intact antibody for antigen binding. Antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv, scFv, Fd, diabodies, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. *See, e.g.*, Hudson et al. (2003) Nat. Med. 9:129-134. In some embodiments, antibody fragments are produced by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antibody fragments are produced by recombinant DNA techniques.
- [037] The term "antigen-binding site" refers to a portion of an antibody capable of specifically binding an antigen. In some embodiments, an antigen-binding site is provided by one or more antibody variable regions.
- [038] The term "binding affinity" refers to a qualitative or quantitative determination of the strength with which an antibody binds to an antigen. In some embodiments, the binding affinity is the dissociation constant (K_D) of the antibody for the antigen. In some embodiments, the binding affinity of an antibody for an antigen is determined qualitatively, such as relative to the binding affinity of a different antibody for an antigen, or relative to the binding affinity of the same antibody for a different antigen (such as the antigen with one or more changes in its amino acid sequence). The binding affinity of an antibody for a first antigen is considered "stronger" than its affinity for a second antigen, for example, when the K_D of the antibody for the first antigen is lower than the K_D of the antibody for the second antigen. In some embodiments, the binding affinity of an antibody for a first antigen is considered "stronger" when the K_D of the antibody for the first antigen is at least 1.5-fold, at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold lower than the K_D of the antibody for the second antigen. Conversely, the binding affinity of an antibody for a first antigen is considered "weaker" than its affinity for a second antigen, for example, when the K_D of the antibody for the first antigen is higher than the K_D of the antibody for the second antigen. In some embodiments, the binding affinity of an antibody for a first antigen is considered

"weaker" when the K_D of the antibody for the first antigen is at least 1.5-fold, at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold higher than the K_D of the antibody for the second antigen.

- [039] A "chimeric" antibody refers to an antibody made up of components from at least two different sources. In some embodiments, a chimeric antibody comprises a portion of an antibody derived from a first species fused to another molecule, e.g., a portion of an antibody derived from a second species. In some such embodiments, a chimeric antibody comprises a portion of an antibody derived from a non-human animal fused to a portion of an antibody derived from a human. In some such embodiments, a chimeric antibody comprises all or a portion of a variable region of an antibody derived from a non-human animal fused to a constant region of an antibody derived from a human.
- [040] The term "epitope" refers to any polypeptide determinant capable of specifically binding to an immunoglobulin or a T-cell receptor. In some embodiments, an epitope is a region of an antigen that is specifically bound by an antibody. In some embodiments, an epitope may include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl groups. In some embodiments, an epitope may have specific three dimensional structural characteristics (e.g., a "conformational" epitope) and/or specific charge characteristics.
- [041] An epitope is defined as "the same" as another epitope if a particular antibody specifically binds to both epitopes. In some embodiments, polypeptides having different primary amino acid sequences may comprise epitopes that are the same. Different antibodies are said to bind to the same epitope if they compete for specific binding to that epitope.
- [042] A "fragment" of a reference polypeptide refers to a contiguous stretch of amino acids from any portion of the reference polypeptide. A fragment may be of any length that is less than the length of the reference polypeptide. In some embodiments, a fragment is a contiguous stretch of amino acids from any portion of the reference polypeptide that has a particular activity or contains a particular epitope.
- [043] The term "human antibody" refers to a monoclonal antibody that contains human antibody sequences and does not contain antibody sequences from a non-human animal. In some embodiments, a human antibody may contain synthetic sequences not found in native antibodies. The term is not limited by the manner in which the antibodies are made. For example, in various embodiments, a human antibody may be made in a transgenic mouse, by phage display, by human B-lymphocytes, or by recombinant methods.
- [044] A "humanized" antibody refers to a non-human antibody that has been modified so that it more closely matches (in amino acid sequence) a human antibody. A humanized antibody is thus a type of chimeric antibody. In some embodiments, amino acid residues outside of the antigen binding residues of the variable region of the non-human antibody are modified. In some embodiments, a humanized antibody is constructed by replacing all or a portion of one or more

complementarity determining region (CDRs) of a human antibody with all or a portion of one or more CDRs from another antibody, such as a non-human antibody, having the desired antigen binding specificity. In some embodiments, a humanized antibody comprises variable regions in which all or substantially all of the CDRs correspond to CDRs of a non-human antibody and all or substantially all of the framework regions (FRs) correspond to FRs of a human antibody. In some embodiments, one or more amino acids within one or more CDRs of the non-human antibody are changed in the humanized antibody, e.g., through a process of affinity maturation. Exemplary methods of affinity maturation are known in the art. In some such embodiments, a humanized antibody further comprises a constant region (Fc) of a human antibody.

- [045] Unless otherwise indicated, the term "include" has the same meaning as "include, but are not limited to," the term "includes" has the same meaning as "includes, but is not limited to," and the term "including" has the same meaning as "including, but not limited to." Similarly, the term "such as" has the same meaning as the term "such as, but not limited to."
- [046] Unless otherwise indicated, the terms "manage," "managing" and "management" encompass preventing the recurrence of the specified disease or disorder in a patient who has already suffered from the disease or disorder, and/or lengthening the time that a patient who has suffered from the disease or disorder remains in remission. The terms encompass modulating the threshold, development and/or duration of the disease or disorder, or changing the way that a patient responds to the disease or disorder.
- [047] The term "monoclonal antibody" refers to an antibody from a substantially homogeneous population of antibodies that specifically bind to the same epitope. In some embodiments, a monoclonal antibody is secreted by a hybridoma. In some such embodiments, a hybridoma is produced according to some methods known to those skilled in the art. *See, e.g.,* Kohler and Milstein (1975) Nature 256: 495-499. In some embodiments, a monoclonal antibody is produced using recombinant DNA methods (*see, e.g.,* U.S. Patent No. 4,816,567). In some embodiments, a monoclonal antibody refers to an antibody fragment isolated from a phage display library. *See, e.g.,* Clackson et al. (1991) Nature 352: 624-628, and Marks et al. (1991) J. Mol. Biol. 222: 581-597. For various other monoclonal antibody production techniques, *see, e.g.,* Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
- [048] The term "neutralizing antibody" or "antibody that neutralizes" refers to an antibody that reduces at least one activity of a polypeptide comprising the epitope to which the antibody specifically binds. In some embodiments, a neutralizing antibody reduces an activity of the polypeptide *in vitro* and/or *in vivo*.
- [049] The term "NOTUM" refers to notum pectinaceylesterase having an amino acid sequence from any vertebrate or mammalian source, including human, bovine, chicken, rodent,

mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise. The term also refers to fragments and variants of native NOTUM that maintain at least one *in vivo* or *in vitro* activity of a native NOTUM. The term encompasses full-length unprocessed precursor forms of NOTUM as well as mature forms resulting from post-translational cleavage of a signal peptide and other forms of proteolytic processing. In some embodiments, a full-length, unprocessed human NOTUM has the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, a full-length, unprocessed mouse NOTUM has the amino acid sequence set forth in SEQ ID NO: 2.

- [050] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers containing naturally occurring amino acids as well as amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid. The amino acid polymers can be of any length. The term "native polypeptide" refers to a naturally occurring polypeptide.
- [051] Unless otherwise indicated, the terms "prevent," "preventing" and "prevention" contemplate an action that occurs before a patient begins to suffer from the specified disease or disorder, which inhibits or reduces the severity of the disease or disorder. In other words, the terms encompass prophylaxis.
- [052] Unless otherwise indicated, a "prophylactically effective amount" of a compound is an amount sufficient to prevent a disease or condition, or one or more symptoms associated with the disease or condition, or prevent its recurrence. A "prophylactically effective amount" of a compound means an amount of therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the disease. The term "prophylactically effective amount" can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent.
- [053] An antibody "specifically binds" an antigen when it preferentially recognizes the antigen in a complex mixture of proteins and/or macromolecules. In some embodiments, an antibody comprises an antigen-binding site that specifically binds to a particular epitope. In some such embodiments, the antibody is capable of binding different antigens so long as the different antigens comprise that particular epitope. In some instances, for example, homologous proteins from different species may comprise the same epitope. In some embodiments, an antibody is said to specifically bind an antigen when the dissociation constant (K_D) is $\leq 1~\mu\text{M}$, in some embodiments, when the dissociation constant is $\leq 100~\text{nM}$, and in some embodiments, when the dissociation constant is $\leq 10~\text{nM}$.
- [054] The terms "subject" and "patient" include both humans and animals. In some embodiments, a subject or patient is a mammal. In some such embodiments, a subject or patient is a human.

[055] Unless otherwise indicated, a "therapeutically effective amount" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment or management of a disease or condition, or to delay or minimize one or more symptoms associated with the disease or condition. A "therapeutically effective amount" of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment or management of the disease or condition. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of a disease or condition, or enhances the therapeutic efficacy of another therapeutic agent.

[056] Unless otherwise indicated, the terms "treat," "treating" and "treatment" contemplate an action that occurs while a patient is suffering from the specified disease or disorder, which reduces the severity of the disease or disorder, or retards or slows the progression of the disease or disorder.

5.2. Antibodies

5.2.1. Exemplary Antibody Structure

[057] A native antibody typically has a tetrameric structure. A tetramer typically comprises two identical pairs of polypeptide chains, each pair having one light chain (In some embodiments, about 25 kDa) and one heavy chain (In some embodiments, about 50-70 kDa). In a native antibody, a heavy chain comprises a variable region, VH, and three constant regions, CH1, CH2, and CH3. The VH domain is at the amino-terminus of the heavy chain, and the CH3 domain is at the carboxy-terminus. In a native antibody, a light chain comprises a variable region, VL, and a constant region, CL. The variable region of the light chain is at the amino-terminus of the light chain. In a native antibody, the variable regions of each light/heavy chain pair typically form the antigen binding site. The constant regions are typically responsible for effector function.

[058] Native human light chains are typically classified as kappa and lambda light chains. Native human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has subclasses, including IgG1, IgG2, IgG3, and IgG4. IgM has subclasses including IgM1 and IgM2. IgA has subclasses including IgA1 and IgA2. Within native human light and heavy chains, the variable and constant regions are typically joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See, e.g., Fundamental Immunology (1989) Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y.).

[059] In a native antibody, the variable regions typically exhibit the same general structure in which relatively conserved framework regions (FRs) are joined by three hypervariable regions, also called complementarity determining regions (CDRs). The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific

epitope. From N-terminus to C-terminus, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The CDRs on the heavy chain are referred to as H1, H2, and H3, while the CDRs on the light chain are referred to as L1, L2, and L3. Typically, CDR3 is the greatest source of molecular diversity within the antigen binding site. H3, for example, in certain instances, can be as short as two amino acid residues or greater than 26. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat et al. (1991) Sequences of Proteins of Immunological Interest (National Institutes of Health, Publication No. 91-3242, vols. 1-3, Bethesda, MD); Chothia, C., and Lesk, A.M. (1987) J. Mol. Biol. 196:901-917; or Chothia, C. et al. Nature 342:878-883 (1989). In the present application, the term "CDR" refers to a CDR from either the light or heavy chain, unless otherwise specified.

- [060] A "Fab" fragment comprises one light chain and the CH1 and variable region of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab'" fragment comprises one light chain and one heavy chain that comprises additional constant region, extending between the CH1 and CH2 domains. An interchain disulfide bond can be formed between two heavy chains of a Fab' fragment to form a "F(ab')2" molecule.
- [061] An "Fv" fragment comprises the variable regions from both the heavy and light chains, but lacks the constant regions. A single-chain Fv (scFv) fragment comprises heavy and light chain variable regions connected by a flexible linker to form a single polypeptide chain with an antigen-binding region. Exemplary single chain antibodies are discussed in detail in WO 88/01649 and U.S. Patent Nos. 4,946,778 and 5,260,203. In certain instances, a single variable region (i.e., a heavy chain variable region or a light chain variable region) may have the ability to recognize and bind antigen.
- [062] As used herein, the term "heavy chain" refers to a polypeptide comprising sufficient heavy chain variable region sequence to confer antigen specificity either alone or in combination with a light chain.
- [063] As used herein, the term "light chain" refers to a polypeptide comprising sufficient light chain variable region sequence to confer antigen specificity either alone or in combination with a heavy chain.

5.2.2. Exemplary Antibodies

- [064] In some embodiments, monoclonal antibodies that specifically bind to NOTUM are provided. In some such embodiments, the monoclonal antibodies are neutralizing antibodies that reduce at least one activity of NOTUM *in vivo* and/or *in vitro*.
- [065] In some embodiments, a neutralizing antibody against NOTUM reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) assay *in vitro*. In some

embodiments, a neutralizing antibody against NOTUM reduces NOTUM activity in a Wnt signaling assay *in vitro*.

PINP levels *in vivo* when administered to a subject in a sufficient amount and for a sufficient duration. Exemplary dosages and dosing schedules for administering a sufficient amount for a sufficient duration are discussed herein. In some embodiments, a neutralizing antibody against NOTUM increases bone mineral density. In some embodiments, a neutralizing antibody against NOTUM increases midshaft femur cortical thickness *in vivo*. In some embodiments, a neutralizing antibody against NOTUM increases midshaft femur bone area *in vivo*. In some embodiments, a neutralizing antibody against NOTUM increases midshaft humerus cortical thickness *in vivo*. In some embodiments, a neutralizing antibody against NOTUM increases endocortical bone formation *in vivo*. In some embodiments, a neutralizing antibody against NOTUM increases the proportion of cortical bone volume in the LV5 vertebral body *in vivo*. By "proportion of cortical bone volume in the LV5 vertebral body" is meant the proportion of cortical bone volume to total volume of the LV5 vertebral body. In some embodiments, a neutralizing antibody against NOTUM increases the proportion of femoral neck bone volume to total volume of the femoral neck *in vivo*.

NOTUM are provided. In some embodiments, neutralizing antibodies that specifically bind to human NOTUM are provided. In some embodiments, neutralizing antibodies that bind to a region from Q47 to M177 of human NOTUM are provided. In some embodiments, neutralizing antibodies that depend upon a region from Q47 to M177 of human NOTUM for binding are provided. In some embodiments, neutralizing antibodies that specifically bind to the same region of NOTUM from different species (i.e., antibodies that demonstrate cross-reactivity) are provided. In some embodiments, neutralizing antibodies that bind to human NOTUM and NOTUM from at least one species selected from mouse, rat, guinea pig, cynomolgus monkey, marmoset, and rhesus macaque, are provided. In some such embodiments, the antibodies specifically bind to both non-human primate NOTUM and human NOTUM. In some embodiments, the antibodies specifically bind to both mouse NOTUM and human NOTUM.

[068] In some embodiments, neutralizing antibodies that bind to a region of human NOTUM from Q47 to M177 are provided. In some embodiments, neutralizing antibodies that depend upon a region of human NOTUM from Q47 to M177 for binding are provided. In some embodiments, NOTUM neutralizing antibodies are provided that bind to human-mouse chimeric NOTUM (SEQ ID NO: 83) with an affinity that is at least 5-fold, at least 10-fold, or at least 20-fold stronger than the affinity for mouse-human chimeric NOTUM (SEQ ID NO: 84). In some embodiments, NOTUM neutralizing antibodies are provided that bind to human-mouse-human chimeric NOTUM (SEQ ID NO: 85) with an affinity that is at least 5-fold, at least 10-fold, or at least

20-fold stronger than the affinity for mouse-human-mouse chimeric NOTUM (SEQ ID NO: 86). In some embodiments, NOTUM neutralizing antibodies are provided that bind to human NOTUM (SEQ ID NO: 1) with an affinity that is at least 5-fold, at least 10-fold, or at least 20-fold stronger than the affinity for NOTUM D141S (SEQ ID NO: 94). In some embodiments, NOTUM neutralizing antibodies are provided that bind to mouse NOTUM S148D (SEQ ID NO: 95) with an affinity that is at least 5-fold, at least 10-fold, or at least 20-fold stronger than the affinity for mouse NOTUM (SEQ ID NO: 2). In some embodiments, NOTUM neutralizing antibodies are provided that bind to human NOTUM (SEQ ID NO: 1) with an affinity that is at least 5-fold, at least 10-fold, or at least 20-fold stronger than the affinity for human NOTUM R144A/R145A (SEQ ID NO: 99).

[069] In some embodiments, a neutralizing antibody against NOTUM binds to human NOTUM (SEQ ID NO: 1) with an affinity (K_D) of less than 100 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 10 nM, less than 5 nM, less than 3 nM, or less than 2 nM, determined as described in Example 6.8. In some embodiments, a neutralizing antibody against NOTUM has an IC₅₀ in an OPTS assay of less than 100 nM, less than 75 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, or less than 10 nM, determined as described in Example 6.4.1. In some embodiments, a neutralizing antibody against NOTUM has an IC₅₀ in a Wnt signaling assay of less than 100 nM, less than 75 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, or less than 10 nM, determined as described in Example 6.4.2. In some embodiments, the IC₅₀ is for human NOTUM. In some embodiments, the IC₅₀ is for mouse NOTUM.

[070] In some embodiments, neutralizing antibodies are non-human monoclonal antibodies. In some such embodiments, neutralizing antibodies are rodent monoclonal antibodies. In some such embodiments, neutralizing antibodies are mouse monoclonal antibodies. In some embodiments, neutralizing antibodies are chimeric monoclonal antibodies. In some embodiments, neutralizing antibodies are humanized monoclonal antibodies. In some embodiments, neutralizing antibodies are human monoclonal antibodies. In some embodiments, chimeric, humanized, and/or human monoclonal antibodies are useful as therapeutic antibodies in humans.

[071] In some embodiments, neutralizing antibodies are antibody fragments. Exemplary antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv, scFv, Fd, diabodies, and the like.

[072] Nonlimiting exemplary NOTUM neutralizing antibodies include MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. Each of MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78 neutralizes at least one activity of NOTUM. Further, at least MAbs 1.802, 1.815, 1.846, and 2.78 are dependent for binding to NOTUM on at least a portion of the region of human NOTUM bounded by amino acids Q47 to M177. In some embodiments, a NOTUM neutralizing antibody

competes for binding to NOTUM with at least one antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. In some embodiments, a NOTUM neutralizing antibody binds to an epitope of NOTUM that at least partially overlaps with the epitope bound by at least one antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. In addition, in some embodiments, an antibody that competes for binding to NOTUM with at least one antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78 is predicted to be a NOTUM neutralizing antibody. The sequences of the CDRs and variable regions of MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78 are shown in Section 7, below.

[073] In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 1.731 binds. In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 1.802 binds. In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 1.815 binds. In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 1.846 binds. In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 2.1029 binds. In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 2.55 binds. In some embodiments, NOTUM neutralizing antibodies are provided that bind to the same epitope to which MAb 2.78 binds.

[074] In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region selected from SEQ ID NOs: 7, 15, 23, 31, 39, and 47. In some embodiments, a NOTUM neutralizing antibody comprises a light chain variable region selected from SEQ ID NOs: 8, 16, 24, 32, 40, and 48. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 7, and a light chain variable region having the amino acid sequence of SEQ ID NO: 8. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 15, and a light chain variable region having the amino acid sequence of SEQ ID NO: 16. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 23, and a light chain variable region having the amino acid sequence of SEQ ID NO: 24. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 31, and a light chain variable region having the amino acid sequence of SEQ ID NO: 32. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 39, and a light chain variable region having the amino acid sequence of SEQ ID NO: 40. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 47, and a light chain variable region having the amino acid sequence of SEQ ID NO: 48.

[075] In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain CDR1 selected from SEQ ID NOs: 9, 17, 25, 33, 41, 49, and 90. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain CDR2 selected from SEQ ID NOs: 10, 18, 26, 34, 42. and 50. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain CDR3 selected from SEQ ID NOs: 11, 19, 27, 35, 43, 51, and 91. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 9, a CDR2 having the amino acid sequence of SEQ ID NO: 10, and a CDR3 having the amino acid sequence of SEQ ID NO: 11. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 17 and 90, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having an amino acid sequence selected from SEQ ID NOs: 19 and 91. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 19. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 25 and 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 25, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 33 and 90, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having an amino acid sequence selected from SEQ ID NOs: 35 and 91. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 35. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 41, a CDR2 having the amino acid sequence of SEQ ID NO: 42, and a CDR3 having the amino acid sequence of SEQ ID NO: 43. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 49, a CDR2 having the amino acid sequence of SEQ ID NO: 50, and a CDR3 having the amino acid sequence of SEQ ID NO: 51. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 59. In some embodiments, X_1 in SEQ ID NO: 90 is selected from Y and F. In some embodiments, X2 in SEQ ID NO: 91 is selected from H and N.

[076] In some embodiments, a NOTUM neutralizing antibody comprises a light chain CDR1 selected from SEQ ID NOs: 12, 20, 28, 36, 44, 52, 60, and 92. In some embodiments, a NOTUM neutralizing antibody comprises a light chain CDR2 selected from SEQ ID NOs: 13, 21, 29, 37, 45, 53, 61, and 93. In some embodiments, a NOTUM neutralizing antibody comprises a light chain CDR3 selected from SEQ ID NOs: 14, 22, 30, 38, 46, 54, and 62. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 12, a CDR2 having the amino acid sequence of SEQ ID NO: 13, and a CDR3 having the amino acid sequence of SEQ ID NO: 14. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 20 and 92, a CDR2 having an amino acid sequence selected from SEQ ID NOs: 21 and 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 22. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 28 and 92, a CDR2 having an amino acid sequence selected from SEQ ID NOs: 29 and 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 30. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 28, a CDR2 having the amino acid sequence of SEQ ID NO: 29, and a CDR3 having the amino acid sequence of SEQ ID NO: 30. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 36 and 92, a CDR2 having an amino acid sequence selected from SEQ ID NOs: 37 and 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 38. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 36, a CDR2 having the amino acid sequence of SEQ ID NO: 37, and a CDR3 having the amino acid sequence of SEQ ID NO: 38. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 44, a CDR2 having the amino acid sequence of SEQ ID NO: 45, and a CDR3 having the amino acid sequence of SEQ ID NO: 46. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 52, a CDR2 having the amino acid sequence of SEQ ID NO: 53, and a CDR3 having the amino acid sequence of SEQ ID NO: 54. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 60, a CDR2 having the amino acid sequence of SEQ ID NO: 61, and a CDR3 having the amino acid sequence of SEQ ID NO: 62. In some embodiments, X3 in SEQ ID NO: 92 is selected from I and S; X4 in SEQ ID NO: 92 is selected from T

and E; and X_5 in SEQ ID NO: 92 is selected from M and I. In some embodiments, X_6 in SEQ ID NO: 93 is selected from D and N.

[077] In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 9, a CDR2 having the amino acid sequence of SEQ ID NO: 10, and a CDR3 having the amino acid sequence of SEQ ID NO: 11; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 12, a CDR2 having the amino acid sequence of SEQ ID NO: 13, and a CDR3 having the amino acid sequence of SEQ ID NO: 14. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 17 and 90, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having an amino acid sequence selected from SEQ ID NOs: 19 and 91; and a light chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 20 and 92, a CDR2 having an amino acid sequence selected from SEQ ID NOs: 21 and 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 22. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 19; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 25 and 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27; and a light chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 28 and 92, a CDR2 having an amino acid sequence selected from SEQ ID NOs: 29 and 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 30. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 25, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 28, a CDR2 having the amino acid sequence of SEQ ID NO: 29, and a CDR3 having the amino acid sequence of SEQ ID NO: 30. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 33 and 90, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having an amino acid sequence selected from SEQ ID NOs: 35 and 91; and a light chain comprising a CDR1 having an amino acid sequence selected from SEQ ID NOs: 36 and 92, a CDR2 having an amino acid sequence selected from SEQ ID NOs: 37 and 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 38. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3

having the amino acid sequence of SEQ ID NO: 35; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 36, a CDR2 having the amino acid sequence of SEQ ID NO: 37, and a CDR3 having the amino acid sequence of SEQ ID NO: 38. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 41, a CDR2 having the amino acid sequence of SEQ ID NO: 42, and a CDR3 having the amino acid sequence of SEQ ID NO: 43; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 44, a CDR2 having the amino acid sequence of SEQ ID NO: 45, and a CDR3 having the amino acid sequence of SEQ ID NO: 46. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 49, a CDR2 having the amino acid sequence of SEQ ID NO: 50, and a CDR3 having the amino acid sequence of SEQ ID NO: 51; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 52, a CDR2 having the amino acid sequence of SEQ ID NO: 53, and a CDR3 having the amino acid sequence of SEQ ID NO: 54. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 59; and a light chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 60, a CDR2 having the amino acid sequence of SEQ ID NO: 61, and a CDR3 having the amino acid sequence of SEQ ID NO: 62. In some embodiments, X1 in SEQ ID NO: 90 is selected from Y and F. In some embodiments, X₂ in SEQ ID NO: 91 is selected from H and N. In some embodiments, X₃ in SEQ ID NO: 92 is selected from I and S; X₄ in SEQ ID NO: 92 is selected from T and E; and X₅ in SEQ ID NO: 92 is selected from M and I. In some embodiments, X₆ in SEQ ID NO: 93 is selected from D and N.

[078] In some embodiments, NOTUM neutralizing antibodies that specifically bind human NOTUM are provided. In some embodiments, NOTUM neutralizing antibodies that specifically bind to the same epitope in NOTUM from different species (i.e., antibodies that demonstrate cross-reactivity) are provided. In some embodiments, NOTUM neutralizing antibodies that specifically bind human NOTUM and also specifically bind at least one species of NOTUM selected from mouse, rat, guinea pig, cynomolgus monkey, marmoset, and rhesus macaque are provided. In some embodiments, NOTUM neutralizing antibodies that specifically bind human NOTUM and NOTUM from at least one species of non-human primate are provided. In some embodiments, NOTUM neutralizing antibodies that specifically bind human NOTUM and mouse NOTUM are provided.

5.2.2.1. Chimerized and humanized monoclonal antibodies

[079] In some embodiments, non-human antibodies are chimerized. In some embodiments, mouse monoclonal antibodies that specifically bind human NOTUM are chimerized. Certain exemplary methods for making chimeric antibodies are provided, for example, in Morrison

et al. (1984) <u>Proc. Nat'l Acad. Sci. USA</u> 81:6851-6855; Neuberger et al. (1984) <u>Nature</u> 312:604-608; Takeda et al. (1985) <u>Nature</u> 314:452-454; and U.S. Patent Nos. 6,075,181 and 5,877,397.

[080] In some embodiments, non-human antibodies are "humanized." In some embodiments, mouse monoclonal antibodies that specifically bind human NOTUM are humanized. In some embodiments, mouse monoclonal antibodies raised against mouse NOTUM, but which specifically bind (i.e., cross react) with human NOTUM, are humanized. In some embodiments, humanized antibodies retain their binding specificity and have reduced immunogenicity (e.g., reduced human anti-mouse antibody (HAMA) response) when administered to a human. In some embodiments, humanization is achieved by methods including CDR grafting and human engineering, as described in detail below.

[081] In some embodiments of humanized antibodies, one or more complementarity determining regions (CDRs) from the light and heavy chain variable regions of an antibody with the desired binding specificity (the "donor" antibody) are grafted onto human framework regions (FRs) in an "acceptor" antibody. Exemplary CDR grafting is described, e.g., in U.S. Patent Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101; Queen et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033. In some embodiments, one or more CDRs from the light and heavy chain variable regions are grafted onto consensus human FRs in an acceptor antibody. To create consensus human FRs, in some embodiments, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence.

[082] In some embodiments, certain FR amino acids in the acceptor antibody are replaced with FR amino acids from the donor antibody. In certain such embodiments, FR amino acids from the donor antibody are amino acids that contribute to the affinity of the donor antibody for the target antigen. *See, e.g.*, in U.S. Patent Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101; Queen et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033. In some embodiments, computer programs are used for modeling donor and/or acceptor antibodies to identify residues that are likely to be involved in binding antigen and/or to contribute to the structure of the antigen binding site, thus assisting in the selection of residues, such as FR residues, to be replaced in the donor antibody.

[083] In some embodiments, CDRs from a donor antibody are grafted onto an acceptor antibody comprising a human constant region. In some such embodiments, FRs are also grafted onto the acceptor. In some embodiments, CDRs from a donor antibody are derived from a single chain Fv antibody. In some embodiments, FRs from a donor antibody are derived from a single chain Fv antibody. In some embodiments, grafted CDRs in a humanized antibody are further modified (e.g., by amino acid substitutions, deletions, or insertions) to increase the affinity of the humanized antibody for the target antigen. In some embodiments, grafted FRs in a humanized

antibody are further modified (e.g., by amino acid substitutions, deletions, or insertions) to increase the affinity of the humanized antibody for the target antigen.

[084] In some embodiments, non-human antibodies may be humanized using a "human engineering" method. *See, e.g.*, U.S. Patent Nos. 5,766,886 and 5,869,619. In some embodiments of human engineering, information on the structure of antibody variable domains (e.g., information obtained from crystal structures and/or molecular modeling) is used to assess the likelihood that a given amino acid residue in a variable region is (a) involved in antigen binding, (b) exposed on the antibody surface (i.e., accessible to solvent), or (c) buried within the antibody variable region (i.e., involved in maintaining the structure of the variable region). Furthermore, in some embodiments, human variable region consensus sequences are generated to identify residues that are conserved among human variable regions. In some embodiments, that information provides guidance as to whether an amino acid residue in the variable region of a non-human antibody should be substituted.

[085] In some embodiments, a humanized NOTUM neutralizing antibody comprises a heavy chain comprising at least one of CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising at least one of CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78. In some embodiments, a NOTUM neutralizing antibody comprises heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 from an antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, and 2.78.

[086] In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 63, 67, 71, 75, and 79. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 64, 68, 72, 76, and 80. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising an amino acid sequence selected from SEQ ID NOs: 65, 69, 73, 77, and 81. In some embodiments, a NOTUM neutralizing antibody comprises a light chain comprising an amino acid sequence selected from SEQ ID NOs: 66, 70, 74, 78, and 82. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 63 and a light chain comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 67 and a light chain comprising the amino acid sequence of SEQ ID NO: 67 and a light chain comprising the amino acid sequence of SEQ ID NO: 67 and a light chain comprising the amino acid sequence of SEQ ID NO: 68 and a light chain comprising the amino acid sequence of SEQ ID NO: 68 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 69 and 60 a

ID NO: 69. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 71 and a light chain comprising the amino acid sequence of SEQ ID NO: 73. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 75 and a light chain comprising the amino acid sequence of SEQ ID NO: 77. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 79 and a light chain comprising the amino acid sequence of SEQ ID NO: 81. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 64 and a light chain comprising the amino acid sequence of SEQ ID NO: 66. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 68 and a light chain comprising the amino acid sequence of SEQ ID NO: 70. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 72 and a light chain comprising the amino acid sequence of SEQ ID NO: 74. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 76 and a light chain comprising the amino acid sequence of SEQ ID NO: 78. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 80 and a light chain comprising the amino acid sequence of SEQ ID NO: 82.

5.2.2.2. Antibody isotypes

[087] In some embodiments, an antibody against NOTUM is of any isotype selected from IgM, IgD, IgG, IgA, and IgE. In some embodiments, an antibody against NOTUM is of the IgG isotype. In certain such embodiments, an antibody is of the subclass IgG1, IgG2, IgG3, or IgG4. In some embodiments, an antibody against NOTUM is of the IgM isotype. In certain such embodiments, an antibody is of the subclass IgM1 or IgM2. In some embodiments, an antibody against NOTUM is of the IgA isotype. In certain such embodiments, an antibody is of the subclass IgA1 or IgA2. An antibody against NOTUM may comprise a lambda or kappa light chain constant region of, e.g., either human or mouse origin. In some embodiments, an antibody against NOTUM comprises a human kappa light chain constant region and a human IgG1, IgG2, or IgG4 heavy chain constant region. In some embodiments, an antibody against NOTUM comprises a mouse kappa light chain and a mouse IgG1 or IgG2 heavy chain.

5.2.2.3. Modified antibodies

[088] In some embodiments, an antibody is modified to alter one or more of its properties. In some embodiments, a modified antibody may possess advantages over an unmodified antibody, such as increased stability, increased time in circulation, or decreased immunogenicity (see, e.g., U.S. Patent No. 4,179,337). In some embodiments, an antibody is

modified by linking it to a nonproteinaceous moiety. In some embodiments, an antibody is modified by altering the glycosylation state of the antibody, e.g., by altering the number, type, linkage, and/or position of carbohydrate chains on the antibody. In some embodiments, an antibody is altered so that it is not glycosylated.

[089] In some embodiments, one or more chemical moieties are linked to the amino acid backbone and/or carbohydrate residues of the antibody. Certain exemplary methods for linking a chemical moiety to an antibody are known to those skilled in the art. Such methods include, but are not limited to, acylation reactions or alkylation reactions. *See, e.g,* EP 0 401 384; Malik et al. (1992), Exp. Hematol., 20:1028-1035; Francis (1992) Focus on Growth Factors 3(2):4-10, published by Mediscript, Mountain Court, Friern Barnet Lane, London N20 OLD, UK; EP 0 154 316; EP 0 401 384; WO 92/16221; WO 95/34326; WO 95/13312; WO 96/11953; WO 96/19459 and WO 96/19459. In some embodiments, any of these reactions are used to generate an antibody that is chemically modified at its amino-terminus.

[090] In some embodiments, an antibody is linked to a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label. In certain such embodiments, a detectable label allows for the detection or isolation of the antibody. In some embodiments, a detectable label allows for the detection of an antigen bound by the antibody.

[091] In some embodiments, an antibody is modified by linking it to one or more polymers. In some embodiments, an antibody is linked to one or more water-soluble polymers. In certain such embodiments, linkage to a water-soluble polymer reduces the likelihood that the antibody will precipitate in an aqueous environment, such as a physiological environment. In some embodiments, a therapeutic antibody is linked to a water-soluble polymer. In some embodiments, one skilled in the art can select a suitable water-soluble polymer based on considerations including whether the polymer/antibody conjugate will be used in the treatment of a patient and, if so, the pharmacological profile of the antibody (e.g., half-life, dosage, activity, antigenicity, and/or other factors).

[092] Certain exemplary clinically acceptable, water-soluble polymers include, but are not limited to, polyethylene glycol (PEG); polyethylene glycol propionaldehyde; copolymers of ethylene glycol/propylene glycol; monomethoxy-polyethylene glycol; carboxymethylcellulose; dextran; polyvinyl alcohol (PVA); polyvinyl pyrrolidone, poly-1, 3-dioxolane; poly-1,3,6-trioxane; ethylene/maleic anhydride copolymer; poly-β-amino acids (either homopolymers or random copolymers); poly(n-vinyl pyrrolidone)polyethylene glycol; polypropylene glycol homopolymers (PPG) and other polyalkylene oxides; polypropylene oxide/ethylene oxide copolymers; polyoxyethylated polyols (POG) (e.g., glycerol) and other polyoxyethylated polyols; polyoxyethylated sorbitol, polyoxyethylated glucose, colonic acids or other carbohydrate polymers; and Ficoll, dextran, or mixtures thereof. Certain exemplary PEGs include, but are not limited to,

certain forms known in the art to be useful in antibody modification, such as mono- (C_1-C_{10}) alkoxyor aryloxy-PEG. In some embodiments, PEG propional dehyde may have advantages in manufacturing due to its stability in water.

[093] In some embodiments, a water-soluble polymer is of any molecular weight. In some embodiments, a water-soluble polymer is branched or unbranched. In some embodiments, a water-soluble polymer has an average molecular weight of about 2 kDa to about 100 kDa, including all points between the end points of the range. In some embodiments, a water-soluble polymer has an average molecular weight of about 5 kDa to about 40 kDa. In some embodiments, a water-soluble polymer has an average molecular weight of about 10 kDa to about 35 kDa. In some embodiments, a water-soluble polymer has an average molecular weight of about 15 kDa to about 30 kDa.

[094] In some embodiments, an antibody is linked to polyethylene glycol (PEG; i.e., an antibody is "pegylated"). In various embodiments, PEG has low toxicity in mammals. See Carpenter et al. (1971) Toxicol. Appl. Pharmacol., 18:35-40. Notably, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. In various embodiments, PEG may reduce the immunogenicity of antibodies. For example, in some embodiments, linkage of PEG to an antibody having non-human sequences may reduce the antigenicity of that antibody when administered to a human.

[095] In some embodiments, a polymer is linked to one or more reactive amino acid residues in an antibody. Certain exemplary reactive amino acid residues include, but are not limited to, the alpha-amino group of the amino-terminal amino acid, the epsilon amino groups of lysine side chains, the sulfhydryl groups of cysteine side chains, the carboxyl groups of aspartyl and glutamyl side chains, the alpha-carboxyl group of the carboxy-terminal amino acid, tyrosine side chains, and activated glycosyl chains linked to certain asparagine, serine or threonine residues. Certain exemplary activated forms of PEG ("PEG reagents") suitable for direct reaction with proteins are known to those skilled in the art. For example, in some embodiments, PEG reagents suitable for linkage to amino groups include, but are not limited to, active esters of carboxylic acid or carbonate derivatives of PEG, for example, those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate. In some embodiments, PEG reagents containing maleimido or haloacetyl groups are used to modify sulfhydryl groups. In some embodiments, PEG reagents containing amino, hydrazine and/or hydrazide groups may be used in reactions with aldehydes generated by periodate oxidation of carbohydrate groups in proteins.

[096] In some embodiments, a water-soluble polymer has at least one reactive group. In some embodiments, an activated derivative of a water-soluble polymer, such as PEG, is created by reacting the water-soluble polymer with an activating group. In some embodiments, an activating group may be monofunctional, bifunctional, or multifunctional. Certain exemplary activating

groups that can be used to link a water-soluble polymer to two or more antibodies include, but are not limited to, the following groups: sulfone (e.g., chlorosulfone, vinylsulfone and divinylsulfone), maleimide, sulfhydryl, thiol, triflate, tresylate, azidirine, oxirane and 5-pyridyl. In some embodiments, a PEG derivative is typically stable against hydrolysis for extended periods in aqueous environments at pHs of about 11 or less. In some embodiments, a PEG derivative linked to another molecule, such as an antibody, confers stability from hydrolysis on that molecule. Certain exemplary homobifunctional PEG derivatives include, but are not limited to, PEG-<u>bis</u>-chlorosulfone and PEG-<u>bis</u>-vinylsulfone (see WO 95/13312).

5.2.3. Certain methods of making monoclonal antibodies

5.2.3.1. Certain hybridoma methods

[097] In some embodiments, monoclonal antibodies are produced by standard techniques. In some embodiments, monoclonal antibodies are produced by hybridoma-based methods. Certain such methods are known to those skilled in the art. *See, e.g.*, Kohler et al. (1975) Nature 256:495-497; Harlow and Lane (1988) Antibodies: A Laboratory Manual Ch. 6 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). In certain such embodiments, a suitable animal, such as a mouse, rat, hamster, monkey, or other mammal, is immunized with an immunogen to produce antibody-secreting cells. In some embodiments, the antibody-secreting cells are B-cells, such as lymphocytes or splenocytes. In some embodiments, lymphocytes (e.g., human lymphocytes) are immunized *in vitro* to generate antibody-secreting cells. *See, e.g.*, Borreback et al. (1988) Proc. Nat'l Acad. Sci. USA 85:3995-3999.

[098] In some embodiments, antibody secreting cells are fused with an "immortalized" cell line, such as a myeloid-type cell line, to produce hybridoma cells. In some embodiments, hybridoma cells that produce the desired antibodies are identified, for example, by ELISA. In some embodiments, such cells can then be subcloned and cultured using standard methods. In some embodiments, such cells can also be grown *in vivo* as ascites tumors in a suitable animal host. In some embodiments, monoclonal antibodies are isolated from hybridoma culture medium, serum, or ascites fluid using standard separation procedures, such as affinity chromatography. Guidance for the production of hybridomas and the purification of monoclonal antibodies according to certain embodiments is provided, for example, in Harlow and Lane (1988) <u>Antibodies: A Laboratory Manual</u> Ch. 8 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[099] In some embodiments, mouse monoclonal antibodies are produced by immunizing genetically altered mice with an immunogen. In certain such embodiments, the mice are NOTUM-deficient mice, which partially or completely lack NOTUM function. In certain such embodiments, the mice are "knockout" mice that lack all or part of a gene encoding NOTUM. In some

embodiments, such knockout mice are immunized with mouse NOTUM. In some embodiments, such knockout mice are immunized with human NOTUM.

[0100] In some embodiments, human monoclonal antibodies are raised in transgenic animals (e.g., mice) that are capable of producing human antibodies. *See, e.g.*, U.S. Patent Nos. 6,075,181 A and 6,114,598 A; and WO 98/24893 A2. For example, in some embodiments, human immunoglobulin genes are introduced (*e.g.*, using yeast artificial chromosomes, human chromosome fragments, or germline integration) into mice in which the endogenous Ig genes have been inactivated. *See, e.g.*, Jakobovits et al. (1993) Nature 362:255-258; Tomizuka et al. (2000) Proc. Nat'l Acad. Sci. USA 97:722-727; and Mendez et al. (1997) Nat. Genet. 15:146-156 (describing the XenoMouse II® line of transgenic mice).

[0101] In some embodiments, such transgenic mice are immunized with an immunogen. In certain such embodiments, lymphatic cells (such as B-cells) from mice that express antibodies are obtained. In certain such embodiments, such recovered cells are fused with an "immortalized" cell line, such as a myeloid-type cell line, to produce hybridoma cells. In certain such embodiments, hybridoma cells are screened and selected to identify those that produce antibodies specific to the antigen of interest. Certain exemplary methods and transgenic mice suitable for the production of human monoclonal antibodies are described, e.g., in Jakobovits et al. (1993) Nature 362:255-258; Jakobovits (1995) Curr. Opin. Biotechnol. 6:561-566; Lonberg et al. (1995) Int'l Rev. Immunol. 13:65-93; Fishwild et al. (1996) Nat. Biotechnol. 14:845-851; Mendez et al. (1997) Nat. Genet. 15:146-156; Green (1999) J. Immunol. Methods 231:11-23; Tomizuka et al. (2000) Proc. Nat'l Acad. Sci. USA 97:722-727; and reviewed in Little et al. (2000) Immunol. Today 21:364-370; and WO 98/24893. In some embodiments, human monoclonal antibodies against NOTUM are suitable for use as therapeutic antibodies. See Part V.G., below.

5.2.3.2. Certain display-based methods

[0102] In some embodiments, human monoclonal antibodies are produced using a display-based method, such as, for example, any of those described below.

[0103] In some embodiments, a monoclonal antibody is produced using phage display techniques. Various antibody phage display methods are known to those skilled in the art and are described, for example, in Hoogenboom, Overview of Antibody Phage-Display Technology and Its Applications, from Methods in Molecular Biology: Antibody Phage Display: Methods and Protocols (2002) 178:1-37 (O'Brien and Aitken, eds., Human Press, Totowa, NJ). For example, in some embodiments, a library of antibodies are displayed on the surface of a filamentous phage, such as the nonlytic filamentous phage fd or M13. In some embodiments, the antibodies are antibody fragments, such as scFvs, Fabs, Fvs with an engineered intermolecular disulfide bond to stabilize the V_H-V_L pair, and diabodies. In some embodiments, antibodies with the desired binding specificity can

then be selected. Nonlimiting exemplary embodiments of antibody phage display methods are described in further detail below.

[0104] In some embodiments, an antibody phage-display library can be prepared using certain methods known to those skilled in the art. *See, e.g.*, Hoogenboom, Overview of Antibody Phage-Display Technology and Its Applications, from Methods in Molecular Biology: Antibody Phage Display: Methods and Protocols (2002) 178:1-37 (O'Brien and Aitken, eds., Human Press, Totowa, NJ). In some embodiments, variable gene repertoires are prepared by PCR amplification of genomic DNA or cDNA derived from the mRNA of antibody-secreting cells. For example, in some embodiments, cDNA is prepared from mRNA of B-cells. In some embodiments, cDNA encoding the variable regions of heavy and light chains is amplified, for example, by PCR.

[0105] In some embodiments, heavy chain cDNA and light chain cDNA are cloned into a suitable vector. In some embodiments, heavy chain cDNA and light chain cDNA are randomly combined during the cloning process, thereby resulting in the assembly of a cDNA library encoding diverse scFvs or Fabs. In some embodiments, heavy chain cDNA and light chain cDNA are ligated before being cloned into a suitable vector. In some embodiments, heavy chain cDNA and light chain cDNA are ligated by stepwise cloning into a suitable vector.

[0106] In some embodiments, cDNA is cloned into a phage display vector, such as a phagemid vector. Certain exemplary phagemid vectors, such as pCES1, are known to those skilled in the art. In some embodiments, cDNA encoding both heavy and light chains is present on the same vector. For example, in some embodiments, cDNA encoding scFvs are cloned in frame with all or a portion of gene III, which encodes the minor phage coat protein pIII. In certain such embodiments, the phagemid directs the expression of the scFv-pIII fusion on the phage surface. Alternatively, in some embodiments, cDNA encoding heavy chain (or light chain) is cloned in frame with all or a portion of gene III, and cDNA encoding light chain (or heavy chain) is cloned downstream of a signal sequence in the same vector. The signal sequence directs expression of the light chain (or heavy chain) into the periplasm of the host cell, where the heavy and light chains assemble into Fab fragments. Alternatively, in some embodiments, cDNA encoding heavy chain and cDNA encoding light chain are present on separate vectors. In certain such embodiments, heavy chain and light chain cDNA is cloned separately, one into a phagemid and the other into a phage vector, which both contain signals for *in vivo* recombination in the host cell.

[0107] In some embodiments, recombinant phagemid or phage vectors are introduced into a suitable bacterial host, such as *E. coli*. In some embodiments using phagemid, the host is infected with helper phage to supply phage structural proteins, thereby allowing expression of phage particles carrying the antibody-plll fusion protein on the phage surface.

[0108] In some embodiments, "synthetic" antibody libraries are constructed using repertoires of variable genes that are rearranged *in vitro*. For example, in some embodiments,

individual gene segments encoding heavy or light chains (V-D-J or V-J, respectively) are randomly combined using PCR. In some embodiments, additional sequence diversity can be introduced into the CDRs, and possibly FRs, e.g., by error prone PCR. In some such embodiments, additional sequence diversity is introduced into CDR3, e.g., H3 of the heavy chain.

[0109] In some embodiments, "naïve" or "universal" phage display libraries are constructed as described above using nucleic acid from an unimmunized animal. In some embodiments, the unimmunized animal is a human. In some embodiments, "immunized" phage display libraries are constructed as described above using nucleic acid from an immunized animal. In some embodiments, the immunized animal is a human, rat, mouse, hamster, or monkey. In certain such embodiments, the animals are immunized with any of the immunogens described below.

[0110] Certain exemplary universal human antibody phage display libraries are available from commercial sources. Certain exemplary libraries include, but are not limited to, the HuCAL* series of libraries from MorphoSys AG (Martinstreid/Munich, Germany); libraries from Crucell (Leiden, the Netherlands) using MAbstract* technology; the n-CoDeR* Fab library from BioInvent (Lund, Sweden); and libraries available from Cambridge Antibody Technology (Cambridge, UK).

[0111] In some embodiments, the selection of antibodies having the desired binding specificity from a phage display library is achieved by successive panning steps. In some embodiments of panning, library phage preparations are exposed to antigen. In certain such embodiments, the phage-antigen complexes are washed, and unbound phage are discarded. In certain such embodiments, bound phage are recovered and subsequently amplified by infecting *E. coli*. In certain such embodiments, monoclonal antibody-producing phage may be cloned by picking single plaques. In some embodiments, the above process is repeated.

[0112] In some embodiments, the antigen used in panning is any of the immunogens described below. In some embodiments, the antigen is immobilized on a solid support to allow purification of antigen-binding phage by affinity chromatography. In some embodiments, the antigen is biotinylated, thereby allowing the separation of bound phage from unbound phage using streptavidin-coated magnetic beads. In some embodiments, the antigen may be immobilized on cells (for direct panning), in tissue cryosections, or on membranes (e.g., nylon or nitrocellulose membranes). Other variations of certain panning procedures may be routinely determined by one skilled in the art.

[0113] In some embodiments, a yeast display system is used to produce monoclonal antibodies. In certain such systems, an antibody is expressed as a fusion protein with all or a portion of the yeast AGA2 protein, which becomes displayed on the surface of the yeast cell wall. In certain such embodiments, yeast cells expressing antibodies with the desired binding specificity can then be identified by exposing the cells to fluorescently labeled antigen. In certain such

embodiments, yeast cells that bind the antigen can then be isolated by flow cytometry. *See, e.g.,* Boder et al. (1997) <u>Nat. Biotechnol.</u> 15:553-557.

5.2.3.3. Certain affinity maturation methods

[0114] In some embodiments, the affinity of an antibody for a particular antigen is increased by subjecting the antibody to affinity maturation (or "directed evolution") *in vitro*. *In vivo*, native antibodies undergo affinity maturation through somatic hypermutation followed by selection. Some *in vitro* methods mimic that *in vivo* process, thereby allowing the production of antibodies having affinities that equal or surpass that of native antibodies.

[0115] In some embodiments of affinity maturation, mutations are introduced into a nucleic acid sequence encoding the variable region of an antibody having the desired binding specificity. See, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; Brekke et al. (2002) Nat. Reviews 2:52-62. In some embodiments, mutations are introduced into the variable region of the heavy chain, light chain, or both. In some embodiments, mutations are introduced into one or more CDRs. In certain such embodiments, mutations are introduced into H3, L3, or both. In some embodiments, mutations are introduced into one or more FRs. In some embodiments, a library of mutations is created, for example, in a phage, ribosome, or yeast display library, so that antibodies with increased affinity may be identified by standard screening methods. See, e.g., Boder et al. (2000) Proc. Nat'l Acad. Sci. USA 97:10701-10705; Foote et al. (2000) Proc. Nat'l Acad. Sci. USA 97:10679-10681; Hoogenboom, Overview of Antibody Phage-Display Technology and Its

Applications, from Methods in Molecular Biology: Antibody Phage Display: Methods and Protocols (2002) 178:1-37 (O'Brien and Aitken, eds., Human Press, Totowa, NJ); and Hanes et al. (1998) Proc. Nat'l Acad. Sci. USA 95:14130-14135.

[0116] In some embodiments, mutations are introduced by site-specific mutagenesis based on information on the antibody's structure, e.g., the antigen binding site. In some embodiments, mutations are introduced using combinatorial mutagenesis of CDRs. In some embodiments, all or a portion of the variable region coding sequence is randomly mutagenized, e.g., using *E. coli* mutator cells, homologous gene rearrangement, or error prone PCR. In some embodiments, mutations are introduced using "DNA shuffling." *See, e.g.*, Crameri et al. (1996) <u>Nat. Med.</u> 2:100-102; Fermer et al. (2004) <u>Tumor Biol.</u> 25:7-13.

[0117] In some embodiments, "chain shuffling" is used to generate antibodies with increased affinity. In some embodiments of chain shuffling, one of the chains, e.g., the light chain, is replaced with a repertoire of light chains, while the other chain, e.g., the heavy chain, is unchanged, thus providing specificity. In certain such embodiments, a library of chain shuffled antibodies is created, wherein the unchanged heavy chain is expressed in combination with each light chain from the repertoire of light chains. In some embodiments, such libraries may then be

screened for antibodies with increased affinity. In some embodiments, both the heavy and light chains are sequentially replaced. In some embodiments, only the variable regions of the heavy and/or light chains are replaced. In some embodiments, only a portion of the variable regions, e.g., CDRs, of the heavy and/or light chains are replaced. *See, e.g.*, Hudson et al. (2003) Nat. Med. 9:129-134; Brekke et al. (2002) Nat. Reviews 2:52-62; Kang et al. (1991) Proc. Nat'l Acad. Sci. USA 88:11120-11123; Marks et al. (1992) Biotechnol. 10:779-83.

[0118] In some embodiments, mouse monoclonal antibodies that specifically bind human NOTUM (including mouse monoclonal antibodies raised against mouse NOTUM but which specifically bind (i.e., cross react) with human NOTUM) are subject to sequential chain shuffling. In some embodiments, for example, the heavy chain of a given mouse monoclonal antibody is combined with a new repertoire of human light chains, and antibodies with the desired affinity are selected. In certain such embodiments, the light chains of the selected antibodies are then combined with a new repertoire of human heavy chains, and antibodies with the desired affinity are selected. Thus, in some embodiments, human antibodies having the desired antigen binding specificity and affinity are selected.

[0119] Alternatively, in some embodiments, the heavy chain of a given mouse monoclonal antibody is combined with a new repertoire of human light chains, and antibodies with the desired affinity are selected from this first round of shuffling. In some embodiments, the light chain of the original mouse monoclonal antibody is combined with a new repertoire of human heavy chains, and antibodies with the desired affinity are selected from this second round of shuffling. In some embodiments, human light chains from the antibodies selected in the first round of shuffling are then combined with human heavy chains from the antibodies selected in the second round of shuffling. Thus, in some embodiments, human antibodies having the desired antigen binding specificity and affinity are selected.

[0120] In some embodiments, a "ribosome display" method is used that alternates antibody selection with affinity maturation. In some embodiments of a ribosome display method, antibody-encoding nucleic acid is amplified by RT-PCR between the selection steps. Thus, in some embodiments, error prone polymerases may be used to introduce mutations into the nucleic acid. A nonlimiting example of such a method is described in detail in Hanes et al. (1998) Proc. Nat'l Acad. Sci. USA 95:14130-14135.

5.2.3.4. Certain recombinant methods

[0121] In some embodiments, a monoclonal antibody is produced by recombinant techniques. *See, e.g.*, U.S. Patent No. 4,816,567. In certain such embodiments, nucleic acid encoding monoclonal antibody chains are cloned and expressed in a suitable host cell. For example, in some embodiments, RNA can be prepared from cells expressing the desired antibody, such as

mature B-cells or hybridoma cells, using standard methods. In some embodiments, the RNA can then be used to make cDNA using standard methods. In some embodiments, cDNA encoding a heavy or light chain polypeptide is amplified, for example, by PCR, using specific oligonucleotide primers. In some embodiments, the cDNA is cloned into a suitable expression vector. In some embodiments, the expression vector is then transformed or transfected into a suitable host cell, such as a host cell that does not endogenously produce antibody. Certain exemplary host cells include, but are not limited to, *E. coli*, COS cells, Chinese hamster ovary (CHO) cells, and myeloma cells. In some embodiments, wherein heavy and light chains are coexpressed in the same host, reconstituted antibody may be isolated.

[0122] In some embodiments, cDNA encoding a heavy or light chain can be modified. For example, in some embodiments, the constant region of a mouse heavy or light chain can be replaced with the constant region of a human heavy or light chain. In this manner, in some embodiments, a chimeric antibody can be produced which possesses human antibody constant regions but retains the binding specificity of a mouse antibody.

[0123] In some embodiments, a nucleic acid molecule comprises a polynucleotide sequence that encodes the heavy chain or the light chain of a NOTUM neutralizing antibody. In some embodiments, a single nucleic acid molecule comprises a first polynucleotide sequence that encodes the heavy chain of a NOTUM neutralizing antibody and a second polynucleotide sequence that encodes the light chain of a NOTUM neutralizing antibody. In some embodiments, for example, when the antibody is a single-chain Fv (scFv), the coding sequence for the heavy chain and the coding sequence for the light chain are part of a continuous coding sequence such that a single polypeptide is expressed, which comprises both the heavy chain and the light chain of the antibody. In some embodiments, a single nucleic acid molecule that encodes both a heavy chain and a light chain is capable of expressing the two chains as separate polypeptides. In some such embodiments, each chain is under the control of a separate promoter. In some embodiments, the two chains are under the control of the same promoter. One skilled in the art can select a suitable configuration and suitable control elements for the heavy and light chain of the NOTUM neutralizing antibody according to the intended application.

[0124] In some embodiments, the nucleic acid is a vector, such as an expression vector suitable for expressing the heavy chain and/or light chain in a particular host cell. One skilled in the art can select a suitable expression vector, or expression vectors, according to the host cell to be used for expression. Many exemplary such vectors are known in the art.

[0125] In some embodiments, a nucleic acid molecule comprises a polynucleotide sequence that encodes a heavy chain of a NOTUM neutralizing antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, 2.78, and humanized versions of such MAbs. In some such embodiments, a nucleic acid molecule comprises a polynucleotide sequence selected from SEQ ID

NOs: 101, 103, 105, 107, 109, 111, 112, 115, 116, 119, 120, 123, 124, 127, and 128. In some embodiments, a nucleic acid molecule comprises a polynucleotide sequence that encodes a light chain of a NOTUM neutralizing antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, 2.78, and humanized versions of such MAbs. In some such embodiments, a nucleic acid molecule comprises a polynucleotide sequence selected from SEQ ID NOs: 102, 104, 106, 108, 110, 113, 114, 117, 118, 121, 122, 125, 126, 129, and 130. In some embodiments, a nucleic acid molecule comprises a first polynucleotide sequence that encodes the heavy chain and a second polynucleotide sequence that encodes the light chain, of a NOTUM neutralizing antibody selected from MAbs 1.731, 1.802, 1.815, 1.846, 2.1029, 2.55, 2.78, and humanized versions of such MAbs.

[0126] In some embodiments, recombinant antibodies can be expressed in certain cell lines. In some embodiments, sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. According to certain embodiments, transformation can be by any known method for introducing polynucleotides into a host cell. Certain exemplary methods include, but are not limited to, packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) and using certain transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. In some embodiments, the transformation procedure used may depend upon the host to be transformed. Certain exemplary methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0127] Certain exemplary mammalian cell lines available as hosts for expression are known in the art and include, but are not limited to, many immortalized cell lines available from the American Type Culture Collection (ATCC), including Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. In some embodiments, cell lines may be selected by determining which cell lines produce high levels of antibodies that specifically bind NOTUM.

5.3. Methods of Treatment

[0128] This invention encompasses a method of stimulating endocortical bone formation in a patient, which comprises administering to a patient in need thereof an effective amount of an antibody of the invention. It also encompasses a method of increasing cortical bone thickness, comprising administering to a patient in need thereof an effective amount of an antibody of the invention.

[0129] This invention encompasses a method of treating, managing, or preventing a disease or disorder associated with bone loss, which comprises administering to a patient in need thereof a therapeutically or prophylactically effective amount of an antibody of the invention. Examples of diseases and disorders include osteoporosis (*e.g.*, postmenopausal osteoporosis, steroid- or glucocorticoid-induced osteoporosis, male osteoporosis, and idiopathic osteoporosis), osteopenia, and Paget's disease.

[0130] Also encompassed by the invention is a method of treating, managing, or preventing bone fractures, which comprises administering to a patient in need thereof a therapeutically or prophylactically effective amount of an antibody of the invention. Particular bone fractures are associated with metastatic bone disease, *i.e.*, cancer that has metastasized to bone. Examples of cancers that can metastasize to bone include prostate, breast, lung, thyroid, and kidney cancer.

[0131] This invention also encompasses a method of treating, managing, or preventing bone loss associated with, or caused by, a disease or disorder, which comprises administering to a patient in need thereof a therapeutically or prophylactically effective amount of an antibody of the invention. Examples of diseases and disorders include celiac disease, Crohn's Disease, Cushing's syndrome, hyperparathyroidism, inflammatory bowel disease, and ulcerative colitis.

[0132] Nonlimiting exemplary patients that may benefit from methods of this invention include men and women aged 55 years or older, post-menopausal women, and patients suffering from renal insufficiency.

[0133] Antibodies of the invention can be administered in combination (*e.g.*, at the same or at different times) with other drugs known to be useful in the treatment, management, or prevention of diseases or conditions affecting the bone. Examples include: androgen receptor modulators; bisphosphonates; calcitonin; calcium sensing receptor antagonists; RANKL antibodies, cathepsin K inhibitors; estrogen and estrogen receptor modulators; integrin binders, antibodies, and receptor antagonists; parathyroid hormone (PTH) and analogues and mimics thereof; and vitamin D and synthetic vitamin D analogues.

[0134] Examples of androgen receptor modulators include finasteride and other 5α -reductase inhibitors, nilutamide, flutamide, bicalutamide, liarozole, and abiraterone acetate.

[0135] Examples of bisphosphonates include alendronate, cimadronate, clodronate, etidronate, ibandronate, incadronate, minodronate, neridronate, olpadronate, pamidronate, piridronate, risedronate, tiludronate, and zolendronate, and pharmaceutically acceptable salts and esters thereof.

[0136] Examples of cathepsin K inhibitors include VEL-0230, AAE581 (balicatib), MV061194, SB-462795 (relacatib), MK-0822 (odanacatib), and MK-1256.

[0137] Examples of estrogen and estrogen receptor modulators include naturally occurring estrogens (*e.g.*, 7-estradiol, estrone, and estriol), conjugated estrogens (*e.g.*, conjugated equine estrogens), oral contraceptives, sulfated estrogens, progestogen, estradiol, droloxifene, raloxifene, lasofoxifene, TSE-424, tamoxifen, idoxifene, LY353381, LY117081, toremifene, fulvestrant, 4-[7-(2,2-dimethyl-1-oxopropoxy-4-methyl-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2H-1-benzopyran-3-yl]-phenyl-2,2-dimethylpropanoate, 4,4'-dihydroxybenzophenone-2,4-dinitrophenyl-hydrazone, and SH646.

[0138] Examples of integrin binders, antibodies, and receptor antagonists include vitaxin (MEDI-522), cilengitide and L-000845704. $^{\circ}$

5.4. Pharmaceutical Formulations

[0139] This invention encompasses pharmaceutical compositions comprising one or more antibodies of the invention, and optionally one or more other drugs, such as those described above.

[0140] In some embodiments, a NOTUM neutralizing antibody may be used as a therapeutic antibody. Exemplary NOTUM neutralizing antibodies to be used as therapeutic antibodies include, but are not limited to, chimeric antibodies, humanized antibodies, and human antibodies. Those skilled in the art are familiar with the use of antibodies as therapeutic agents.

[0141] In some embodiments, a pharmaceutical composition is provided that comprises an effective amount of an antibody to NOTUM and a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. In some embodiments, a pharmaceutical composition is provided that comprises an effective amount of an antibody to NOTUM and an effective amount of at least one additional therapeutic agent, together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. In some embodiments, at least one additional therapeutic agent is selected from those described above.

[0142] In some embodiments, formulation materials for pharmaceutical compositions are nontoxic to recipients at the dosages and concentrations employed.

[0143] In some embodiments, the pharmaceutical composition comprises formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In some embodiments, suitable formulation materials include, but are not limited to, amino acids (for example, glycine, glutamine, asparagine, arginine and lysine); antimicrobials; antioxidants (for example, ascorbic acid, sodium sulfite and sodium hydrogen-sulfite); buffers (for example, borate, bicarbonate, Tris-HCl, citrates, phosphates and other organic acids); bulking agents (for example, mannitol and glycine); chelating agents (for example, ethylenediamine tetraacetic acid (EDTA)); complexing agents (for example, caffeine, polyvinylpyrrolidone, beta-cyclodextrin, and hydroxypropyl-beta-cyclodextrin); fillers;

monosaccharides, disaccharides, and other carbohydrates (for example, glucose, mannose and dextrins); proteins (for example, serum albumin, gelatin and immunoglobulins); coloring, flavoring, and diluting agents; emulsifying agents; hydrophilic polymers (for example, polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (for example, sodium); preservatives (for example, benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and hydrogen peroxide); solvents (for example, glycerin, propylene glycol and polyethylene glycol); sugar alcohols (for example, mannitol and sorbitol); suspending agents; surfactants or wetting agents (for example, pluronics, PEG, sorbitan esters, polysorbates (for example, polysorbate 20 and polysorbate 80), triton, tromethamine, lecithin, cholesterol, and tyloxapal); stability enhancing agents (for example, sucrose and sorbitol); tonicity enhancing agents (for example, alkali metal halides (for example, sodium or potassium chloride), mannitol, and sorbitol); delivery vehicles; diluents; excipients; and pharmaceutical adjuvants. (*Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company (1990).

[0144] In some embodiments, an antibody to NOTUM or other therapeutic molecule is linked to a half-life extending vehicle. Nonlimiting exemplary half-life extending vehicles include those known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Exemplary such vehicles are described, e.g., in published PCT Application No. WO 99/25044.

[0145] In some embodiments, an optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. *See, e.g., Remington's Pharmaceutical Sciences, supra*. In some embodiments, such compositions may influence the physical state, stability, rate of *in vivo* release, or rate of *in vivo* clearance of a neutralizing antibody.

[0146] In some embodiments, a primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, in some embodiments, a suitable vehicle or carrier may be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Exemplary vehicles include, but are not limited to, neutral buffered saline and saline mixed with serum albumin. In some embodiments, pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In some embodiments, a composition comprising an antibody to NOTUM, with or without at least one additional therapeutic agents, may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences*, *supra*) in the form of a lyophilized cake or an aqueous solution. In some embodiments, a composition comprising an antibody to NOTUM.

with or without at least one additional therapeutic agent, may be formulated as a lyophilizate using appropriate excipients such as sucrose.

[0147] In some embodiments, a pharmaceutical composition is selected for parenteral delivery. In some embodiments, a pharmaceutical composition is selected for inhalation or for delivery through the digestive tract, such as orally. Various techniques for preparing pharmaceutically acceptable compositions are within the skill of one skilled in the art.

[0148] In some embodiments, formulation components are present in concentrations that are acceptable to the site of administration. In some embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

[0149] In some embodiments, when parenteral administration is contemplated, a pharmaceutical composition may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired antibody to NOTUM, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In some embodiments, a vehicle for parenteral injection is sterile distilled water in which the antibody to NOTUM, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In some embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that may provide for the controlled or sustained release of the product which may then be delivered via a depot injection. In some embodiments, hyaluronic acid may also be used, and may have the effect of promoting sustained duration in the circulation. In some embodiments, implantable drug delivery devices may be used to introduce the desired molecule.

[0150] In some embodiments, a pharmaceutical composition may be formulated for inhalation. In some embodiments, an antibody to NOTUM, with or without at least one additional therapeutic agent, may be formulated as a dry powder for inhalation. In some embodiments, an inhalation solution comprising an antibody to NOTUM, with or without at least one additional therapeutic agent, may be formulated with a propellant for aerosol delivery. In some embodiments, solutions may be nebulized.

[0151] In some embodiments, a formulation may be administered orally. In some embodiments, an antibody to NOTUM, with or without at least one additional therapeutic agent, that is administered in this fashion may be formulated with or without carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In some embodiments, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. In some embodiments, at least one additional agent can be included to facilitate absorption of the

antibody to NOTUM with or without any additional therapeutic agents. In some embodiments, diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and/or binders may also be employed.

[0152] In some embodiments, a pharmaceutical composition comprises an effective amount of an antibody to NOTUM, with or without at least one additional therapeutic agent, in a mixture with non-toxic excipients which are suitable for the manufacture of tablets. In some embodiments, by dissolving the tablets in sterile water, or another appropriate vehicle, solutions may be prepared in unit-dose form. Exemplary excipients include, but are not limited to, inert diluents (for example, calcium carbonate, sodium carbonate, sodium bicarbonate, lactose, and calcium phosphate); binding agents (for example, starch, gelatin, and acacia); and lubricating agents (for example, magnesium stearate, stearic acid, and talc).

[0153] Additional pharmaceutical compositions will be evident to those skilled in the art. including formulations comprising an antibody to NOTUM, with or without at least one additional therapeutic agent, in sustained- or controlled-delivery formulations. Exemplary sustained- or controlled-delivery formulations include, but are not limited to, liposome carriers, bio-erodible microparticles, porous beads, and depot injections. Various techniques for preparing formulations are known to those skilled in the art. In some embodiments, sustained-release preparations may include semipermeable polymer matrices in the form of shaped articles, e.g. films or microcapsules. Exemplary sustained release matrices include, but are not limited to, polyesters, hydrogels. polylactides (see, e.g., U.S. Patent No. 3,773,919 and EP 058,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (see, e.g., Sidman et al. (1983) Biopolymers 22:547-556), poly (2hydroxyethyl-methacrylate) (see, e.g., Langer et al. (1981) J. Biomed. Mater. Res. 15:167-277 and Langer (1982) Chem. Tech. 12:98-105), ethylene vinyl acetate (Langer et al., supra), and poly-D(-)-3hydroxybutyric acid (EP 133,988). In some embodiments, sustained release compositions may include liposomes, which can be prepared, in some embodiments, by any of several methods known in the art. See e.g., Eppstein et al. (1985) Proc. Natl. Acad. Sci. USA, 82:3688-3692; EP 036,676; EP 088,046; and EP 143,949.

[0154] In some embodiments, a pharmaceutical composition to be used for *in vivo* administration typically is sterile. In some embodiments, this may be accomplished by filtration through sterile filtration membranes. In some embodiments, where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. In some embodiments, the composition for parenteral administration may be stored in lyophilized form or in a solution. In some embodiments, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0155] In some embodiments, once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. In some embodiments, such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

[0156] In some embodiments, kits for producing a single-dose administration unit are provided. In some embodiments, the kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. In some embodiments, kits containing single or multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are included.

[0157] In some embodiments, the effective amount of a pharmaceutical composition comprising an antibody to NOTUM, with or without at least one additional therapeutic agent, to be employed therapeutically will depend, for example, upon the context and objectives of treatment. One skilled in the art will appreciate that the appropriate dosage levels for treatment, according to some embodiments, will thus vary depending, in part, upon the molecule delivered, the indication for which the antibody to NOTUM, with or without at least one additional therapeutic agent, is being used, the route of administration, and the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient. In some embodiments, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. In some embodiments, a typical dosage may range from about 0.1 µg/kg of patient body weight, up to about 100 mg/kg or more, depending on the factors mentioned above. In some embodiments, the dosage may range from 0.1 µg/kg up to about 100 mg/kg; 1 µg/kg up to about 100 mg/kg; or 5 μg/kg up to about 100 mg/kg, including all points (including fractions) between any of the foregoing endpoints. In some embodiments, the dosage is between about 1 mg/kg body weight and about 60 mg/kg body weight. In some embodiments, the dosage is about 1 mg/kg body weight, about 3 mg/kg body weight, about 5 mg/kg body weight, about 10 mg/kg body weight. about 20 mg/kg body weight, about 30 mg/kg body weight, about 40 mg/kg body weight, about 50 mg/kg body weight, or about 60 mg/kg body weight.

[0158] In some embodiments, a human dose of a neutralizing antibody against NOTUM is determined based on the efficacious dose of the same antibody in another species, such as mice, dogs, monkeys, etc. In some embodiments, a human dose of a neutralizing antibody against NOTUM is determined using "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers," U.S. Department of Health and Human Services, Food and Drug Administration, and Center for Drug Evaluation and Research (CDER), July 2005 (Pharmacology and Toxicology).

[0159] In some embodiments, a suitable dosage may be determined by one skilled in the art, for example, based on animal studies.

[0160] In various embodiments, a neutralizing antibody against NOTUM is administered to a patient twice per week, once per week, once every two weeks, once per month, once every other month, or even less frequently.

[0161] In some embodiments, the frequency of dosing will take into account the pharmacokinetic parameters of an antibody to NOTUM and, if applicable, any additional therapeutic agents in the formulation used. In some embodiments, a clinician will administer the composition until a dosage is reached that achieves the desired effect. In some embodiments, the composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. In some embodiments, further refinement of the appropriate dosage is routinely made by those skilled in the art and is within the ambit of tasks routinely performed by them. In some embodiments, appropriate dosages may be ascertained through use of appropriate dose-response data. In some embodiments, a patient receives one dose of a pharmaceutical composition comprising an antibody to NOTUM. In some embodiments, a patient receives one, two, three, or four doses per day of a pharmaceutical composition comprising an antibody to NOTUM. In some embodiments, a patient receives one, two, three, four, five, or six doses per week of a pharmaceutical composition comprising an antibody to NOTUM. In some embodiments, a patient receives one, two, three, or four doses per month of a pharmaceutical composition comprising an antibody to NOTUM.

[0162] In some embodiments, the route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by subcutaneous, intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, or intralesional routes; by sustained release systems or by implantation devices. In some embodiments, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

[0163] In some embodiments, the composition may be administered locally via implantation of a membrane, sponge or another appropriate material onto which the desired molecule has been absorbed or encapsulated. In some embodiments, where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed-release bolus, or continuous administration.

[0164] In some embodiments, an antibody to NOTUM, with or without at least one additional therapeutic agent, is delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptides. In some embodiments, such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. In some embodiments, the cells may be immortalized. In some embodiments, in order to decrease the chance of an immunological response, the cells may

be encapsulated to avoid infiltration of surrounding tissues. In some embodiments, the encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

6. **EXAMPLES**

6.1. Knock-out Mouse

[0165] Mice homozygous for a genetically engineered mutation in the murine ortholog of the human NOTUM gene were generated using corresponding mutated embryonic stem (ES) cell clones from the OMNIBANK collection of mutated murine ES cell clones (see generally, U.S. Patent No. 6,080,576). In brief, ES cell clones containing a mutagenic viral insertion into the murine NOTUM locus were microinjected into blastocysts which were in turn implanted into pseudopregnant female hosts and carried to term. The resulting chimeric offspring were subsequently bred to C57 black 6 female mice and the offspring checked for the germline transmission of the knocked-out NOTUM allele. Animals heterozygous for the mutated NOTUM allele were subsequently bred to produce offspring that were homozygous for the mutated NOTUM allele, heterozygous for the mutated NOTUM allele, heterozygous for the mutated NOTUM allele, or wild type offspring at an approximate ratio of 1:2:1.

[0166] Mice homozygous (-/-) for the disruption of the NOTUM gene were studied in conjunction with mice heterozygous (+/-) for the disruption of the NOTUM gene and wild-type (+/+) litter mates. During this analysis, the mice were subject to a medical work-up using an integrated suite of medical diagnostic procedures designed to assess the function of the major organ systems in a mammalian subject. By studying the homozygous (-/-) "knockout" mice in the described numbers and in conjunction with heterozygous (+/-) and wild-type (+/+) litter mates, more reliable and repeatable data were obtained.

[0167] As shown in Figure 1, male mice having homozygous disruption of the NOTUM gene ("homs") exhibited greater cortical thicknesses at various bone sites, compared to their wildtype littermates at 16 weeks of age (number of mice N = 10 for both groups). These differences, which were measured by microCT (Scanco μ CT40), were: 28% (p < 0.001) at midshaft femur; 19% (p < 0.001) at midshaft humerus; 17% (p < 0.001) at midshaft tibia; and 11% (p < 0.001) at tibia-fibula junction. As shown in Figure 2, at 16 weeks of age, the midshaft femur cortical bone thickness of mice heterozygous for the NOTUM mutation ("hets") was also greater than that of their wildtype littermates: male hets (N = 50) exhibited a 6% (p = 0.007) increase compared to their wildtype littermates (N = 23); and female hets (N = 57) exhibited a 9% (p < 0.001) increase compared to their wildtype littermates (N = 22).

[0168] Practical manifestations of the observed redistribution of bone formation in NOTUM animals are reflected in Figures 3 and 4, which show results of femur breaking strength tests (performed by SkeleTech, now Ricerca Biosciences) using a standard 4-point bending test. As shown in Figure 3, which provides results obtained for male mice at 16 weeks of age, hets (N = 20) exhibited a 5% (p = 0.54) increase in femur breaking strength compared to their wildtype littermates (N = 23), whereas homs (N = 17) exhibited a 28% (p < 0.001) increase. On the other hand, spine compression tests of both NOTUM homs and hets did not show a significant reduction in maximum spine compression loads as compared to wildtype controls. Similar results were obtained for female mice at 16 weeks of age. As shown in Figure 4, hets (N = 20) exhibited a 12% (p = 0.04) increase in femur breaking strength compared to their wildtype littermates (N = 21), whereas homs (N = 18) exhibited a 28% (p < 0.001) increase. Analysis of these and other data revealed a strong correlation between cortical thickness and femur breaking strength.

6.2. <u>Production and Purification of Recombinant NOTUM Proteins</u>

[0169] The full-length coding sequences for human, catalytically inactive human (S232A), mouse, catalytically inactive mouse (S239A), rat, guinea pig, cynomolgus monkey, and rhesus monkey NOTUM, each with a C-terminal 6XHis epitope tag, were subcloned into the expression vector pIRESpuro2 (Clontech). The expression constructs can be used to generate conditioned medium containing secreted NOTUM protein by transient transfection, or to establish stable transfectants for the generation of larger quantities of conditioned medium, e.g., for subsequent purification of NOTUM protein.

[0170] HEK293F cells were transfected using Lipofectamine2000 (Invitrogen) and grown in suspension culture in Freestyle 293 Expression Medium (Invitrogen) in shaker flasks. For transient transfections, conditioned medium was harvested four days after transfection, sterile filtered and stored at 4°C. For the generation of cell lines stably expressing NOTUM protein, genomic integration of the expression plasmid was selected for in the presence of puromycin.

[0171] Expression and secretion of NOTUM protein was confirmed by Western blot of cell lysates and/or conditioned medium, using an anti-His antibody. Subcloning of NOTUM-producing bulk stable transfectants by limiting dilution enabled the identification by anti-His Western blot of individual clones expressing NOTUM at relatively high levels.

[0172] To produce purified mouse and human NOTUM proteins at 10-20 mg scale, clonal HEK293F cell lines expressing either mouse or human NOTUM were expanded in suspension culture to a volume of 3L. When the cell density at this volume reached 1x10^6 viable cells per ml, the cells were pelleted by centrifugation and resuspended in fresh Freestyle 293 Expression Medium and maintained in culture for a further 96 hours without additional medium changes. After 96 hours,

cultures were harvested, cells were pelleted by centrifugation, and the conditioned medium was sterile filtered and stored at 4°C for subsequent processing.

[0173] Immediately prior to purification, NOTUM-containing conditioned medium was concentrated from 3L to 1L and then buffer exchanged into nickel immobilized metal affinity chromatography (IMAC) buffer (20 mM Tris-HCl, 10 mM imidazole, 0.5 M NaCl, pH 7.4) by tangential flow filtration using a membrane with a 10kDa nominal molecular weight cut off. Concentrated, buffer exchanged conditioned medium was then applied to an equilibrated, nickel charged, metal chelating column. Bound protein was washed and eluted using an imidazole concentration gradient. Elution fractions containing pure NOTUM protein were pooled and dialyzed against phosphate buffered saline to remove the elution buffer. Purified, dialyzed protein was aliquotted and frozen at -80°C.

[0174] For each batch of protein, one aliquot was used to determine protein concentration by bicinchoninic acid (BCA) assay (Thermo Scientific, Rockford, IL), purity by SDS PAGE followed by Coomassie or silver staining, activity in both the cell-free OPTS enzymatic assay (described in Example 6.4.1, below) and the cell-based Wnt signaling assay (described in Example 6.4.2, below), and endotoxin concentration by Limulus Amoebocyte Lysate (LAL) assay (Lonza, Basel, Switzerland).

6.3. Generation of Mouse Monoclonal Antibodies to NOTUM

[0175] Antibodies were raised against purified recombinant human and mouse NOTUM proteins in two separate immunization campaigns.

[0176] In Campaign 1, mice homozygous for a gene trap insertion in the NOTUM gene and therefore lacking endogenous NOTUM protein were immunized with human NOTUM protein as follows. Mice were primed with 20µg human NOTUM protein in complete Freund's adjuvant injected intraperitoneally. Mice were boosted with 20µg human NOTUM protein in incomplete Freund's adjuvant injected intraperitoneally every two to three weeks. Mice exhibiting a robust serum titer against human NOTUM as determined by ELISA received a final boost of 10µg human NOTUM protein in PBS injected intravenously (i.v.).

[0177] In Campaign 2, mice homozygous for a gene trap insertion in the NOTUM gene were immunized via the hind footpads with a priming immunization of 10µg mouse NOTUM protein in TiterMax adjuvant with CpG DNA followed by ten boosts of 10µg mouse NOTUM protein in Alum adjuvant with CpG DNA at three or four day intervals. Inguinal and popliteal lymph nodes were harvested from high titer mice after a final footpad boost with 10µg mouse NOTUM protein in PBS.

[0178] Spleens from i.v. boosted mice or lymph nodes from footpad immunized mice were collected four days after the final boost and were minced and strained to yield a cell suspension. Red blood cells were lysed and the cell suspension was enriched for B-cells by negative selection using magnetic beads coated with antibodies specific for non-B-cell populations. Hybridomas were

generated by electro-cell fusion of enriched B-cells with mouse NS1 myeloma cells and were seeded onto 96-well plates in hybridoma medium containing hypoxanthine and aminopterin to select for viable B-cell/myeloma cell hybridomas.

[0179] Hybridomas were screened for the production of NOTUM-specific antibodies by assaying hybridoma conditioned medium for immunoreactivity with passively adsorbed NOTUM protein in an ELISA format. Hundreds of hybridomas secreting antibody specific for mouse and/or human NOTUM were found from both immunization campaigns.

6.4. NOTUM Neutralization Assays

6.4.1. OPTS Assay

[0180] In the OPTS assay, trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS), a water soluble enzyme substrate for fluorimetric assays of esterases and lipases, is used to measure NOTUM activity. Enzymatic cleavage of the ester bond in OPTS yields a fluorescent product.

[0181] It was found that hybridoma conditioned medium in general interfered in the OPTS assay perhaps due to the release from dying cells of hydrolases that could also cleave the OPTS. For this reason, additional hybridoma conditioned medium was generated for those lines originally showing the highest level of binding activity by ELISA and antibody was purified in a 96-well format by affinity chromatography using protein A beads. These purified antibodies were then tested in the OPTS assay at a four-fold dilution without prior quantitation.

[0182] Antibodies were tested in quadruplicate in 384-well plates. 12.5 μ l containing 125 ng of purified NOTUM in 4X reaction buffer (20 mM CaCl2, 2mM MgCl2, 50mM Tris-HCl, pH7.4) was added to 12.5 μ l of purified antibody. After mixing, antibody and NOTUM were incubated at room temperature for 20 minutes followed by addition of 25 μ l of 1.25 μ M OPTS (Sigma, catalog # 74875) in 50 mM Tric-HCl, pH7.4. After mixing, the enzyme reaction was allowed to proceed at room temperature for 10 minutes before being stopped by addition of 25 μ l of 3% SDS. Plates were read on an Envision plate reader with an excitation wavelength of 485nm and emission wavelength of 535 nm to quantify the amount of cleavage product.

[0183] Screening of 1,135 human NOTUM immunoreactive hybridomas from Campaign 1 yielded three antibodies that showed greater than 70% inhibition of human NOTUM. These three together with an additional five hybridomas exhibiting some degree of neutralization in the OPTS assay were selected for subcloning by limiting dilution and small scale purified antibody production by protein A affinity chromatography using 50ml conditioned medium from clonal hybridomas.

[0184] OPTS assay screening of 1,056 mouse NOTUM immunoreactive hybridomas identified from Campaign 2 yielded six antibodies that showed greater than 50% inhibition of mouse NOTUM. These six together with an additional six hybridomas exhibiting some degree of neutralization in the OPTS assay were selected for subcloning by limiting dilution and small scale

purified antibody production by protein A affinity chromatography using 50ml conditioned medium from clonal hybridomas.

6.4.2. Wnt Signaling Assay

[0185] NOTUM can act as a negative regulator of Wnt signaling. Antibody neutralizing activity, determined through the effect on Wnt signaling, was determined in a Wnt signaling assay, which uses CellSensor® technology and conditioned media prepared as follows. Plasmid containing human NOTUM in pcDNA3.1(+) vector was transfected into HEK293 cells and clones were selecting by growing in presence of 400 μ g/mL of G418. Condition media from these cells was used for the assay. L cells overexpressing and secreting Wnt3a into the conditioned media were purchased from ATCC.

[0186] The assay protocol was as follows. CellSensor®LEF/TCF-bla FreeStyle™ 293F cells (Invitrogen) were grown to near confluency in 15-cm plates in DMEM with 10% Dialyzed FBS, 5 µg/ml Blasticidin (Invitrogen, R210-01), 0.1 mM NEAA, 25 mM HEPES and 1×GPS. Cells were trypsinized by first rinsing with PBS, followed by addition of 5 mL trypsin and incubation of plates at room temperature for two minutes. A total of 10 mL of assay media (Opti-MEM, plus 0.5% dialyzed FBS, 0.1 mM NEAA, 1mM sodium pyruvate, 10 mM HEPES, 1x GPS) was then added per 15 cm plate. Cells were counted and suspended at 50,000 cells per mL. Cells were seeded into Biocoat 384-well plates (Fisher, Catalogue #356663) at a density of 10000 cells per 20 µL per well. After incubation of cells at 37°C for 3 hours, 10 µL of 30 mM LiCl in assay medium was added per well, followed by incubation at 37°C overnight. The next day, 15 μL of antibody and 15 μL of purified NOTUM, both in assay medium, were coincubated in a total volume of 45 µL assay medium at room temperature for 30 minutes in a 96-well plate. NOTUM was used in a concentration previously determined to give 50% inhibition in the assay, typically 25 nM. Following the 30 minute incubation, 15 µL of undiluted L-Wnt3a conditioned medium was added to the 45 μL antibody/NOTUM mixture, and 10 μl of the resulting mixture was added to the wells of the 384-well plate containing the CellSensor® cells, in quadruplicate. Controls included wells lacking any cells, wells lacking NOTUM, and wells lacking L-Wnt3a conditioned medium. The assay plate was incubated for 5 hours at 37°C to enable Wnt-mediated beta-lactamase upregulation, and then 8 µl LiveBLAzer™-FRET B/G Substrate (CCF4-AM, Invitrogen) was added to each well and the plate incubated in the dark at room temperature for 3 hours. Plates were then read on an Envision plate reader using an excitation wavelength of 400 nm and emission wavelengths of 460 nm and 535 nm.

6.5. <u>Characterization of NOTUM Neutralizing Antibodies</u>

[0187] Antibodies purified from clonal hybridomas were characterized with respect to their species cross-reactivity by ELISA, their ability to recognize reduced, denatured NOTUM protein

by Western blot, and their neutralizing potency in the cell-free OPTS assay and the cell-based Wnt signaling assay, both of which are described above in Example 6.4.

[0188] Functional testing of monoclonal antibodies from Campaign 1 revealed three antibodies, 1.802, 1.815, 1.846, that neutralize human NOTUM in both the OPTS and Wnt signaling assays with an IC₅₀ in the range of 1 to 10nM. These antibodies do not have any effect on the activity of mouse NOTUM and were shown by ELISA to bind human NOTUM but not mouse NOTUM. Furthermore, these antibodies recognized human NOTUM only weakly when NOTUM protein was passively adsorbed to the assay plate and were much more sensitive to anti-His displayed human NOTUM protein.

[0189] Table 1 shows the results of various characterization experiments for certain antibodies from Campaign 1. The data in the "Bin" column was generated using the method described in Example 6.6, below.

Table 1: Characterization of certain antibodies raised against human NOTUM

Antibody	Isotype	<u>Bin</u>	OPTS IC ₅₀ (nM; human NOTUM)	Wnt signaling IC ₅₀ (nM; human NOTUM)	OPTS IC ₅₀ (nM; mouse NOTUM)	Wnt signaling IC ₅₀ (nM; mouse NOTUM)	Mouse NOTUM binding	Western blot binding
1.802	lgG1	1	6.44	5.71	No inhibition	No inhibition	No	No
1.815	lgG1	1	7.62	6.88	No inhibition	nd	No	No
1.846	lgG2b	1	10.07	1.70	No inhibition	nd	No	No
1.731	lgG1	3	>166.67	15.52	196.74	No inhibition	Yes	Yes
1.655	lgG1	3	>166.67	nd	>166.67	nd	nd	Yes
1.168	lgG2a	4	56.61	No inhibition	No inhibition	nd	Yes	Yes
1.712	lgG2a	2	125.36	58.49	No inhibition	nd	Yes	Yes
1.807	lgG2a	2	nd	No inhibition	No inhibition	nd	Yes	Yes

[0190] Functional testing of monoclonal antibodies from Campaign 2 revealed interesting activity profiles. In particular, MAb 2.78 neutralized both mouse and human NOTUM in both the OPTS and Wnt signaling assays with an IC_{50} in the range of 3 to 50 nM while MAb 2.1029 neutralized both mouse and human NOTUM in the OPTS assay with an IC_{50} in the range of 5 to 30nM but only human NOTUM in the Wnt signaling assay with an IC_{50} of 14 nM. This latter observation was ascribed to there being some difference in the quality of the recombinant mouse and human NOTUM proteins. One known difference between the proteins is that recombinant mouse NOTUM exists as multimers/aggregates to a much greater extent than does recombinant human NOTUM. Neither 2.78 nor 2.1029 recognized reduced, denatured NOTUM protein by Western blotting and both were substantially more immunoreactive with anti-His displayed NOTUM than with passively adsorbed NOTUM.

[0191] Table 2 shows the results of various characterization experiments for certain antibodies from Campaign 2. The data in the "Bin" column was generated using the method described in Example 6.6, below.

Table 2: Characterization of certain antibodies raised against mouse NOTUM

Antibody	Isotype	Bin	OPTS IC ₅₀	Wnt signaling	OPTS IC50	<u>Wnt</u>	Human	Western
			(nM; mouse	IC ₅₀	(nM; human	signaling IC ₅₀	NOTUM	blot
			NOTUM)	(nM; mouse	NOTUM)	(nM; human	binding	binding
				NOTUM)		NOTUM)		
2.78	IgG2b	2	35.65	3.75	15.49	45.94	Yes	No
2.1029	IgG2a	3	29.19	No inhibition	5.77	14.02	Yes	No
2.816	IgG2a	3	31.70	No inhibition	No inhibition	39.11	Yes	No
2.856	lgG2b	3	37.70	No inhibition	No inhibition	No inhibition	Yes	No
2.1001	lgG2b	3	>166.67	No inhibition	No inhibition	No inhibition	No	Yes
2.55	lgG2a	1	26.13	No inhibition	No inhibition	No inhibition	Yes	Yes
2.1002	lgG2a	1	42.39	No inhibition	No inhibition	No inhibition	Yes	Yes
2.497	IgG2a	1	54.95	No inhibition	No inhibition	No inhibition	Yes	Yes
2.341	lgG2a	1	56.95	No inhibition	No inhibition	No inhibition	Yes	Yes
2.236	lgG2a	1	64.54	No inhibition	No inhibition	No inhibition	Yes	Yes
2.688	IgG2a	4	No inhibition	No inhibition	12.84 [‡]	No inhibition	Yes	No
2.1006	IgG2a	5	>166.67	No inhibition	>166.67 [‡]	No inhibition	Yes	Yes

[‡] Maximum inhibition ≈50%.

6.6. <u>Binding Competition Studies Using NOTUM Neutralizing Antibodies</u>

[0192] Antibodies from both immunization campaigns were assessed for their ability to interfere with each other's binding to NOTUM protein in an epitope binning assay. This assay was performed in an ELISA format using anti-His captured NOTUM protein. The captured NOTUM protein was incubated with an excess of an unlabelled NOTUM-specific antibody (the 'blocking' antibody) followed by addition of a biotinylated NOTUM-specific antibody (the 'probe' antibody). Binding of the probe antibody was measured using HRP conjugated to streptavidin. If the two antibodies compete for binding in the same epitope space or if the blocking antibody otherwise affects the ability of the probe antibody to bind, e.g., by allosteric interference, no signal is generated. If the two antibodies do not interfere with one another, a signal similar to that of the biotinylated antibody tested in the absence of blocking antibody is generated. Antibodies are tested in a reciprocal matrix format. Typically, a pair of antibodies will show the same level of interference regardless of which of the two is the blocking antibody and which is the probe antibody. Antibodies exhibiting similar profiles are assigned to the same epitope 'bin'.

[0193] Using this methodology it was shown that MAbs 1.802, 1.815, 1.846, 2.78, and 2.1029 all interfere with each other's binding to human NOTUM while they do not interfere with the binding of several other less potent neutralizers or non-neutralizers.

6.7. Epitope Mapping of NOTUM Neutralizing Antibodies

[0194] In an effort to map the amino acids involved in binding of human NOTUM-specific MAbs 1.802, 1.815, and 1.846, human/mouse chimeric NOTUM proteins were produced by transient transfection in HEK293F of expression constructs encoding NOTUM open reading frames with a mixture of human and mouse sequences. By Western blotting with anti-His antibody and by OPTS assay it was shown that conditioned media from these transfections contained functional NOTUM chimeras.

[0195] Figure 5 shows schematic representations of the human/mouse chimeric NOTUM proteins used in this experiment. The sequences of those proteins are shown in Section 7 (Table of Sequences). The conditioned media were used in ELISA format to determine antibody specificity. Based on loss of human-specific MAb binding to particular chimeras it was determined that MAbs 1.802, 1.815, and 1.846 (all of which are "Bin 1" antibodies) depend on human NOTUM amino acids between Q47 and M177 for binding. See Figure 5. Within this region, mouse and human NOTUM differ at five positions (R115K, D141S. R150K, R154H, and Y171H, based on the human sequence numbering). Human NOTUM point mutants were generated by transient transfection of constructs expressing human NOTUM with the mouse amino acid at each of these five positions and the point mutants were all shown to be functional in the OPTS assay. By ELISA, MAbs 1.802, 1.815, and 1.846 bound all point mutants except human NOTUM D141S, indicating that this amino acid is important for their binding to human NOTUM. Mouse NOTUM with the reciprocal point mutation, mouse NOTUM S148D was generated by transient transfection, shown to be active in the OPTS assay, and was shown to support binding of the human NOTUM-specific MAbs. Therefore, the species specificity of MAbs 1.802, 1.815, and 1.846 appears to be dependent upon the amino acid at position 141 in human NOTUM, which is aspartic acid in the native human NOTUM protein.

[0196] The chimera approach could not be used to map amino acids involved in binding of MAbs 2.78 or 2.1029 because those cross-react with both human and mouse NOTUM. Based on the finding that MAbs 1.802, 1.815, 1.846, 2.78, and 2.1029 interfere with one another's binding, alanine scanning mutagenesis of charged amino acid residues in the vicinity of human D141 was performed. Five human NOTUM mutants were constructed, each with a pair of charged residues mutated to alanines: human NOTUM N132A/R133A (SEQ ID NO: 96); human NOTUM E134A/N135A (SEQ ID NO: 97); human NOTUM D137A/R139A (SEQ ID NO: 98); human NOTUM R144A/R145A (SEQ ID NO: 99); and human NOTUM R150A/D151A (SEQ ID NO: 100). All five human mutants were effectively expressed and secreted after transient transfection. Four of the five mutants exhibited significant activity in the OPTS assay while the fifth (human NOTUM D137A/R139A) showed little to no activity. All five mutants were detected in ELISA format by at least some of the Campaign 1 and Campaign 2 MAbs. MAb 2.78 failed to bind human NOTUM D137A/R139A and human NOTUM R144A/R145A, while MAbs 1.802, 1.815, and 1.846 failed to bind only NOTUM R144A/R145A. MAb 2.1029 was immunoreactive with all five of the alanine mutants.

6.8. Binding Affinities of NOTUM Neutralizing Antibodies

[0197] Binding affinities of certain anti-NOTUM MAbs was determined using a Biacore 3000. In order to obtain meaningful affinity values for binding to multimeric mouse NOTUM protein, antibody FAb fragments were generated by digestion of whole IgG with the protease Ficin, followed by removal of undigested IgG and Fc fragments by protein A affinity chromatography.

Affinity values for binding of FAbs and whole IgG to human NOTUM corresponded, and their affinity values were in the single to low double digit nM range, as shown in Table 3.

Table 3: Binding affinity of certain antibodies raised against human and mouse NOTUM

Affinity for human NOTUM				
Antibody or	<u>K_D (nM)</u>	<u>k_{on} (M⁻¹sec⁻¹)</u>	k_{off} (M ⁻¹ sec ⁻¹)	
<u>fragment</u>				
1.802 lgG	1.42	2.57 x 10 ⁵	3.65 x 10 ⁻⁴	
1.802 Fab	0.91	8.99 x 10 ⁵	8.20 x 10 ⁻⁴	
2.78 lgG	17.6	4.79 x 10 ⁴	8.41 x 10 ⁻⁴	
2.78 Fab	15.4	8.77×10^4	1.36 x 10 ⁻³	
2.1029 lgG	5.99	1.51 x 10 ⁵	9.08 x 10 ⁻⁴	
	Affinity for mo	use NOTUM	***************************************	
Antibody or	<u>K_D (nM)</u>	k _{on} (M ⁻¹ sec ⁻¹)	$k_{off} (M^{-1} sec^{-1})$	
<u>fragment</u>				
1.802 Fab No binding observed				
2.78 Fab	4.99	3.91 x 10 ⁴	1.95 x 10 ⁻⁴	

6.9. Administration of NOTUM Neutralizing Antibodies to Mice

6.9.1. Administration of NOTUM Neutralizing Antibodies Weekly for 8 Weeks

[0198] Eight week old male F1 hybrid (129 x C57) mice were administered NOTUM neutralizing antibody 2.1029 or 2.78b, or a control antibody, by intraperitoneal injection at 30 mg/kg once per week for eight weeks. There were 12 mice per group. At the end of the study, the mice were sacrificed. Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0199] As shown in Figure 6, midshaft femur cortical thickness increased by 12% (P < 0.001) with administration of NOTUM neutralizing antibody 2.1029, and 16% (P < 0.001) with administration of NOTUM neutralizing antibody 2.78b, as compared to the control antibody.

6.9.2. Administration of NOTUM Neutralizing Antibody 2.1029 Weekly for 4 Weeks

[0200] Eight week old male F1 hybrid (129 x C57) mice were administered NOTUM neutralizing antibody 2.1029 by intraperitoneal injection at 3 mg/kg, 10 mg/kg, or 30 mg/kg once per week for four weeks. There were 10 mice per group. At the end of the study, the mice were sacrificed. Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0201] 'As shown in Figure 7, midshaft femur cortical thickness increased by 5% (P = 0.12) with administration of 30 mg/kg NOTUM neutralizing antibody 2.1029, relative to administration of control antibody.

6.9.3. Administration of NOTUM Neutralizing Antibody 2.78b Weekly for 4 Weeks

[0202] Eight week old male F1 hybrid (129 x C57) mice were administered NOTUM neutralizing antibody 2.78b by intraperitoneal injection at 3 mg/kg, 10 mg/kg, or 30 mg/kg once per week for four weeks. There were 10 mice per group in the first experiment. In a second experiment, NOTUM neutralizing antibody 2.78b was administered by intraperitoneal injection at 0.3 mg/kg, 1 mg/kg, or 3 mg/kg once per week for four weeks. There were 12 mice per group in the second experiment. At the end of each study, the mice were sacrificed. Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0203] As shown in Figure 8A, midshaft femur cortical thickness increased by 13% (P < 0.001), 17% (P < 0.001), and 16% (P < 0.001) with administration of 3 mg/kg, 10 mg/kg, and 30 mg/kg, respectively, of NOTUM neutralizing antibody 2.78b, relative to administration of control antibody, in the first experiment. As shown in Figure 8B, midshaft femur cortical thickness increased by 3% (P=0.46), 7% (P = 0.01), and 10% (P < 0.001) with administration of 0.3 mg/kg, 1 mg/kg, and 3 mg/kg, respectively, of NOTUM neutralizing antibody 2.78b, relative to administration of control antibody, in the second experiment.

6.9.4. Administration of NOTUM Neutralizing Antibody 2.78b Weekly for 4 Weeks with Zoledronate Pretreatment

[0204] 28-week old male F1 hybrid mice (129 x C57) were administered a single dose 50 μ g/kg zoledronate by intraperitoneal injection. Four weeks after the dose of zolendronate, the mice were administered 10 mg/kg NOTUM neutralizing antibody 2.78b by i.p. injection weekly for 4 weeks. At the end of each study, the mice were sacrificed. There were 11 or 12 mice per group. Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV. In addition, serum levels of PINP, which is a marker of bone formation, were measured using a commercially available ELISA assay (Immunodiagnostic Systems, Scottsdale, AZ) at day 7 after the first dose of Mab 2.78b.

[0205] As shown in Figure 9A, the midshaft femur cortical thickness increased by 10 μ m, or 4% (P = 0.31), in mice administered zoledronate and control antibody, relative to mice administered saline and control antibody. Midshaft femur cortical thickness increased by 23 μ m, or 9% (P < 0.001), in mice administered NOTUM neutralizing antibody 2.78b without zoledronate pretreatment, relative to mice administered saline and control antibody, and increased by 14 μ m, or 5% (P = 0.06), in mice administered NOTUM neutralizing antibody 2.78b with zoledronate pretreatment, relative to mice administered zeledronate and control antibody. Figure 9B shows that serum PINP levels decreased by 15 ng/mL, or 50% (P < 0.001) in mice administered zolendronate treatment and control antibody, relative to mice administered saline and control

antibody. PINP levels increased by 14 ng/mL, or 47% (P < 0.001) in mice administered NOTUM neutralizing antibody 2.78b without zoledronate pretreatment, relative to mice administered saline and control antibody, and increased by 12 ng/mL, or 79% (P < 0.001) in mice administered NOTUM neutralizing antibody 2.78b with zoledronate pretreatment, relative to mice administered zeledronate and control antibody.

6.9.5. Administration of NOTUM Neutralizing Antibody 2.78a for 4 Weeks

[0206] For this experiment, Mab 2.78 (also referred to as "2.78b"), which is an IgG2b antibody, was reformatted as an IgG2a antibody (IgG2a antibodies often have longer half-lives than IgG2b antibodies). Reformatted Mab 2.78 is referred to as "2.78a."

[0207] 13-week old male F1 hybrid mice (129 x C57) were administered NOTUM neutralizing antibody 2.78a by intraperitoneal injection at 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg once per week for four weeks. There were 10 or 12 mice per group. At the end of each study, the mice were sacrificed. Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0208] As shown in Figure 10, midshaft femur cortical thickness increased by 3% (P = 0.57), 7% (P = 0.02), 9% (P = 0.002), and 10% (P < 0.001) with administration of 0.3 mg/gk, 1 mg/kg, 3 mg/kg, and 10 mg/kg, respectively, of NOTUM neutralizing antibody 2.78a in that experiment.

6.9.6. Administration of NOTUM Neutralizing Antibody 2.78a Weekly or Biweekly for 12 Weeks

[0209] Ten week old male F1 hybrid mice (129 x C57) were administered a control antibody, 0.3 mg/kg NOTUM neutralizing antibody 2.78a by i.p. injection weekly for 12 weeks, or 1 mg/kg NOTUM neutralizing antibody 2.78a by i.p. injection every other week (biweekly) for 12 weeks or 24 weeks. There were twelve mice per administration group. At the end of each study, the mice were sacrificed. Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0210] As shown in Figure 11A, the midshaft femur cortical thickness increased by 6% (P < 0.001) and 9% (P < 0.001) in mice administered 0.3 mg/kg weekly and 1 mg/kg biweekly, respectively, of NOTUM neutralizing antibody 2.78a for 12 weeks. Similarly, as shown in Figure 11B, the midshaft humerus cortical thickness increased by 5% (P = 0.007) and 7% (P < 0.001) in mice administered 0.3 mg/kg weekly and 1 mg/kg biweekly, respectively, of NOTUM neutralizing antibody 2.78a for 12 weeks.

[0211] As shown in Figure 12A, the midshaft femur cortical thickness increased by 7% (P = 0.002) and 9% (P < 0.001) in mice administered 0.3 mg/kg weekly and 1 mg/kg biweekly, respectively, of NOTUM neutralizing antibody 2.78a for 24 weeks. As shown in Figure 12B, the

midshaft humerus cortical thickness increased by 3% (P = 0.09) and 8% (P < 0.001) in mice administered 0.3 mg/kg weekly and 1 mg/kg biweekly, respectively, of NOTUM neutralizing antibody 2.78a for 24 weeks. Finally, as shown in Figure 12C, the ninth rib cortical thickness increased by 7% (P = 0.02) and 9% (P = 0.003) in mice administered 0.3 mg/kg weekly and 1 mg/kg biweekly, respectively, of NOTUM neutralizing antibody 2.78a for 24 weeks.

6.10. Administration of NOTUM Neutralizing Antibodies to Ovariectomized Mice 6.10.1. Ovariectomy

[0212] Sixteen-week-old albino C57BL/6J female mice were ovariectomized or given sham surgery. Serum levels of PINP, which is a marker of bone formation, and CTX, which is a marker of bone resorption, were measured using a commercially available ELISA assay (Immunodiagnostic Systems, Scottsdale, AZ) in the interval after ovariectomy and before administration of NOTUM neutralizing antibody, to confirm that increased bone remodelling was occuring after ovariectomy.

[0213] Following surgery and prior to the start of treatment, ovariectomized mice showed increased bone remodeling relative to sham surgery mice, as shown in Table 4. Since trabecular bone contains many more bone cells than cortical bone, these data likely reflect primarily increased trabecular bone remodeling.

Table 4:	Bone marke	er levels i	following	surgery

Marker	Weeks after surgery	Sham surgery (N=10)	OVX surgery (N=10)	Statistics
PINP (ng/ml)	1	36.4 ± 0.9	50.6 ± 5.3	$\Delta = 39\%$ P = 0.02
CTX (ng/ml)	2	10.5 ± 0.9	14.1 ± 0.9	$\Delta = 33\%$ P = 0.01
PINP (ng/ml)	4	41.2 ± 2.3	54.8 ± 2.5	$\Delta = 33\%$ P = 0.001

6.10.2. Administration of NOTUM Neutralizing Antibody 2.78b to Ovariectomized Mice

[0214] NOTUM neutralizing antibody 2.78b or a control antibody was administered at 10 mg/kg by intraperitoneal injection once per week for 4 weeks, starting 8 weeks after surgery. The study included the treatment groups shown in Table 5.

Table 5: Treatment groups in ovariectomy (OVX) study

Number of mice	Surgery	Antibody
13	Sham	Control
13*	Sham	NOTUM
10	OVX	Control
11	OVX	NOTUM

^{*} There were originally 14 mice in this group, but one mouse died during the study.

[0215] To assess the location and extent of new bone formation, fluorochrome bone labels were administered on treatment days 7, 14, and 21 (i.e., with the 2nd, 3rd, and 4th treatments). Calcein, which fluoresces green, was administered on day 7; alizarin, which fluoresces red, was administered on day 14; and tetracycline, which fluoresces yellow, was administered on day 21.

The mice were sacrificed at the end of the 4 week treatment. Uterine weight at necropsy confirmed that the ovariectomy surgery was successful. (Data not shown.)

6.10.3. Bone Mass and Architecture in NOTUM Neutralizing Antibody-Treated Ovariectomized Mice

[0216] Bone mass and architecture were determined by microCT following necropsy, using a Scanco μ CT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV. The midshaft femur, LV5 vertebral body, and the femoral neck were scanned.

[0217] As shown in Figure 13A, the midshaft femur cortical thickness increased by 22 μ m, or 9%, in sham surgery mice administered NOTUM neutralizing antibody 2.78b, relative to sham surgery mice administered control antibody, and increased by 26 μ m, or 12%, in ovariectomized mice administered NOTUM neutralizing antibody 2.78b, relative to ovariectomized mice administered control antibody. As shown in Figure 13B, the midshaft femur mineralized bone area increased by 0.1 mm², or 11%, in sham surgery mice administered NOTUM neutralizing antibody 2.78b, relative to sham surgery mice administered control antibody, and increased by 0.08 mm², or 10%, in ovariectomized mice administered NOTUM neutralizing antibody 2.78b, relative to ovariectomized mice administered control antibody.

[0218] As shown in Figure 14A, the proportion in the LV5 vertebral body of total (cortical plus trabecular) bone volume to total volume increased by 9% in sham surgery mice administered NOTUM neutralizing antibody 2.78b, relative to sham surgery mice administered control antibody, and increased by 3% in ovariectomized mice administered NOTUM neutralizing antibody 2.78b, relative to ovariectomized mice administered control antibody. As shown in Figure 14B, the proportion in the LV5 vertebral body of cortical bone volume to total volume increased by 13% in sham surgery mice administered NOTUM neutralizing antibody 2.78b, relative to sham surgery mice administered control antibody, and increased by 9% in ovariectomized mice administered NOTUM neutralizing antibody 2.78b, relative to ovariectomized mice administered control antibody. As shown in Figure 14C, the proportion in the LV5 vertebral body of trabecular bone volume to total volume was not significantly affected by administration of NOTUM neutralizing antibody 2.78b in either the sham surgery mice or the ovariectomized mice.

[0219] Finally, as shown in Figure 15, the proportion of femoral neck bone volume to total volume increased by 4% in sham surgery mice administered NOTUM neutralizing antibody 2.78b, relative to sham surgery mice administered control antibody, and increased by 6% in ovariectomized mice administered NOTUM neutralizing antibody 2.78b, relative to ovariectomized mice administered control antibody.

6.10.4. Bone Histomorphometry in NOTUM Neutralizing Antibody-Treated Ovariectomized Mice

[0220] Femur shafts were embedded in methylmethacrylate using a rapid embedding protocol. *See* Brommage and Vafai, *Calcified Tissue Int'l* 67: 479 (2000). Midshaft cross-sections with a thickness of about 80 μm were prepared using a Leica SP1600 bone saw. Sections were then examined with an Olympus BX60 fluorescent microscope. Various bone histomorphometric parameters were determined using OsteoMeasure^{τM} software (OsteoMetrics, Decatur, GA). Both static parameters (such as bone area and thickness) and dynamic parameters (such as single label surface (SLS), mineral aposition rater (MAR), and bone formation rate (BFR)) were measured at 100x magnification.

[0221] Figure 16 shows the percentage of the endocortical surface of the midshaft femur cross-sections that were labeled with calcein, which was administered on day 7, with alizarin, which was administered on day 14, and with tetracycline, which was administered on day 21. Table 6 shows the statistical analysis of the data in Figure 16. Mice administered NOTUM neutralizing antibody 2.78b showed a significantly higher percentage of endocortical labeling at days 7 and 14 compared to mice administered control antibody.

Table 6: Two-factor ANOVA of Single-Label Surface %

Two-Factor ANOVA	<u>Day 7</u>	<u>Day 14</u>	<u>Day 21</u>
Effect of Ovariectomy	P = 0.16	P = 0.65	P = 0.28
Effect of Treatment	P < 0.001	P < 0.001	P = 0.02
Effect of Interaction	P = 0.66	P = 0.74	P = 0.77

[0222] Figure 17 shows the mineral appositional rate (A) and the volume-referent bone formation rate (B) of sham surgery and ovariectomized mice that were administered control antibody or NOTUM neutralizing antibody 2.78b. The mineral appositional rate (Figure 17A) was determined by measuring the distance between the calcein label (day 7) and the alizarin label (day 14) and dividing by 7 to obtain the "days 7 to 14 rate," and measuring the distance between the alizarin label (day 14) and the tetracycline label (day 21) and dividing by 7 to obtain the "days 14 to 21 rate." Table 7 shows the statistical analysis of the data in Figure 17A. Mice administered NOTUM neutralizing antibody 2.78b showed a greater rate of mineral apposition than mice administered control antibody during the time period from days 7 to 14.

Table 7: Two-factor ANOVA of Mineral Appositional Rate

Two-Factor ANOVA	Days 7 to 14	Days 14 to 21
Effect of Ovariectomy	P = 0.80	P = 0.70
Effect of Treatment	P < 0.001	P = 0.82
Effect of Interaction	P = 0.86	P = 0.02

[0223] The volume-referent bone formation rate (Figure 17B) was determined by standard calculations involving multiplying the endocortical mineralization surface (percentage of double-labeled surface plus one-half of the single labeled surface, derived from Figure 16) by the mineral apposition rate (see Figure 17A). The result is the bone formation rate divided by the bone volume, expressed as a percentage per 7 days. Table 8 shows the statistical analysis of the data in Figure 17B. As evident in Figure 17B, the bone formation rate per bone volume is significantly higher in mice administered NOTUM neutralizing antibody 2.78b than in mice administered control antibody.

Table 8: Two-factor ANOVA of Volume-Referent Bone Formation Rate

Two-Factor ANOVA	Days 7 to 14	Days 14 to 21
Effect of Ovariectomy	P = 0.95	P = 0.80
Effect of Treatment	P < 0.001	P < 0.001
Effect of Interaction	P = 0.39	P = 0.30

6.11. <u>Identification of Species Suitable for Testing NOTUM Neutralizing Antibodies</u>

[0224] Based upon multi-species protein sequence alignments taken from the public domain, it was predicted that MAbs 1.802, 1.815, and 1.846 would bind to guinea pig NOTUM and that this species might therefore be suitable for preclinical studies. To test this hypothesis, guinea pig NOTUM was cloned and expressed by transient transfection, and shown to be active in the OPTS assay. MAbs 1.802, 1.815, and 1.846 were found to bind to guinea pig NOTUM by ELISA and MAb 1.802 was shown to neutralize guinea pig NOTUM activity in the OPTS assay. MAb 2.78 bound guinea pig NOTUM with lower affinity than MAb 1.802, and had correspondingly lower inhibiting activity in the OPTS assay. MAb 2.1029 bound guinea pig NOTUM only weakly, and did not significantly inhibit it in the OPTS assay.

[0225] Cynomolgus and rhesus monkey NOTUM were cloned from cDNA preparations from those species. Analysis of the sequences revealed that the amino acid at the position equivalent to human NOTUM D141 is an asparagine, which is different from the amino acid at that position in both mouse and human NOTUM. Active (as determined by OPTS assay) cynomolgus and rhesus NOTUM proteins were generated by transient transfection, and it was found that MAb 1.802 neither binds nor inhibits either protein. An active human NOTUM point mutant, human NOTUM D141N, was generated by transient transfection, and it was found that MAb 1.802 does not bind to that human NOTUM point mutant.

[0226] MAb 2.78 bound both cynomolgus and rhesus NOTUM weakly by ELISA, but did not inhibit either protein significantly in the OPTS assay. In contrast, MAb 2.1029 bound both cynomolgus and rhesus monkey NOTUM by ELISA as well as it binds human NOTUM, and also inhibited both proteins in the OPTS assay as well as it inhibited human NOTUM.

6.12. Antibody Sequencing and Humanization

[0227] Heavy and light chain variable regions were sequenced by specific RT-PCR using total RNA from the relevant hybridoma cell line followed by sequencing of the PCR product. The heavy and light chain variable regions from four Campaign 1 antibodies: 1.731, 1.802, 1.815, and 1.846, and three Campaign 2 antibodies: 2.1029, 2.55, and 2.78, were sequenced. The variable region sequences, without signal sequences, for each of those antibodies are shown in Section 7 (Table of Sequences), below. Section 7 also shows the sequences for the heavy and light chain CDR1, CDR2, and CDR3 for each of those antibodies. The following table shows the SEQ ID NOs corresponding to the heavy and light chain variable regions, and to CDR1, CDR2, and CDR3, for each of those antibodies.

Table 9: SEQ ID NOs for heavy and light chain variable regions and CDRs

,	The state of the s	
Mouse antibody	Heavy chain variable	<u>Light chain variable</u>
	region SEQ ID NO	region SEQ ID NO
	(CDR1, CDR2, CDR3	(CDR1, CDR2, CDR3
	SEQ ID NOs)	SEQ ID NOs)
1.731	7 (9, 10, 11)	8 (12, 13, 14)
1.802	15 (17, 18, 19)	16 (20, 21, 22)
1.815	23 (25, 26, 27)	24 (28, 29, 30)
1.846	31 (33, 34, 35)	32 (36, 37, 38)
2.1029	39 (41, 42, 43)	40 (44, 45, 46)
2.55	47 (49, 50, 51)	48 (52, 53, 54)
2.78	55 (57, 58, 59)	56 (60, 61, 62)

[0228] Certain heavy and light chain CDRs were found to have high homology among two or more of the sequenced antibodies. MAbs 1.802 and 1.846 share an identical heavy chain CDR1 (GFTFSDYGMH; SEQ ID NOs: 17 and 33), while heavy chain CDR1 of MAb 1.815 (GFTFSDFGMH; SEQ ID NO: 25) differs from MAbs 1.802 and 1.846 by only one conservative amino acid substitution (Phenylalanine (F) in place of Tyrosine (Y)). The consensus sequence for the heavy chain CDR1 for those antibodies is therefore GFTFSDX₁GMH (SEQ ID NO: 90), wherein X₁ is F or Y. Heavy chain CDR3 of MAbs 1.802 and 1.846 differ by only one conservative amino acid substitution (histidine (H) versus asparagine (N)). The consensus sequence for the heavy chain CDR3 for those antibodies is therefore KX₂YNGGYFDV (SEQ ID NO: 91), wherein X₂ is H or N. MAbs 1.802 and 1.846 share an identical light chain CDR2 (LASNLES; SEQ ID NOs: 21 and 37), while light chain CDR2 of MAb 1.815 (LASDLES; SEQ ID NO: 29) differs from MAbs 1.802 and 1.846 by only one conservative amino acid substitution (aspartic acid (D) in place of asparagine (N)). The consensus sequence for the light chain CDR2 for those antibodies is therefore LASX₆LES (SEQ ID NO: 93), wherein X₆ is D or N. Finally, a consensus sequence for the light chain CDR1 for the three antibodies from Campaign 1, 1.802, 1.846, and 1.815, is RASKX₃VSX₄SGYSYX₅H (SEQ ID NO: 92), wherein X_3 is I or S, X_4 is T or E, and X_5 is M or I.

[0229] BLAST searching was performed against public databases to identify the human germline variable region sequences with greatest similarity to each of the mouse heavy and light

chain variable regions. Using the AbM definition, CDRs from the mouse variable regions were then grafted in silico into these human germline variable sequences in place of the human germline CDRs. The resulting humanized variable regions for five of the mouse antibodies (2.78, 2.1029, 1.802, 1.815, and 1.846) were synthesized with a 5' leader sequence encoding an in-frame signal peptide and cloned upstream of sequence encoding human IgG2 constant regions in the case of the heavy chain variable sequences or human kappa constant region in the case of the light chain variable sequences. The sequences for each of the humanized variable regions are shown in Section 7 (Table of Sequences), below, along with the sequences for the full-length humanized heavy and light chains (without the signal peptide).

[0230] Coding sequences for full length humanized heavy and light chains were subcloned into mammalian expression vectors and corresponding heavy and light chain constructs were cotransfected into CHO-S cells. The resulting conditioned media were checked by Western blotting with an anti-human secondary antibody to confirm expression and secretion of intact humanized antibody. The conditioned media were then tested in ELISA format to determine whether the humanized antibodies retained the capacity to bind human NOTUM protein. Humanized MAbs 1.802, 1.815, 1.846, and 2.1029 bound human NOTUM while humanized MAb 2.78 exhibited little to no binding to either human or mouse NOTUM.

[0231]

7. <u>Table of Sequences</u>

SEQ ID NO	Description	Sequence				
1	Human NOTUM		LSLLHCAGGS		~~	-
			GNMDSFMAQV			
		CNDGSPAGYY	LKESRGSRRW	LLFLEGGWYC	FNRENCDSRY	DTMRRLMSSR
			ILSSQPEENP			
		AFMGALIIQE	VVRELLGRGL	SGAKVLLLAG	SSAGGTGVLL	NVDRVAEQLE
			${\tt GLADSGWFLD}$			
			QEGEEWNCFF			
	1		RLYIQNLGRE			
		QVKGTSLPRA	LHCWDRSLHD	SHKASKTPLK	GCPVHLVDSC	PWPHCNPSCP
		TVRDQFTGQE	MNVAQFLMHM	GFDMQTVAQP	QGLEPSELLG	MLSNGS
2	Mouse NOTUM	MGGEVRVLLL	LGLLHWVGGS	EGRKTWRRRG	QQPPQPPPPP	PLPQRAEVEP
_		GAGQPVESFP	LDFTAVEGNM	DSFMAQVKSL	AQSLYPCSAQ	QLNEDLRLHL
		LLNTSVTCND	GSPAGYYLKE	SKGSRRWLLF	LEGGWYCFNR	ENCDSRYSTM
		RRLMSSKDWP	HTRTGTGILS	SQPEENPHWW	NANMVFIPYC	SSDVWSGASP
		KSDKNEYAFM	GSLIIQEVVR	ELLGKGLSGA	KVLLLAGSSA	GGTGVLLNVD
		RVAELLEELG	YPSIQVRGLA	DSGWFLDNKQ	YRRSDCIDTI	NCAPTDAIRR
		GIRYWSGMVP	ERCQRQFKEG	EEWNCFFGYK	VYPTLRCPVF	VVQWLFDEAQ
		LTVDNVHLTG	QPVQEGQWLY	IQNLGRELRG	TLKDVQASFA	PACLSHEIII
		RSYWTDVQVK	GTSLPRALHC	WDRSFHDSHK	ASKTPMKGCP	FHLVDSCPWP
ì		HCNPSCPTIR	DQFTGQEMNV	AQFLMHMGFD	VQTVAQQQGM	EPSKLLGMLS
		NGN				
3	Human NOTUM	MGRGVRVLLL	LSLLHCAGGS	EGRKTWRRRG	QQPPPPPRTE	AAPAAGQPVE
	S232A	SFPLDFTAVE	GNMDSFMAQV	KSLAQSLYPC	SAQQLNEDLR	LHLLLNTSVT
		CNDGSPAGYY	LKESRGSRRW	LLFLEGGWYC	FNRENCDSRY	DTMRRLMSSR
}		DWPRTRTGTG	ILSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNEY
		AFMGALIIQE	VVRELLGRGL	SGAKVLLLAG	SAAGGTGVLL	NVDRVAEQLE
		KLGYPAIQVR	GLADSGWFLD	NKQYRHTDCV	DTITCAPTEA	IRRGIRYWNG

VVPERCERGE QUGGENNOYF QYKVYETLEC PYETVYMED EROTTON CHOOPEVGEL RIYTQNIAGE LERHILDVEA SEARALESE LITESING OVKITSLIPRA LECTURSLIP SERASITILE LITESING OVKITSLIPRA LECTURSLIP SERASITILE LITESING TRADQPTGGE MYNAOPLAMIM GEOGRAPHY OF PRINCIPS SUBSESSION MAGGEVERULL LICLLEHVOGS BORKTHRIRG OCPPOPPEPP PLECARE GAGGOVESEP LIPTIAVENIM BEPRAOVISL AGSLYCCAQ QUINDELIR KINTSVICTOR SEPRATYLIKE RESISSERNLE LIDEGAMYON RENCOSKY KRUMSSKIMP HIRTOTICLIS SOPERPHIWM NANWFIPYC SEDUNGON KRUMELLERLG YPSIQVEGIA BEGNELDSKQ VYPELDCEPY VOWNLED LIVIDWHUTG QEVGEOGILY TONIGERIG TILKOVAGSEA PACLISHED REVYMTOVOK STISLPRAILIC MORFITIGHER ASKYPHKOLY VYPOUNCED LIVIDWHUTG QEVGEOGILY TONIGERIG TILKOVAGSEA PACLISHED RESYMTOVOK STISLPRAILIC MORFITIGHER ASKYPHKOLY FELLODE HCNPSCPTLE QPTGGENNY AQPLAMMING VOTVAQQQM BESKLIGA NOR SUBJECT OF THE PROPERTY OF THE PRO	2012/	7/1301				FC1/US2	4011/001/85
QUKSTSLPRA LIKCMESSLHD SHARSKTELK GCPHILVOSC PRIPECTOR TYROQPTSGE MWAQCHAIM GPROTYCAQ GGLEREALIS BLISMSS S29A mutant							
Mouse NOTUM MGGEVRYLLL LEGLEWYGGS EGRETWEREG OOFPOPPPP PLYORABE							
S239A mutont GAGGYEESP LDFIAVEGMM DEFMACYKEL LEGGMYCONE ENDESSIST RELINISTATION GSPAGYYLLE SKORRNELTHE LEGGMYCHEN ENDESSIST RELINISTATION GSPAGYYLLE SKORRNELTHE LEGGMYCHEN ENDESSIST STATEMING TREATMY GELLIGERY GELTAME							
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### TOYTONDGS ### AGYYLESKG SRRWLIFLEG GWYCFRENC DRYDTWS ### MSSKDWPOTE TOTGLISSOP EMPYWNANAN WYFPYCSSD WSGASSE KNEYVFMGAL IIREVVQELL GRGLSGAKVL LLAGSSAGGT GVLLNVDF EGLECLGYPA 10VRGLADSG WFLDNNGYRR TDCVDTVTCA PTEATRK YNNGWVSKOTS LPRALHCWR SLIDSHKASK THEOFYFVVQ WFDDADI DNAHLTGGPV OEGQMLVION LGHELRNTIK DVPASFAPAC LSHEITIF WTDVQVKGTS LPRALHCWR SLIDSHKASK THEORGYPHLV VBCPWPF PSCPTIRDQF TGQEMNVAQF LMHMGFDVQT VAQQQGLEPS KLLGMLSS Cynomolgus monkey NOTUM ### MGRGWPVILL LGLLHCAGGS EGKKTWRERG QQPPPPPRTE AAPAAGGO SFFLDFTAVE GMUDSPAQV KLAGGLYPC SAQQLMEDLR LHLLINTS CNDGSPAGYY LKESRGSRRW LLFLEGGTUS GASGGTGVLL NDRVABE ELGYPAIQVE GLADSGMPLD NKQYRHTDCV DTITCAPTEA IRRGITW VVPERCROP GEGEWENCFF GYKLYPTILGE CYPVQWMLPD EAQLTVOM LTGQPVQESQ RLYJONLGRE LRHPLKDVFA SAGGGTGVL NDRVABE ELGYPAIQVE GLADSGMPLD NKQYRHTDCV DTITCAPTEA IRRGITW VVPERCROP GREWNORD WKSLAGSLYPC SAQQLMEDLR LHLLINTS CNDGSPAGYY LKESRGSRRW LLFLEGGTWY CFNENCDGRY NTWRLMS ELGYPAIQVE GLADSGMPLD NKQYRHTDCV DTITCAPTEA IRRGITW VVPERCROP GREWNORD WKSLAGSLYPC SAGGGTGVL NDRVABE ELGYPAIQVE GLADSGMPLD NKQYRHTDCV DTITCAPTEA IRRGITW VVPERCROP GREWNORD WKSLAGSLYPC SAQQLMEDLR LHLLINTS CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FRENCODSRY NTWRRLMS AFMGALIUGE VVRELLEGGTWY GFNENCDGRY NTWRRLMS AFMGALIUGE VRELTHALVOPA SFAPACLSHE IIRSHWI VVPERCROP GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGITW VVPERCROP GRANGHTHM GFDVQTVAQQ GPEPSKLIG LPSDGS ### MAD 1.731 leavy chain variable region ### GTOLEVKRAD AAP ### WAD 1.731 heavy chain CDR3 ### MAD 1.731 heavy chain CDR3 ### MAD 1.731 heavy chain CDR3 ### MAD 1.731 light chain CD		1 0	QPVESFPLDF	TAVEGNMDSF	MAQVKSLAQS	LYPCSAQQLN	EDLRLHLLL
MSSKUMPOTR TOTGILSSOP EENPYWNNAN WYFIPYCSSD VWSGASSY KREYVPMOAL LIREWVOELL GRLGGAKVL LLAGSSAGGT GVLINVDE EQLEQLGYPA 1QVRGLABGS WFLDNGYRR TDCVDTVTCA PTEAIRK KREYVPMOAL TIREWVOELL GRLGGAKVL LLAGSSAGGT GVLINVDE EQLEQLGYPA 1QVRGLABGS WFLDNGYRR TDCVDTVTCA PTEAIRK WNDOVCKGTS DNAHLTGOPV OR WIFT TOCVDTVTCA PTEAIRK WNDOVCKGTS DRALHGWDR SLHDSHKASK TPLKGCPTFUV WRDGEDBAD WNDOVCKGTS DFRALHGWDR SLHDSHKASK TPLKGCPTFUV WRDGEDBAD WNDOWN SLHDBADQU KLHHUGFDVQT VAQQGLEPS KLLGMLSS GOMMSFWAQV LLHLLLAGGS EGKTWRERG QQPPPPRTE AAPAAGGT CNGSPAGYY LKESRGSRRN LLFLEGGWVC FNRENCDERY NTWRKLMS DMPRTRTGTC LISSOPEENP YWMNANNVEI PYCSSDVWSG ASKSEK AFMGALIIQE VVRELLGGGL SGAKVLLLAG SSAGGTGVLL NVDRVABE ELGYPATQVR GLADSGMFLD NKQVRHTDCV DTTCAPTEA ITRGITYP VVPECKROF OEGEBNOCFF GYKLYPTLRC PVFVVQWLFD EAQLTVDN LTGQPVQESQ RLYIQNLGRE LRHTLKDVPA SFAPACLSHE ITIRSHWY VVPECKROF OEGEBNOCFF GYKLYPTLRC PVFVVQWLFD EAQLTVDN TVRDQFTGQE WNVAQFLMHM GFDVQTVAQQ QGPEPSKLIG LPBDGS Rhesus macaque NOTUM MRGGVRVLLL LCLLHCAGGS EGRKTWRRRG QQPPPPRTE AAPAAGGT CNGGSPAGYY LKESRGSRRW LLFLEGGWVC FNRENCDSRY NTWRLMS DWPRTRTGTG ILSOPEENP YWNNANNVFI PYCSSDVWSG ASSKEN AFMGALIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVABE ELGYPATQVR GLADSGMFLD NKGYRRTDCV DTTTCAPTEA IRRGITYP VVPECKROF QEGEBNOCFF GYKLYPTLKC PVFVVQWLFD EAQLTVDN LTGQPVQESQ RLYTQNLGRE LRHTLKDVPA SFAPACLSHE ILINSTEN AFMGALIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVABE ELGYPATQVR GLADSGMFLD NKGYRRTDCV DTTTCAPTEA IRRGITYP VVPECKROF QEGEBNOCFF GYKLYPTLKC PVFVVQWLFD EAQLTVDN LTGQPVQESQ RLYTQNLGRE LRHTLKDVPA SFAPACLSHE ILINSTEN QVKGTSLPRA LHCWBRSLHD SKRNKYPLK GCPVHLVDSC PWPHCNPE TVRDQFTGGE MVVAQFIMM GFDVQTVAQQ QGPEPSKLIG LPSDGS MAD 1.731 light Chain variable region SVYMDYMG QGTSVTVSSA KTTP GYNGYSMDYMG QGTSVTVSSA KTTP SVWTQTFFF LLVSAGDRVT ITCKASQSVG DDVAWYQQKP GQSPTLLI GNYSMDYMG QGTSVTVSSA KTTP SVWTQTFFF LLVSAGDRVT ITCKASQSVG DDVAWYQQKP GGYPTLIG GNYSMDYMG QGTSVTVSSA KTTP SVWTQTFFF LLVSAGDRVT ITCKASQSVG DDVAWYQQKP GGYPTLIG MAD 1.731 leavy Chain CDR1 MAD 1.731 leavy Chain CDR2 MAD 1.731 leavy Chain CDR3 MAD 1.731 light Chain CDR2							
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Monkey NOTUM			i .				
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chain variable ISSGSRTVYY ADTVKGRFTI SRDNAKNTLS LQMTSLRSED TAMYYCAR	12	1					

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	region	YNGGYFDVWG TGTTVTVSSA KTTP
16	MAb 1.802 light chain variable region	DIVLTQSPAS LAVSLGQRAT ISCRASKIVS TSGYSYMHWY QQKPGQPPK LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSRELF TFGSGTKLEI KRADAAP
17	MAb 1.802 heavy chain CDR1	GFTFSDYGMH
18	MAb 1.802 heavy	YISSGSRTVY
19	MAb 1.802 heavy	KHYNGGYFDV
20	MAb 1.802 light chain CDR1	RASKIVSTSGYSYMH
21	MAb 1.802 light chain CDR2	LASNLES
22	MAb 1.802 light chain CDR3	QHSRELPPT
23	MAb 1.815 heavy	DVQLLESGGG LVQPGGSRKL SCAASGFTFS DFGMHWVRQA PEKGLEWVA
	chain variable region	SSSGGTTVYY ADTVKGRLTL SRDNSKNTLF LEMTSLRSED TAMYYCARA YDGGYFDCWG QGTSLTVSSA KTTPP
24	MAb 1.815 light	DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYSYIHWY QQKPGQPPK
	chain variable region	LIYLASDLES GVPARFSGSG SGAAFTLNIH PVEEEDAATY YCHHSRELP TFGSGTKLEI KRADAAP
25	MAb 1.815 heavy chain CDR1	GFTFSDFGMH
26	MAb 1.815 heavy chain CDR2	YSSSGGTTVY
27	MAb 1.815 heavy chain CDR3	ASYDGGYFDC
28	MAb 1.815 light chain CDR1	RASKSVSTSGYSYIH
29	MAb 1.815 light chain CDR2	LASDLES
30	MAb 1.815 light chain CDR3	HHSRELPFT
31	MAb 1.846 heavy chain variable region	EVQLVESGGD LVKPGGSLKL SCAASGFTFS DYGMHWLRQA PEKGLEWVA ISSGSTTLSY ANTMKGRFTI SRDNAKKTLS LQMTSLRSED TAIYYCARK YNGGYFDVWG TGTTVTVSSA KTTPP
32	MAb 1.846 light chain variable region	DIVLTQSPAS LVVSLGQRAT ISCRASKSVS ESGYSYMHWY QQKPGQPPK LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEGDATTY YCQHSRVLP TFGSGTKLEI KRADAAP
33	MAb 1.846 heavy chain CDR1	GFTFSDYGMH
34	MAb 1.846 heavy chain CDR2	YISSGSTTLS
35	MAb 1.846 heavy chain CDR3	KNYNGGYFDV
36	MAb 1.846 light chain CDR1	RASKSVSESGYSYMH
37	MAb 1.846 light chain CDR2	LASNLES
38	MAb 1.846 light : chain CDR3	QHSRVLPPT
39	MAb 2.1029 heavy chain variable region	QVQLKESGFG LVAPSQSLSI TCTVSGFSLT SYGVHWVRQP PGKGLEWLG IWAGGSTNYN SALMSRLSIS KDNSKSQVFL KMNSLQTDDT AIYFCARDG YGTIYAMDYW GQGTSVTVSS AKTTAPS
40	MAb 2.1029 light chain variable region	DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIY TSRLHSGVPS RFTGSGSGTD YSLTISNLEQ EDIATYFCQQ GKTLPRTFGG GTMLEIKRAD AAP
41	MAb 2.1029 heavy chain CDR1	GFSLTSYGVH
	MAb 2.1029 heavy	VIWAGGSTN

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	chain CDR2	
43	MAb 2.1029 heavy	DGDYGTIYAMDY
	chain CDR3	
44	MAb 2.1029 light chain CDR1	RASQDISNYLN
45	MAb 2.1029 light chain CDR2	YTSRLHS
46	MAb 2.1029 light chain CDR3	QQGKTLPRT
47	MAb 2.55 heavy	EVQLQQSGTV LARPGALVKM SCKASGYTFT SYWMHWVKQR PGQGLEWIGA
	chain variable region	IYPGKSDTRY NQKFKDKAKL TAVTSTSTAY MDLSSLTDED SAVYYCSRRY GNFYAMDYWG QGTSVTVSSA KTTAPS
48	MAb 2.55 light	SIVMTQTPKF LLVSAGDRVT MTCKASQSVS NDVAWYQQKP GQSPELLIYY
	chain variable region	ASDRYTGVPD RFTGSGYGTD FTLTISTVQA EDLAVYFCQQ DYSSPYTFGG GTKLETKRAD AAP
49	MAb 2.55 heavy chain CDR1	GYTFTSYWMH
50	MAb 2.55 heavy chain CDR2	AIYPGKSDTR
51	MAb 2.55 heavy chain CDR3	RYGNFYAMDY
52	MAb 2.55 light chain CDR1	KASQSVSNDVA
53	MAb 2.55 light chain CDR2	YASDRYT
54	MAb 2.55 light chain CDR3	QQDYSSPYT
55	MAb 2.78 heavy	DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWVRQA PEKGLEWVAY
	chain variable region	ITSGSGAIYY ADTVRGRFTI SRDTPKNTLF LQMTSLRSED TAMYYCARSA DGLDYWGQGT SVTVSSAKTT PPS
		DIOMEGODIA I VIJAVARENIM IMADIA GRATIA GIVARIA GIVARIA GIVARIA
56	MAb 2.78 light chain variable region	DIQMTQSPAS LYVSVGETVT ITCRASENIY SNLAWYQQKQ GKSPQLLVYG ATNLADGVPS RFSGSGSGTQ YSLKINSLKS EDFGSYYCQH FWGTPFTFGS GTKLEIKRAD AAP
57	MAb 2.78 heavy chain CDR1	GFTFSSFGMH
58	MAb 2.78 heavy chain CDR2	YITSGSGAIY
59	MAb 2.78 heavy chain CDR3	SADGLDY
60	MAb 2.78 light chain CDR1	RASENIYSNLA
61	MAb 2.78 light chain CDR2	GATNLAD
62	MAb 2.78 light chain CDR3	QHFWGTPFT
63	Humanized Ab (HumAb) 2.78 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFGMHWVRQA PGKGLEWVSY ITSGSGAIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARSA DGLDYWGQGT TVTVSS
64	HumAb 2.78 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFGMHWVRQA PGKGLEWVSY ITSGSGAIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARSA DGLDYWGQGT TVTVSSDVWG QGTTVTVSSA STKGPSVFPL APCSRSTSES TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV TSSNFGTQTY TCNVDHKPSN TKVDKTVERK CCVECPPCPA PPVAGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VQFNWYVDGM EVHNAKTKPR EEQFNSTFRV VSVLTVVHQD WLNGKEYKCK VSNKGLPAPI EKTISKTKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK
65	HumAb 2.78 light	TTPPMLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK DIQMTQSPSS LSASVGDRVT ITCRASENIY SNLAWYQQKP GKAPKLLIYG
	chain variable region	ATNLADGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQH FWGTPFTFGQ GTKVEI
66	HumAb 2.78 light	DIQMTQSPSS LSASVGDRVT ITCRASENIY SNLAWYQQKP GKAPKLLIYG

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	chain	GTKVEIKRTV	AAPSVFIFPP ESVTEQDSKD	FTLTISSLQP SDEQLKSGTA STYSLSSTLT	SVVCLLNNFY	PREAKVQWK
67	HumAb 2.1029 heavy chain variable region	IWAGGSTNYN		TCTVSGFSLT VDTSKNQFSL		
68	HumAb 2.1029 heavy chain	IWAGGSTNYN YGTIYAMDYW TSESTAALGC VVTVTSSNFG SVFLFPPKPK TKPREEQFNS TKGQPREPQV	PSLKSRVTIS GQGTLVTVSS LVKDYFPEPV TQTYTCNVDH DTLMISRTPE TFRVVSVLTV YTLPPSREEM	TCTVSGFSLT VDTSKNQFSL DVWGQGTTVT TVSWNSGALT KPSNTKVDKT VTCVVVDVSH VHQDWLNGKE TKNQVSLTCL KLTVDKSRWQ	KLSSVTAADT VSSASTKGPS SGVHTFPAVL VERKCCVECP EDPEVQFNWY YKCKVSNKGL VKGFYPSDIA	AVYYCARDGI VFPLAPCSR: QSSGLYSLS: PCPAPPVAG: VDGMEVHNAI PAPIEKTISI VEWESNGQPI
69	HumAb 2.1029 light chain variable region	DIQMTQSPSS TSRLHSGVPS GTKVEI	LSASVGDRVT RFSGSGSGTD	ITCRASQDIS FTFTISSLQP	NYLNWYQQKP EDIATYYCQQ	GKAPKLLIY GKTLPRTFG0
70	HumAb 2.1029 light chain	TSRLHSGVPS GTKVEIKRTV	RFSGSGSGTD AAPSVFIFPP ESVTEQDSKD	ITCRASQDIS FTFTISSLQP SDEQLKSGTA STYSLSSTLT	EDIATYYCQQ SVVCLLNNFY	GKTLPRTFG PREAKVQWK
71	HumAb 1.802 heavy chain variable region	EVQLVESGGG ISSGSRTVYY YNGGYFDVWG	ADSVKGRFTI	SCAASGFTFS SRDNAKNSLY	DYGMHWVRQA LQMNSLRDED	PGKGLEWVS TAVYYCARK
72	HumAb 1.802 heavy chain	ISSGSRTVYY YNGGYFDVWG SESTAALGCL VTVTSSNFGT VFLFPPKPKD KPREEQFNST KGQPREPQVY	ADSVKGRFTI QGTLVTVSSD VKDYFPEPVT QTYTCNVDHK TLMISRTPEV FRVVSVLTVV TLPPSREEMT	SCAASGFTFS SRDNAKNSLY VWGQGTTVTV VSWNSGALTS PSNTKVDKTV TCVVVDVSHE HQDWLNGKEY KNQVSLTCLV LTVDKSRWQQ	LQMNSLRDED SSASTKGPSV GVHTFPAVLQ ERKCCVECPP DPEVQFNWYV KCKVSNKGLP KGFYPSDIAV	TAVYYCARK FPLAPCSRS' SSGLYSLSS' CPAPPVAGP, DGMEVHNAK' APIEKTISK' EWESNGOPE
73	HumAb 1.802 light chain variable region	DIVMTQSPDS LIYLASNLES TFGQGTKLEI	LAVSLGERAT GVPDRFSGSG	INCRASKIVS SGTDFTLTIS	TSGYSYMHWY SLQAEDVAVY	QQKPGQPPK YCQHSRELP
74	HumAb 1.802 light chain	LIYLASNLES TFGQGTKLEI	GVPDRFSGSG KRTVAAPSVF GNSQESVTEQ	INCRASKIVS SGTDFTLTIS IFPPSDEQLK DSKDSTYSLS	SLQAEDVAVY SGTASVVCLL	YCQHSRELP: NNFYPREAK
75	HumAb 1.815 heavy chain variable region	QVQLVESGGG SSSGGTTVYY YDGGYFDCWG	ADSVKGRFTI	SCAASGFTFS SRDNAKNSLY	DFGMHWIRQA LQMNSLRAED	PGKGLEWVS TAVYYCARA:
76	HumAb 1.815 heavy chain	SSSGGTTVYY YDGGYFDCWG SESTAALGCL VTVTSSNFGT VFLFPPKPKD KPREEQFNST KGQPREPQVY NYKTTPPMLD SLSLSPGK	ADSVKGRFTI QGTTVTVSSD VKDYFPEPVT QTYTCNVDHK TLMISRTPEV FRVVSVLTVV TLPPSREEMT SDGSFFLYSK	SCAASGFTFS SRDNAKNSLY VWGQGTTVTV VSWNSGALTS PSNTKVDKTV TCVVVDVSHE HQDWLNGKEY KNQVSLTCLV LTVDKSRWQQ	LQMNSLRAED SSASTKGPSV GVHTFPAVLQ ERKCCVECPP DPEVQFNWYV KCKVSNKGLP KGFYPSDIAV GNVFSCSVMH	TAVYYCARAS FPLAPCSRS: SSGLYSLSSV CPAPPVAGPS DGMEVHNAK: APIEKTISK: EWESNGQPEN EALHNHYTQI
77	HumAb 1.815 light chain variable region	DIVMTQSPDS LIYLASDLES TFGQGTKLEI	LAVSLGERAT GVPDRFSGSG	INCRASKSVS SGTDFTLTIS	TSGYSYIHWY SLQAEDVAVY	QQKPGQPPKI YCHHSRELPI
78	HumAb 1.815 light	DIVMTQSPDS	LAVSLOPPAT	TMCDAGKGUG	TECVEVIIIIV	OOKDGODDKI

	chain	LIYLASDLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHSRELF TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAK QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACE THQGLSSPVT KSFNRGEC
79	HumAb 1.846 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA PGKGLEWVS ISSGSTTLSY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARK YNGGYFDVWG QGTLVTVSS
80	HumAb 1.846 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA PGKGLEWVS ISSGSTTLSY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARK YNGGYFDVWG QGTLVTVSSD VWGQGTTVTV SSASTKGPSV FPLAPCSRS SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSS VTVTSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGF VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGMEVHNAK KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISK KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPE NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQ SLSLSPGK
81	HumAb 1.846 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASKSVS ESGYSYMHWY QQKPGQPPK LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSRVLE TFGQGTKLEI
82	HumAb 1.846 light chain	DIVMTQSPDS LAVSLGERAT INCRASKSVS ESGYSYMHWY QQKPGQPPK LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSRVLF TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAK QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACE THQGLSSPVT KSFNRGEC
90	Campaign 1 heavy chain CDR1 consensus	GFTFSDX ₁ GMH
91	Campaign 1 heavy chain CDR3 consensus	$\mathrm{KX}_{2}\mathrm{YNGGYFDV}$
92	Campaign 1 light chain CDR1 consensus	RASKX ₃ VSX ₄ SGYSYX ₅ H
93	Campaign 1 light chain CDR2 consensus	LASX ₆ LES
83	Human-mouse chimeric NOTUM	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPV SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLINTSV CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSS DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNE AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQL KLGYPAIQVR GLADSGWFLD NKQYRRSDCI DTINCAPTDA IRRGIRYWS MVPERCQRQF KEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNV LTGQPVQEGQ WLYIQNLGRE LRGTLKDVQA SFAPACLSHE IIIRSYWTD QVKGTSLPRA LHCWDRSFHD SHKASKTPMK GCPFHLVDSC PWPHCNPSC TIRDQFTGQE MNVAQFLMHM GFDVQTVAQQ QGMEPSKLLG MLSNGN
84	Mouse-human chimeric NOTUM	MGGEVRVLLL LGLLHWVGGS EGRKTWRRRG QQPPQPPPPP PLPQRAEVE GAGQPVESFP LDFTAVEGNM DSFMAQVKSL AQSLYPCSAQ QLNEDLRLH LLNTSVTCND GSPAGYYLKE SKGSRRWLLF LEGGWYCFNR ENCDSRYST RRLMSSKDWP HTRTGTGILS SQPEENPHWW NANMVFIPYC SSDVWSGAS KSDKNEYAFM GSLIIQEVVR ELLGKGLSGA KVLLLAGSSA GGTGVLLNV RVAELLEELG YPSIQVRGLA DSGWFLDNKQ YRHTDCVDTI TCAPTEAIR GIRYWNGVVP ERCRRQFQEG EEWNCFFGYK VYPTLRCPVF VVQWLFDEA LTVDNVHLTG QPVQEGLRLY IQNLGRELRH TLKDVPASFA PACLSHEII RSHWTDVQVK GTSLPRALHC WDRSLHDSHK ASKTPLKGCP VHLVDSCPW HCNPSCPTVR DQFTGQEMNV AQFLMHMGFD MQTVAQPQGL EPSELLGML NGS
85	Human-mouse- human chimeric NOTUM	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPV SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSV CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSS DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASPKSDKNE AFMGSLIIQE VVRELLGKGL SGAKVLLLAG SSAGGTGVLL NVDRVAELL ELGYPSIQVR GLADSGWFLD NKQYRRSDCI DTINCAPTDA IRRGIRYWS MVPERCQRQF KEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNV

0 2012	2/0/1381 PC1/US2011/061/85					2011/001/05
		LTGQPVQEGL F				
		QVKGTSLPRA I	LHCWDRSLHD	SHKASKTPLK	GCPVHLVDSC	PWPHCNPSCP
		TVRDQFTGQE N	MNVAQFLMHM	GFDMQTVAQP	QGLEPSELLG	MLSNGS
86	Mouse-human-	MGGEVRVLLL I	LGLLHWVGGS	EGRKTWRRRG	OOPPOPPPP	PLPCRAEVEP
	mouse chimeric	GAGQPVESFP I	LDFTAVEGNM	DSFMAQVKSL	AQSLYPCSAO	OLNEDLRLHL
	NOTUM	LLNTSVTCND (SPAGYYLKE	SKGSRRWLLF	LEGGWYCFNR	ENCDSRYSTM
		RRLMSSKDWP H	HTRTGTGILS	SQPEENPHWW	NANMVFIPYC	SSDVWSGASS
		KSEKNEYAFM (GALIIQEVVR	ELLGRGLSGA	KVLLLAGSSA	GGTGVLLNVD
		RVAEQLEKLG Y	/PAIQVRGLA	DSGWFLDNKQ	YRHTDCVDTI	TCAPTEAIRR
		GIRYWNGVVP E	ERCRRQFQEG	EEWNCFFGYK	VYPTLRCPVF	VVQWLFDEAQ
		LTVDNVHLTG Ç)PVQEGQWLY	IQNLGRELRG	TLKDVQASFA	PACLSHEIII
		RSYWTDVQVK C	STSLPRALHC	WDRSFHDSHK	ASKTPMKGCP	FHLVDSCPWP
		HCNPSCPTIR I	QFTGQEMNV	AQFLMHMGFD	VQTVAQQQGM	EPSKLLGMLS
		NGN				
87	Human NOTUM	MPLLLLLPLL V	VAGALAOPVE	SEPLDETAVE	GNMDSEMAOV	KGI VOGI VDC
-	(Δ1-46); CD33	SAQQLNEDLR I	HLLLNTSVT	CNDGSPAGYY	LKESEGSERW	I.I.FI.ECCWVC
	signal peptide in	FNRENCDSRY I	TMRRLMSSR	DWPRTRTGTG	TISSOPEEND	THIMINAMINET
	italics	PYCSSDVWSG A	ASSKSEKNEY	AFMGALITIOE	VVRELLGRGI.	SCAKVIJIAC
	italics	SSAGGTGVLL N	IVDRVAEOLE	KLGYPATOVR	GLADSGWELD	DOUG THE PORT OF T
		DTITCAPTEA I	RRGTRYWNG	VVPERCRROF	OEGEEWNCEE	CAKAADATEC
		PVFVVQWLFD E	HVMOVTJOAS	LTGOPVOEGL	PLVIONI.CPF	I.DUTT KOMMA
		SFAPACLSHE I	IIRSHWTDV	OVKGTSLPRA	I.HCWDRSI.HD	SHKY SKADI'K
		GCPVHLVDSC P	WPHCNPSCP	TVRDOFTGOE	MNVAOFLMHM	CEDMOTVAOD
		QGLEPSELLG M		1111211021	. moving i millio	GI DNQI VAQI
		Manathan				
88	Human NOTUM	MGRGVRVLLL I	SLLHCAGGS	EGRKTWRRRG	QQPPPPPRTE	AAPAAGQPVE
	N96D	SFPLDFTAVE G	MINDSFMAQV	KSLAQSLYPC	SAQQLNEDLR	LHLLLDTSVT
		CNDGSPAGYY L	KESRGSRRW	LLFLEGGWYC	FNRENCDSRY	DTMRRLMSSR
		DWPRTRTGTG I	LSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNEY
		AFMGALIIQE V	VKELLGRGL	SGAKVLLLAG	SSAGGTGVLL	NVDRVAEQLE
		KLGYPAIQVR G	LADSGWFLD	NKQYRHTDCV	DTITCAPTEA	IRRGIRYWNG
		VVPERCRRQF Q	EGEEWNCFF	GYKVYPTLRC	PVFVVQWLFD	EAQLTVDNVH
		LTGQPVQEGL R	TITONDOLITO	TKHILKUVPA	SFAPACLSHE	IIIRSHWTDV
		QVKGTSLPRA I TVRDQFTGQE M	INTO A DES MUM	CEDMOTINOD	GCPVHLVDSC	PWPHCNPSCP
89	Human NOTUM	QPVE SFPLDFT	AVE GNMDSF	MAQV KSLAQS	SLYPC SAQQLI	IEDLR
	Q47-M177	LHLLLNTSVT C	NDGSPAGYY	LKESRGSRRW	LLFLEGGWYC	FNRENCDSRY
		DTMRRLMSSR D	WPRTRTGTG	ILSSQPEENP	YWWNANM	
94	Human NOTUM	MGRGVRVLLL L	SLLHCAGGS	EGRKTWRRRG	QQPPPPPRTE	AAPAAGOPVE
	D141S	SFPLDFTAVE G	NMDSFMAQV	KSLAQSLYPC	SAQQLNEDLR	LHLLLNTSVT
		CNDGSPAGYY L	KESRGSRRW	LLFLEGGWYC	FNRENCDSRY	STMRRLMSSR
		DWPRTRTGTG I	LSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNEY
		AFMGALIIQE V	VRELLGRGL	SGAKVLLLAG	SSAGGTGVLL	NVDRVAEQLE
		KLGYPAIQVR G	LADSGWFLD	NKQYRHTDCV	DTITCAPTEA	IRRGIRYWNG
		VVPERCRRQF Q	EGEEWNCFF	GYKVYPTLRC	PVFVVQWLFD	EAQLTVDNVH
		LTGQPVQEGL R	LYIQNLGRE	LRHTLKDVPA	SFAPACLSHE	IIIRSHWTDV
		QVKGTSLPRA L	HCWDRSLHD	SHKASKTPLK	GCPVHLVDSC	PWPHCNPSCP
		TVRDQFTGQE M	NVAQFLMHM	GFDMQTVAQP	QGLEPSELLG	MLSNGS
95	Mouse NOTUM	MGGEVRVLLL L	GLLHWVGGS	ECEKTWEEEG	OODDODDDD	DI DODARIJED
55	S148D	GAGQPVESFP L	DELTARECAM	DGEMPOAKET	AUGI ADGGAO	OLNEDIDIU
	31460	LLNTSVTCND G	SDAGVVLKE	SKGSDDWIJE	I ECCMVCEND	OPNEDTKPHP
		RRLMSSKDWP H	TRICTIERE	SUDDEMBRING	DEGGWICINK	ENCUSKIDIM
		KSDKNEYAFM G	STITTOEVVD	FILGEGIAGA	NAMMOLIPIC	COMOUTING
		RVAELLEELG Y	PSTOVEGLA	DSGWET DNKO	VADDODOTOUT	AGIGATTINAD
		GIRYWSGMVP E	BCOBOEKEG	PEGMICEEGAN	TKKPDCIDII	NCAPIDAIRK
		LTVDNVHLTG Q	PVOECOWI.V	TONLOPFIE	TINDUONCEN	DAGLGHELLT
		RSYWTDVQVK G	TSLPRALHC	WDRSFHDSHK	AGREDANKGCD	PACTOUETTI
		HCNPSCPTIR D	OFTGOEMNY	AOFLMHMGFD	VOTVACOOCM	EDGALICMIC
		NGN	z- TOSTILITA	TAT DIMINOLD	A A T A POO TO ONLY	CTMPHT7C 3 T
0.0	11 1/07:1-		OT 7 7 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		-79	
96	Human NOTUM	MGRGVRVLLL L	SLLHCAGGS	EGRKTWRRRG	QQPPPPPRTE	AAPAAGQPVE
	N132A/R133A	SFPLDFTAVE G	NMDSFMAQV	KSLAQSLYPC	SAQQLNEDLR	LHLLLNTSVT
		CNDGSPAGYY L	KESRGSRRW	LLFLEGGWYC	FAAENCDSRY	DTMRRLMSSR
		DWPRTRTGTG I	LSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNEY
		AFMGALIIQE V	VRELLGRGL	SGAKVLLLAG	SSAGGTGVLL	NVDRVAEQLE
		KLGYPAIQVR G	LADSGWFLD	NKQYRHTDCV	DTITCAPTEA	IRRGIRYWNG
		VVPERCRRQF Q	EGEEWNCFF	GYKVYPTLRC	PVFVVQWLFD	EAQLTVDNVH
	1	LTGQPVQEGL R	LYIQNLGRE	LRHTLKDVPA	SFAPACLSHE	IIIRSHWTDV

		1		SHKASKTPLK		
		TVRDQFTGQE	MNVAQFLMHM	GFDMQTVAQP	QGLEPSELLG	MLSNGS
97	Human NOTUM	MGRGVRVLLL	LSLLHCAGGS	EGRKTWRRRG	QQPPPPPRTE	AAPAAGOPV
	E134A/N135A	SFPLDFTAVE	GNMDSFMAQV	KSLAQSLYPC	SAQQLNEDLR	LHLLLNTSV
		CNDGSPAGYY	LKESRGSRRW	LLFLEGGWYC	FNRAACDSRY	DTMRRLMSS
		DWPRTRTGTG	ILSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNE
				SGAKVLLLAG		
				NKQYRHTDCV		
				GYKVYPTLRC		
				LRHTLKDVPA		
		QVKGTSLPRA	LHCWDRSLHD	SHKASKTPLK	GCPVHLVDSC	PWPHCNPSC
				GFDMQTVAQP		
98	Human NOTUM	MGRGVRVLLL	LSLLHCAGGS	EGRKTWRRRG	OOPPPPPRTE	AAPAAGOPV
	D137A/R139A			KSLAQSLYPC		
		CNDGSPAGYY	LKESRGSRRW	LLFLEGGWYC	FNRENCASAY	DTMRRLMSS
				YWWNANMVFI		
				SGAKVLLLAG		
		KLGYPAIQVR	GLADSGWFLD	NKQYRHTDCV	DTITCAPTEA	IRRGIRYWN
				GYKVYPTLRC		
				LRHTLKDVPA		
				SHKASKTPLK		
				GFDMQTVAQP		
99	Human NOTUM			EGRKTWRRRG		
	R144A/R145A	SFPLDFTAVE	GNMDSFMAQV	KSLAQSLYPC	SAQQLNEDLR	LHLLLNTSV
				LLFLEGGWYC		
		DWPRTRTGTG	ILSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNE
		AFMGALIIQE	VVRELLGRGL	SGAKVLLLAG	SSAGGTGVLL	NVDRVAEQI
		KLGYPAIQVR	GLADSGWFLD	NKQYRHTDCV	DTITCAPTEA	IRRGIRYWN
		VVPERCRRQF	QEGEEWNCFF	GYKVYPTLRC	PVFVVQWLFD	EAQLTVDNV
		LTGQPVQEGL	RLYIQNLGRE	LRHTLKDVPA	SFAPACLSHE	IIIRSHWTI
				SHKASKTPLK		
				GFDMQTVAQP		
100	Human NOTUM	MGRGVRVLLL	LSLLHCAGGS	EGRKTWRRRG	QQPPPPPRTE	AAPAAGQPV
	R150A/D151A			KSLAQSLYPC		
				LLFLEGGWYC		
		AWPRTRTGTG	ILSSQPEENP	YWWNANMVFI	PYCSSDVWSG	ASSKSEKNE
		AFMGALIIQE	VVRELLGRGL	SGAKVLLLAG	SSAGGTGVLL	NVDRVAEQI
				NKQYRHTDCV		
		VVPERCRRQF	QEGEEWNCFF	GYKVYPTLRC	PVFVVQWLFD	EAQLTVDNV
				LRHTLKDVPA		
		QVKGTSLPRA	LHCWDRSLHD	SHKASKTPLK	GCPVHLVDSC	PWPHCNPSC
		TVRDQFTGQE	MNVAQFLMHM	GFDMQTVAQP	QGLEPSELLG	MLSNGS
101	1.802 heavy chain			AGTTTTCCTT		
	variable region			TGGAGTCTGG		
	polynucleotide	GAGGGTCCCT	GAAACTCTCC	TGTGCAGCCT	CTGGATTCAC	TTTCAGTGA
	sequence			TCAGGCTCCA		
				GTAGAACCGT		
				${\tt AGAGACAATG}$		
				TGAGGACACG		
				ACTTCGATGT		
				ACGACACCCC		
		CCTGGATCTG	CTGCCCAAAC	TAACTCCATG	GTGACCCTGG	GATGC
102	1.802 light chain	1		GAGATGGAGA		
102			T	Δ CCTTCC Δ CT	CCTCACATTC	TCCTCACAC
	variable region	GTACTGCTGC				
		GTCTCCTGCT	TCCTTAGCTG	TATCTCTGGG	GCAGAGGGCC	ACCATCTCA
	variable region	GTCTCCTGCT GCAGGGCCAG	TCCTTAGCTG CAAAATTGTC	TATCTCTGGG AGTACATCTG	GCAGAGGGCC GCTATAGTTA	ACCATCTCA TATGCACTG
	variable region polynucleotide	GTCTCCTGCT GCAGGGCCAG TACCAACAGA	TCCTTAGCTG CAAAATTGTC AACCAGGACA	TATCTCTGGG AGTACATCTG GCCGCCCAAA	GCAGAGGGCC GCTATAGTTA CTCCTCATCT	ACCATCTCA TATGCACTC ATCTTGCAT
	variable region polynucleotide	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG
	variable region polynucleotide	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG TGCTGCAAC
	variable region polynucleotide	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC TATTACTGTC	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC AGCACAGTAG	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG GGAGCTTCCT	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA CCCACGTTCG	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG TGCTGCAAC GCTCGGGGA
	variable region polynucleotide	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC TATTACTGTC AAAGTTGGAA	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC AGCACAGTAG ATAAAACGGG	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG GGAGCTTCCT CTGATGCTGC	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA CCCACGTTCG ACCAACTGTA	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG TGCTGCAAC GCTCGGGGA
	variable region polynucleotide	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC TATTACTGTC AAAGTTGGAA	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC AGCACAGTAG ATAAAACGGG	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG GGAGCTTCCT	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA CCCACGTTCG ACCAACTGTA	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG TGCTGCAAC GCTCGGGGA
103	variable region polynucleotide sequence	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC TATTACTGTC AAAGTTGGAA CACCATCCAG	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC AGCACAGTAG ATAAAACGGG TGAGCAGTTA	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG GGAGCTTCCT CTGATGCTGC ACATCTGGAG CTGGATTCCC	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA CCCACGTTCG ACCAACTGTA GT	ACCATCTCA TATGCACTC ATCTTGCAT GGGTCTGGG TGCTGCAAC GCTCGGGGA TCCATCTTC ATTCAGTGA
103	variable region polynucleotide sequence	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC TATTACTGTC AAAGTTGGAA CACCATCCAG TCTGACAGAG CAGCACTGAA	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC AGCACAGTAG ATAAAACGGG TGAGCAGTTA GAGCCAAGCC CACAGACCAC	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG GGAGCTTCCT CTGATGCTGC ACATCTGGAG CTGGATTCCC TCACCATGGA	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA CCCACGTTCG ACCAACTGTA GT AGGTCCTCAC CTCCAGGCTC	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG TGCTGCAAC GCTCGGGGA TCCATCTTC ATTCAGTGA AATTTAGTT
103	variable region polynucleotide sequence	GTCTCCTGCT GCAGGGCCAG TACCAACAGA CAACCTAGAA CAGACTTCAC TATTACTGTC AAAGTTGGAA CACCATCCAG TCTGACAGAG CAGCACTGAA	TCCTTAGCTG CAAAATTGTC AACCAGGACA TCTGGGGTCC CCTCAACATC AGCACAGTAG ATAAAACGGG TGAGCAGTTA GAGCCAAGCC CACAGACCAC TATTTTAAAA	TATCTCTGGG AGTACATCTG GCCGCCCAAA CTGCCAGGTT CATCCTGTGG GGAGCTTCCT CTGATGCTGC ACATCTGGAG CTGGATTCCC	GCAGAGGGCC GCTATAGTTA CTCCTCATCT CAGTGGCAGT AGGAGGAGGA CCCACGTTCG ACCAACTGTA GT AGGTCCTCAC CTCCAGGCTC GTGATGTGCA	ACCATCTCA TATGCACTG ATCTTGCAT GGGTCTGGG TGCTGCAAC GCTCGGGGA TCCATCTTC ATTCAGTGA AATTTAGTT ACTGCTGGA

<i>J</i> 2012/(0/1301				FC1/05	2011/001/85
	sequence	AGCCTCTGGA	TTCACTTTCA	GTGACTTTGG	AATGCACTGG	GTTCGTCAG
		CTCCAGAGAA	GGGGCTGGAG	TGGGTCGCAT	ACAGTAGTAG	TGGCGGTAC
		ACCGTCTACT	ATGCAGACAC	GGTGAAGGGC	CGACTCACCC	TCTCCAGAG
				TCCTGGAAAT		
				GCAAGAGCGT		
		GACTGCTGGG	GCCAAGGCAC	CTCTCTCACA	GTCTCCTCAG	CCAAAACGA
		ACCCCCATCT	GTCTATCCAC	TGGCCCCTGG	ATCTGCTGCC	CAAACTAAC
		CCATGGTGAC	CCTGGGATGC			
104	1.815 light chain	ATCCTCTCTT	CCAGCTCTCA	GAGATGGAGA	CAGACACACT	CCTGTTATG
	variable region	GTACTGCTGC	TCTGGGTTCC	AGGTTCCACT	GGTGACATTG	TGCTGACAC
	polynucleotide	GTCTCCTGCT	TCCTTAGCTG	TATCTCTGGG	GCAGAGGGCC	ACCATCTCA
	sequence			AGTACATCTG		
	Jequence			GCCACCCAAA		
		CGACCTAGAA	TCTGGGGTCC	CTGCCAGGTT	CAGTGGCAGT	GGATCTGGG
		CAGCCTTCAC	CCTCAACATC	CATCCTGTGG	AGGAGGAGGA	TGCTGCAAC
		TATTACTGTC	ACCACAGTAG	GGAGCTTCCA	TTCACGTTCG	GCTCGGGGA
		AAAGTTGGAA	ATAAAACGGG	CTGATGCTGC	ACCAACTGTA	TCCATCTTC
				ACATCTGGAG		
105	1.846 heavy chain	AGAGGAGCCA	AACCCTGGAT	TCCCAGGTCC	TCACATTCAG	TGATCAGCA
	variable region			TGGACTCCAG		
	polynucleotide	TCCTTATTTT	AAAAGGTGTC	CAGTGTGAGG	TGCAGCTGGT	GGAGTCTGG
	sequence	GGAGACTTAG	TGAAGCCTGG	AGGGTCCCTG	AAACTCTCCT	GTGCAGCCT
	sequence			ATGGAATGCA		
		AGAAGGGGCT	GGAGTGGGTT	GCATATATTA	GTAGTGGCAG	TACTACCCT
				GGGCCGATTC		
		CAAGAAAACC	CTGTCCCTGC	AAATGACCAG	TCTGAGGTCT	GAGGACACO
		CCATTTATTA	CTGTGCGCGG	AAAAATTACA	ACGGTGGTTA	CTTCGATGT
		TGGGGCACAG	GGACCACGGT	CACCGTCTCC	TCAGCCAAAA	CAACACCC
				CTGGGTGTGG		
			ATGCCTGGTC			
106	1.846 light chain	ATCCTCTCTT	CCAGCTCTCA	GAGATGGAGA	CAGACACACT	ССТСТТАТС
	variable region			AGGTTCCACT		
	polynucleotide	GTCTCCTGCT	TCCTTAGTTG	TATCTCTGGG	GCAGAGGGCC	ACCATCTC
	1	GCAGGGCCAG	CAAAAGTGTC	AGTGAATCTG	GCTATAGTTA	TATGCACTO
	sequence	TACCAACAGA	AACCAGGACA	GCCACCCAAA	CTCCTCATCT	ATCTTGCAT
		CAACCTAGAG	TCTGGGGTCC	CTGCCAGGTT	CAGTGGCAGT	GGGTCTGGG
		CAGACTTCAC	CCTCAACATC	CATCCTGTGG	AGGAGGGGGA	TGCTACAAC
				GGTCCTTCCT		
		AAAGTTGGAA	ATAAAACGGG	CTGATGCTGC	ACCAACTGTA	TCCATCTTC
				ACATCTGGAG		
107	2.78 heavy chain	GACAGAGGAG	CCAAGCCCTG	GATTCCCAGG	TCCTCACATT	CAGTGATCA
20,	variable region			CCATGGACTC		
	polynucleotide	TTGTCCTTAT	TTTAAAAGGT	GTCCAGTGTG	ATGTGCAGCT	GGTGGAGTC
	1 '	GGGGGAGGCT	TAGTGCAGCC	TGGAGGGTCC	CGGAAACTCT	CCTGTGCAG
	sequence	CTCTGGATTC	ACTTTCAGTA	GCTTTGGCAT	GCACTGGGTT	CGTCAGGCT
		CAGAGAAGGG	ACTGGAGTGG	GTCGCATACA	TTACTAGTGG	CAGTGGTGC
		ATCTACTATG	CAGACACAGT	GAGGGGCCGA	TTCACCATCT	CCAGAGACA
		TCCCAAGAAC	ACCCTGTTCC	TGCAGATGAC	CAGTCTAAGG	TCTGAGGAC
		CGGCCATGTA	TTACTGTGCA	AGATCGGCTG	ATGGTTTGGA	CTACTGGGG
		CAAGGAACCT	CAGTCACCGT	CTCCTCAGCC	AAAACAACAC	CCCCATCAG
				GTGGAGATAC		00001110110
108	2.78 light chain	CAGCCTCACA	CTGATCACAC	ACAGACATGA	GTGTGGCCAC	TCACCTCCT
100	variable region	GGGTTGCTGC	TGCTGTGGCT	TACAGATGCC	AGATGTGACA	TCCAGATGA
	polynucleotide	TCAGTCTCCA	GCCTCCCTAT	ATGTATCTGT	GGGAGAAACT	GTCACCATC
	1 ' '	CATGTCGAGC	AAGTGAGAAT	ATTTACAGTA	ATTTAGCATG	GTATCAGCA
	sequence	AAACAGGGAA	AATCTCCTCA	GCTCCTGGTC	TATGGTGCAA	CALACTTAC
		AGATGGTGTG	CCATCAAGGT	TCAGTGGCAG	TGGATCAGGC	DCDCDCuvu CTTVC
				AAGTCTGAAG		
		CAACATTTTT	GGGGTACTCC	ATTCACGTTC	GGCTCGGGGA	CAAAGTTGG
		AATAAAACGG	GCTGATGCTG	CACCAACTGT	ATCCATCTTC	CCACCATCC
		GTGAGCAGTT	AACATCTGGA	GGTGCCTCAG	TCGTGTGC	CCACCAICC
	1		The Change of th	ATCACACCAT	GGCTGTCCTG	CTCCTCTTC
109	2 1029 heavy chain	L AdiGilicicilicaci	I M(2M)			
109	2.1029 heavy chain					
109	variable region	TCTGCCTGGT	TGCATTTCCA	AGCTGTGTCC	TGTCCCAGGT	GCAGCTGAA
109	variable region polynucleotide	TCTGCCTGGT GAGTCAGGAC	TGCATTTCCA CTGGCCTGGT	AGCTGTGTCC GGCGCCCTCA	TGTCCCAGGT CAGAGCCTGT	GCAGCTGAA CCATCACTT
109	variable region	TCTGCCTGGT GAGTCAGGAC CACTGTCTCT	TGCATTTCCA CTGGCCTGGT GGGTTTTCAT	AGCTGTGTCC	TGTCCCAGGT CAGAGCCTGT TGGTGTACAC	GCAGCTGAA CCATCACTT TGGGTTCGC

CACATICORAG AGICAAGTTT TETTAAAATAT GARCHTTTE GARCATGA TETTACTTE GCTATGACTT CTACATTCACTTE GCOLAGAAGTGA GOGLACIGGS TETTACTCATTEGG COCATOGATCA ACCAGAGATCA ACCAGATCACAT COTOCOTTOT ACCACTGACATTA ACCAGAGATCA ACCAGATCACAT COTOCOTTOT ACCACTGACATTA ACCAGAGATCA ACCAGATCACAT COTOCOTTOT ACCACTGACATTA ACCAGATCACAT ACCAGAGATCA ACCAGATCACAT ACCACAGATCA ACCAGATCACAT ACCACACACTACAT ACCACACTGA ACCAGATCAT ACCACACTGA ACCAGATCAT ACCACACTGACATCA ACCACATCACAT	0 2012/0	,,,1001				101,002	.022,002,00
SCHARGSACT ACTOSCIPCT GARACCTTC GERAGOSTE CARAGOSTE CARAGOSTE ANDRAKTORG CONTOCRITO GARACTECT GARACTECT GARACTECT GARACTECT COTOCACCA ARCAGOSTE CARAGOSTE GARACTECT GARACTECT GARACTECT GARACTECT GARACTECT GARACTECT GARACTECT COTOCACCACT ACTOCACCACT CARACTECT COTOCACCACT CARACTECT CARACTECT CARACTECT CARACTECT CARACTECT ACTACACACT CARACTECT ACTACACACT CARACTECT CA			CAACTCCAAG	AGCCAAGTTT	TCTTAAAAAT	GAACAGTCTG	CAAACTGAT
AACAGAGGC CCATCGGTC ATCCATGGG CCCTGTGTG GAGATACO CTGGGTCCTC GGTTACTCTA GAGTGCCTG TCAGATACO CTGGGTCCTC GGTTACTCTA GAGTGCCTGT CAGTTCCTT Variable region polyrucleotide GCCCTCTGTC CCCTGTCT TCCCTGTTTC GGGGAGAGAG CAGATGAC TGCAGAGACA CAGATGACA TGCAGAGACA TGCAGAGACA CAGATGACA TGCAGAGACA			ACACAGCCAT	CTACTTCTGT	GCCAGAGATG	GCGACTACGG	TACTATCTA
110 2.1029 light chain variable region polymucleotide sequence							
2.1029 light chain variable region polymuclestide sequence							GGAGATACA
variable region polymucleotide sequence AGACTACAT COTCOCTORT COTCOCTORTS GAGACAGAS TACCAGATACA CAGATACA CAGATACA COTCAGATACA CAGATACAC			CTGGCTCCTC	GGTGACTCTA	GGATGCCTGG	TCAAGG	
polynucleotide sequence ### Processor	110	1					
sequence THIGGAGGICA ANTCAGGACA THAGGARTITA THARACTICS TATCAGGAGTICA AACCAGAGTICA CATCAGGATTC AGCAGATTCA CACACTACACAT CAGATTACT CATCAGAGTICA CATCAGGAGT CAGTIGGAGAGAGA ATTACACCATT ACCACTACTG AGCAGAGAGA ATTACCACT CACACTACACAGGAGAGA ATTACACCATT CACACACCACAC		variable region					
AACCAGAMIGA ACCOUNTANA CITICTIGATICT ACTACACATIC AAGATTANT TCACCATT AACCAGCTG AGCAGAGAGA TATTECCACT TACTACATT ACCATCCACATT AGCAGCAGT GGGTCGGA CACATTANT TCTCACCATT ACCATCCACTCACACAGAGAGA TATTECCACT TACCATTCACACAGAGAGAGA TATTECCACT TACCATCACACACACACACACACACACACACACA		polynucleotide					
TCAGGAGTC CATCAAGGT CACTGCAGA GACAGAGAGA TATTGCCACT TACTACCATT AGCACAGTT GAGAGAGAGA TATTACCACA CACTGCTAGA AACAGGTTAA CACTGCAGT ACACTGCAGAACAGGAGACACAGGAGAGACACAGGAGAGACACAGGAGAGAACAGGAGACACAGAGAGAACAGAGAGAACAGAGAGAACAGAGAGAC		sequence					
TCTCACCATT AGCARCTGG AGCARGAGGA TATTGCCACT TACTTCCC AACAAGGG CTGATGCTGC ACCAACTGTA TCCATCTTCC ATCAAACGG CTGATGCTGC ACCACGTCA TCCATCTTCC TCACCATCAA ACCATCTGAG GTGCCTCAGT CGTGTGC 111 Humanized Ab (HumAb) 2.78 heavy chain variable region polynucleotide sequence 112 HumAb 2.78 heavy chain polynucleotide sequence 113 HumAb 2.78 heavy chain polynucleotide sequence 114 HumAb 2.78 heavy chain polynucleotide sequence 115 ATGCGTACTC TGCGTATCCT TCCACCTATC CTCTCTCTCTC CAGAGGAGA TCCACGCAGC AGCGCAGCAGCACACCACAC			AACCAGATGG	AACTGTTAAA	CTCCTGATCT	ACTACACATC	AAGATTACA
AACAGGGTAA AACGCTTCCT CGGAGGTTCG GTGGAGGTCC CATCCTCCACATCCA TCAACOGG CTGATGTCG ACCAATCTGAT CCGTCTTCC CACCATCCA TCAACOGG CTGATGTCG ACCAATCTGAT CGGTGTC							
ATCARACOGO GEGATTA ACATCTOGA GRACACTOTA TECATCTEC CACCACCA TA TRACACCACTOR TAGACACTOR TA			TCTCACCATT	AGCAACCTGG	AGCAAGAAGA	TATTGCCACT	TACTTTTGC
Humanized Ab							
heavy chain variable region polynucleotide sequence							CACCATCCA
heavy chain variable region polynucleotide sequence	111	Humanized Ah	gaggtgcagc	taataaaaa	caacaacaac	ctaatacaac	ccaacaaca
heavy chain variable region polynucleotide sequence atcacageg gagagaggagagagagagagagagagagagagaga			cctgagactg	agctgcgccg	ccagcggctt	caccttcage	agettegge
variable region polynucleotide sequence atteaceatc agacagaga cagecragaa edgecagaga cagecragaa acagecragaa cagecragaaca acagecragaa acagecragaaacaacaacaacaacaacaacaacaacaacaacaaca			tgcactgggt	dadacaddcc	cccdacaaaaa	acctagaata	agereegge
polynucleotide sequence acagectaga acagecagaa caccegtaga cacagagaga cacagagagagagagagagagagag		'	atcaccagcg	acaacaacac	catctactac	accascacca	taaaaaaaa
sequence Sequence		_					
Humab 2.78 heavy chain		1 ' '					
Humab 2.78 heavy chain		sequence	gacggcctgg	actactgggg	ccadadcacc	accataacca	tgaggagege
chain polynucleotide sequence GGGCAGCCT GGGCAGCCT GGGCAGCCCC GGGAGGGCCCC GGGAGGGCCCC GGGAGGGCCCC GGGAGGGCCCC GGGAGGGCCCC GGGAGGCCCC GGGAGGGCCCC CTCCAGCCGC AGGGCAGTC CACCATCAGCC CAGCAGCCCC CAGCAGCACC CACCAGCACC CACCACCACC CACCAGCACC CACCAGCACC CACCAGCACC CACCACCACC CACCACCACC CACCACCACC CACCAC	112	11					
polynucleotide sequence GCGGCAGCCT ACTGGCATCA ACTGGGTAGA ACAGGCCCCC GGAAAGGCC GGAAGGCC AGCGCACACATC ACCAGCGGCACA AGGGCAGATT CACATCAGC AGCGACACATC CACATCAGCC AGCGCACACC AGGGCAGATT CACATCAGCC AGCGCACACC CCAAGAACAC CCAGTGACCC AGCGCCGACC AGCGCCGACC AGCGCCGCC AGCGCCGACC AGCGCCGACC AGCGCCGACC ACCACCACCG GCACCACCC GGACCACCC GGACCACCC GGCACCACCC GTGACCTG ACCACAGGGCC ACCACCGGCCGTC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC GCGCCGTCC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCGAGACCACC CCCACCACCC GACCACCCC GACCACCCC CCCAGCACCAC ATCCACAGCCC CCTCCCCCACC CCTCACACCC CCTCCCCCACCC CCCACCACCC CCTCCCCCCC CCCACCACCC CCTCCCCCCCC	112						
TCGGCATCA ACTGGTAGA ACAGGCCCC GGCAAGGGCC TGAGATGCC GAGCTACATC ACCAGCACACA CACAGCACCA CACAGAACACA CAGGACACACA CACAGACACACA ACAGCCCGAC GCCTGAGACACACACACACACACACACACACACACACACA		1	GCGGCAGCCT	GAGACTGAGC	TGCGCCGCCA	GCGGCTTCAC	CTTCACCAC
GAGCTACATC ACCACCATCAGC AGAGCACCAT CACATAGGCC GACAGGACACACC CACAGAACAG CACACACCACC GACAGAACAG CACACACCACC GACAGAACAG GCAGCACACCC GACAGACA			TTCGGCATGC	ACTGGGTGAG	ACAGGCCCCC	GGCAAGGGCC	TGGAGTGGG
AGGGCAGATT CACCATCAGC AGAGACAAGG CCAGAGACAG CAGGCTGTACT ACTGGCCA AAGCCAGCAGA GCCTGTACT ACTGGCCAA AACCGCCGAC GCCTGTACT ACTGGCCAA AACCGCCGAC GCCAGGACACC GCCGTGTACT ACTGGCCAA AACCGCCGAC GCAGCACCAC GCAGCACACC GCAGGACACAC GCAGCACGAC ACCAAGGGCC CACCAGGGCCTT CCCCCTGGCC CCTGGTCCA GCAGCACCAC CCGAGACACAC GCGTGCACCAC GCGAGACACCC CCGAGACACAC CCGTGCACCAC CCGCCACACAC CCGCCACACAC CCGCCACACAC CCTCAGCACCA CCTCAGCACCAC CCTCAGCACCAC CCTCAGCACCAC CCTCAGCACCAC CCTCAGCACCAC CCTCAGCACCAC CCTCAGCACCACCACCACCACCACCACCACCACCACCACCACC		sequence					
CAGATAACA GCCTGACAGC GCGAGGACACC GCGCTTACT ACTGGGCCA AAGCGCCACA CGCCGCACCACC GTGACCCTTG GCAGGGATT GTGGGGCAG GGCACCACC GTGACCCTTG GCAGGGATT GTGGGGCAG GGCACCACC GTGACCCTTG GCAGGGATT GCCGAGAGCACAC GTGACCGTTG GCAGGGACACAC CCTAGAGGCCAC ACCAGAGGCACAC CCTAGAGGCACAC CCTAGAGGCCACAC CCTAGAGGCCACAC CCTAGAGGCACAC CCTAGAGGCACAC CCTAGAGGCACAC CCTAGAGGCACAC TCCAGGACACAC ACCAGAGCACAC ACCAGAGCACAC ACCAGAGCACAC CCTAGAGGCACAC CTTAGAGCACCAC ACCAGAGCACAC CCTAGAGCACAC CTTAGAGCACAC CCTAGAGCACAC CCTGAGCACACACACACACACACACACACACACACACACA			AGGGCAGATT	CACCATCAGC	AGAGACAACG	CCAAGAACAG	CCTGTACCT
AAGCGCCACA ACCAAGGGCC ACCAGCACTA ACCAAGGGCC ACCAGCGATT ACCAAGGGCC ACCAGCGCTTG ACCAAGGGCC CGAGGCCTGG ACCAGCGCCTGG CCGGGCCTGG CCGGGCCTGG CCGGGCCTGG CCGGGCCTGG CCGGGCCTGG CCGGGCCTGG CCGGCCTGG CCGGGCCTGG CCGGCCTGG CCGGCCTGG CCGCCTGGC CTCACCCGCG CCGCGCCTGG CCGCCCCC CCCCCAACC CCCCCACACC CCGCCCACC CCCCCACACC CCCCCACACC CCCCCCCC							
GCAGGATAT GTGGGCCAG GGCACCACCG TGACCTTCAG ACCACGGCCCACCCACACGACCACCG ACCACAGGCCCACCCA			AAGCGCCGAC	GGCCTGGACT	ACTGGGGCCA	GGGCACCACC	GTGACCGTG
ACCAAGGGCC CATCGGTCT CCCCCGGG CCCTGCTCCA GGAGCACCT CGAGAGCACA GCGGCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAA CGGTGACCGT GTCCTGGAAC TCAGGCGCTC TGACCAGGG GGTGCACCAC TTCCCGGCTG TCCTACAGTC TCAGGCGCTC TGACCAGGGG GGTGCACCAC GACCGTGACC TCCAGCACT TCGGCACCCA GACCTACACC TGCAACCTA ATCACAAGCC CAGCAACACC AGGTGGACCA AGCCTACACC TGCAACCTA CTTCTCTCTC CCCCCAAAAC CAGGACCAC AGCTACACC AGCCTACACC CTGAGGTCAC GTGCGTGGTG GTGACCAGAG CCCCGAGACC CAGGTTCAACT GGTACGTGG CCCCAGAACA CCTCATGATC TCCCGGACC CAGTTCAACT GGTACGTGG CGCACATAG CCCCGAGAGA CCCCGAGAGA CCGCGGGGA GACCACCAC AGGCACCA CCTCATGATC TCCCGGACC CCGTCGTGCA CCGCGTGGTG GTGACACCA AGCCACACAA ACCCCGAAGA CCCCGAGGACAC CAGGACACAC AGGCACACAC AGCCCCCAACAC CAGGACCAC AGCCTCCACACACACACACACACACACACACACACACACA			GCAGCGATGT	GTGGGGCCAG	GGCACCACCG	TGACCGTGAG	CACCCCCTC
CGAGAGCACA GGGGCCTGG GCTGCTGGT CAAGGACTAC TTCCCGAA CGGTGACGGT GTCCTGGAAC TCAGGCGCTC TGACCAGGG GGTGCACAC TTCCCGGCTG TCCTACAGTC CTCAGGACCT TACTCCCTCA GAGGGTGG GACCGTGACC TCCAGCACC TCAGGCCCA GACCTACACC TGCAACGTA ATCACAAGCC CAGCAACACC AGCTGCACCA GACCTACACC TGCAACGTA ATCACAAGCC CAGCACACC AGCTGCACCA GACCTTGGCAG GACCGTCAG CTTCCTCTTC CCCCCAAAAC CCAAGGACCA CCTGTGGCAG GACCGTCAG CTGAAGTCAAC GTGCGTGGTG GTGGACGTGA GCCACGAGA CCCTGAGGTCAG CCGGCGGGAG GAGCAGTTCA ACAGCACCTA CCGTGTGTC AGCGCCCCA GCCTCAACAC GTGAACGGA GTGAACGGA GCCACGAGA CCCCGAGGT CCGTCGTGCA CGCACGTAC CCGCACGAG GCCACGAGA CCCCGAGGT CCGTCGTGCA CGCAGGACC CGCATGGAG GTGCATATAG CCAAGGCAC GCGCGGGAG GAGCAGTTCA ACAGCACCTA CCGTTGGTCA AGCGTCCTC CCGTCGTGCA CCAGGACTGA CCCCATCGAG GTGCATATAG CCAAGGCA AGGCAGCCC CGAGAACAC AGGTGTACAC CCTGCTGGTCA AGCGTCCTC CCGTCGTGCA GACCACCAC AGCTTACACC CCTGCCCCA TCCCCGGAG AGATGACCAA GACCACCAC AGCTGTACCA CCTGCCCCA TCCCCGGGAG AGATGACCAA GACCACCAC AGCTGTACCA CCTGCCCCA TCCCCGGGAG AGATGACCAA CACCTCCCA TGCTGGACT CCCTGGTCAA AGGGTTCTA CCCAGGGACA TCGCGTGGAA GGCAGAGAC AATGGGCAGC CGGAGAACA CTACAAGACC ACACCTCCCA TGCTGGACT CCCTGGTCAA AGGGTTCTA CCCAGGCACA CACCTCCCA TCCTGGACT CCCTGGCTC TTCTTCTCT ACAGCAACCT CACCGTGGAC AAGAGCAGG GAACGTCTTT TCATGCTCCG TGATGCATG GCCTGCAC ACCACCACA CACAGAAGA CCTTCCCCTT TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence gcaccacacc cggcaccgac tcacctacta cggcaccgac ccacctacta ctgcagcac ggcaccgac ccacctacta ctgcagcac ggcaccgac ccacctacta ctgcagcac ggcaccgac ccacctacta ctgcagcac ccacctacc ctgcagcac ccacctacc ccacctacta ctgcagcac ccacctacc ccacctacta ctgcagcac ccaccacc ctgcagcac ccaccacc ctgcagcac ccaccacc ctgcagcac ccaccacc ctgcagcac ccaccacc ctgcagcac ccaccacc ccaccacc ccaccacc ccaccacc ccacca							
CGGTGACGGT GTCGTGGAAC TCAGGCGCTC TGACCAGCGG CGTGCACAC TTCCCCGGCTT TCCTACAGTC CTCAGGACTC TACCTCCTCA GACGCGTTG GACCGTGACC TCCACCAACT TGGGCACCCA GACCTACACC TGCAACGTA ATCACAAGCC CAGCACACT AAGGTGGACA AGACAGTTGA GCCCAACTAC ATCACAAGCC CAGCACCACCTG CCCAAGCACA CCTGTGGAG GACCGTCAG CTTCCTCTCTC CCCCCAACAC CCAGGACCA CCTGTGGAG GACCGTCAG CTGAGGTCAC GTGCGTGGTG CTCAAGGACCA CCTGTGGAG GACCGTCAG CTGAGGTCAC GTGCGTGGTG GTGGACGTGA GCCACGAGAG CCCCGAGGAC CCGTCGTGCA GACCAGTCA ACAGCACCT CCCGTGTGGTC CCGTGTGGTC CCGTGTGGAC GACCACAAGA CCCCGAGGACA GCCCCGGGGAG GACCATTCA ACAGCACCTT CCGTGTGGTC CCGTCGTGACACAACAA GGCCCCCCA TCCAGGACACA AGGGCACACA AGGGCACACAACACA			CGAGAGCACA	GCGGCCCTGG	GCTGCCTGGT	CAAGGACTAC	TTCCCCCAA
TTCCCGGCTG TCCTACAGTC CTCAGGACTC TACTCCCTCA GACCTGGGACGGACCGACCGACCGACCGACCACCT TCGGCACCCA GACCTACACC TGCACCCTACAGCC TGCACCCTACAGCC TGCACCCTACAGCC TGCACCCTACAGCC TGCACCCTACAGCC TGCACCCTACAGCC TGCACCCTACAGCC TGCACCCTACCCT			CGGTGACGGT	GTCGTGGAAC	TCAGGCGCTC	TGACCAGCGG	CGTGCACAC
GACCGTGACC ATCACAGGC ATCACAGGC ATCACAGGC ATCACAGGC CAGCAACACC CAGCACACC CCTGTGGCA GGCCACATG TGTGTCGAGT GCCACCGTG CCCACGCACA CCTGTGGCA GGCCACATG CCTGTGCAG CCTCTCTCTC CCCCCAAAAC CCAAGGACAC CCTCATGATC CTGAGGTCAC CTGAGGTCAC CTGAGGTCAC CTGAGGTCAC CTGAGGTCAC CTGAGGTCAC CTGAGGTCAC CTGAGGTCAC CCGAGACTG CCAGTTCAACT GGTACGTGGA GCCACGAGG CCCCTATGATC CCAAGACAA GCCCGCGGAG GGCAGTTCA ACAGCACGTT CCAAGCACA GCCCGCAGGAC CCGTCTGCA CCGTCGTGCA CCGTCGTGCA CCGTCGTGCA CCGAGAACCAC CCGACAACCAC AGGGCACCAC CCGACAACCAC AGGGCACCA AGGGCACCAC AGGGCACCA AGGGCACCAC CCAACAACCA AGGCCACCC CCGACAACCAC AGGTGACCA AGGGCACCAC CCACACAACCA AGGCCACCAC ACACCTCCCA ACACCTCCCA ACACCACCAC CCACCACACAC CCACCACCAC CCACCA			TTCCCGGCTG	TCCTACAGTC	CTCAGGACTC	TACTCCCTCA	GCAGCGTGG
ATCACAAGCC CAGCACACCA AAGGTGGACA AGACAGTGA GCCACATG TGTGTGAGT GCCCACCGTG CCCAGCACCA CCTGTGGCAG GACCGTCAG CTTCCTCTCTC CCCCCAAAAC CCAAGGACAC CCTCAGAGCA CCTCAGAGCACA CTGAGGTCAC GTGCGTGGTG GTGGACGTGA GCCACGAGGA CCCCGAGGACA CAGTTCAACT GGTACGTGGA CGCATGGAG GTGCATAATC CCCAAGACAA GCCGGGGGAG GAGCAGTTCA ACCGACGACGA GGCCTCTGCCCC CCGTCGTGCA CCAGGACTGG CTGAACGGAC AGGACTACAA GGCCTCTCAGC CCGTCGTGCA CCAGGACTGG CTCAACCGACA AGGACTACAA GTCCAAGACAA AGGGCAGCC CGAGAACCAC AGGTGTACAC CCCCCCATCGAG AGATGACCAA AGGCCACCAC AGCTTGACCCCCAC CCCCCCAC AGGCCACACACACACACACAC			GACCGTGACC	TCCAGCAACT	TCGGCACCCA	GACCTACACC	TGCAACGTA
TGTGTCGAGT GCCCACCGTG CCCAGCACCA CCTGTGGCAG GACCGTCAG CTTCCTCTTC CCCCCAAAAC CCCAAGGACAC CCTCATGATC TCCCGGAGC CTGAGGTCAC GTGCGTGGTG GTGGACGTGA GCCACGAAGA CCCCAAGACA GCCGCGGGAG GGCACGAGAG CCCCAAGACACA GCCGCGGGAG GACAGTTCA ACAGCACGT CCGTGTGGTC AGCTCTCCAGCACGACGA GCCACGAGAG GCCACGAGACACA GCCGCGGGAG GACAGTTCA ACAGCACGT CCGTGTGGTC AGCGTCTCC CCGTCGTGCA CCAGAACGAC CCCCATCGAG AAAACCATCT CCAAAACCA AGGGCACCC CGAGAACCAC AGGTGTACAC CCTGCCCCA TCCCAGGAGACAA AGAGCACCA GAACCACCT AGCTGACACGACA AAACCATCT CCAAAACCA AGATGACCAA GAACCAGGTC AGCCTGACCT CCCAGGCAGACA CCACAAGACC AGACCACCTCCA GCCTGACCA CCTGCTCCA TCCCGGGAGACACA CCTACAAGACC ACACCTCCCA TGCTGGACT CCACACGACCA CCACAAGACC ACACCTCCCA TGCTGGACT CCACACGACC CCACAAGACA CCTACAAGACC ACACCTCCCA TGCTGGACT CCACACGACC CCACAAGACA CCTCCCCTG TCACGGTGA AAAGAGCAGGT GCACGACGC CCTTCCTCCTG TCCCGGGTA AATGA 113 HumAb 2.78 light Chain variable region polynucleotide sequence cctggtacca gcacacctga gagcaagga gaacatctac agcaacctga ccacaccacc tggccaaga ctacacctga gagcaaggac agattcagcg gagcaaccaac tggccaaacc tggccaagac ccacaccacc tggccaagac ccacaccacc ggcacacacc ggcacacga ccacaccacc tggccaaga ccacaccacc ggcacacacc ggcacacacc ggcacacacc tggccaagac ccacaccacc ggcacacacc ggcacacacc ggcacacacc ggcacacacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacacacc aggcacacacc aggcacacacc aggcacacacc aggcacacacc aggcacacacc aggcacacacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacacacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacaccacc aggcacacacc aggcacaccacc aggcacaccacc aggcacaccacc aggcaccaccacc aggcacaccacc aggcacaccacc aggcaccaccacc aggcaccaccacc aggcaccaccacc aggcaccaccacc aggcaccaccacc aggcaccaccaccacc aggcaccaccacc aggcaccaccacc aggcaccaccacc aggcaccaccacc aggcaccaccaccaccaccaccaccaccaccaccaccacc							
CTTCCTCTC CCCCAAAAC CAAGGACAC CCTCATGATC TCCCGACCC CTGAGGTCAC GTGCGTGGTG GTGACGTGA GCACGAAGA CCCCAAGGT CAGTTCAACT GGTACGTGGA CGCACTGAGA GCACGAAGAC CCCCAAGGAC GCCGCGGGAG GAGCAGTTCA ACAGCACAT CCCGTGTGTC AGCGTCCTC CCGTCGTGCA CCAGGACTGG CTGAACGGCA AGGAGTACAA GCCCCCAACGAC AGGCAGCCC CAGGACACAC AGGATGTACA ACAGCACAT CCCAAAACCAA AGGCAGCCC CAGAAACCAA AGGTGTACAC CCCTGCCCCA TCCCAGGAGACACA AGGTGACACA AGGTGACACA AGGCACACAC AGACTGACA AAAACCAACA CCCCAGCACACAC ACACCTCCCA TGCTGGACACAC CCCAGCAGCAC CCCAACGACAC ACACCACCACACAC ACACCACCAC ACACCACCA			TGTGTCGAGT	GCCCACCGTG	CCCAGCACCA	CCTGTGGCAG	GACCGTCAG'
CTGAGGTCAC GTGCGTGGTG GTGGACGTGA GCCACGAAGA CCCCGAGGT CAGTTCAACT GGTACGTGA CGGCATGGAC GTGCATAATG CCAAGACAA GCCGCGGGAG GAGCAGTCA ACAGCACGT CCGTGTGGTC ACCGTCTGCA CCGTCGTGCA CCAGGACTGG CTGAACGCA AGAGGACAA AGAGCACCAC CCCCATCGAG AAAACCATCT CCAAAACCA AGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA TCCCGGGAG AGATGACCAA GAACCAAGGTC AGCGTGTACAC CCTGCCCCCA TCCCGGGAGACAC CCACGCACAA TCGCCGTGGA GTGGAGAGC AATGGGCAGC CTACAAGACA ACACCTCCCA TCCCGGGAGACAC ACACCAAGACCA ACACCTCCCA TCCTGGACTC CCACGGACACAC ACACGAAGCT CACCGTGGA GTGGGAGACA CACCGCTCCA TCCAGGAGACA ACACCACCACCA TCCCCGTGGA GTGGGAGACA CACCGCTCCA TCCACGAGACA ACACCACCACCA TCCCCGTGGA GTGGGAGACA CACCGCTCC TCTTCTCTC ACACGAAGCC ACCCTCCCA TCCTCGGACT CCACGGCGCC TTCTTCTCTC TCATGCTCCG TGATCCATGA GGCTCTGCAC AACCACTACA CACAGAAGA 113 HumAb 2.78 light Chain variable region cctggtacca gcagaagcc ccaagcagc cctggacgc ccaagcagc cctggacgc ccagcagc ccagcagc cctggacgc ccaagcagc cctggacgcac gcaccacac ccggcaccacac ccggcaccacac ccggcaccacac ccggcaccacac ccggcacacac ccaccacacac cggcaccacacac cggcaccacacaca			CTTCCTCTTC	CCCCCAAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACC
CAGTTCAACT GGTACGTGGA CGGCATGAG GTGCATAATG CCAAGACAA GCCGCGGGAG GAGCAGTTCA ACAGCACGTT CCGTGGTGC AGCGTCTC CCGTCGTGCA CCAGGACTG CTGAACGGCA AGAGTACAA GTGCAAGCA TCCAACAAAG GCCTCCCAGC CCCCATCGAG AAAACCATCT CCAAAACCA AGGGCAGCC CGAGAACCAC AGGTTACAC CCTGCCCCA TCCCGGGAG AGATGACCAA GAACCACCA AGGTTACAC CCTGCCCCA TCCCGGGAG AGATGACCAA TCGCCGTGGA GTGGAAGAC CACACCTCCA TGCTGGAGACCA CTACAAGACC CACCCTCCA TGCTGGACT CGACGAGCC CGAGGAACA CTACAAGACC CACCCTCCA TGCTGGACT CGACGAGCAC CACACAGCTC ACACCAAGCT CACCCTGGAC AAGCCAGGT GGCAGCAGG GAACGTCTT TCATGCTCCC TGATCCATGA GGCTCTCAC ACCACTACA CACACACTCCA TCATGCTCCC TGCTCCAGA AGACCAGGT GGCAGCAGG GAACGTCTT TCATGCTCCC TCCCGGGTA AATGA 113 HumAb 2.78 light chain variable cagagtacc atcacctgca gagccagca gaacatctac agcaacctgregion polynucleotide sequence ccacctacta ctgccagag cgtgccagc agattcagcg gagcaccagac ccaccaaga tggagacc ccaccaacac ttcaccctga ccatcagcag cctgcagcc gaggacttccaccaccaccaccaccaccaccaccaccaccacca			CTGAGGTCAC	GTGCGTGGTG	GTGGACGTGA	GCCACGAAGA	CCCCGAGGT
GCCGCGGGAG GAGCAGTTCA ACAGCACGTT CCGTGTGGTC AGCGTCCTCC CCGTCGTGCA CCAGGACTGG CTGAACGGCA AGGAGTACAA GTGCAAGGCA AGGGCAGCCC CGAGAACCAC CCCCATCGAG AAAACCATCT CCAAAACCA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCA TCCCGGGAG AGATGACCAA GAACCACCAC AGGTGTACAC CCTGGCCCCA TCCCGGGAG AGATGACCAA GAACCACCAC AGGTGTACAC CCTGGCCCCA TCCCGGGAG ACACCACCAC AGCTGACCT GCCTGGTCAA AGGCTTCTA CCCAGCGACA TCGCCGTGGA GTGGGAGAGC CGACGGCTC TTCTTCCTC ACAGCAAGCC ACACCTCCCA TGCTGGACTC CGACGGCTC TTCTTCCTC ACAGCAAGCC CCCCTGGTCAA AAGACCACCACCAC CCCCCCAACACAGG GGCCCTCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence cggcaccacc ctgccagcac ctggaggcc agacatctac agcacctacta ggcaccaagg tggagacc caactagcag cccactacac ctgccagcac ctggaggca cccactacac ggcaccaaga ttcacctga gagcacaga ccccatcac ggcaccaaga ttcacctga cactcagcag cccccatcac ggcaccaaga ttcacctga cactcagcag cccccttcac cttccggcaaga ccccccttcac cttccggcaaga ccccccttcac cttccggcaaga ccccccttcac cttccggcaaga ccccccttcac cttccggcaaga ccccccttcac cttccggcaaga ccccccccttcac cttccggcaaga cccccccccacacacac ctgcaagacacacacacacacacacacacacacacacaca							
CCGTCGTGCA CCAGGACTGG CTGAACGGCA AGGAGTACAA GTGCAAGGGT TCCAACAAAG GCCTCCCAGC CCCCATCGAG AAAACCATCT CCAAAAACCA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCA TCCCGGGAGG AGATGACCAA GACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTA CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGACAGACCA CTACAAGACC ACACCTCCCA TGCTGGACTG GCAGCGCTCC TTCTTCTCTC ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCACGGG GAACGATTCAAGCACACCTCCCA TCCTGGACTG CACCGTGGAC AACCACTACA CACCACTACA CCCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence ccacctacta ctgccagcag gcacagcga gaacatctac agcaacctgg ccaccaagag ccaccagag ccaccagagagag			GCCGCGGGAG	GAGCAGTTCA	ACAGCACGTT	CCGTGTGGTC	AGCGTCCTC
TCCAACAAAG GCCTCCCAGC CCCCATCGAG AAAACCATCT CCCAAAACCA AGGGCAGCC CAGAGAACCAC AGGTGTACAC CCTGCCCCA TCCCGGAGA AGATGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTA CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CTACAAGACCA CCTACAAGACC ACACCTCCCA TGCTGGACT CGACGGCTCC TTCTTCTCTC ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTT- TCATGCTCCG TGATGCATGA AGCCACTACA CACACTACA CACAGAAGA- CCTCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence ccacctacta cgagagacc cgagagacc cgagagacc cgagagacc cgagagacc cacagagac ccaccagag ccaccagag ccaccaga gagacatctac gagacaccaga ttcacctga gagacatcaga gacatctac gagacaccaga ttcacctga ccacctacta ctgccagaac ttctaggagac ccaccagagac ccaccagagac ccaccagagac ccaccagagac ccaccagagac ccaccagaac ccaccaacac gagaacctgac ccacctacta ctgccagaac ttctaggagac ccaccatacta ctgccagaac ttctaggagac ccaccatacta gagaactc ccacctacta ctgccagaac ttctgggagac ccaccatacta gagaactc ccacctacta ctgccagaac ttctgggagac ccaccatacta gagaactc ccacctacta ctgccagaac ccaccagaac ccaccatacta gagaactc ccacctacta ctgccagaac ccaccagaac ccaccatacta gagaactc ccacctacta ctgccagaac ccaccatacta gagaactc ccacctacta ctgccagaac ccaccatacta ctgccagaac ccaccatacta ctgccagaac ccaccatacta ctgccagaac ccaccatacta gagaactc ccacctacacaacaacaacaacaacaacaacaacaacaac							
AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCA TCCCGGGAG AGATGACCAA GAACCAGGTC AGCCTGACCT GCCTGACCT AGGCTTCTA CCCAGGGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGAGGAACA CTACAAGACC ACACCTCCCA TGCTGGACTC CGACGGCTCC TTCTTCCTC ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTT CATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGAC CCTCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light Chain variable region polynucleotide sequence ccacctacta ggcaccagac ctgacagca gacactctac agacacctgc ggcaccagac ctgacagca gacactctac agacacctgc ggcaccagac ctgacagca gacactctac agacacctgc ggcaccagac ctgacagca ccacagcag ccaagcagc ccaagcagca ggacacctaca agcaacctga ggacacctaca agcaagcagca ccaagcagca ccaagcagc ccaagcagc ccaagcagc ccaagcagc ccaagcagc ccaagcagca ccaccaagcag ccaaccagag ccaacaaccagag ccaaccagag ccaacaacagag ccaacagag ccaacaacagag ccaacaacagag ccaacaacagag ccaacaacagag ccaacaa			TCCAACAAAG	GCCTCCCAGC	CCCCATCGAG	AAAACCATCT	
AGATGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTA CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACA CTACAAGACC ACACCTCCCA TGCTGCACT CGACGGCTCC TTCTTCCTC ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTT TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGAC CCTCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 HumAb 2.78 light chain polynucleotide sequence 116 GGCGCAGGGC CGGCCAGCAG GGCAGCAGG GAACGACAGAGAGACAGAGAGAG			AGGGCAGCCC	CGAGAACCAC	AGGTGTACAC	CCTGCCCCCA	TCCCGGGAG
CCCAGCGACA CTACAAGACC CTACAAGACC ACACCTCCCA ACAGCAGCT ACAGCAAGCT ACAGCAAGCT ACAGCATGA CTACTCCCG ACAGCAGCT ACAGCAAGCT TCATGCTCCG ACAGCAGCT TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGACA CCTCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 HumAb 2.78 light chain polynucleotide sequence 116 ATGAAAAATCC TGATTCTCG TATCTCTCG TATCTCTCG TATCTTCTCT TTTCTCTCT TTTCTCTCT TTTCTCTCC TATCTCCAGC TCTCCCGGTA AACCACTACA CACAGAAGACA CACAGAAGAC CACAGAAGAC CACAGAAGAC CACAGAAGAC CACAGAAGAC CACAGAAGAC CACAGAAGAC CACAGAAGAC CACAGACGC CAGCAGCCC CAGCAGCCC CAGCAGACC CAGCAGAGC CCAGCAGACC CCAGCAGACC CCAGCAGACC CCAGCAGACC CCAGCAGACC CCAGCAGCC CCAGCCAG			AGATGACCAA	GAACCAGGTC	AGCCTGACCT	GCCTGGTCAA	AGGCTTCTA
CTACAAGACC ACACCTCCA TGCTGGACTC CGACGGCTCC TTCTTCCTC ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTT TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGA CCTCTCCCTG TCTCCGGGTA AATGA 113 HumAb 2.78 light chain variable region polynucleotide sequence cacactacta cacactacta cacactactactactactactactactactactactacta			CCCAGCGACA	TCGCCGTGGA	GTGGGAGAGC	AATGGGCAGC	CGGAGAACA
ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTT- TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGA 113 HumAb 2.78 light chain variable region polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGG ACCTGCAGAGGA ACCTGCAGAGGA CATCTACAGGCGG CAGCAGGAG CAGCAGGAGA CATCTACAGG GACCTTC ACCTGCAGG GACCTTC TGGGGCAGCC GCAGCAGCAG TTCAGCGGGG GACCTTCACGT CCGGCCAGGGG ACCAACCTG CCAGCAGCCT GCAGCCCGAGACCC CCTTCACCTT CCGGCCAGCCCGACACCC CCTTCACCTT CCGGCCAGCCCGACACCC CCTTCACCTT CCGGCCAGCCCGACCCC CCTTCACCTT CCGGCCAGCCCC CCGCCCACCCCCACCCCCACCCCCACCCCCACCCCCACCCCCC			CTACAAGACC	ACACCTCCCA	TGCTGGACTC	CGACGGCTCC	TTCTTCCTC
TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGA 113 HumAb 2.78 light chain variable region polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGG ACCTGCAGAGAC ACCACAGGC ACCAACCTG CCGACGGCG AGGCCCCA AGGCCCCA AGGCCCCCA AGGCCCCCA AGCTGCTGACA CCTACCAGG GACTTCCAGG GACTTCCAGGC CCGGCCAGGCC GCAGCAGCCT GCAGCCCGGC ACCAACCTG CCAGCAGCCT GCAGCCCGACCCCACCCC			ACAGCAAGCT	CACCGTGGAC	AAGAGCAGGT	GGCAGCAGGG	GAACGTCTT
HumAb 2.78 light chain variable region polynucleotide sequence 114 HumAb 2.78 light chain variable region polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 HumAb 2.78 light chain polynucleotide sequence 116 HumAb 2.78 light chain polynucleotide sequence 117 HumAb 2.78 light chain polynucleotide sequence 118 HumAb 2.78 light chain polynucleotide sequence 119 HumAb 2.78 light chain polynucleotide sequence 110 HumAb 2.78 light chain polynucleotide sequence 110 HumAb 2.78 light chain polynucleotide sequence 111 HumAb 2.78 light chain polynucleotide sequence 112 HumAb 2.78 light chain polynucleotide sequence 113 HumAb 2.78 light chain polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 HumAb 2.78 light chain polynucleotide sequence 116 HumAb 2.78 light chain polynucleotide sequence 117 HumAb 2.78 light chain polynucleotide sequence 118 HumAb 2.78 light chain polynucleotide sequence 119 HumAb 2.78 light chain polynucleotide sequence 110 HumAb 2.78 light chain polynucleotide sequence 111 HumAb 2.78 light chain polynucleotide sequence 112 HumAb 2.78 light chain polynucleotide sequence 113 HumAb 2.78 light chain polynucleotide sequence 114 HumAb 2.78 light chain polynucleotide sequence 115 HumAb 2.78 light chain polynucleotide sequence 116 HumAb 2.78 light chain polynucleotide sequence 117 HumAb 2.78 light chain polynucleotide sequence 118 HumAb 2.78 light chain polynucleotide sequence 119 HumAb 2.78 light chain polynucleotide sequence 110 HumAb 2.78 light chain polynucleotide sequence 115 HumAb 2.78 light chain polynucleotide sequence 116 HumAb 2.78 light chain polynucleotide sequence 117 HumAb 2.78 light chain polynucleotide sequence 118 HumAb 2.78 light chain polynucleotide sequence 119			TCATGCTCCG	TGATGCATGA	GGCTCTGCAC	AACCACTACA	CACAGAAGA
chain variable region polynucleotide sequence			CCTCTCCCTG	TCTCCGGGTA	AATGA		
region polynucleotide sequence THUMAD 2.78 light chain polynucleotide sequence THEGGCAGAC ACCTGGCCT GCGGCAGGGC GCGGCAGGGC GCGCAGGGC GCGGCAGGGC GCGCCAGGCC GCGCCAGCCC GCGCCAGCCC GCGCCAGCCC GCGCCAGCCC GCGCCCGC GCGCCCGC GCGCCCGC GCGCCCGC GCGCCCGC GCGCCCC CCTCCCCC CCGCCCCC CCTCCCCC CCGCCCCC CCTCCCCC CCCCCCCC	113	HumAb 2.78 light	gacatccaga	tgacccagag	ccccagcagc	ctgagcgcca	gcgtgggcg
polynucleotide sequence Polynucleotide sequence gcaccaacc cggcaccaacc cggcaccaacc cggcaccaacc cggcaccaacc cggcaccaacc ccacctacta ctaccctga ccatcaagcag cctgcagcac cccccttcac cttcggcaac cccccttcac cttcggcaaccaagg cccccttcac cttcggcaaccaagg cccccttcaccaccaagg cccccaccaagg cccccaccaagg cccccaccaagg cccccaccaagg cccccaccaagg ccccccttcac cttcggcaaccaagg ccccccttcaccaccaagg ccccccttcaccaccaagg ccccccaccaagg ccccccttcaccaccaccaagg ccccccttcaccaccaccaagg ccccccaccaccaagg ccccccaccaagg ccccccttcaccaccaccaccaccaccaccaccaccacca		chain variable	cagagtgacc	atcacctgca	gagccagcga	gaacatctac	agcaacctg
sequence ccacctacta ctgccagcac ccatcagcag cctgcagccc gaggacttcg ccacctacta ctgccagcac ttctggggca cccccttcac cttcggccag ggcaccaagg tggagatc 114 HumAb 2.78 light chain polynucleotide sequence TGGCGACAGC GGTACCAGCAG ACCAACCTG GAAGCCCCA AGCGCAGAACCCA AGCTGCAGAGCCC CTACGGCGC ACCAACCTG GCAGCAGCAG AGCGCCCAAGCCCA AGCTGCAGAG CTACGGCGC ACCAACCTG CCAGCAGCAG AGCGCCCAAGCCCA AGCTGCAGAG CTACGGCGC ACCAACCTG CCAGCAGCAG ACCAGCAGCAG ACCAACCTG CCAGCAGCAG TTCAGCGGCG ACCTTCGCCAGCAG ACCTTCACCTC CCGGCCAGGGCC ACCAACCTTC ACCCTGACCA TCAGCAGCCC CCTTCACCTT CCGGCCAGGGCC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC CCGGCCAGCAGCT TCACGCTCTC CCGGCCAGGGCC ACCAACGTG AGATCAAACG TACGGTGGCT GCACCATCTC CCGGCCAGCAGCT TCACCTTCACCTT CCGGCCAGCAGCT TCACCTTCACCTT TCAGCAGCCT TCACCTT TCAGCAGCCT TCACCTT TCAGCAGCCT TCACCTT TCAGCAGCT TCAGCACCT TCACCTT TCAGCAGCT TCAGCAGCT TCAGCAGCT TCAGCAGCT TCAGCAGCT TCACCTT TCAGCAGCT TCACCTT TCAGCAGCT TCACCTT TCAGCAGCT TCACCT TCAGCAGCT TCAGCAG			cctggtacca	gcagaagccc	ggcaaggccc	ccaagctgct	gatctacgg
sequence cggcacgac ttcaccctga ccatcagcag cctgcagccc gaggacttcg ccacctacta ctgccagcac ttctggggaa cccccttcac cttcggccag ggcaccaagg tggagatc 114 HumAb 2.78 light chain polynucleotide sequence cTACGGCCC ACCAACCTGG CTACGGCCC AGCGGGGAGAA CATCTACAGG CTACGGCGCC ACCAACCTGG CCGACGGGGAGAA CATCTACAGG GCGCAGGGG CACCGACTTC ACCTGACGA TTCAGCGGGCG GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC GCAGCCCGACGACGACCCCAACCTGG CCGACGACTTC TGGGGCACCCC CCTTCACCTT CGGCCAGGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC		polynucleotide	gccaccaacc	tggccgacgg	cgtgcccagc	agattcagcg	gcagcggcag
ggcaccaagg tggagatc 114 HumAb 2.78 light chain polynucleotide sequence 126 ACCTGGCGC CACCAGACCC CAGCAGCAGCA AGCTGCAGAGCCTG AGCGCCAGAGCCTG AGCGCCAGAGCCTG AGCGCCAGAGCCTG AGCGCCAGAGCCCG AACCTGCAGAG CAGCAGCAGCAGA CATCTACAGG CAGCAGCAGA AACCTGGCAGA GAAGCCCGGA AAGCCCGGA AGCTGCAGAGA CATCTACAGG CAGCAGGAGA CATCTACAGG CAGCAGCAGA ACCTACCAGCAGA GAAGCCCGAACGAGA TACAGCAGAGAGA ACCAACCTGG CCGACCAGCAGA TACAGCAGCAGA TACAGCAGCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA			cggcaccgac	ttcaccctga	ccatcagcag	cctgcagccc	gaggacttcg
114 Humab 2.78 light chain TTGGGCAGAC ATCCAGATGA CCCAGAGCCC CAGCAGCCT AGCGCCAGCCC GOLynucleotide sequence TGGGCGCAGCC ACCACACCTG CTACCTGCAGCCCC AGCGCAGAAC CATCTACAGCCCCAGCCCCAGCAGCCCCAGCCCCAGCCCCAGCCCCAACCCCCAACCCCCAACCCCCC					ttctggggca	ccccttcac	cttcggccag
chain polynucleotide sequence TTGGGCAGAC ACCTGAGATGA CCCAGAGCCC CAGCAGCCTG AGCGCCAGCC AACCTGGCACAG CCAGCAGAGA CATCTACAGG CTACGGCGCC GGTACCAGCA GAAGCCCGGC AAGGCCCCA AGCTGCTGAC CTACGGCGCC ACCAACCTGG CCGACGGCGT GCCCAGCAGA TTCAGCGGCC GCGCCAGCGG CACCGACTTC ACCCTGACCA TCAGCAGCCT GCAGCCCGAC GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC	111	Lium Ab 2 70 liebe			TATOTTO COM	mmmamamam	ama ama
polynucleotide sequence TGGGCGACAG AGTGACCATC ACCTGCAGAG CCAGCGAGAA CATCTACAGG AACCTGGCCT GGTACCAGCA GAAGCCCCGA AAGGCCCCCA AGCTGCTGAC CTACGGCGCC ACCAACCTGG CCGACGGCT GCCCAGCAGA TTCAGCGGCC GCGGCAGCGG CACCGACTTC ACCCTGACCA TCAGCAGCCT GCAGCCCGAC GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC	114	_	TTCCCCAAATCC	ATCALICICGG	CCCACACCCC	CAGGAGGGTG	AGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
sequence AACCTGGCCT GGTACCAGCA GAAGCCCCGC AAGGCCCCCA AGCTGCTGA' CTACGGCGCC ACCAACCTGG CCGACGGCGT GCCCAGCAGA TTCAGCGGCC GCGGCAGCGG CACCGACTTC ACCCTGACCA TCAGCAGCCT GCAGCCCGAC GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC		1	TCCCCCAGAC	ALCCAGATGA	A CCTCCAGAGCCC	CAGCAGCCTG	AGCGCCAGC
CTACGGCGCC ACCAACCTGG CCGACGGCT GCCCAGCAGA TTCAGCGGCC GCGGCAGCGG CACCGACTTC ACCCTGACCA TCAGCAGCCT GCAGCCCGAC GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC		1 ' '	A A COTTO COTT	COMMODACCATC	ACCIGCAGAG	LCAGCGAGAA	LATCTACAGO
GCGGCAGCGG CACCGACTTC ACCCTGACCA TCAGCAGCCT GCAGCCCGAC GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC		sequence	CTACCCCCCC	A CCA A CCTCC	CCCACCCCGGC	AAGGCCCCCA	AGCTGCTGAT
GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC			GUGGGGGGG	CACCAACCIGG	A CCCACGCGCGT	BCACCAGCAGA	TTCAGCGGCI
CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTC							
TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTC			CCCCCACCCA	ACCAACCTCC	ACATCANACC	TAGAGCACCC	CCTTCACCTT
TETTEMTETT CECECUATET GATGAGCAGT TGAAATUTGG AACTGCCTC			TOGCCAGGGC	CCCCCCO TO	CATCACACCA	TACGGTGGCT	GCACCATCTC
			refreateff	CCCGCCATCT	GATGAGCAGT	TGAAATCTGG	AACTGCCTC

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				CTTCTATCCC		
				AATCGGGTAA		
				ACCTACAGCC		
				ACACAAAGTC		
			AGCTCGCCCG	TCACAAAGAG	CTTCAACAGG	GGAGAGTGTT
		GA				
115	HumAb 2.1029	caggtgcagc	tgcaggagag	cggccccggc	ctggtgaagc	ccagcgagac
	heavy chain	cctgagcctg	acctgcaccg	tgagcggctt	cagcctgacc	agctacggcg
	variable region	tgcactggat	cagacagccc	cccggcaagg	gcctggagtg	gatcggcgtg
	polynucleotide	atctgggccg	gcggcagcac	caactacaac	cccagcctga	agagcagagt
	sequence	gaccatcagc	gtggacacca	gcaagaacca	gttcagcctg	aagctgagca
		gcgtgaccgc	cgccgacacc	gccgtgtact	actgcgccag	agacggcgac
		cgtgagcagc	tetaegeeat	ggactactgg	ggccagggca	ccctggtgac
116	HumAb 2.1029			TGCAGCTATT		
	heavy chain	TCAAGCGCAG	GTGCAGCTGC	AGGAGAGCGG	CCCCGGCCTG	GTGAAGCCCA
	polynucleotide	GCGAGACCCT	GAGCCTGACC	TGCACCGTGA	GCGGCTTCAG	CCTGACCAGC
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		AGCAGCGCGT	CGACCAAGGG	CCCATCGGTC	TTCCCCCTCC	CCCCCCCCCCC
		CAGGAGCACC	TCCGAGAGCA	CAGCGGCCCT	GGGCTGCCTG	CGCCCTGCTC
		ACTTCCCCGA	ACCGGTGACG	GTGTCGTGGA	ACTCAGGGGG	TCTCACCACC
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		CTCCAAAACC	AAAGGGCAGC	CCCGAGAACC	ACAGGTGTAC	ACCCTGCCCC
		CATCCCGGGA	GGAGATGACC	AAGAACCAGG	TCAGCCTGAC	CTGCCTGGTC
		AAAGGCTTCT	ACCCCAGCGA	CATCGCCGTG	GAGTGGGAGA	GCAATGGGCA
		GCCGGAGAAC	AACTACAAGA	CCACACCTCC	CATGCTGGAC	TCCGACGGCT
		CCTTCTTCCT	CTACAGCAAG	CTCACCGTGG	ACAAGAGCAG	GTGGCAGCAG
				CGTGATGCAT		ACAACCACTA
		CACACAGAAG	AGCCTCTCCC	TGTCTCCGGG	'I'AAA'I'GA	
117	HumAb 2.1029 light	gacatccaga	tgacccagag	ccccagcagc	ctgagcgcca	gcgtgggcga
	chain variable	cagagtgacc	atcacctgca	gagccagcca	ggacatcagc	aactacctga
	region	actggtacca	gcagaagccc	ggcaaggccc	ccaagctgct	gatctactac
	polynucleotide	accagcagac	tgcacagcgg	cgtgcccagc	agattcagcg	gcagcggcag
	sequence	cggcaccgac	ttcaccttca	ccatcagcag	cctgcagccc	gaggacatcg
				ggcaagaccc	tgcccagaac	cttcggcggc
		ggcaccaagg	tggagatc			
118	HumAb 2.1029 light	ATGAAAATCC	TGATTCTCGG	TATCTTCCTG	TTTCTCTGTT	CTACTCCAGC
	chain	TTGGGCAGAC	ATCCAGATGA	CCCAGAGCCC	CAGCAGCCTG	AGCGCCAGCG
	polynucleotide	TGGGCGACAG	AGTGACCATC	ACCTGCAGAG	CCAGCCAGGA	CATCAGCAAC
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		GACATCGCCA	CCTACTACTG	CCAGCAGGGC	AAGACCCTGC	CCAGAACCTT
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		AGCAGGACAG	CAAGGACAGC	ACCTACAGCC	TCAGCAGCAC	CCTGACGCTG
		AGCAAAGCAG	ACTACGAGAA	ACACAAAGTC	TACGCCTGCG	AAGTCACCCA
			AGCTCGCCCG	TCACAAAGAG	CTTCAACAGG	GGAGAGTGTT
		GA				
119	HumAb 1.802	gaggtgcagc	tggtggagag	cggcggcggc	ctggtgcagc	ccggcggcag
	heavy chain	cctgagactg	agctgcgccg	ccagcggctt	caccttcagc	gactacggca

	, 1001				101/002	.011.001.00
	variable region	tgcactgggt	gagacaggcc	cccggcaagg	gcctggagtg	ggtgagcta
	polynucleotide	atcagcagcg	gcagcagaac	cgtgtactac	gccgacagcg	tgaagggca
	sequence	attcaccatc	agcagagaca	acgccaagaa	cagcctgtac	ctgcagatg
				accgccgtgt		
		i .		cgtgtggggc		
		gagcagc	2 3	3 3 3332		- 33 - 34-6 - 3
						W10000000
120	HumAb 1.802			TGCAGCTATT		
	heavy chain			TGGAGAGCGG		
	polynucleotide			TGCGCCGCCA		
	sequence	TACGGCATGC	ACTGGGTGAG	ACAGGCCCCC	GGCAAGGGCC	TGGAGTGGG'
	Jequence			GCAGAACCGT		
		AGGGCAGATT	CACCATCAGC	AGAGACAACG	CCAAGAACAG	CCTGTACCT
				CGAGGACACC		
		AAAGCACTAC	AACGGCGGCT	ACTTCGACGT	GTGGGGCCAG	GGCACCCTG
				TGGGGCCAGG		
				ATCGGTCTTC		
				CGGCCCTGGG		
		TCCCCCAACC	COTTON COOTTO	TCGTGGAACT	CIGCCIGGIC	AAGGACTAC
		GIGCACACCT	1 CCCGGCTGT	CCTACAGTCC	TCAGGACTCT	ACTCCCTCA
				CCAGCAACTT		
		GCAACGTAGA	TCACAAGCCC	AGCAACACCA	AGGTGGACAA	GACAGTTGA
		CGCAAATGTT	GTGTCGAGTG	CCCACCGTGC	CCAGCACCAC	CTGTGGCAG
		ACCGTCAGTC	TTCCTCTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATC'
		CCCGGACCCC	TGAGGTCACG	TGCGTGGTGG	TGGACGTGAG	CCACGAAGA
		CCCGAGGTCC	AGTTCAACTG	GTACGTGGAC	GGCATGGAGG	TGCATAATG
		CAAGACAAAG	CCGCGGGAGG	AGCAGTTCAA	CAGCACGTTC	CGTGTGGTC
		GCGTCCTCAC	CGTCGTGCAC	CAGGACTGGC	TGAACGGCAA	GGAGTACAA
		TGCAAGGTCT	CCAACAAAGG	CCTCCCAGCC	CCCATCGAGA	AAACCATCT
		CAAAACCAAA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	CTGCCCCA'
		CCCGGGAGGA	GATGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAA
				CGCCGTGGAG		
		GGAGAACAAC	TACAAGACCA	CACCTCCCAT	CCTCCACTCC	CACCCCTCC
				ACCGTGGACA		
		A A COMOMINAM	CAGCAAGCIC	GATGCATGAG	AGAGCAGGTG	GCAGCAGGG
				CTCCGGGTAA		ACCACTACA
		ACAGAAGAGC	CICICCCIGI	CICCGGGIAA	ATGA	
121	HumAb 1.802 light	gacatcgtga	tgacccagag	ccccgacagc	ctggccgtga	gcctgggcg
	chain variable	gagagecace	atcaactqca	gagccagcaa	gatcgtgagc	accageggel
	region	acagctacat	gcactggtac	cagcagaagc	ccqqccaqcc	ccccaaget
	polynucleotide	ctgatctacc	tggccagcaa	cctggagagc	gacataccca	acagattca
	1 ' '	cadcadcadc	agcggcaccg	acttcaccct	gaccatcagc	agectgeag
	sequence	ccgaggacgt	agccatatac	tactgccagc	acagcagaga	actaccccc
		accttcggcc	agggcaccaa	actagagate	acajoajaja	300300000
122	HumAb 1.802 light	ATGAAAATCC	TGATTCTCGG	TATCTTCCTG	TTTCTCTGTT	CTACTCCAG
	chain	TTGGGCAGAC	ATCGTGATGA	CCCAGAGCCC	CGACAGCCTG	GCCGTGAGC
	polynucleotide	TGGGCGAGAG	AGCCACCATC	AACTGCAGAG	CCAGCAAGAT	CGTGAGCAC
	sequence	AGCGGCTACA	GCTACATGCA	CTGGTACCAG	CAGAAGCCCG	GCCAGCCCC
	Jequellee		ATCTACCTGG			
	1	1				
		GATTCAGCGG	CAGCGGCAGC	GGCACCGACT	TCACCCTGAC	CATCAGCAGG
		GATTCAGCGG CTGCAGGCCG	CAGCGGCAGC AGGACGTGGC	GGCACCGACT	TCACCCTGAC	CATCAGCAGO GCAGAGAGO
		CTGCAGGCCG	AGGACGTGGC	GGCACCGACT CGTGTACTAC	TCACCCTGAC TGCCAGCACA	GCAGAGAGC:
		CTGCAGGCCG GCCCCCCACC	AGGACGTGGC TTCGGCCAGG	GGCACCGACT CGTGTACTAC GCACCAAGCT	TCACCCTGAC TGCCAGCACA GGAGATCAAA	GCAGAGAGCT CGTACGGTGC
		CTGCAGGCCG GCCCCCACC CTGCACCATC	AGGACGTGGC TTCGGCCAGG TGTCTTCATC	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA	GCAGAGAGCT CGTACGGTGC GTTGAAATCT
		CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC	GCAGAGAGCCCGTACGGTGCGTTGAAATCCCCAGAGAGGGC
		CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT	GCAGAGAGCCCGTACGGTGCGTGAAATCCCCAGAGAGGCCAACTCCCAGC
		CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGGAC	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG	GCAGAGAGCT CGTACGGTGC GTTGAAATCT CCAGAGAGGC AACTCCCAGC
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		CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGGAC TGAGCAAAGC CATCAGGGCC TTGA	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG	GCAGAGAGCCCGTACGGTGCGGTGCAGAGAGAGGCCTCAGCAGCAGCAGCAGCAGCAGCAGCCTCAGCAGCAGCAGCCTCAGCAGCAGCAGCTTCAACACAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA
123	HumAb 1.815	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG Caggtgcagc	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGGAC TGAGCAAAGC CATCAGGGCC TTGA tggtggagag	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG	GCAGAGAGCT CGTACGGTGC GTTGAAATCT CCAGAGAGAGGC AACTCCCAGC CCTCAGCAGC TCTACGCCTC AGCTTCAACA
123	HumAb 1.815 heavy chain	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGGAC TGAGCAAAGC CATCAGGGCC TTGA tggtggagag agctgcgccg	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC cggcggcggc ccagcggctt	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc	GCAGAGAGCT CGTACGGTGC GTTGAAATCT CCAGAGAGAGGC AACTCCCAGC CCTCAGCAGC TCTACGCCTC AGCTTCAACA CCGGGGGGGGG
123	1	CTGCAGGCCG GCCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg tgcactggat	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGGAC TGAGCAAAGC CATCAGGGCC TTGA tggtggagag agctgcgccg cagacaggcc	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC ccagcggctt cccggcaagg	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc gcctggagtq	GCAGAGAGCCCGTACGGTGCGGTGCAGAGAGAGAGAGAGA
123	heavy chain variable region	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGAGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcg	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGGAC TGAGCAAAGC CATCAGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC ccagcggcgc ccagcggcaagg cgtgtactac	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc gcctggagtg	GCAGAGAGC CGTACGGTGC GTTGAAATC CCAGAGAGAGGC AACTCCCAGC TCTACGCCTC AGCTTCAACA ccggcggcag gacttcggcag ggtgagctac tgaaggcag
123	heavy chain variable region polynucleotide	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcagcagcagcagcagcagcagcagcagcagca	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC TGAGCAAAGC CATCAGGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC CCagcggcgt cccagcggctt cccggcaagg cgtgtactac acgccaagaa	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac	GCAGAGAGC CGTACGGTGG GTTGAAATC CCAGAGAGAGGG AACTCCCAG TCTACGCCT AGCTTCAACA ccggcggcag gacttcggcag ggtgagctag tgaaggcag ctgcagatga
123	heavy chain variable region	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcagcagcagcagcagcagcagcagcagcagca	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC TGAGCAAAGC CATCAGGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC CCagcggcgt cccagcggctt cccggcaagg cgtgtactac acgccaagaa	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac	GCAGAGAGC CGTACGGTGC GTTGAAATC CCAGAGAGAGGC AACTCCCAG TCTACGCCT AGCTTCAACA ccggcggcac gacttcggcac ggtgagctac tgaaggcac ctgcagatga
123	heavy chain variable region polynucleotide	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGAAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcg attcaccatc acagcctgag	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC CATCAGGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca agccgaggac	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGGGGGC CCGGCGGGCGC ccagcaggctt cccggcaagg cgtgtactac acgccaagaa accgccgtgt	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac actactgcgc	GCAGAGAGC CGTACGGTGC GTTGAAATC CCAGAGAGAGGC AACTCCCAGC TCTACGCCT AGCTTCAACA ccggcggcac gacttcggca ggtgagctac tgaaggcac ctgcagatga cagagccag
123	heavy chain variable region polynucleotide	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGAGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcagcagcagcagcagcagcagcagcagcagca	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC CATCAGGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca agccgaggac	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC CCagcggcgt cccagcggctt cccggcaagg cgtgtactac acgccaagaa	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG ctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac actactgcgc	GCAGAGAGC CGTACGGTGC GTTGAAATC CCAGAGAGAGGC AACTCCCAGC TCTACGCCT AGCTTCAACC CCGGCGGGGGGG gacttcggcag ggtgagctac tgaaggcag ctgcagatga
	heavy chain variable region polynucleotide sequence	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcagcagcagcagcagcagcagcagcagcagca	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC TGACCACAGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca agccgagaca gctacttcga	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC CGGCGGCGC ccagcggctt cccggcaagg cgtgtactac acgccaagaa accgccgtgt ctgctgggc	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG CGtCACAAAG cctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac actactgcgc cagggcacca	GCAGAGAGCC CGTACGGTGC GTTGAAATCC CCAGAGAGAGGC AACTCCCAGC CCTCAGCAGC TCTACGCCTC AGCTTCAACA CCGGCGGGGGGG GacttcgGCA GacttcgGCA GacttcgGCA GacttcgGCA CCGGAGAGGCCAGC CCGGAGAGGCCAGC CCGGGGAGAGGCCGGGGGCCGGGCCGGGCCGGCGGCCGGGCCGG
123	heavy chain variable region polynucleotide	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcagcagcagcagcagcagcagcagcagcagca	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC TGACCACAGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca agccgagaca gctacttcga TGGCTATCCT	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT CCTGCTGAAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC CGGCGGCGC ccagcggctt cccggcaagg cgtgtactac acgccaagaa accgccgtgt ctgctgggc	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG cctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac actactgcgc cagggcacca	GCAGAGAGCCCGTACGGTGCGGTGCAGAGAGAGAGAGAGA
	heavy chain variable region polynucleotide sequence	CTGCAGGCCG GCCCCCACC CTGCACCATC GGAACTGCCT CAAAGTACAG AGAGTGTCAC ACCCTGACGC CGAAGTCACC GGGGAGAGTG caggtgcagc cctgagactg tgcactggat agcagcagcagc attcaccatc acagcctgag tacgacggg gagcagc ATGCGTACTC TCAAGCGCAG	AGGACGTGGC TTCGGCCAGG TGTCTTCATC CTGTTGTGTG TGGAAGGTGG AGAGCAGAC TGACCACAGGCC ATCAGGGCC TTGA tggtggagag agctgcgccg cagacaggcc gcggcaccac agcagagaca agccgaggac gctacttcga TGGCTATCCT GTGCAGCTGG	GGCACCGACT CGTGTACTAC GCACCAAGCT TTCCCGCCAT ATAACGCCCT AGCAAGGACA AGACTACGAG TGAGCTCGCC CGGCGGCGGC CGGCGGCGC ccagcggctt cccggcaagg cgtgtactac acgccaagaa accgccgtgt ctgctgggc	TCACCCTGAC TGCCAGCACA GGAGATCAAA CTGATGAGCA AACTTCTATC CCAATCGGGT GCACCTACAG AAACACAAAG CGTCACAAAG cctggtgaagc caccttcagc gcctggagtg gccgacagcg cagcctgtac actactgcgc cagggcacca CTGCTTGTTG CGGCGGCCTG	GCAGAGAGCCCCGTACGGTGCGGTGCAGAGAGAGCCCCCAGCAGCAGCAGCAGCAGCAGCAGCA

11 () 2012/	/1361 PC1/US2011/001/65					
	sequence				GGCAAGGGCC	
					GTACTACGCC	
					CCAAGAACAG	
					GCCGTGTACT	
					CTGGGGCCAG	
		TGACCGTGAG	CAGCGATGTG	TGGGGCCAGG	GCACCACCGT	GACCGTGAGC
					CCCCTGGCGC	
					CTGCCTGGTC	
		TCCCCGAACC	GGTGACGGTG	TCGTGGAACT	CAGGCGCTCT	GACCAGCGGC
					TCAGGACTCT	
		CAGCGTGGTG	ACCGTGACCT	CCAGCAACTT	CGGCACCCAG	ACCTACACCT
		GCAACGTAGA	TCACAAGCCC	AGCAACACCA	AGGTGGACAA	GACAGTTGAG
		CGCAAATGTT	GTGTCGAGTG	CCCACCGTGC	CCAGCACCAC	CTGTGGCAGG
		ACCGTCAGTC	TICCTCTTCC	CCCCAAAACC	CAAGGACACC	CTCATGATCT
		CCCGGACCCC	TGAGGTCACG	TGCGTGGTGG	TGGACGTGAG	CCACGAAGAC
					GGCATGGAGG	
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		GCGTCCTCAC	CGTCGTGCAC	CAGGACTGGC	TGAACGGCAA	GGAGTACAAG
		TGCAAGGTCT	CCAACAAAGG	CCTCCCAGCC	CCCATCGAGA	AAACCATCTC
		CAAAACCAAA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	CTGCCCCCAT
		CCCGGGAGGA	GATGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA
		GGCTTCTACC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC
		GGAGAACAAC	TACAAGACCA	CACCTCCCAT	GCTGGACTCC	GACGGCTCCT
		TCTTCCTCTA	CAGCAAGCTC	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG
					GCTCTGCACA	ACCACTACAC
		ACAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGA	
125	HumAb 1.815 light	gacatcgtga	tgacccagag	ccccgacage	ctggccgtga	gcctgggcga
	chain variable	gagagccacc	atcaactgca	gagccagcaa	gagcgtgagc	accagegget
	region	acagctacat	ccactggtac	cagcagaagc	ccggccagcc	ccccaagctg
	polynucleotide	ctgatctacc	tggccagcga	cctggagagc	ggcgtgcccg	acagattcag
	sequence	cggcagcggc	agcggcaccg	acttcaccct	gaccatcagc	agcctgcagg
		ccgaggacgt	ggccgtgtac	tactgccacc	acagcagaga	gctgcccttc
		accttcggcc	agggcaccaa	gctggagatc		
126	HumAb 1.815 light	ATGAAAATCC	TGATTCTCGG	TATCTTCCTG	TTTCTCTGTT	CTACTCCAGC
	chain	TTGGGCAGAC	ATCGTGATGA	CCCAGAGCCC	CGACAGCCTG	GCCGTGAGCC
	polynucleotide	TGGGCGAGAG	AGCCACCATC	AACTGCAGAG	CCAGCAAGAG	CGTGAGCACC
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	'	CAAGCTGCTG	ATCTACCTGG	CCAGCGACCT	GGAGAGCGGC	GTGCCCGACA
		GATTCAGCGG	CAGCGGCAGC	GGCACCGACT	TCACCCTGAC	CATCAGCAGC
		CTGCAGGCCG	AGGACGTGGC	CGTGTACTAC	TGCCACCACA	GCAGAGAGCT
		GCCCTTCACC	TTCGGCCAGG	GCACCAAGCT	GGAGATCAAA	CGTACGGTGG
		CTGCACCATC	TGTCTTCATC	TTCCCGCCAT	CTGATGAGCA	GTTGAAATCT
		GGAACTGCCT	CTGTTGTGTG	CCTGCTGAAT	AACTTCTATC	CCAGAGAGGC
					CCAATCGGGT	
		AGAGTGTCAC	AGAGCAGGAC	AGCAAGGACA	GCACCTACAG	CCTCAGCAGC
		ACCCTGACGC	TGAGCAAAGC	AGACTACGAG	AAACACAAAG	TCTACGCCTG
				TGAGCTCGCC	CGTCACAAAG	AGCTTCAACA
		GGGGAGAGTG	TTGA			
127	HumAb 1.846	gaggtqcaqc	tggtggagag	cggcggcgac	ctggtgcagc	ccaacaacaa
	heavy chain	cctgagactq	agctgcqccq	ccagcqqctt	caccttcagc	gactacggca
	variable region	tgcactgggt	gagacaqqcc	cccggcaagg	gcctggagtg	ggtgaggtag
	polynucleotide	atcagcagcg	gcagcaccac	cctgaqctac	gccgacagcg	tgaagggcag
	sequence	attcaccatc	agcagagaca	acgccaaqaa	cagcctgtac	ctgcagatga
	sequence	acagcctqaq	agacgaqqac	accgccqtqt	actactgcgc	cagaaagaar
		tacaacggcg	gctacttcqa	catatagaac	cagggcaccc	taataaccat
		gagcagc			333	. 3 3 - 3 3 -
128	HumAb 1.846	ATGCGTACTC	TGGCTATCCT	ТССАССТАТТ	CTGCTTGTTG	CACTCCACCC
	heavy chain	TCAAGCGGAG	GTGCAGCTGG	TGGAGAGCCC	CGGCGGCCTG	CACIGCAGGC
	polynucleotide	GCGGCAGCCT	GAGACTGAGC	TGCGCCGCCA	GCGGCTTCAC	CTTCAGCCCG
	' '	TACGGCATGC	ACTGGGTGAG	ACAGGCCCCC	GGGAAGGGCC	TCCACCCAC
	sequence	GAGCTACATC	AGCAGCGGCA	GCACCACCCT	GAGCTACGCC	TOGUGIGGGI
		AGGGCAGATT	CACCATCAGC	AGAGACAACC	CCAAGAACAG	COTOTA COTO
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		TGACCGTGAG	CAGCGATGTG	TGGGGCCACGI	GCACCACCGT	GGCACCCIGG CACCCCCACA
		AGCGCGTCGA	CCAAGGGCCC	ATCCCTCTC	CCCCTGGCGC	COMOCHICAN C
		GAGCACCTCC	GAGAGCACAG	CGGCCCTGGG	CTGCCTGGTC .	ANCONCENCE
		TCCCCGAACC	GGTGACGGTG	TCGTGGAACT	CAGGCGCTCT	CDCCDCCCCC
	_L			CAACI	CAGGCGCICI	CACCAGCGGC

		· •			-	
		GTGCACACCT	TCCCGGCTGT	CCTACAGTCC	TCAGGACTCT	ACTCCCTCAG
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		ACAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGA	
129	HumAb 1.846 light	gacatcgtga	tgacccagag	ccccgacage	ctggccgtga	gcctgggcga
	chain variable	gagagccacc	atcaactgca	gagccagcaa	gagcgtgagc	gagagcggct
	region	acagctacat	gcactggtac	cagcagaagc	ccggccagcc	ccccaagctg
	polynucleotide	ctgatctacc	tggccagcaa	cctggagagc	ggcgtgcccg	acagattcag
	sequence	cggcagcggc	agcggcaccg	acttcaccct	gaccatcagc	agcctgcagg
	'			tactgccagc	acagcagagt	getgeeeec
		accttcggcc	agggcaccaa	gctggagatc		
130	HumAb 1.846 light	ATGAAAATCC	TGATTCTCGG	TATCTTCCTG	TTTCTCTGTT	CTACTCCAGC
	chain			CCCAGAGCCC		
	polynucleotide	TGGGCGAGAG	AGCCACCATC	AACTGCAGAG	CCAGCAAGAG	CGTGAGCGAG
	sequence	AGCGGCTACA	GCTACATGCA	CTGGTACCAG	CAGAAGCCCG	GCCAGCCCCC
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		GATTCAGCGG	CAGCGGCAGC	GGCACCGACT	TCACCCTGAC	CATCAGCAGC
		CTGCAGGCCG	AGGACGTGGC	CGTGTACTAC	TGCCAGCACA	GCAGAGTGCT
		GCCCCCCACC	TTCGGCCAGG	GCACCAAGCT	GGAGATCAAA	CGTACGGTGG
		CTGCACCATC	TGTCTTCATC	TTCCCGCCAT	CTGATGAGCA	GTTGAAATCT
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				ATAACGCCCT		
				AGCAAGGACA		
				AGACTACGAG		
				TGAGCTCGCC		
		GGGGAGAGTG				

CLAIMS

What is claimed is:

- 1. A use of an effective amount of a monoclonal antibody for stimulating endocortical bone formation in a patient, wherein the antibody binds human notum pectinacetylesterase (NOTUM) and neutralizes at least one activity of NOTUM, wherein the antibody reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1, 3, 6-trisulfonate (OPTS) assay *in vitro* and reduces NOTUM activity in Wnt signaling assay *in vitro*.
- 2. A use of an effective amount of a monoclonal antibody for the preparation of a medicament for stimulating endocortical bone formation in a patient, wherein the antibody binds human notum pectinacetylesterase (NOTUM) and neutralizes at least one activity of NOTUM, wherein the antibody reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1, 3, 6-trisulfonate (OPTS) assay *in vitro* and reduces NOTUM activity in Wnt signaling assay *in vitro*.
- 3. A use of an effective amount of a monoclonal antibody for treating, managing, or preventing a disease or disorder characterized by bone loss in a patient, wherein the antibody binds human notum pectinacetylesterase (NOTUM) and neutralizes at least one activity of NOTUM, wherein the antibody reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1, 3, 6-trisulfonate (OPTS) assay *in vitro* and reduces NOTUM activity in Wnt signaling assay *in vitro*.
- 4. A use of an effective amount of a monoclonal antibody for the preparation of a medicament for treating, managing, or preventing a disease or disorder characterized by bone loss in a patient, wherein the antibody binds human notum pectinacetylesterase (NOTUM) and neutralizes at least one activity of NOTUM, wherein the antibody reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1, 3, 6-trisulfonate (OPTS) assay *in vitro* and reduces NOTUM activity in Wnt signaling assay *in vitro*.

- 5. The use of claim 3 or claim 4, wherein the disease or disorder is selected from osteoporosis, osteopenia, and Paget's disease.
- 6. The use of any one of claims 1 to 5, wherein upon administration to a subject the antibody increases serum PINP levels *in vivo*, increases bone mineral density *in vivo*, increases midshaft femur cortical thickness *in vivo*, increases midshaft femur bone area *in vivo*, increases midshaft humerus cortical thickness *in vivo*, increases endocortical bone formation in vivo, increases the proportion of cortical bone volume in the LV5 vertebral body *in vivo*, and/or increases the proportion of femoral neck bone volume to femoral neck total volume *in vivo*.
- 7. The use of any one of claims 1 to 6, wherein the antibody binds to a polypeptide having the amino acid sequence of SEQ ID NO: 1 with K_D of less than 50 nM.
- 8. The use of claim 7, wherein the K_D is less than 20 nM.
- 9. The use of claim 8, wherein the K_D is less than 10 nM.
- 10. The use of any one of claims 1 to 9, wherein the antibody is selected from a mouse antibody, a chimeric antibody, a humanized antibody, and a human antibody.
- 11. The use of any one of claims 1 to 10, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein:
 - a) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 91, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; or
 - b) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino

- acid sequence of SEQ ID NO: 19, and the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; or
- the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; or
- d) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 25, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 28, a CDR2 having the amino acid sequence of SEQ ID NO: 29, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; or
- e) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 91, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; or
- f) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino

- acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 35, and the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 36, a CDR2 having the amino acid sequence of SEQ ID NO: 37, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; or
- g) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 41, a CDR2 having the amino acid sequence of SEQ ID NO: 42, and a CDR3 having the amino acid sequence of SEQ ID NO: 43, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 44, a CDR2 having the amino acid sequence of SEQ ID NO: 45, and a CDR3 having the amino acid sequence of SEQ ID NO: 46; or
- h) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 59, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 60, a CDR2 having the amino acid sequence of SEQ ID NO: 61, and a CDR3 having the amino acid sequence of SEQ ID NO: 62.

12. The use of claim 11, wherein:

- a) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 15 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16; or
- b) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 71 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73; or

- the heavy chain comprises the amino acid sequence of SEQ ID
 NO: 72 and the light chain comprises the amino acid sequence of SEQ ID NO: 74; or
- d) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 23 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 24; or
- e) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 75 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 77; or
- f) the heavy chain comprises the amino acid sequence of SEQ ID NO: 76 and the light chain comprises the amino acid sequence of SEQ ID NO: 78; or
- g) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 31 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 32; or
- h) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 79 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 81; or
- the heavy chain comprises the amino acid sequence of SEQ ID
 NO: 80 and the light chain comprises the amino acid sequence of SEQ ID NO: 82; or
- j) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 39 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 40; or
- k) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 67 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 69; or
- the heavy chain comprises the amino acid sequence of SEQ ID NO: 68 and the light chain comprises the amino acid sequence of SEQ ID NO: 70; or

- m) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 55 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 56.
- 13. A monoclonal antibody that binds human notum pectinacetylesterase (NOTUM), wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein:
 - a) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 91, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; or
 - b) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 17, a CDR2 having the amino acid sequence of SEQ ID NO: 18, and a CDR3 having the amino acid sequence of SEQ ID NO: 19, and the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; or
 - c) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; or

- d) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 25, a CDR2 having the amino acid sequence of SEQ ID NO: 26, and a CDR3 having the amino acid sequence of SEQ ID NO: 27, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 28, a CDR2 having the amino acid sequence of SEQ ID NO: 29, and a CDR3 having the amino acid sequence of SEQ ID NO: 30; or
- e) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 90, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 91, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 92, a CDR2 having the amino acid sequence of SEQ ID NO: 93, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; or
- f) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 33, a CDR2 having the amino acid sequence of SEQ ID NO: 34, and a CDR3 having the amino acid sequence of SEQ ID NO: 35, and the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 36, a CDR2 having the amino acid sequence of SEQ ID NO: 37, and a CDR3 having the amino acid sequence of SEQ ID NO: 38; or
- g) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 41, a CDR2 having the amino acid sequence of SEQ ID NO: 42, and a CDR3 having the amino acid sequence of SEQ ID NO: 43, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 44, a CDR2 having the amino acid sequence of

- SEQ ID NO: 45, and a CDR3 having the amino acid sequence of SEQ ID NO: 46; or
- h) the heavy chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 57, a CDR2 having the amino acid sequence of SEQ ID NO: 58, and a CDR3 having the amino acid sequence of SEQ ID NO: 59, and wherein the light chain variable region comprises a CDR1 having the amino acid sequence of SEQ ID NO: 60, a CDR2 having the amino acid sequence of SEQ ID NO: 61, and a CDR3 having the amino acid sequence of SEQ ID NO: 62.

14. The antibody of claim 13, wherein:

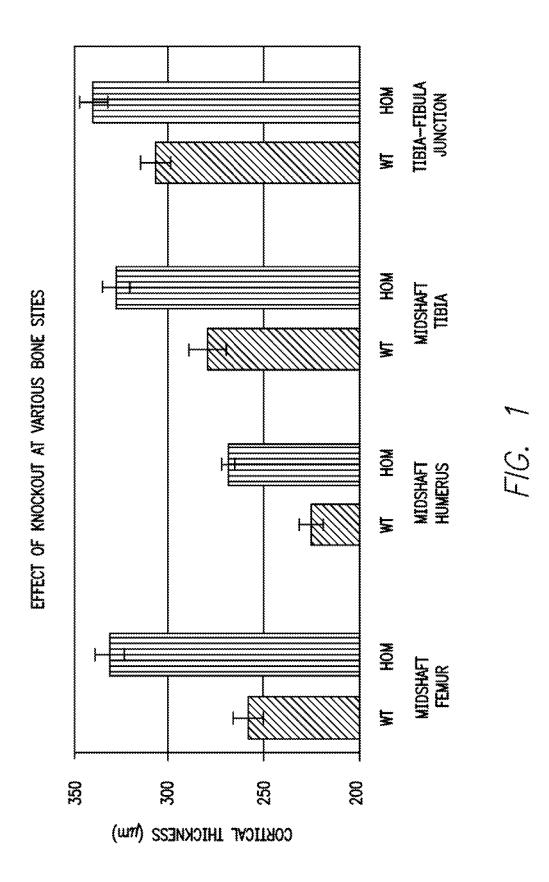
- a) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 15 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16; or
- b) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 71 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73; or
- the heavy chain comprises the amino acid sequence of SEQ ID
 NO: 72 and the light chain comprises the amino acid sequence of SEQ ID NO: 74; or
- d) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 23 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 24; or
- e) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 75 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 77; or
- f) the heavy chain comprises the amino acid sequence of SEQ ID NO: 76 and the light chain comprises the amino acid sequence of SEQ ID NO: 78; or

- g) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 31 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 32; or
- h) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 79 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 81; or
- the heavy chain comprises the amino acid sequence of SEQ ID
 NO: 80 and the light chain comprises the amino acid sequence of SEQ ID NO: 82; or
- j) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 39 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 40; or
- k) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 67 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 69; or
- the heavy chain comprises the amino acid sequence of SEQ ID NO: 68 and the light chain comprises the amino acid sequence of SEQ ID NO: 70; or
- m) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 55 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 56.
- 15. The antibody of claim 13 or claim 14, wherein the antibody neutralizes at least one activity of NOTUM.
- 16. The antibody of claim 15, wherein the antibody reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1, 3, 6-trisulfonate (OPTS) assay *in vitro*.
- 17. The antibody of claim 15 or claim 16, wherein the antibody reduces NOTUM activity in Wnt signaling assay *in vitro*.
- 18. The antibody of any one of claims 13 to 17, wherein upon administration to a subject the antibody increases serum PINP levels *in vivo*, increases bone mineral density *in vivo*, increases midshaft femur cortical thickness *in vivo*, increases midshaft femur bone area *in vivo*, increases midshaft

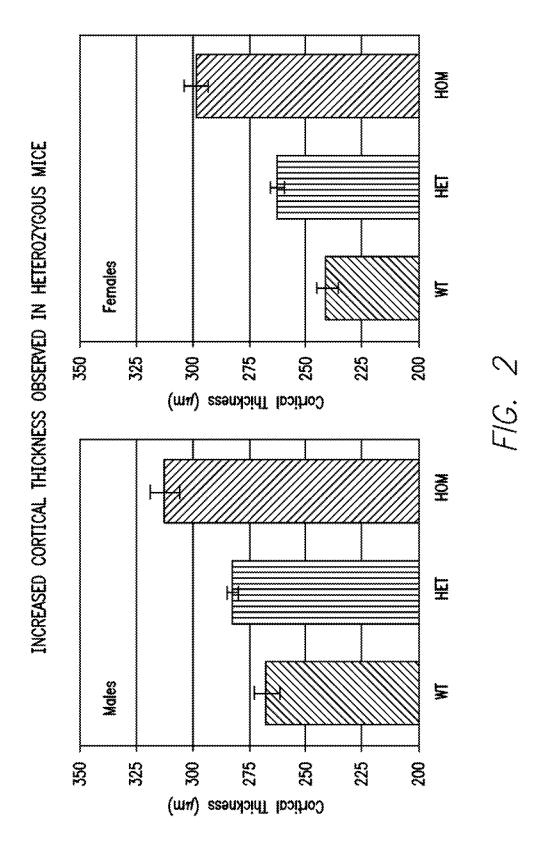
- humerus cortical thickness *in vivo*, increases endocortical bone formation in vivo, increases the proportion of cortical bone volume in the LV5 vertebral body *in vivo*, and/or increases the proportion of femoral neck bone volume to femoral neck total volume *in vivo*.
- 19. The antibody of any one of claims 13 to 16, wherein the antibody binds to a polypeptide having the amino acid sequence of SEQ ID NO: 1 with K_D of less than 50 nM.
- 20. The antibody of claim 19, wherein the K_D is less than 20 nM.
- 21. The antibody of claim 20, wherein the K_D is less than 10 nM.
- 22. The antibody of any one of claims 13 to 21, wherein the antibody is selected from a mouse antibody, a chimeric antibody, and a humanized antibody.
- 23. A pharmaceutical composition comprising the antibody of any one of claims 13 to 22 and a pharmaceutically acceptable carrier.
- 24. A nucleic acid molecule comprising a polynucleotide sequence that encodes a heavy chain or a light chain of the antibody of any one of claims 13 to 22.
- 25. The nucleic acid molecule of claim 24, wherein the nucleic acid molecule comprises a first polynucleotide sequence that encodes the heavy chain, and a second polynucleotide sequence that encodes the light chain.
- 26. The nucleic acid molecule of claim 24 or claim 25, wherein the nucleic acid molecule is a vector.
- 27. A host cell comprising the nucleic acid molecule of any one of claims 24 to 26.
- 28. The host cell of claim 27, wherein the host cell comprises a first nucleic acid molecule comprising a polynucleotide sequence that encodes a heavy chain, and a second nucleic acid molecule comprising a polynucleotide sequence that encodes a light chain.
- 29. The host cell of claim 27, wherein the nucleic acid molecule comprises a first polynucleotide sequence that encodes the heavy chain, and a second polynucleotide sequence that encodes the light chain.

30. A method of producing the antibody of any one of claims 13 to 22 comprising incubating the host cell of any one of claims 27 to 29 under conditions sufficient to express the antibody.

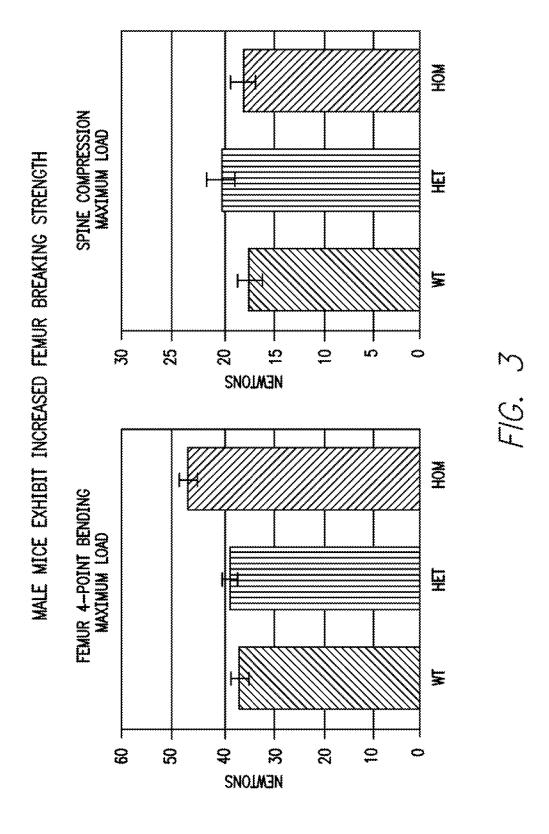
1/15



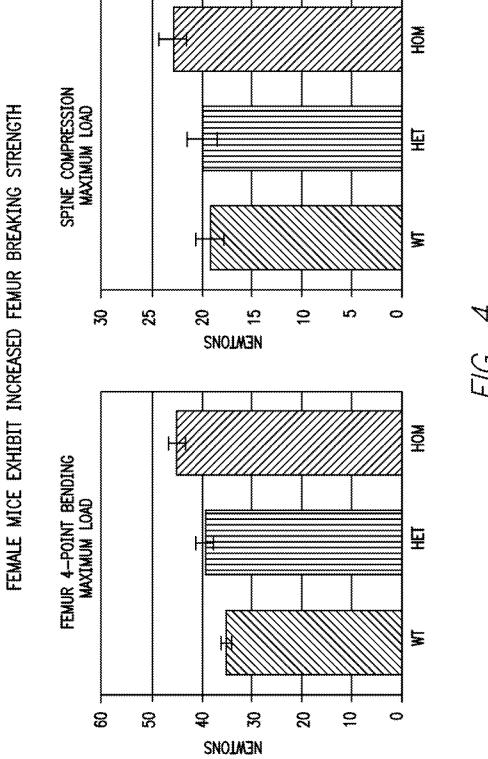
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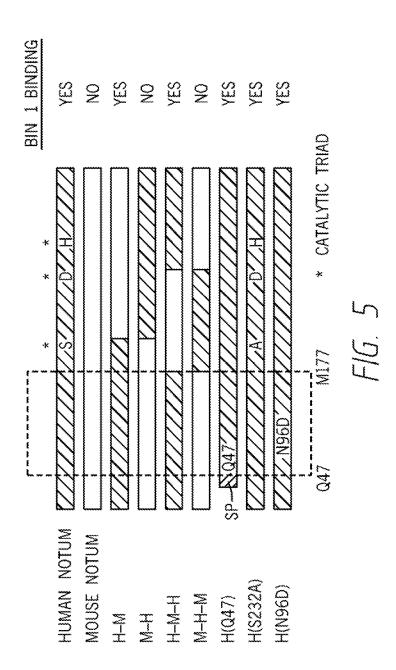
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4/15

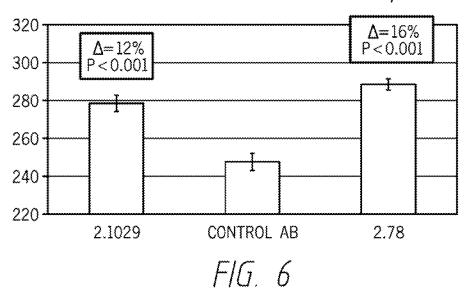


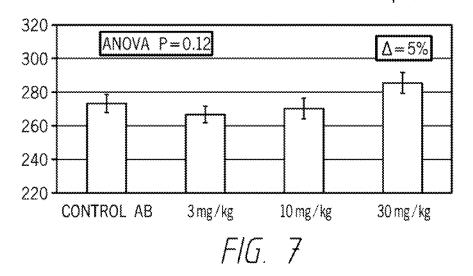
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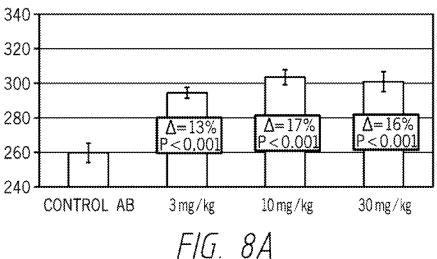
MIDSHAFT FEMUR CORTICAL THICKNESS (µm)





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MIDSHAFT FEMUR CORTICAL THICKNESS (µm)



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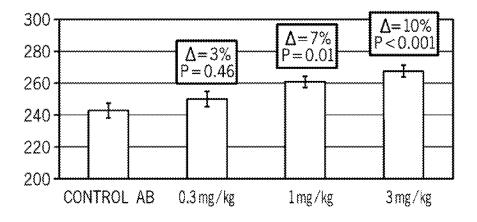
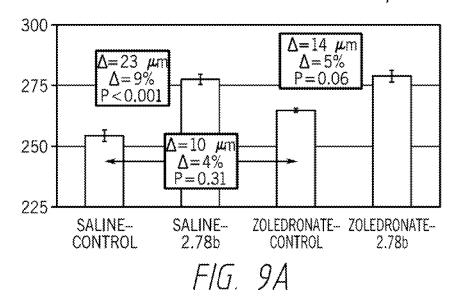
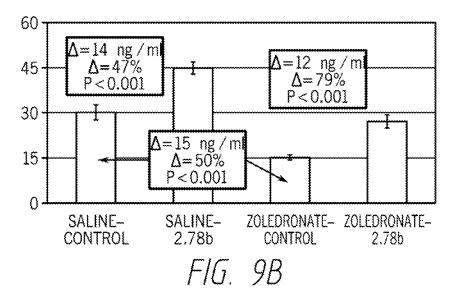


FIG. 8B

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MIDSHAFT FEMUR CORTICAL THICKNESS (µm)

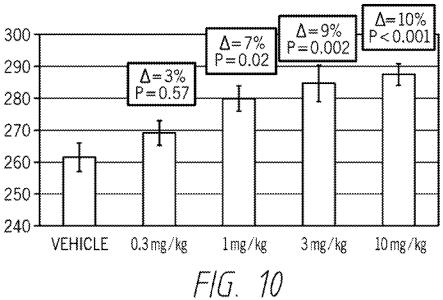


SERUM PINP (ng/ml)



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MIDSHAFT FEMUR CORTICAL THICKNESS (µm)



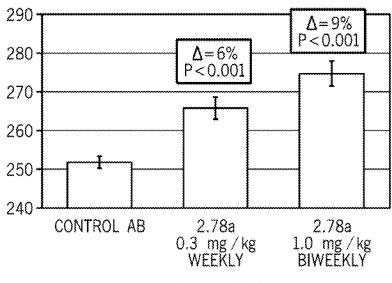
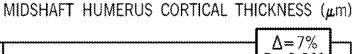


FIG. 11A

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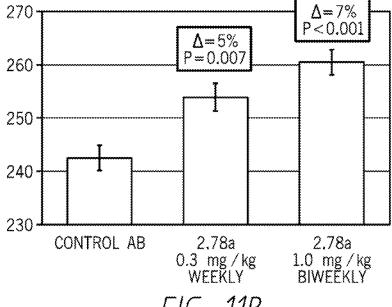
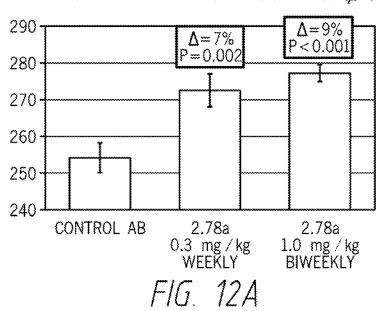
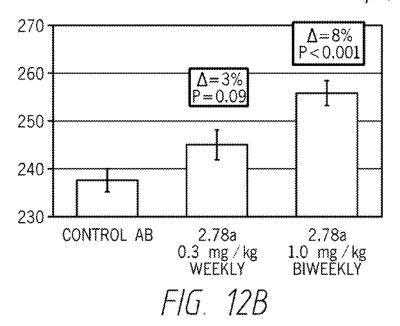


FIG. 11B

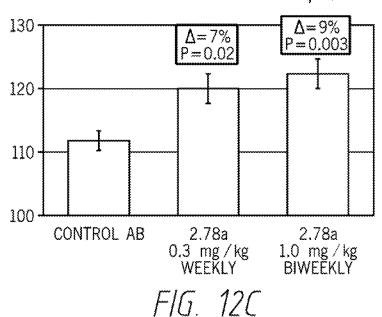


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MIDSHAFT HUMERUS CORTICAL THICKNESS (µm)

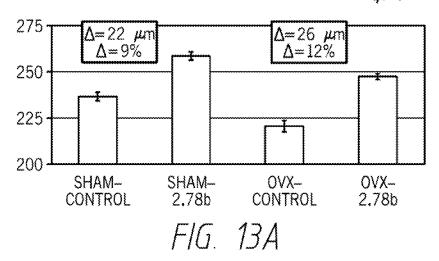


9th RIB CORTICAL THICKNESS (µm)

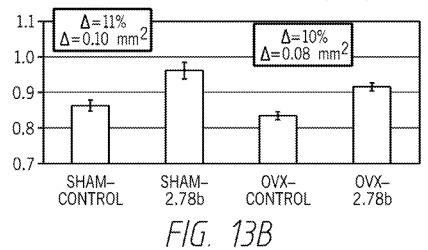


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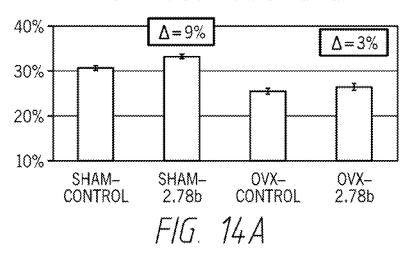
MIDSHAFT FEMUR CORTICAL THICKNESS (µm)



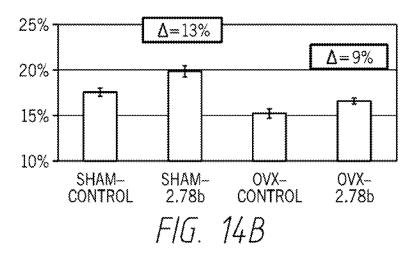
MIDSHAFT FEMUR BONE AREA (mm²)



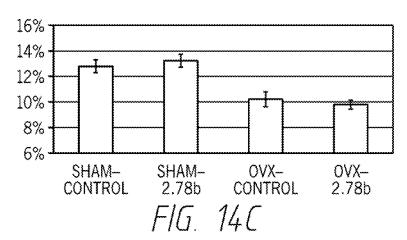
13 /15
LV5 TOTAL BV /TOTAL VOLUME



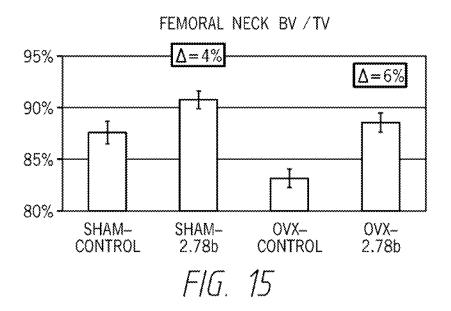
LV5 CORTICAL BV / TOTAL VOLUME



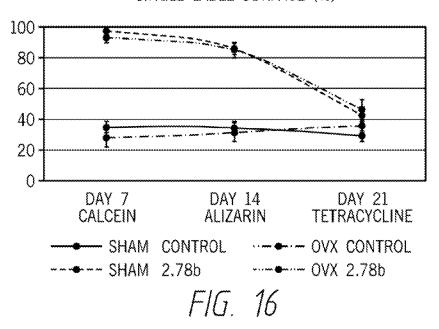
LV5 TRABECULAR BV / TOTAL VOLUME



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SINGLE-LABEL SURFACE (%)



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