(19) DANMARK

(10) DK/EP 2643019 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 61 K 39/00 (2006.01)* *A 61 K 39/395 (2006.01)* *C 07 K 16/40 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2019-04-15**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2019-01-02**

(86) Europæisk ansøgning nr.: **11843652.6**

(86) Europæisk indleveringsdag: **2011-11-22**

(87) Den europæiske ansøgnings publiceringsdag: **2013-10-02**

(86) International ansøgning nr.: **US2011061785**

(87) Internationalt publikationsnr.: **WO2012071381**

(30) Prioritet: **2010-11-24 US 416927 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Lexicon Pharmaceuticals, Inc., 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA**

(72) Opfinder: **BROMMAGE, Robert, Joseph, Jr., 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA**
FENG, Xiao, 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA
HONG, Seokjoo, 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA
LANDES, Gregory, 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA
LIU, Jeff, 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA
POTTER, David, George, 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA
POWELL, David, Reed, 8800 Technology Forest Place, The Woodlands, TX 77381-1160, USA

(74) Fuldmægtig i Danmark: **Larsen & Birkeholm A/S Skandinavisk Patentbureau, Banegårdspladsen 1, 1570 København V, Danmark**

(54) Benævnelse: **ANTISTOFFER MOD NOTUM PECTINACETYLESTERASE**

(56) Fremdragne publikationer:
EP-A1- 1 652 923
WO-A1-2012/027723
WO-A2-03/062410
CATHERINE CAVARD ET AL: "Gene expression profiling provides insights into the pathways involved in solid pseudopapillary neoplasm of the pancreas", JOURNAL OF PATHOLOGY, JOHN WILEY & SONS LTD, GB, vol. 218, no. 2, 1 June 2009 (2009-06-01), pages 201-209, XP002662070, ISSN: 0022-3417, DOI: 10.1002/PATH.2524 [retrieved on 2009-01-20]
ALEXANDRA TRAISTER ET AL: "Mammalian Notum induces the release of glypicans and other GPI-anchored proteins from the cell surface", BIOCHEMICAL JOURNAL, PORTLAND PRESS LTD, GB, vol. 410, 30 October 2007 (2007-10-30), pages 503-511, XP002662072, ISSN: 0264-6021, DOI: 10.1042/BJ20070511

Fortsættes ...

ROUX ET AL: "New treatment targets in osteoporosis", JOINT BONE SPINE, ELSEVIER, PARIS, FR, vol. 77, no. 3, 1 May 2010 (2010-05-01), pages 222-228, XP027060878, ISSN: 1297-319X [retrieved on 2010-04-08]
TORISU ET AL.: 'Human homolog of NOTUM, overexpressed in hepatocellular carcinoma, is regulated transcriptionally by beta-catenin/TCF' CANCER SCI vol. 99, no. 6, June 2008, pages 1139 - 1146, XP002662071

DESCRIPTION

1. FIELD OF THE INVENTION

[0001] This invention relates to antibody inhibitors of Notum Pectinacetylesterase, compositions comprising them, and methods of their use.

2. BACKGROUND OF THE INVENTION

[0002] 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.*

[0003] 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.

[0004] "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.

[0005] 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.*

[0006] Recent analysis of gene expression data has led to the identification of new targets of WNT signaling. *See, e.g.,* Torisu, Y., et al., Cancer Sci., 99(6):1139-1146, 1143 (2008). One such target is Notum Pectinacetylesterase, also known as NOTUM and LOC174111.

3. SUMMARY OF THE INVENTION

[0007] The subject-matter of the invention is as set out in the appended claims. In one aspect the invention provides a monoclonal antibody that binds human notum pectinacetylesterase (NOTUM) and reduces human NOTUM activity in a trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) assay *in vitro,* for use in a method of treating or preventing a disease or disorder characterized by bone loss in a patient. In some embodiments, the antibody binds to a NOTUM selected from mouse NOTUM, guinea pig NOTUM, cynomolgus monkey NOTUM, and rhesus monkey NOTUM. The antibody for use according to the invention reduces human NOTUM activity in a trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) assay *in vitro.*

[0008] In some embodiments, the antibody for use according to the invention 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 for use according to the invention 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.

[0009] In some embodiments, the antibody for use according to the invention 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) 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; f) 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; g) 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; h) 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; i) and 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.

[0010] In some embodiments, the antibody for use according to the invention is selected from a mouse antibody, a chimeric antibody, a humanized antibody, and a human antibody.

[0011] In some embodiments, an antibody for use according to the invention 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: 17, 25, 33 and 41; b) a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 18, 26, 34 and 42; and c) a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 19, 27, 35 and 43. 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: 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;

b) 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;

c) a set 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; d) 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;

and e) a set 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, the heavy chain comprises a heavy chain variable regions comprising an amino acid sequence selected from SEQ ID NOs: 15, 23, 31, 39, 63, 67, 71, 75, and 79.

[0012] In some embodiments, an antibody for use according to the invention 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: 20, 28, 36, 44 and ; b) a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 21, 29, 37, 45 and 61; and c) a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 22, 30, 38, 46, 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: 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;

b) 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;

c) 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; d) 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;

and e) 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 NOs: 16, 24, 32, 40, 56, 65, 69, 73, 77, and 81.

[0013] In some embodiments, an antibody for use according to the invention 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: 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 b) 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 c) 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 d) 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 e) 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.

[0014] In some embodiments, an antibody for use according to the invention 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: 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 c) 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 h) 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 i) 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

l) 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.

[0015] A further aspect of the invention provides a monoclonal antibody that binds to human notum pectinacetylesterase (NOTUM) and that neutralizes at least one activity of 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: 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 b) 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. In some embodiments of the inventive antibody according to (a), 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 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. In some embodiments of the inventive antibody according to (b), 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.

[0016] In a further aspect the invention provides a nucleic acid molecule comprising 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. A further aspect of the invention provides, a host cell comprising the above 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 a further aspect the invention provides a method of producing the above antibody of the invention, comprising incubating the above host cell under conditions sufficient to express the antibody.

[0017] A further aspect of the invention provides a pharmaceutical composition comprising an above antibody of the invention. In some embodiments, the antibody for use according to the invention stimulates endocortical bone formation in a patient, and the method comprises administering an effective amount of a pharmaceutical composition comprising an antibody for

use according to the invention. In some embodiments of the antibody for use according to the invention, said method comprises administering an effective amount of a pharmaceutical composition comprising the antibody for use according to the invention. 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

[0018]

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").

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.

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.

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

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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. DETAILED DESCRIPTION OF THE INVENTION

[0019] 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.

[0020] 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. Definitions

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] 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."

[0031] 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.

[0032] 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).

[0033] 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*.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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 ≤ 1 μM, in some embodiments, when the dissociation constant is ≤ 100 nM, and in some embodiments, when the dissociation constant is ≤ 10 nM.

[0039] 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.

[0040] 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.

[0041] 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

[0042] 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.

[0043] 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.).

[0044] 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.

[0045] 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.

[0046] 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.

[0047] 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.

[0048] 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

[0049] One aspect of the invention provides a monoclonal antibody that binds human notum pectinacetylesterase (NOTUM) and reduces human NOTUM activity in a trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) assay *in vitro,* for use in a method of treating or preventing a disease or disorder characterized by bone loss in a patient.

[0050] A neutralizing antibody against NOTUM for use according to the invention reduces NOTUM activity in a trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) assay *in vitro.*

[0051] In some embodiments, a neutralizing antibody against NOTUM for use according to the invention increases serum 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 for use according to the invention increases bone mineral density. In some embodiments, a neutralizing antibody against NOTUM for use according to the invention increases midshaft femur cortical thickness *in vivo*. In some embodiments, a neutralizing antibody against NOTUM for use according to the invention increases midshaft femur bone area *in vivo*. In some embodiments, a neutralizing antibody against NOTUM for use according to the invention increases midshaft humerus cortical thickness *in vivo*. In some embodiments, a neutralizing antibody against NOTUM for use according to the invention increases endocortical bone formation *in vivo*. In some embodiments, a neutralizing antibody against NOTUM for use according to the invention 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 for use according to the invention increases the proportion of femoral neck bone volume to total volume of the femoral neck *in vivo*.

[0052] 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 for use according to the invention. In some embodiments, neutralizing antibodies that depend upon a region from Q47 to M177 of human NOTUM for binding are provided for use according to the invention. 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 for use according to the invention. 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 for use according to the invention. In some such embodiments, the antibodies for use according to the invention specifically bind to both non-human primate NOTUM and human NOTUM. In some embodiments, the antibodies for use according to the invention specifically bind to both mouse NOTUM and human NOTUM.

[0053] In some embodiments, neutralizing antibodies that bind to a region of human NOTUM from Q47 to M177 are provided for use according to the invention. In some embodiments, neutralizing antibodies that depend upon a region of human NOTUM from Q47 to M177 for binding are provided for use according to the invention. In some embodiments, NOTUM neutralizing antibodies for use according to the inveniton 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 for use according to the invention 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 for use according to the invention 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 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).

[0054] In some embodiments, a neutralizing antibody against NOTUM for use according to the invention 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 for use according to the invention has an IC_{50} 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 IC_{50} 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. The IC_{50} is for human NOTUM.

[0055] In some embodiments, neutralizing antibodies for use according to the invention are non-human monoclonal antibodies. In some such embodiments, neutralizing antibodies are rodent monoclonal antibodies. In some such embodiments, neutralizing antibodies for use according to the invention are mouse monoclonal antibodies. In some embodiments, neutralizing antibodies for use according to the invention are chimeric monoclonal antibodies. In some embodiments, neutralizing antibodies for use according to the invention are humanized monoclonal antibodies. In some embodiments, neutralizing antibodies for use according to the invention are human monoclonal antibodies. In some embodiments, chimeric, humanized, and/or human monoclonal antibodies are useful as therapeutic antibodies in humans.

[0056] In some embodiments, neutralizing antibodies for use according to the invention are antibody fragments. Exemplary antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, Fv, scFv, Fd, diabodies, and the like.

[0057] Nonlimiting exemplary NOTUM neutralizing antibodies for use according to the invention include MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78. Each of MAbs 1.802, 1.815, 1.846, 2.1029, 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 for use according to the invention competes for binding to NOTUM with at least one antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78. In some embodiments, a NOTUM neutralizing antibody for use according to the invention binds to an epitope of NOTUM that at least partially overlaps with the epitope bound by at least one antibody selected from MAbs 1,802, 1.815, 1.846, 2.1029, and 2.78. In addition, in some

embodiments, an antibody for use according to the invention that competes for binding to NOTUM with at least one antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78 is predicted to be a NOTUM neutralizing antibody. The sequences of the CDRs and variable regions of MAbs 1.731 (comparative), 1.802, 1.815, 1.846, 2.1029, 2.55 (comparative), and 2.78 are shown in Section 7, below.

[0058] In some embodiments, NOTUM neutralizing antibodies for use according to the invention are provided that bind to the same epitope to which MAb 1.802 binds. In some embodiments, NOTUM neutralizing antibodies for use according to the invention are provided that bind to the same epitope to which MAb 1.815 binds. In some embodiments, NOTUM neutralizing antibodies for use according to the invention are provided that bind to the same epitope to which MAb 1.846 binds. In some embodiments, NOTUM neutralizing antibodies for use according to the invention are provided that bind to the same epitope to which MAb 2.1029 binds. In some embodiments, NOTUM neutralizing antibodies for use according to the invention are provided that bind to the same epitope to which MAb 2.78 binds.

[0059] In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a heavy chain variable region selected from SEQ ID NOs: 15, 23, 31 and 39. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a light chain variable region selected from SEQ ID NOs: 16, 24, 32 and 40. In some embodiments, a NOTUM neutralizing antibody for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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.

[0060] In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a heavy chain CDR1 selected from SEQ ID NOs: 17, 25, 33 and 41. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a heavy chain CDR2 selected from SEQ ID NOs: 18, 26, 34 and 42. In some embodiments, a NOTUM neutralizing antibody comprises a heavy chain CDR3 selected from SEQ ID NOs: 19, 27, 35 and 43. In some embodiments, a NOTUM neutralizing antibody for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention comprtses 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.

[0061] In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a light chain CDR1 selected from SEQ ID NOs: 20, 28, 36, 44, and 60. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a light chain CDR2 selected from SEQ ID NOs: 21, 29, 37, 45, and 61. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a light chain CDR3 selected from SEQ ID NOs: 22, 30, 38, 46, and 62. In some embodiments, a NOTUM neutralizing antibody for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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.

[0062] In some embodiments, a NOTUM neutralizing antibody for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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.

[0063] NOTUM neutralizing antibodies that specifically bind human NOTUM are provided for use according to the invention. 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 for use according to the invention. 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 for use according to the invention. In some embodiments, NOTUM neutralizing antibodies that specifically bind human NOTUM and NOTUM from at least one species of non-human primate are provided for use according to the invention. In some embodiments, NOTUM neutralizing antibodies that specifically bind human NOTUM and mouse NOTUM are provided for use according to the invention.

[0064] A further aspect of the invention provides a monoclonal antibody that binds to human notum pectinacetylesterase (NOTUM) and that neutralizes at least one activity of 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: 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 b) 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. In some embodiments of the inventive antibody according to (a), 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 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. In some embodiments of the inventive antibody according to (b), 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.

5.2.2.1. Chimerized and humanized monoclonal antibodies

[0065] In some embodiments, non-human antibodies for use according to the invention are chimerized. In some embodiments, mouse monoclonal antibodies that specifically bind human NOTUM for use according to the invention are chimerized. Certain exemplary methods for making chimeric antibodies are provided, for example, in Morrison et al. (1984) Proc. Nat'l Acad. Sci. USA 81:6851-6855; Neuberger et al. (1984) Nature 312:604-608; Takeda et al. (1985) Nature 314:452-454; and U.S. Patent Nos. 6,075,181 and 5,877,397.

[0066] In some embodiments, non-human antibodies for use according to the invention are "humanized." In some embodiments, mouse monoclonal antibodies for use according to the invention that specifically bind human NOTUM are humanized. In some embodiments, mouse monoclonal antibodies for use according to the invention, that are raised against mouse NOTUM, but which specifically bind (i.e., cross react) with human NOTUM, are humanized. In some embodiments, humanized antibodies for use according to the invention 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.

[0067] In some embodiments of humanized antibodies for use according to the invention, 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.

[0068] 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.

[0069] 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.

[0070] In some embodiments, non-human antibodies for use according to the invention 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.

[0071] In some embodiments, a humanized NOTUM neutralizing antibody for use according to the invention comprises a heavy chain comprising at least one of CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a heavy chain comprising CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises a light chain comprising at least one of CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78. In some embodiments, a

NOTUM neutralizing antibody for use according to the invention comprises a light chain comprising CDR1, CDR2, and CDR3 of an antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78. In some embodiments, a NOTUM neutralizing antibody for use according to the invention comprises heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 from an antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029, and 2.78.

[0072] In some embodiments, a NOTUM neutralizing antibody for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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: 69. In some embodiments, a NOTUM neutralizing antibody for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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 for use according to the invention 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

[0073] 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 constatnt 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

[0074] 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.

[0075] 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 401384; 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.

[0076] 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.

[0077] 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).

[0078] 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-p-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})alkoxy- or aryloxy-PEG. In some embodiments, PEG propionaldehyde may have advantages in manufacturing due to its stability in water.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] 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-bis-chlorosulfone and PEG-bis-vinylsulfone (see WO 95/13312).

5.2.3. Certain methods of making monoclonal antibodies

5.2.3.1. Certain hybridoma methods

[0083] 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 non-human 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.

[0084] 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) Antibodies: A Laboratory Manual Ch. 8 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0085] 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 human NOTUM.

[0086] 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).

[0087] 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

[0088] In some embodiments, human monoclonal antibodies are produced using a display-based method, such as, for example, any of those described below.

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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-pIII fusion protein on the phage surface.

[0094] 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.

[0095] 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 non-human animals are immunized with any of the immunogens described below.

[0096] 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).

[0097] 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.

[0098] 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.

[0099] 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) Nat. Biotechnol. 15:553-557.

5.2.3.3. Certain affinity maturation methods

[0100] 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.

[0101] 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.

[0102] 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) Nat. Med. 2:100-102; Fermer et al. (2004) Tumor Biol. 25:7-13.

[0103] 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.

[0104] 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.

[0105] 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.

[0106] 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

[0107] 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.

[0108] 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.

[0109] 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.

[0110] 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.

[0111] In some embodiments, a nucleic acid molecule comprises a polynucleotide sequence that encodes a heavy chain of a NOTUM neutralizing antibody selected from MAbs 1.802, 1.815, 1.846, 2.1029 and 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, 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 1.802, 1.815, 1.846, 2.1029, and 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, 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 1.802, 1.815, 1.846, 2.1029, and 2.78, and humanized versions of such MAbs.

[0112] 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.

[0113] 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. <u>Monoclonal antibodies for use in treatment</u>

[0114] 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.

[0115] This invention encompasses a monoclonal antibody that binds to human NOTUM and reduces human NOTUM activity in an OPTS assay *in vitro,* for use in a method of treating, or preventing a disease or disorder associated with bone loss. The method comprises administering to a patient in need thereof a therapeutically or prophylactically effective amount of an antibody for use according to 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.

[0116] Also disclosed herein 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 described herein. 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.

[0117] This invention also encompasses a monoclonal antibody that binds to human NOTUM and reduces human NOTUM activity in an OPTS assay *in vitro,* for use in a method of treating, managing, or preventing bone loss associated with, or caused by, a disease or disorder. The method comprises administering to a patient in need thereof a therapeutically or prophylactically effective amount of an antibody for use according to the invention. Examples of diseases and disorders include celiac disease, Crohn's Disease, Cushing's syndrome, hyperparathyroidism, inflammatory bowel disease, and ulcerative colitis.

[0118] Nonlimiting exemplary patients that may benefit from the antibodies and antibodies for use according to this invention include men and women aged 55 years or older, post-menopausal women, and patients suffering from renal insufficiency.

[0119] Antibodies of the invention and antibodies for use according to 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.

[0120] Examples of androgen receptor modulators include finasteride and other 5α-reductase inhibitors, nilutamide, flutamide, bicalutamide, liarozole, and abiraterone acetate.

[0121] 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.

[0122] Examples of cathepsin K inhibitors include VEL-0230, AAE581 (balicatib), MV061194, SB-462795 (relacatib), MK-0822 (odanacatib), and MK-1256.

[0123] 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.

[0124] Examples of integrin binders, antibodies, and receptor antagonists include vitaxin (MEDI-522), cilengitide and L-000845704.

5.4. Pharmaceutical Formulations

[0125] 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.

[0126] 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.

[0127] 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.

[0128] In some embodiments, formulation materials for pharmaceutical compositions are nontoxic to recipients at the dosages and concentrations employed.

[0129] 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 hydrogensulfite); 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).

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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.

[0137] 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.

[0138] 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).

[0139] 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 (2-hydroxyethyl-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(-)-3-hydroxybutyric 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.

[0140] 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.

[0141] 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.

[0142] 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.

[0143] 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.

[0144] 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).

[0145] In some embodiments, a suitable dosage may be determined by one skilled in the art, for example, based on animal studies.

[0146] 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.

[0147] 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.

[0148] 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.

[0149] 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.

[0150] 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

[0151] 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, or wild type offspring at an approximate ratio of 1:2:1.

[0152] 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.

[0153] 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 pCT40), 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).

[0154] 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>

[0155] 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.

[0156] 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.

[0157] 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.

[0158] 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.

[0159] 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.

[0160] 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

[0161] Antibodies were raised against purified recombinant human and mouse NOTUM proteins in two separate immunization campaigns.

[0162] 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.).

[0163] 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.

[0164] 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.

[0165] 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

[0166] 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.

[0167] 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.

[0168] Antibodies were tested in quadruplicate in 384-well plates. 12.5 µl 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 µl of purified antibody. After mixing, antibody and NOTUM were incubated at room temperature for 20 minutes followed by addition of 25µl of 1.25 µM 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 25µl 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.

[0169] 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.

[0170] 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

[0171] 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 µg/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.

[0172] 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. Characterization of NOTUM Neutralizing Antibodies

[0173] 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.

[0174] 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 IC_{50} 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.

[0175] 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	Bin	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	IgG1	1	6.44	5.71	No inhibition	No inhibition	No	No
1.815	IgG1	1	7.62	6.88	No inhibition	nd	No	No
1.846	IgG2b	1	10.07	1.70	No inhibition	nd	No	No
1.731 *	IgG1	3	>166.67	15.52	196.74	No inhibition	Yes	Yes
1.655 *	IgG1	3	>166.67	nd	>166.67	nd	nd	Yes
1.168 *	IgG2a	4	56.61	No inhibition	No inhibition	nd	Yes	Yes
1.712 *	IgG2a	2	125.36	58.49	No inhibition	nd	Yes	Yes
1.807 *	IgG2a	2	nd	No inhibition	No inhibition	nd	Yes	Yes

[0176] 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.

[0177] 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} (nM; mouse NOTUM)	Wnt signaling IC_{50} (nM; mouse NOTUM)	OPTS IC50 (nM; human NOTUM)	Wnt signaling IC_{50} (nM; human NOTUM)	Human NOTUM binding	Western blot binding
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 *	IgG2b	3	37.70	No inhibition	No inhibition	No inhibition	Yes	No
2.1001 *	IgG2b	3	>166.67	No inhibition	No inhibition	No inhibition	No	Yes
2.55 *	IgG2a	1	26.13	No inhibition	No inhibition	No inhibition	Yes	Yes
2.1002 *	IgG2a	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 *	IgG2a	1	56.95	No inhibition	No inhibition	No inhibition	Yes	Yes
2.236 *	IgG2a	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%.
* comparative antibody

6.6. Binding Competition Studies Using NOTUM Neutralizing Antibodies

[0178] 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'.

[0179] 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

[0180] 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.

[0181] 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.

[0182] 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

[0183] 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 fragment	K_D (nM)	k_{on} ($M^{-1}sec^{-1}$)	k_{off} (M^{-1} sec^{-1})
1.802 IgG	1.42	2.57×10^5	3.65×10^{-4}
1.802 Fab	0.91	8.99×10^5	8.20×10^{-4}
2.78 IgG	17.6	4.79×10^4	8.41×10^{-4}
2.78 Fab	15.4	8.77×10^4	1.36×10^{-3}
2.1029 IgG	5.99	1.51×10^5	9.08×10^{-4}
Affinity for mouse NOTUM			
Antibody or fragment	k_D (nM)	k_{on} ($M^{-1}sec^{-1}$)	k_{oof} ($M^{-1}sec^{-1}$)
1.802 Fab	*No binding observed*		
2.78 Fab	4.99	3.91×10^4	1.95×10^{-4}

6.9. Administration of NOTUM Neutralizing Antibodies to Mice

6.9.1. Administration of NOTUM Neutralizing Antibodies Weekly for 8 Weeks

[0184] 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 µCT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0185] 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

[0186] 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 µCT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0187] 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

[0188] 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 µCT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0189] 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

[0190] 28-week old male F1 hybrid mice (129 x C57) were administered a single dose 50 µg/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 µCT40 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.

[0191] As shown in Figure 9A, the midshaft femur cortical thickness increased by 10 µm, 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 µm, 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 µm, 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

[0192] 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."

[0193] 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 μCT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0194] 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

[0195] 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 μCT40 with a threshold value of 240, an integration time of 200 milliseconds, and an X-ray tube voltage of 55 keV.

[0196] 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.

[0197] 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

[0198] 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.

[0199] 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 marker levels 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	Δ = 39%
				P = 0.02
CTX (ng/ml)	2	10.5 ± 0.9	14.1 ± 0.9	Δ = 33%
				P = 0.01
PINP (ng/ml)	4	41.2 ± 2.3	54.8 ± 2.5	Δ = 33%
				P = 0.001

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

[0200] 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.		

[0201] 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

[0202] Bone mass and architecture were determined by microCT following necropsy, using a Scanco μCT40 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.

[0203] As shown in Figure 13A, the midshaft femur cortical thickness increased by 22 μm, or 9%, in sham surgery mice administered NOTUM neutralizing antibody 2.78b, relative to sham surgery mice administered control antibody, and increased by 26 μm, 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 mm^2, 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 mm^2, or 10%, in ovariectomized mice administered NOTUM neutralizing antibody 2.78b, relative to ovariectomized mice administered control antibody.

[0204] 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.

[0205] 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

[0206] 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™ 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.

[0207] 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	Day 7	Day 14	Day 21
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

[0208] 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

Two-Factor ANOVA	Days 7 to 14	Days 14 to 21

[0209] 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. Identification of Species Suitable for Testing NOTUM Neutralizing Antibodies

[0210] 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.

[0211] 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.

[0212] 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

[0213] 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

Mouse antibody	Heavy chain variable region SEQ ID NO (CDR1, CDR2, CDR3 SEQ ID NOs).	Light chain variable region SEQ ID NO (CDR1, CDR2, CDR3 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)
* comparative antibody		

[0214] 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 GFTFSDX_1GMH (SEQ ID NO: 90), wherein X_1 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 KX_2YNGGYFDV (SEQ ID NO: 91), wherein X_2 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 LASX$_6$LES (SEQ ID NO: 93), wherein X$_6$ 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 RASKX$_3$VSX$_4$SGYSYX$_5$H (SEQ ID NO: 92), wherein X$_3$ is I or S, X$_4$ is T or E, and X$_5$ is M or I.

[0215] 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).

[0216] 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.

7. Table of Sequences

SEQ ID NO	Description	Sequence
1	Human NOTUM	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV 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

SEQ ID NO	Description	Sequence
		RSYWTDVQVK GTSLPRALHC WDRSFHDSHK ASKTPMKGCP FHLVDSCPWP HCNPSCPTIR DQFTGQEMNV AQFLMHMGFD VQTVAQQQGM EPSKLLGMLS NGN
3	Human NOTUM S232A	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE 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
		VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
4	Mouse NOTUM S239A mutant	MGGEVRVLLL LGLLHWVGGS EGRKTWRRRG QQPFQPPPPP PLPQRAEVEP GAGQPVESFP LDFTAVEGNM DSFMAQVKSL AQSLYPCSAQ QLNEDLRLHL LLNTSVTCND GSPAGYYLKE SKGSRRWLLF LEGGWYCFNR ENCDSRYSTM RRLMSSKDWP HTRTGTGILS SQPEENPHWW NANMVFIPYC SSDVWSGASP KSDKNEYAFM GSLIIQEVVR ELLGKGLSGA KVLLLAGSAA GGTGVLLNVD RVAELLEELG YPSIQVRGLA DSGWFLDNKQ YRRSDCIDTI NCAPTDAIRR GIRYWSGMVP ERCQRQFKEG EEWNCFFGYK VYPTLRCPVF VVQWLFDEAQ LTVDNVHLTG QPVQEGQWLY IQNLGRELRG TLKDVQASFA PACLSHEIII RSYWTDVQVK GTSLPRALHC WDRSFHDSHK ASKTPMKGCP FHLVDSCPWP HCNPSCPTIR DQFTGQEMNV AQFLMHMGFD VQTVAQQQGM EPSKLLGMLS NGN
5	Guinea pig NOTUM	MGRGVRVLFL LGLLHWAGGG EGRKTWRRRG QQPAPAPLPP QRTEAAPGTG QPVESFPLDF TAVEGNMDSF MAQVKSLAQS LYPCSAQQLN EDLRLHLLLN TSVTCNDGSP AGYYLKESKG SRRWLLFLEG GWYCFSRENC DSRYDTMRRL MSSKDWPQTR TGTGILSSQP EENPYWWNAN MVFIPYCSSD VWSGASSKSE KNEYVFMGAL IIREVVQELL GRGLSGAKVL LLAGSSAGGT GVLLNVDRVA EQLEQLGYPA IQVRGLADSG WFLDNKQYRR TDCVDTVTCA PTEAIRRGIR YWNGMVPERC RSQFKEGEEW NCFLGYKVYP TLRCPVFVVQ WLFDEAQLTA DNAHLTGQPV QEGQWLYIQN LGHELRNTLK DVPASFAPAC LSHEIIIRSH WTDVQVKGTS LPRALHCWDR SLHDSHKASK TPLKGCPIHL VDSCPWPHCN PSCPTIRDQF TGQEMNVAQF LMHMGFDVQT VAQQQGLEPS KLLGMLSSGS
	Cynomolgus monkey NOTUM	MGRGVRVLLL LGLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY NTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE ELGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKIYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQESQ RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKTSKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDVQTVAQQ QGPEPSKLLG LPSDGS
6	Rhesus macaque NOTUM	MGRGVRVLLL LGLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY NTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE ELGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKIYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQESQ RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKNSKTPLK GCPVHLVDSC PWPHCNPSCP

SEQ ID NO	Description	Sequence
		TVRDQFTGQE MNVAQFLMHM GFDVQTVAQQ QGPEPSKLLG LPSDGS
7	MAb 1.731 heavy chain variable region	EVQLQQSGPE LVKPGASVKV SCKASGYPFT DYFIHWVKQT HGKSLEWIGY FFPKNGANGY NQKFEGKVTL TVDKSSSTAY MELRSLTSED SAVYYCARRY GNYYSMDYWG QGTSVTVSSA KTTPP
8	MAb 1.731 light chain variable region	SFVMTQTPKF LLVSAGDRVT ITCKASQSVG DDVAWYQQKP GQSPTLLIYR VSNRYTGVPD RFTGSGYGTD FTFTINTVQA EDLAVYFCQQ DYSSPYTFGG GTQLEVKRAD AAP
9	MAb 1.731 heavy chain CDR1	GYPFTDYFIH
10	MAb 1.731 heavy chain CDR2	YFFPKNGANG
11	MAb 1.731 heavy chain CDR3	RYGNYYSMDY
12	MAb 1.731 light chain CDR1	KASQSVGDDVA
13	MAb 1.731 light chain CDR2	RVSNRYT
14	MAb 1.731 light chain CDR3	QQDYSSPYT
15	MAb 1.802 heavy chain variable region	EVQLVESGGG LVKPGGSLKL SCAASGFTFS DYGMHWFRQA PEKGLEWVAY ISSGSRTVYY ADTVKGRFTI SRDNAKNTLS LQMTSLRSED TAMYYCARKH
		YNGGYFDVWG TGTTVTVSSA KTTP
16	MAb 1.802 light chain variable region	DIVLTQSPAS LAVSLGQRAT ISCRASKIVS TSGYSYMHWY QQKPGQPPKL LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSRELPP TFGSGTKLEI KRADAAP
17	MAb 1.802 heavy chain CDR1	GFTFSDYGMH
18	MAb 1.802 heavy chain CDR2	YISSGSRTVY
19	MAb 1.802 heavy chain CDR3	KHYNGGYFDV
20	MAb 1.802 light chain CDR1	RASKIVSTSGYSYMH
21	MAb 1.802 light chain CDR2	LASNLES

SEQ ID NO	Description	Sequence
22	MAb 1.802 light chain CDR3	QHSRELPPT
23	MAb 1.815 heavy chain variable region	DVQLLESGGG LVQPGGSRKL SCAASGFTFS DFGMHWVRQA PEKGLEWVAY SSSGGTTVYY ADTVKGRLTL SRDNSKNTLF LEMTSLRSED TAMYYCARAS YDGGYFDCWG QGTSLTVSSA KTTPP
24	MAb 1.815 light chain variable region	DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYSYIHWY QQKPGQPPKL LIYLASDLES GVPARFSGSG SGAAFTLNIH PVEEEDAATY YCHHSRELPF 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 PEKGLEWVAY ISSGSTTLSY ANTMKGRFTI SRDNAKKTLS LQMTSLRSED TAIYYCARKN YNGGYFDVWG TGTTVTVSSA KTTPP
32	MAb 1.846 light chain variable region	DIVLTQSPAS LVVSLGQRAT ISCRASKSVS ESGYSYMHWY QQKPGQPPKL LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEGDATTY YCQHSRVLPP 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	QHSRVLPPT

SEQ ID NO	Description	Sequence
	chain CDR3	
39	MAb 2.1029 heavy chain variable region	QVQLKESGPG LVAPSQSLSI TCTVSGFSLT SYGVHWVRQP PGKGLEWLGV IWAGGSTNYN SALMSRLSIS KDNSKSQVFL KMNSLQTDDT AIYFCARDGD YGTIYAMDYW GQGTSVTVSS AKTTAPS
40	MAb 2.1029 light chain variable region	DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY TSRLHSGVPS RFTGSGSGTD YSLTISNLEQ EDIATYFCQQ GKTLPRTFGG GTMLEIKRAD AAP
41	MAb 2.1029 heavy chain CDR1	GFSLTSYGVH
42	MAb 2.1029 heavy chain CDR2	VIWAGGSTN
43	MAb 2.1029 heavy chain CDR3	DGDYGTIYAMDY
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 chain variable region	EVQLQQSGTV LARPGALVKM SCKASGYTFT SYWMHWVKQR PGQGLEWIGA IYPGKSDTRY NQKFKDKAKL TAVTSTSTAY MDLSSLTDED SAVYYCSRRY GNFYAMDYWG QGTSVTVSSA KTTAPS
48	MAb 2.55 light chain variable region	SIVMTQTPKF LLVSAGDRVT MTCKASQSVS NDVAWYQQKP GQSPELLIYY 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

SEQ ID NO	Description	Sequence
55	MAb 2.78 heavy chain variable region	DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWVRQA PEKGLEWVAY ITSGSGAIYY ADTVRGRFTI SRDTPKNTLF LQMTSLRSED TAMYYCARSA DGLDYWGQGT SVTVSSAKTT PPS
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 TTPPMLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
65	HumAb 2.78 light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASENIY SNLAWYQQKP GKAPKLLIYG ATNLADGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQH FWGTPFTFGQ GTKVEI
66	HumAb 2.78 light chain	DIQMTQSPSS LSASVGDRVT ITCRASENIY SNLAWYQQKP GKAPKLLIYG
		ATNLADGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQH FWGTPFTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

SEQ ID NO	Description	Sequence
67	HumAb 2.1029 heavy chain variable region	QVQLQESGPG LVKPSETLSL TCTVSGFSLT SYGVHWIRQP PGKGLEWIGV IWAGGSTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARDGD YGTIYAMDYW GQGTLVTVSS
68	HumAb 2.1029 heavy chain	QVQLQESGPG LVKPSETLSL TCTVSGFSLT SYGVHWIRQP PGKGLEWIGV IWAGGSTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARDGD YGTIYAMDYW GQGTLVTVSS DVWGQGTTVT VSSASTKGPS VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVTSSNFG TQTYTCNVDH KPSNTKVDKT VERKCCVECP PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVQFNWY VDGMEVHNAK TKPREEQFNS TFRVVSVLTV VHQDWLNGKE YKCKVSNKGL PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPML DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
69	HumAb 2.1029 light chain variable region	DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY TSRLHSGVPS RFSGSGSGTD FTFTISSLQP EDIATYYCQQ GKTLPRTFGG GTKVEI
70	HumAb 2.1029 light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY TSRLHSGVPS RFSGSGSGTD FTFTISSLQP EDIATYYCQQ GKTLPRTFGG GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
71	HumAb 1.802 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA PGKGLEWVSY ISSGSRTVYY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARKH YNGGYFDVWG QGTLVTVSS
72	HumAb 1.802 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA PGKGLEWVSY ISSGSRTVYY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARKH YNGGYFDVWG QGTLVTVSSD VWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVTSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGMEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
73	HumAb 1.802 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASKIVS TSGYSYMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSRELPP TFGQGTKLEI
74	HumAb 1.802 light chain	DIVMTQSPDS LAVSLGERAT INCRASKIVS TSGYSYMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSRELPP TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

SEQ ID NO	Description	Sequence
75	HumAb 1.815 heavy chain variable region	QVQLVESGGG LVKPGGSLRL SCAASGFTFS DFGMHWIRQA PGKGLEWVSY SSSGGTTVYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARAS YDGGYFDCWG QGTTVTVSS
76	HumAb 1.815 heavy chain	QVQLVESGGG LVKPGGSLRL SCAASGFTFS DFGMHWIRQA PGKGLEWVSY SSSGGTTVYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARAS YDGGYFDCWG QGTTVTVSSD VWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVTSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGMEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
77	HumAb 1.815 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASKSVS TSGYSYIHWY QQKPGQPPKL LIYLASDLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHSRELPF TFGQGTKLEI
78	HumAb 1.815 light chain	DIVMTQSPDS LAVSLGERAT INCRASKSVS TSGYSYIHWY QQKPGQPPKL
		LIYLASDLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHSRELPF TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
79	HumAb 1.846 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA PGKGLEWVSY ISSGSTTLSY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARKN YNGGYFDVWG QGTLVTVSS
80	HumAb 1.846 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA PGKGLEWVSY ISSGSTTLSY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARKN YNGGYFDVWG QGTLVTVSSD VWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVTSSNFGT QTYTCNVDHK PSNTKVDKTV ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGMEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
81	HumAb 1.846 light chain variable region	DIVMTQSPDS LAVSLGERAT INCRASKSVS ESGYSYMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSRVLPP TFGQGTKLEI
82	HumAb 1.846 light chain	DIVMTQSPDS LAVSLGERAT INCRASKSVS ESGYSYMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHSRVLPP TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

SEQ ID NO	Description	Sequence
90	Campaign 1 heavy chain CDR1 consensus	GFTFSDX$_1$GMH
91	Campaign 1 heavy chain CDR3 consensus	KX$_2$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 AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRRSDCI DTINCAPTDA IRRGIRYWSG MVPERCQRQF KEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGQ WLYIQNLGRE LRGTLKDVQA SFAPACLSHE IIIRSYWTDV QVKGTSLPRA LHCWDRSFHD SHKASKTPMK GCPFHLVDSC PWPHCNPSCP TIRDQFTGQE MNVAQFLMHM GFDVQTVAQQ QGMEPSKLLG MLSNGN
84	Mouse-human chimeric 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 YRHTDCVDTI TCAPTEAIRR GIRYWNGVVP ERCRRQFQEG EEWNCFFGYK VYPTLRCPVF VVQWLFDEAQ LTVDNVHLTG QPVQEGLRLY IQNLGRELRH TLKDVPASFA PACLSHEIII RSHWTDVQVK GTSLPRALHC WDRSLHDSHK ASKTPLKGCP VHLVDSCPWP HCNPSCPTVR DQFTGQEMNV AQFLMHMGFD MQTVAQPQGL EPSELLGMLS NGS
85	Human-mouse-human chimeric NOTUM	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASPKSDKNEY AFMGSLIIQE VVRELLGKGL SGAKVLLLAG SSAGGTGVLL NVDRVAELLE ELGYPSIQVR GLADSGWFLD NKQYRRSDCI DTINCAPTDA IRRGIRYWSG MVPERCQRQF KEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH
		LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
86	Mouse-human-mouse chimeric NOTUM	MGGEVRVLLL LGLLHWVGGS EGRKTWRRRG QQPPQPPPPP PLPQRAEVEP GAGQPVESFP LDFTAVEGNM DSFMAQVKSL AQSLYPCSAQ QLNEDLRLHL LLNTSVTCND GSPAGYYLKE SKGSRRWLLF LEGGWYCFNR ENCDSRYSTM RRLMSSKDWP HTRTGTGILS SQPEENPHWW NANMVFIPYC SSDVWSGASS

SEQ ID NO	Description	Sequence
		KSEKNEYAFM GALIIQEVVR ELLGRGLSGA KVLLLAGSSA GGTGVLLNVD RVAEQLEKLG YPAIQVRGLA DSGWFLDNKQ YRHTDCVDTI TCAPTEAIRR GIRYWNGVVP ERCRRQFQEG EEWNCFFGYK VYPTLRCPVF VVQWLFDEAQ LTVDNVHLTG QPVQEGQWLY IQNLGRELRG TLKDVQASFA PACLSHEIII RSYWTDVQVK GTSLPRALHC WDRSFHDSHK ASKTPMKGCP FHLVDSCPWP HCNPSCPTIR DQFTGQEMNV AQFLMHMGFD VQTVAQQQGM EPSKLLGMLS NGN
87	Human NOTUM (Δ1-46); CD33 signal peptide in italics	*MPLLLLLPLL WAGALAQPVE* SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
88	Human NOTUM N96D	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLDTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
89	Human NOTUM Q47-M177	QPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANM
94	Human NOTUM D1415	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY STMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
95	Mouse NOTUM S148D	MGGEVRVLLL LGLLHWVGGS EGRKTWRRRG QQPPQPPPPP PLPQRAEVEP GAGQPVESFP LDFTAVEGNM DSFMAQVKSL AQSLYPCSAQ QLNEDLRLHL LLNTSVTCND GSPAGYYLKE SKGSRRWLLF LEGGWYCFNR ENCDSRYDTM 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 HCNPSCPTIR DQFTGQEMNV AQFLMHMGFD VQTVAQQQGM EPSKLLGMLS NGN
96	Human NOTUM N132A/R133A	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FAAENCDSRY DTMRRLMSSR

SEQ ID NO	Description	Sequence
		DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV
		QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
97	Human NOTUM E134A/N135A	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRAACDSRY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
98	Human NOTUM D137A/R139A	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCASAY DTMRRLMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
99	Human NOTUM R144A/R145A	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMAALMSSR DWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
100	Human NOTUM R150A/D151A	MGRGVRVLLL LSLLHCAGGS EGRKTWRRRG QQPPPPPRTE AAPAAGQPVE SFPLDFTAVE GNMDSFMAQV KSLAQSLYPC SAQQLNEDLR LHLLLNTSVT CNDGSPAGYY LKESRGSRRW LLFLEGGWYC FNRENCDSRY DTMRRLMSSA AWPRTRTGTG ILSSQPEENP YWWNANMVFI PYCSSDVWSG ASSKSEKNEY AFMGALIIQE VVRELLGRGL SGAKVLLLAG SSAGGTGVLL NVDRVAEQLE KLGYPAIQVR GLADSGWFLD NKQYRHTDCV DTITCAPTEA IRRGIRYWNG VVPERCRRQF QEGEEWNCFF GYKVYPTLRC PVFVVQWLFD EAQLTVDNVH LTGQPVQEGL RLYIQNLGRE LRHTLKDVPA SFAPACLSHE IIIRSHWTDV QVKGTSLPRA LHCWDRSLHD SHKASKTPLK GCPVHLVDSC PWPHCNPSCP TVRDQFTGQE MNVAQFLMHM GFDMQTVAQP QGLEPSELLG MLSNGS
101	1.802 heavy chain variable region polynucleotide sequence	ATGGACTCCA GGCTCAATTT AGTTTTCCTT GTCCTTATTT TAAAAGGTGT CCAGTGTGAG GTGCAGCTGG TGGAGTCTGG GGGAGGCTTA GTGAAGCCTG GAGGGTCCCT GAAACTCTCC TGTGCAGCCT CTGGATTCAC TTTCAGTGAC TATGGAATGC ACTGGTTTCG TCAGGCTCCA GAGAAGGGGC TGGAGTGGGT TGCATATATT AGTAGTGGCA GTAGAACCGT CTACTATGCA GACACAGTGA AGGGCCGATT CACCATCTCC AGAGACAATG CCAAGAACAC CCTGTCCCTG

SEQ ID NO	Description	Sequence
		CAAATGACCA GTCTGAGGTC TGAGGACACG GCCATGTATT ACTGTGCGAG GAAACATTAC AACGGTGGAT ACTTCGATGT CTGGGGCACA GGGACCACGG TCACCGTCTC CTCAGCCAAA ACGACACCCC CATCTGTCTA TCCACTGGCC CCTGGATCTG CTGCCCAAAC TAACTCCATG GTGACCCTGG GATGC
102	1.802 light chain variable region polynucleotide sequence	ATCCTCTCTT CCAGCTCTCA GAGATGGAGA CAGACACACT CCTGTTATGG GTACTGCTGC TCTGGGTTCC AGGTTCCACT GGTGACATTG TGCTGACACA GTCTCCTGCT TCCTTAGCTG TATCTCTGGG GCAGAGGGCC ACCATCTCAT GCAGGGCCAG CAAAATTGTC AGTACATCTG GCTATAGTTA TATGCACTGG TACCAACAGA AACCAGGACA GCCGCCCAAA CTCCTCATCT ATCTTGCATC CAACCTAGAA TCTGGGGTCC CTGCCAGGTT CAGTGGCAGT GGGTCTGGGA CAGACTTCAC CCTCAACATC CATCCTGTGG AGGAGGAGGA TGCTGCAACC TATTACTGTC AGCACAGTAG GGAGCTTCCT CCCACGTTCG GCTCGGGGAC AAAGTTGGAA ATAAAACGGG CTGATGCTGC ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAGTTA ACATCTGGAG GT
103	1.815 heavy chain variable region polynucleotide sequence	TCTGACAGAG GAGCCAAGCC CTGGATTCCC AGGTCCTCAC ATTCAGTGAT CAGCACTGAA CACAGACCAC TCACCATGGA CTCCAGGCTC AATTTAGTTT TCCTTGTCCT TATTTTAAAA GGTGTCCAGT GTGATGTGCA ACTGCTGGAA TCTGGGGGAG GCTTAGTGCA GCCTGGAGGG TCCCGGAAAC TCTCCTGTGC
		AGCCTCTGGA TTCACTTTCA GTGACTTTGG AATGCACTGG GTTCGTCAGG CTCCAGAGAA GGGGCTGGAG TGGGTCGCAT ACAGTAGTAG TGGCGGTACT ACCGTCTACT ATGCAGACAC GGTGAAGGGC CGACTCACCC TCTCCAGAGA CAATTCCAAG AACACCCTGT TCCTGGAAAT GACCAGTCTA AGGTCTGAGG ACACGGCCAT GTATTACTGT GCAAGAGCGT CCTATGATGG AGGGTACTTT GACTGCTGGG GCCAAGGCAC CTCTCTCACA GTCTCCTCAG CCAAAACGAC ACCCCCATCT GTCTATCCAC TGGCCCCTGG ATCTGCTGCC CAAACTAACT CCATGGTGAC CCTGGGATGC
104	1.815 light chain variable region polynucleotide sequence	ATCCTCTCTT CCAGCTCTCA GAGATGGAGA CAGACACACT CCTGTTATGG GTACTGCTGC TCTGGGTTCC AGGTTCCACT GGTGACATTG TGCTGACACA GTCTCCTGCT TCCTTAGCTG TATCTCTGGG GCAGAGGGCC ACCATCTCAT GCAGGGCCAG CAAAAGTGTC AGTACATCTG GCTATAGTTA TATACACTGG TACCAACAGA AACCAGGACA GCCACCCAAA CTCCTCATCT ATCTTGCATC CGACCTAGAA TCTGGGGTCC CTGCCAGGTT CAGTGGCAGT GGATCTGGGG CAGCCTTCAC CCTCAACATC CATCCTGTGG AGGAGGAGGA TGCTGCAACC TATTACTGTC ACCACAGTAG GGAGCTTCCA TTCACGTTCG GCTCGGGGAC AAAGTTGGAA ATAAAACGGG CTGATGCTGC ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAGTTA ACATCTGGAG GTGCCTCAGT CGTGTGC
105	1.846 heavy chain variable region polynucleotide sequence	AGAGGAGCCA AACCCTGGAT TCCCAGGTCC TCACATTCAG TGATCAGCAC TGAACACAGA CCACTCACCA TGGACTCCAG GCTCAATTTA GTTTTCCTTG TCCTTATTTT AAAAGGTGTC CAGTGTGAGG TGCAGCTGGT GGAGTCTGGG GGAGACTTAG TGAAGCCTGG AGGGTCCCTG AAACTCTCCT GTGCAGCCTC TGGATTCACT TTCAGTGACT ATGGAATGCA CTGGCTTCGT CAGGCTCCAG AGAAGGGGCT GGAGTGGGTT GCATATATTA GTAGTGGCAG TACTACCCTC TCCTATGCAA ACACAATGAA GGGCCGATTC ACCATCTCCA GAGACAATGC CAAGAAAACC CTGTCCCTGC AAATGACCAG TCTGAGGTCT GAGGACACGG CCATTTATTA CTGTGCGCGG AAAAATTACA ACGGTGGTTA CTTCGATGTC TGGGGCACAG GGACCACGGT CACCGTCTCC TCAGCCAAAA CAACACCCCC ATCAGTCTAT CCACTGGCCC CTGGGTGTGG AGATACAACT GGTTCCTCTG TGACTCTGGG ATGCCTGGTC AAGGG
106	1.846 light chain variable region polynucleotide sequence	ATCCTCTCTT CCAGCTCTCA GAGATGGAGA CAGACACACT CCTGTTATGG GTACTGCTGC TCTGGGTTCC AGGTTCCACT GGTGACATTG TGCTGACACA GTCTCCTGCT TCCTTAGTTG TATCTCTGGG GCAGAGGGCC ACCATCTCAT GCAGGGCCAG CAAAAGTGTC AGTGAATCTG GCTATAGTTA TATGCACTGG TACCAACAGA AACCAGGACA GCCACCCAAA CTCCTCATCT ATCTTGCATC CAACCTAGAG TCTGGGGTCC CTGCCAGGTT CAGTGGCAGT GGGTCTGGGA

SEQ ID NO	Description	Sequence
		CAGACTTCAC CCTCAACATC CATCCTGTGG AGGAGGGGGA TGCTACAACC TATTACTGTC AGCACAGTAG GGTCCTTCCT CCCACGTTCG GCTCGGGGAC AAAGTTGGAA ATAAAACGGG CTGATGCTGC ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAGTTA ACATCTGGAG GTGC
107	2.78 heavy chain variable region polynucleotide sequence	GACAGAGGAG CCAAGCCCTG GATTCCCAGG TCCTCACATT CAGTGATCAG CACTGAACAC AGACCACTCA CCATGGACTC CAGGCTCAAT TTAGTTTTCC TTGTCCTTAT TTTAAAAGGT GTCCAGTGTG ATGTGCAGCT GGTGGAGTCT GGGGGAGGCT TAGTGCAGCC TGGAGGGTCC CGGAAACTCT CCTGTGCAGC CTCTGGATTC ACTTTCAGTA GCTTTGGCAT GCACTGGGTT CGTCAGGCTC CAGAGAAGGG ACTGGAGTGG GTCGCATACA TTACTAGTGG CAGTGGTGCC ATCTACTATG CAGACACAGT GAGGGGCCGA TTCACCATCT CCAGAGACAC TCCCAAGAAC ACCCTGTTCC TGCAGATGAC CAGTCTAAGG TCTGAGGACA CGGCCATGTA TTACTGTGCA AGATCGGCTG ATGGTTTGGA CTACTGGGGT CAAGGAACCT CAGTCACCGT CTCCTCAGCC AAAACAACAC CCCCATCAGT CTATCCACTG GCCCCTGGGT GTGGAGATAC AACTG
108	2.78 light chain variable region polynucleotide sequence	CAGCCTCACA CTGATCACAC ACAGACATGA GTGTGGCCAC TCAGGTCCTG GGGTTGCTGC TGCTGTGGCT TACAGATGCC AGATGTGACA TCCAGATGAC TCAGTCTCCA GCCTCCCTAT ATGTATCTGT GGGAGAAACT GTCACCATCA CATGTCGAGC AAGTGAGAAT ATTTACAGTA ATTTAGCATG GTATCAGCAG AAACAGGGAA AATCTCCTCA GCTCCTGGTC TATGGTGCAA CAAACTTAGC AGATGGTGTG CCATCAAGGT TCAGTGGCAG TGGATCAGGC ACACAGTATT CCCTCAAGAT CAACAGCCTG AAGTCTGAAG ATTTTGGGAG TTATTACTGT CAACATTTTT GGGGTACTCC ATTCACGTTC GGCTCGGGGA CAAAGTTGGA AATAAAACGG GCTGATGCTG CACCAACTGT ATCCATCTTC CCACCATCCA GTGAGCAGTT AACATCTGGA GGTGCCTCAG TCGTGTGC
109	2.1029 heavy chain variable region polynucleotide sequence	ATCTCCTCAC TAGAGCCCCC ATCAGAGCAT GGCTGTCCTG GTGCTGTTCC TCTGCCTGGT TGCATTTCCA AGCTGTGTCC TGTCCCAGGT GCAGCTGAAG GAGTCAGGAC CTGGCCTGGT GGCGCCCTCA CAGAGCCTGT CCATCACTTG CACTGTCTCT GGGTTTTCAT TAACCAGCTA TGGTGTACAC TGGGTTCGCC AGCCTCCAGG AAAGGGTCTG GAGTGGCTGG GAGTAATATG GGCTGGTGGA AGCACAAATT ATAATTCGGC TCTCATGTCC AGACTGAGCA TCAGCAAAGA
		CAACTCCAAG AGCCAAGTTT TCTTAAAAAT GAACAGTCTG CAAACTGATG ACACAGCCAT CTACTTCTGT GCCAGAGATG GCGACTACGG TACTATCTAC GCTATGGACT ACTGGGGTCA AGGAACCTCA GTCACCGTCT CCTCAGCCAA AACAACAGCC CCATCGGTCT ATCCACTGGC CCCTGTGTGT GGAGATACAA CTGGCTCCTC GGTGACTCTA GGATGCCTGG TCAAGG
110	2.1029 light chain variable region polynucleotide sequence	ATTGAAGTCA AGACTCAGCC TGGACATGAT GTCCTCTGCT CAGTTCCTTG GTCTCCTGTT GCTCTGTTTT CAAGGTACCA GATGTGATAT CCAGATGACA CAGACTACAT CCTCCCTGTC TGCCTCTCTG GGAGACAGAG TCACCATCAG TTGCAGGGCA AGTCAGGACA TTAGCAATTA TTTAAACTGG TATCAGCAGA AACCAGATGG AACTGTTAAA CTCCTGATCT ACTACACATC AAGATTACAC TCAGGAGTCC CATCAAGGTT CACTGGCAGT GGGTCTGGAA CAGATTATTC TCTCACCATT AGCAACCTGG AGCAAGAAGA TATTGCCACT TACTTTTGCC AACAGGGTAA AACGCTTCCT CGGACGTTCG GTGGAGGCAC CATGCTGGAA ATCAAACGGG CTGATGCTGC ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAGTTA ACATCTGGAG GTGCCTCAGT CGTGTGC
111	Humanized Ab (HumAb) 2.78 heavy chain variable region polynucleotide	gaggtgcagc tggtggagag cggcggcggc ctggtgcagc ccggcggcag cctgagactg agctgcgccg ccagcggctt caccttcagc agcttcggca tgcactgggt gagacaggcc cccggcaagg gcctggagtg ggtgagctac atcaccagcg gcagcggcgc catctactac gccgacagcg tgaagggcag attcaccatc agcagagaca acgccaagaa cagcctgtac ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc cagaagcgcc

SEQ ID NO	Description	Sequence
	sequence	gacggccrgg actacrgggg ccagggcacc accgrgaccg rgagcagc
112	HumAb 2.78 heavy chain polynucleotide sequence	ATGCGTACTC TGGCTATCCT TGCAGCTATT CTGCTTGTTG CACTGCAGGC TCAAGCGGAG GTGCAGCTGG TGGAGAGCGG CGGCGGCCTG GTGCAGCCCG GCGGCAGCCT GAGACTGAGC TGCGCCGCCA GCGGCTTCAC CTTCAGCAGC TTCGGCATGC ACTGGGTGAG ACAGGCCCCC GGCAAGGGCC TGGAGTGGGT GAGCTACATC ACCAGCGGCA GCGGCGCCAT CTACTACGCC GACAGCGTGA AGGGCAGATT CACCATCAGC AGAGACAACG CCAAGAACAG CCTGTACCTG CAGATGAACA GCCTGAGAGC CGAGGACACC GCCGTGTACT ACTGCGCCAG AAGCGCCGAC GGCCTGGACT ACTGGGGCCA GGGCACCACC GTGACCGTGA GCAGCGATGT GTGGGGCCAG GGCACCACCG TGACCGTGAG CAGCGCGTCG ACCAAGGGCC CATCGGTCTT CCCCCTGGCG CCCTGCTCCA GGAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCTC TGACCAGCGG CGTGCACACC TTCCCGGCTG TCCTACAGTC CTCAGGACTC TACTCCCTCA GCAGCGTGGT GACCGTGACC TCCAGCAACT TCGGCACCCA GACCTACACC TGCAACGTAG ATCACAAGCC CAGCAACACC AAGGTGGACA AGACAGTTGA GCGCAAATGT TGTGTCGAGT GCCCACCGTG CCCAGCACCA CCTGTGGCAG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC GTGCGTGGTG GTGGACGTGA GCCACGAAGA CCCCGAGGTC CAGTTCAACT GGTACGTGGA CGGCATGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTTCA ACAGCACGTT CCGTGTGGTC AGCGTCCTCA CCGTCGTGCA CCAGGACTGG CTGAACGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG GCCTCCCAGC CCCCATCGAG AAAACCATCT CCAAAACCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAC CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACACCTCCCA TGCTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGA
113	HumAb 2.78 light chain variable region polynucleotide sequence	gacatccaga tgacccagag ccccagcagc ctgagcgcca gcgtgggcga cagagtgacc atcacctgca gagccagcga gaacatctac agcaacctgg cctggtacca gcagaagccc ggcaaggccc ccaagctgct gatctacggc gccaccaacc tggccgacgg cgtgcccagc agattcagcg gcagcggcag cggcaccgac ttcaccctga ccatcagcag cctgcagccc gaggacttcg ccacctacta ctgccagcac ttctggggca ccccccttcac cttcggccag ggcaccaagg tggagatc
114	HumAb 2.78 light chain polynucleotide sequence	ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGC TTGGGCAGAC ATCCAGATGA CCCAGAGCCC CAGCAGCCTG AGCGCCAGCG TGGGCGACAG AGTCACCATC ACCTGCAGAG CCAGCGAGAA CATCTACAGC AACCTGGCCT GGTACCAGCA GAAGCCCGGC AAGGCCCCCA AGCTGCTGAT CTACGGCGCC ACCAACCTGG CCGACGGCGT GCCCAGCAGA TTCAGCGGCA GCGGCAGCGG CACCGACTTC ACCCTGACCA TCAGCAGCCT GCAGCCCGAG GACTTCGCCA CCTACTACTG CCAGCACTTC TGGGGCACCC CCTTCACCTT CGGCCAGGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTG TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTCT
		GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTCACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA TCAGGGCCTG AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT GA
115	HumAb 2.1029 heavy chain variable region polynucleotide sequence	caggtgcagc tgcaggagag cggccccggc ctggtgaagc ccagcgagac cctgagcctg acctgcaccg tgagcggctt cagcctgacc agctacggcg tgcactggat cagacagccc cccggcaagg gcctggagtg gatcggcgtg atctgggccg gcggcagcac caactacaac cccagcctga agagcagagt gaccatcagc gtggacacca gcaagaacca gttcagcctg aagctgagca gcgtgaccgc cgccgacacc gccgtgtact actgcgccag agacggcgac

SEQ ID NO	Description	Sequence
		tacggcacca tctacgccac ggactactgg ggccagggca ccctggtgac cgtgagcagc
116	HumAb 2.1029 heavy chain polynucleotide sequence	ATGCGTACTC TGGCTATCCT TGCAGCTATT CTGCTTGTTG CACTGCAGGC TCAAGCGCAG GTGCAGCTGC AGGAGAGCGG CCCCGGCCTG GTGAAGCCCA GCGAGACCCT GAGCCTGACC TGCACCGTGA GCGGCTTCAG CCTGACCAGC TACGGCGTGC ACTGGATCAG ACAGCCCCCC GGCAAGGGCC TGGAGTGGAT CGGCGTGATC TGGGCCGGCG GCAGCACCAA CTACAACCCC AGCCTGAAGA GCAGAGTGAC CATCAGCGTG GACACCAGCA AGAACCAGTT CAGCCTGAAG CTGAGCAGCG TGACCGCCGC CGACACCGCC GTGTACTACT GCGCCAGAGA CGGCGACTAC GGCACCATCT ACGCCATGGA CTACTGGGGC CAGGGCACCC TGGTGACCGT GAGCAGCGAT GTGTGGGGCC AGGGCACCAC CGTGACCGTG AGCAGCGCGT CGACCAAGGG CCCATCGGTC TTCCCCCTGG CGCCCTGCTC CAGGAGCACC TCCGAGAGCA CAGCGGCCCT GGGCTGCCTG GTCAAGGACT ACTTCCCCGA ACCGGTGACG GTGTCGTGGA ACTCAGGCGC TCTGACCAGC GGCGTGCACA CCTTCCCGGC TGTCCTACAG TCCTCAGGAC TCTACTCCCT CAGCAGCGTG GTGACCGTGA CCTCCAGCAA CTTCGGCACC CAGACCTACA CCTGCAACGT AGATCACAAG CCCAGCAACA CCAAGGTGGA CAAGACAGTT GAGCGCAAAT GTTGTGTCGA GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACGTGCGTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA CTGGTACGTG GACGGCATGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGGCCTCCCA GCCCCCATCG AGAAAACCAT 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 GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACACAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA
117	HumAb 2.1029 light chain variable region polynucleotide sequence	gacatccaga tgacccagag ccccagcagc ctgagcgcca gcgtgggcga cagagtgacc atcacctgca gagccagcca ggacatcagc aactacctga actggtacca gcagaagccc ggcaaggccc ccaagctgct gatctactac accagcagac tgcacagcgg cgtgcccagc agattcagcg gcagcggcag cggcaccgac ttcaccttca ccatcagcag cctgcagccc gaggacatcg ccacctacta ctgccagcag ggcaagaccc tgcccagaac cttcggcggc ggcaccaagg tggagatc
118	HumAb 2.1029 light chain polynucleotide sequence	ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGC TTGGGCAGAC ATCCAGATGA CCCAGAGCCC CAGCAGCCTG AGCGCCAGCG TGGGCGACAG AGTGACCATC ACCTGCAGAG CCAGCCAGGA CATCAGCAAC TACCTGAACT GGTACCAGCA GAAGCCCGGC AAGGCCCCCA AGCTGCTGAT CTACTACACC AGCAGACTGC ACAGCGGCGT GCCCAGCAGA TTCAGCGGCA GCGGCAGCGG CACCGACTTC ACCTTCACCA TCAGCAGCCT GCAGCCCGAG GACATCGCCA CCTACTACTG CCAGCAGGGC AAGACCCTGC CCAGAACCTT CGGCGGCGGC ACCAAGGTGG AGATCAAACG TACGGTGGCT GCACCATCTG TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTCT GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTCACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA TCAGGGCCTG AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT GA
119	HumAb 1.802 heavy chain variable region polynucleotide sequence	gaggtgcagc tggtggagag cggcggcggc ctggtgcagc ccggcggcag cctgagactg agctgcgccg ccagcggctt caccttcagc gactacggca
		[illegible]

SEQ ID NO	Description	Sequence
		tgcatctgggc gagacaggcc cccggcaagg gcctggagtg ggtgagctac atcagcagcg gcagcagaac cgtgtactac gccgacagcg tgaagggcag attcaccatc agcagagaca acgccaagaa cagcctgtac ctgcagatga acagcctgag agacgaggac accgccgtgt actactgcgc cagaaagcac tacaacggcg gctacttcga cgtgtggggc cagggcaccc tggtgaccgt gagcagc
120	HumAb 1.802 heavy chain polynucleotide sequence	ATGCGTACTC TGGCTATCCT TGCAGCTATT CTGCTTGTTG CACTGCAGGC TCAAGCGGAG GTGCAGCTGG TGGAGAGCGG CGGCGGCCTG GTGCAGCCCG GCGGCAGCCT GAGACTGAGC TGCGCCGCCA GCGGCTTCAC CTTCAGCGAC TACGGCATGC ACTGGGTGAG ACAGGCCCCC GGCAAGGGCC TGGAGTGGGT GAGCTACATC AGCAGCGGCA GCAGAACCGT GTACTACGCC GACAGCGTGA AGGGCAGATT CACCATCAGC AGAGACAACG CCAAGAACAG CCTGTACCTG CAGATGAACA GCCTGAGAGA CGAGGACACC GCCGTGTACT ACTGCGCCAG AAAGCACTAC AACGGCGGCT ACTTCGACGT GTGGGGCCAG GGCACCCTGG TGACCGTGAG CAGCGATGTG TGGGGCCAGG GCACCACCGT GACCGTGAGC AGCGCGTCGA CCAAGGGCCC ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG TCGTGGAACT CAGGCGCTCT GACCAGCGGC GTGCACACCT TCCCGGCTGT CCTACAGTCC TCAGGACTCT ACTCCCTCAG CAGCGTGGTG ACCGTGACCT CCAGCAACTT CGGCACCCAG ACCTACACCT GCAACGTAGA TCACAAGCCC AGCAACACCA AGGTGGACAA GACAGTTGAG CGCAAATGTT GTGTCGAGTG CCCACCGTGC CCAGCACCAC CTGTGGCAGG ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACG TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCCGAGGTCC AGTTCAACTG GTACGTGGAC GGCATGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTTCAA CAGCACGTTC CGTGTGGTCA 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 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC ACAGAAGAGC CTCTCCCTGT CTCCGGGTAA ATGA
121	HumAb 1.802 light chain variable region polynucleotide sequence	gacatcgtga tgacccagag ccccgacagc ctggccgtga gcctgggcga gagagccacc atcaactgca gagccagcaa gatcgtgagc accagcggct acagctacat gcactggtac cagcagaagc ccggccagcc ccccaagctg ctgatctacc tggccagcaa cctggagagc ggcgtgcccg acagattcag cggcagcggc agcggcaccg acttcaccct gaccatcagc agcctgcagg ccgaggacgt ggccgtgtac tactgccagc acagcagaga gctgccccct accttcggcc agggcaccaa gctggagatc
122	HumAb 1.802 light chain polynucleotide sequence	ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGC TTGGGCAGAC ATCGTGATGA CCCAGAGCCC CGACAGCCTG GCCGTGAGCC TGGGCGAGAG AGCCACCATC AACTGCAGAG CCAGCAAGAT CGTGAGCACC AGCGGCTACA GCTACATGCA CTGGTACCAG CAGAAGCCCG GCCAGCCCCC CAAGCTGCTG ATCTACCTGG CCAGCAACCT GGAGAGCGGC GTGCCCGACA GATTCAGCGG CAGCGGCAGC GGCACCGACT TCACCCTGAC CATCAGCAGC CTGCAGGCCG AGGACGTGGC CGTGTACTAC TGCCAGCACA GCAGAGAGCT GCCCCCCACC TTCGGCCAGG GCACCAAGCT GGAGATCAAA CGTACGGTGG CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAACTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG TTGA
123	HumAb 1.815 heavy chain variable region polynucleotide	caggtgcagc tggtggagag cggcggcggc ctggtgaagc ccggcggcag cctgagactg agctgcgccg ccagcggctt caccttcagc gacttcggca tgcactggat cagacaggcc cccggcaagg gcctggagtg ggtgagctac agcagcagcg gcggcaccac cgtgtactac gccgacagcg tgaagggcag attcaccatc agcagagaca acgccaagaa cagcctgtac ctgcagatga

SEQ ID NO	Description	Sequence
	sequence	acagcctgag agccgaggac accgccgtgt actactgcgc cagagccagc tacgacggcg gctacttcga ctgctggggc cagggcacca ccgtgaccgt gagcagc
124	HumAb 1.815 heavy chain polynucleotide	ATGCGTACTC TGGCTATCCT TGCAGCTATT CTGCTTGTTG CACTGCAGGC TCAAGCGCAG GTGCAGCTGG TGGAGAGCGG CGGCGGCCTG GTGAAGCCCG GCGGCAGCCT GAGACTGAGC TGCGCCGCCA GCGGCTTCAC CTTCAGCGAC
	sequence	TTCGGCATGC ACTGGATCAG ACAGGCCCCC GGCAAGGGCC TGGAGTGGGT GAGCTACAGC AGCAGCGGCG GCACCACCGT GTACTACGCC GACAGCGTGA AGGGCAGATT CACCATCAGC AGAGACAACG CCAAGAACAG CCTGTACCTG CAGATGAACA GCCTGAGAGC CGAGGACACC GCCGTGTACT ACTGCGCCAG AGCCAGCTAC GACGGCGGCT ACTTCGACTG CTGGGGCCAG GGCACCACCG TGACCGTGAG CAGCGATGTG TGGGGCCAGG GCACCACCGT GACCGTGAGC AGCGCGTCGA CCAAGGGCCC ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG TCGTGGAACT CAGGCGCTCT GACCAGCGGC GTGCACACCT TCCCGGCTGT CCTACAGTCC TCAGGACTCT ACTCCCTCAG CAGCGTGGTG ACCGTGACCT CCAGCAACTT CGGCACCCAG ACCTACACCT GCAACGTAGA TCACAAGCCC AGCAACACCA AGGTGGACAA GACAGTTGAG CGCAAATGTT GTGTCGAGTG CCCACCGTGC CCAGCACCAC CTGTGGCAGG ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACG TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCCGAGGTCC AGTTCAACTG GTACGTGGAC GGCATGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTTCAA CAGCACGTTC CGTGTGGTCA 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 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC ACAGAAGAGC CTCTCCCTGT CTCCGGGTAA ATGA
125	HumAb 1.815 light chain variable region polynucleotide sequence	gacatcgtga tgacccagag ccccgacagc ctggccgtga gcctgggcga gagagccacc atcaactgca gagccagcaa gagcgtgagc accagcggct acagctacat ccactggtac cagcagaagc ccggccagcc ccccaagctg ctgatctacc tggccagcga cctggagagc ggcgtgcccg acagattcag cggcagcggc agcggcaccg acttcaccct gaccatcagc agcctgcagg ccgaggacgt ggccgtgtac tactgccacc acagcagaga gctgcccttc accttcggcc agggcaccaa gctggagatc
126	HumAb 1.815 light chain polynucleotide sequence	ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGC TTGGGCAGAC ATCGTGATGA CCCAGAGCCC CGACAGCCTG GCCGTGAGCC TGGGCGAGAG AGCCACCATC AACTGCAGAG CCAGCAAGAG CGTGAGCACC AGCGGCTACA GCTACATCCA CTGGTACCAG CAGAAGCCCG GCCAGCCCCC 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 CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG TTGA
127	HumAb 1.846 heavy chain variable region	gaggtgcagc tggtggagag cggcggcggc ctggtgcagc ccggcggcag cctgagactg agctgcgccg ccagcggctt caccttcagc gactacggca tgcactgggt gagacaggcc cccggcaagg gcctggagtg ggtgagctac

SEQ ID NO	Description	Sequence
	polynucleotide sequence	atcagcagcg gcagcaccac cctgagctac gccgacagcg tgaagggcag attcaccatc agcagagaca acgccaagaa cagcctgtac ctgcagatga acagcctgag agacgaggac accgccgtgt actactgcgc cagaaagaac tacaacggcg gctacttcga cgtgtggggc cagggcaccc tggtgaccgt gagcagc
128	HumAb 1.846 heavy chain polynucleotide sequence	ATGCGTACTC TGGCTATCCT TGCAGCTATT CTGCTTGTTG CACTGCAGGC TCAAGCGGAG GTGCAGCTGG TGGAGAGCGG CGGCGGCCTG GTGCAGCCCG GCGGCAGCCT GAGACTGAGC TGCGCCGCCA GCGGCTTCAC CTTCAGCGAC TACGGCATGC ACTGGGTGAG ACAGGCCCCC GGCAAGGGCC TGGAGTGGGT GAGCTACATC AGCAGCGGCA GCACCACCCT GAGCTACGCC GACAGCGTGA AGGGCAGATT CACCATCAGC AGAGACAACG CCAAGAACAG CCTGTACCTG CAGATGAACA GCCTGAGAGA CGAGGACACC GCCGTGTACT ACTGCGCCAG AAAGAACTAC AACGGCGGCT ACTTCGACGT GTGGGGCCAG GGCACCCTGG TGACCGTGAG CAGCGATGTG TGGGGCCAGG GCACCACCGT GACCGTGAGC AGCGCGTCGA CCAAGGGCCC ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG TCGTGGAACT CAGGCGCTCT GACCAGCGGC
		GTGCACACCT TCCCGGCTGT CCTACAGTCC TCAGGACTCT ACTCCCTCAG CAGCGTGGTG ACCGTGACCT CCAGCAACTT CGGCACCCAG ACCTACACCT GCAACGTAGA TCACAAGCCC AGCAACACCA AGGTGGACAA GACAGTTGAG CGCAAATGTT GTGTCGAGTG CCCACCGTGC CCAGCACCAC CTGTGGCAGG ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACG TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCCGAGGTCC AGTTCAACTG GTACGTGGAC GGCATGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTTCAA CAGCACGTTC CGTGTGGTCA 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 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC ACAGAAGAGC CTCTCCCTGT CTCCGGGTAA ATGA
129	HumAb 1.846 light chain variable region polynucleotide sequence	gacatcgtga tgacccagag ccccgacagc ctggccgtga gcctgggcga gagagccacc atcaactgca gagccagcaa gagcgtgagc gagagcggct acagctacat gcactggtac cagcagaagc ccggccagcc ccccaagctg ctgatctacc tggccagcaa cctggagagc ggcgtgcccg acagattcag cggcagcggc agcggcaccg acttcaccct gaccatcagc agcctgcagg ccgaggacgt ggccgtgtac tactgccagc acagcagagt gctgcccccc accttcggcc agggcaccaa gctggagatc
130	HumAb 1.846 light chain polynucleotide sequence	ATGAAAATCC TGATTCTCGG TATCTTCCTG TTTCTCTGTT CTACTCCAGC TTGGGCAGAC ATCGTGATGA CCCAGAGCCC CGACAGCCTG GCCGTGAGCC TGGGCGAGAG AGCCACCATC AACTGCAGAG CCAGCAAGAG CGTGAGCGAG AGCGGCTACA GCTACATGCA CTGGTACCAG CAGAAGCCCG GCCAGCCCCC CAAGCTGCTG ATCTACCTGG CCAGCAACCT GGAGAGCGGC GTGCCCGACA GATTCAGCGG CAGCGGCAGC GGCACCGACT TCACCCTGAC CATCAGCAGC CTGCAGGCCG AGGACGTGGC CGTGTACTAC TGCCAGCACA GCAGAGTGCT GCCCCCCACC TTCGGCCAGG GCACCAAGCT GGAGATCAAA CGTACGGTGG CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAACTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG TTGA

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US4816567A [0032] [0107]
- WO8801649A [0046]
- US4946778A [0046]
- US5260203A [0046]
- US6075181A [0065] [0086]
- US5877397A [0065]
- US6180370B [0067] [0068]
- US5693762A [0067] [0068]
- US5693761A [0067] [0068]
- US5585089A [0067] [0068]
- US5530101A [0067] [0068]
- US5766886A [0070]
- US5869619A [0070]
- US4179337A [0074]
- EP0401384A [0075] [0075]
- EP0154316A [0075]
- WO9216221A [0075]
- WO9534326A [0075]
- WO9513312A [0075] [0082]
- WO9611953A [0075]
- WO9619459A [0075] [0075]
- US6114598A [0086]
- WO9824893A2 [0086]
- WO9824893A [0087]
- US4399216A [0112]
- US4912040A [0112]
- US4740461A [0112]
- US4959455A [0112]
- WO9925044A [0130]
- US3773919A [0139]
- EP058481A [0139]

- EP133988A [0139]
- EP036676A [0139]
- EP088046A [0139]
- EP143949A [0139]
- US6080576A [0151]

Non-patent literature cited in the description

- **ALLEN, J.G. et al.**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 circulationJ. Med. Chem., 2010, vol. 53, 4332-43534332- [0002]
- **GUO, H. et al.**J. Med. Chem., 2010, vol. 53, 1819-18291819- [0003]
- **TORISU, Y. et al.**Cancer Sci., 2008, vol. 99, 61139-11461143- [0006]
- **HUDSON et al.**Nat. Med., 2003, vol. 9, 129-134 [0021] [0101] [0103]
- **KOHLERMILSTEIN**Nature, 1975, vol. 256, 495-499 [0032]
- **CLACKSON et al.**Nature, 1991, vol. 352, 624-628 [0032]
- **MARKS et al.**J. Mol. Biol., 1991, vol. 222, 581-597 [0032]
- **HARLOWLANE**Antibodies: A Laboratory ManualCold Spring Harbor Laboratory19880000 [0032] [0083] [0084]
- Fundamental ImmunologyRaven Press19890000 [0043]
- **KABAT et al.**Sequences of Proteins of Immunological InterestNational Institutes of Health19910000vol. 1-3, [0044]
- **CHOTHIA, C.LESK, A.M.**J. Mol. Biol., 1987, vol. 196, 901-917 [0044]
- **CHOTHIA, C. et al.**Nature, 1989, vol. 342, 878-883 [0044]
- **MORRISON et al.**Proc. Nat'l Acad. Sci. USA, 1984, vol. 81, 6851-6855 [0065]
- **NEUBERGER et al.**Nature, 1984, vol. 312, 604-608 [0065]
- **TAKEDA et al.**Nature, 1985, vol. 314, 452-454 [0065]
- **QUEEN et al.**Proc. Nat'l Acad. Sci. USA, 1989, vol. 86, 10029-10033 [0067] [0068]
- **MALIK et al.**Exp. Hematol., 1992, vol. 20, 1028-1035 [0075]
- **FRANCIS**Focus on Growth FactorsMediscript, Mountain Court, Friern Barnet Lane19920000vol. 3, 4-10 [0075]
- **CARPENTER et al.T**oxicol. Appl. Pharmacol., 1971, vol. 18, 35-40 [0080]
- **KOHLER et al.**Nature, 1975, vol. 256, 495-497 [0083]
- **BORREBACK et al.**Proc. Nat'l Acad. Sci. USA, 1988, vol. 85, 3995-3999 [0083]
- **JAKOBOVITS et al.**Nature, 1993, vol. 362, 255-258 [0086] [0087]
- **TOMIZUKA et al.**Proc. Nat'l Acad. Sci. USA, 2000, vol. 97, 722-727 [0086] [0087]
- **MENDEZ et al.**Nat. Genet., 1997, vol. 15, 146-156 [0086] [0087]
- **JAKOBOVITS**Curr. Opin. Biotechnol., 1995, vol. 6, 561-566 [0087]
- **LONBERG et al.**Int'l Rev. Immunol., 1995, vol. 13, 65-93 [0087]

- **FISHWILD et al.**Nat. Biotechnol., 1996, vol. 14, 845-851 [0087]
- **GREEN**J. Immunol. Methods, 1999, vol. 231, 11-23 [0087]
- **LITTLE et al.**Immunol. Today, 2000, vol. 21, 364-370 [0087]
- **HOOGENBOOM**Overview of Antibody Phage-Display Technology and Its Applications, from Methods in Molecular Biology: Antibody Phage Display: Methods and ProtocolsHuman Press20020000vol. 178, 1-37 [0089] [0101]
- Overview of Antibody Phage-Display Technology and Its Applications**HOOGENBOOM**Methods in Molecular Biology: Antibody Phage Display: Methods and ProtocolsHuman Press20020000vol. 178, 1-37 [0090]
- **BODER et al.**Nat. Biotechnol, 1997, vol. 15, 553-557 [0099]
- **BREKKE et al.**Nat. Reviews, 2002, vol. 2, 52-62 [0101] [0103]
- **BODER et al.**Proc. Nat'l Acad. Sci. USA, 2000, vol. 97, 10701-10705 [0101]
- **FOOTE et al.**Proc. Nat'l Acad. Sci. USA, 2000, vol. 97, 10679-10681 [0101]
- **HANES et al.**Proc. Nat'l Acad. Sci. USA, 1998, vol. 95, 14130-14135 [0101] [0106]
- **CRAMERI et al.**Nat. Med., 1996, vol. 2, 100-102 [0102]
- **FERMER et al.**Tumor Biol., 2004, vol. 25, 7-13 [0102]
- **KANG et al.**Proc. Nat'l Acad. Sci. USA, 1991, vol. 88, 11120-11123 [0103]
- **MARKS et al.**Biotechnol., 1992, vol. 10, 779-83 [0103]
- Remington's Pharmaceutical SciencesMack Publishing Company19900000 [0129]
- **SIDMAN et al.**Biopolymers, 1983, vol. 22, 547-556 [0139]
- **LANGER et al.**J. Biomed. Mater. Res., 1981, vol. 15, 167-277 [0139]
- **LANGER**Chem. Tech., 1982, vol. 12, 98-105 [0139]
- **EPPSTEIN et al.**Proc. Natl. Acad. Sci. USA, 1985, vol. 82, 3688-3692 [0139]
- Pharmacology and ToxicologyGuidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy VolunteersU.S. Department of Health and Human Services, Food and Drug Administration, and Center for Drug Evaluation and Research (CDER)20050700 [0144]

P A T E N T K R A V

1. Monoklonalt antistof, som binder human notum pectinacetylesterase (NOTUM) og reducerer human NOTUM-aktivitet i et trinatrium 8-octanoyloxy-pyren-1,3,6-trisulfonat (OPTS) assay *in vitro,* til anvendelse ved en fremgangsmåde til behanding eller forebyggelse af en sygdom eller en lidelse, **kendetegnet ved** knogletab hos en patient.

2. Monoklonalt antistof til anvendelse ifølge krav 1, hvor sygdommen eller lidelsen er valgt blandt gruppen bestående af osteoporose, osteopeni og Pagets sygdom.

3. Monoklonalt antistof til anvendelse ifølge krav 2, hvor osteoporosen er valgt blandt gruppen bestående af postmenopausal osteoporose, steroid- eller glukokortikoid-induceret osteoporose, mandlig osteoporose og idiopatisk osteoporose.

4. Monoklonalt antistof til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet efter indgift til en person øger niveauer af serum PINP *in vivo,* øger knoglemineraltætheden *in vivo,* øger den kortikale tykkelse *in vivo* af lårbenets midtskaft, øger knogleområdet *in vivo* af lårbenets midtskaft, øger den kortikale tykkelse *in vivo* af overarmsknoglens midtskaft, øger endokortikal knogledannelse *in vivo,* øger andelen af kortikal knoglevolumen i LV5-hvirvellegemet *in vivo* og/eller øger andelen af lårbenshalsknoglevoluminet i forhold til det samlede lårbenshalsvolumen *in vivo.*

5. Monoklonalt antistof til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet binder til et polypeptid med aminosyresekvensen af SEQ ID NO: 1 med KD på mindre end 50 nM, fortrinsvis mindre end 20 nM eller mere foretrukket mindre end 10 nM.

6. Monoklonalt antistof til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet har mindst en bindingskarakteristik valgt blandt:

a) binder til et polypeptid med aminosyresekvensen af SEQ ID NO: 83 med en bindingsaffinitet, som er mindst 5 gange stærkere end bindingsaffiniteten af antistoffet for et polypeptid med aminosyresekvensen af SEQ ID NO: 84;

b) binder til et polypeptid med aminosyresekvensen af SEQ ID NO: 85 med en bindingsaffinitet, som er mindst 5 gange stærkere end bindingsaffiniteten af antistoffet for et polypeptid med aminosyresekvensen af SEQ ID NO: 86;

c) binder til et polypeptid med aminosyresekvensen af SEQ ID NO: 1 med en bindingsaffinitet, som er mindst 5 gange stærkere end bindingsaffiniteten af antistoffet for et polypeptid med aminosyresekvensen af SEQ ID NO: 94;

d) binder til et polypeptid med aminosyresekvensen af SEQ ID NO: 1 med en bindingsaffinitet, som er mindst 5 gange stærkere end bindingsaffiniteten af antistoffet for et polypeptid med aminosyresekvensen af SEQ ID NO: 99;

e) konkurrerer om at binde til NOTUM med et antistof, som omfatter en variabel region af en tung kæde med en aminosyresekvens af SEQ ID NO: 15 og en variabel region af en let kæde med aminosyresekvensen af SEQ ID NO: 16;

f) konkurrerer om at binde til NOTUM med et antistof, som omfatter en variabel region af en tung kæde med en aminosyresekvens af SEQ ID NO: 23 og en variabel region af en let kæde med aminosyresekvensen af SEQ ID NO: 24;

g) konkurrerer om at binde til NOTUM med et antistof, som omfatter en variabel region af en tung kæde med en aminosyresekvens af SEQ ID NO: 31 og en variabel region af en let kæde med aminosyresekvensen af SEQ ID NO: 32;

h) konkurrerer om at binde til NOTUM med et antistof, som omfatter en variabel region af en tung kæde med en aminosyresekvens af SEQ ID NO: 39 og en variabel region af en let kæde med aminosyresekvensen af SEQ ID NO: 40;

i) konkurrerer om at binde til NOTUM med et antistof, som omfatter en variabel region af en tung kæde med en aminosyresekvens af SEQ ID NO: 55 og en variabel region af en let kæde med aminosyresekvensen af SEQ ID NO: 56.

7. Monoklonalt antistof til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet er valgt blandt et antistof fra mus, et kimært antistof, et humaniseret antistof og et humant antistof.

8. Monoklonalt antistof til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet omfatter en variabel region af en tung kæde og en variabel region af en let kæde, hvor:

a) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 17, en CDR2 med aminosyresekvensen af SEQ ID NO: 18 og en CDR3 med aminosyresekvensen af SEQ ID NO: 19, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 20, en CDR2 med aminosyresekvensen af SEQ ID NO: 21 og en CDR3 med aminosyresekvensen af SEQ ID NO: 22; eller

b) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 25, en CDR2 med aminosyresekvensen af SEQ ID NO: 26 og en CDR3 med aminosyresekvensen af SEQ ID NO: 27, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 28, en CDR2 med aminosyresekvensen af SEQ ID NO: 29 og en CDR3 med aminosyresekvensen af SEQ ID NO: 30; eller

c) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 33, en CDR2 med aminosyresekvensen af SEQ ID NO: 34 og en CDR3 med aminosyresekvensen af SEQ ID NO: 35, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 36, en CDR2 med aminosyresekvensen af SEQ ID NO: 37 og en CDR3 med aminosyresekvensen af SEQ ID NO: 38; eller

d) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 41, en CDR2 med aminosyresekvensen af SEQ ID NO: 42 og en CDR3 med aminosyresekvensen af SEQ ID NO: 43, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 44, en CDR2 med aminosyresekvensen af SEQ ID NO: 45 og en CDR3 med aminosyresekvensen af SEQ ID NO: 46; eller

e) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 57, en CDR2 med aminosyresekvensen af SEQ ID NO: 58 og en CDR3 med aminosyresekvensen af SEQ ID NO: 59, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 60, en CDR2 med aminosyresekvensen af SEQ ID NO: 61 og en CDR3 med aminosyresekvensen af SEQ ID NO: 62.

9. Monoklonalt antistof til anvendelse ifølge krav 8, hvor

a) i antistoffet nævnt i krav 8, del (a): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 15, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 16; eller den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 71, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 73; eller den tunge kæde omfatter aminosyre-

sekvensen af SEQ ID NO: 72 og den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 74; eller

b) i antistoffet nævnt i krav 8, del (b): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 23, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 24; eller den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 75, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 77; eller den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 76, og den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 78; eller

c) i antistoffet nævnt i krav 8, del (c): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 31, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 32; eller den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 79, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 81; eller den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 80, og den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 82; eller

d) i antistoffet nævnt i krav 8, del (d): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 39, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 40; eller den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 67, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 69; eller den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 68, og den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 70; eller

e) i antistoffet nævnt i krav 8, del (e): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 55, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 56.

10. Monoklonalt antistof, som binder til human notum pectinacetylesterase

(NOTUM), og som neutraliserer mindst en aktivitet af NOTUM, hvor antistoffet omfatter en variabel region af en tung kæde og en variabel region af en let kæde, hvor:

a) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 41, en CDR2 med aminosyresekvensen af SEQ ID NO: 42 og en CDR3 med aminosyresekvensen af SEQ ID NO: 43, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 44, en CDR2 med aminosyresekvensen af SEQ ID NO: 45 og en CDR3 med aminosyresekvensen af SEQ ID NO: 46; eller

b) den variable region af den tunge kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 57, en CDR2 med aminosyresekvensen af SEQ ID NO: 58 og en CDR3 med aminosyresekvensen af SEQ ID NO: 59, og hvor den variable region af den lette kæde omfatter en CDR1 med aminosyresekvensen af SEQ ID NO: 60, en CDR2 med aminosyresekvensen af SEQ ID NO: 61 og en CDR3 med aminosyresekvensen af SEQ ID NO: 62.

11. Monoklonalt antistof ifølge krav 10 hvor

a) i antistoffet ifølge krav 10, del (a): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 39, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 40; eller den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 67, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 69; eller den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 68, og den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 70; eller

b) i antistoffet ifølge krav 10, del (b): den variable region af den tunge kæde omfatter aminosyresekvensen af SEQ ID NO: 55, og den variable region af den lette kæde omfatter aminosyresekvensen af SEQ ID NO: 56.

12. Farmaceutisk sammensætning, omfattende antistoffet ifølge et hvilket som helst af kravene 10 eller 11.

13. Nukleinsyremolekyle, omfattende første og andre polynukleotidsekvenser, som koder de respektive tunge og lette kæder af antistoffet ifølge et hvilket som helst af kravene 10 eller 11.

14. Værtscelle, omfattende nukleinsyremolekylet ifølge krav 13.

15. Fremgangsmåde til fremstilling af et antistof ifølge et hvilket som helst af kravene 10 eller 11, omfattende at inkubere værtscellen ifølge krav 14 under omstændigheder, som er tilstrækkelige til at eksprimere antistoffet.

DRAWINGS

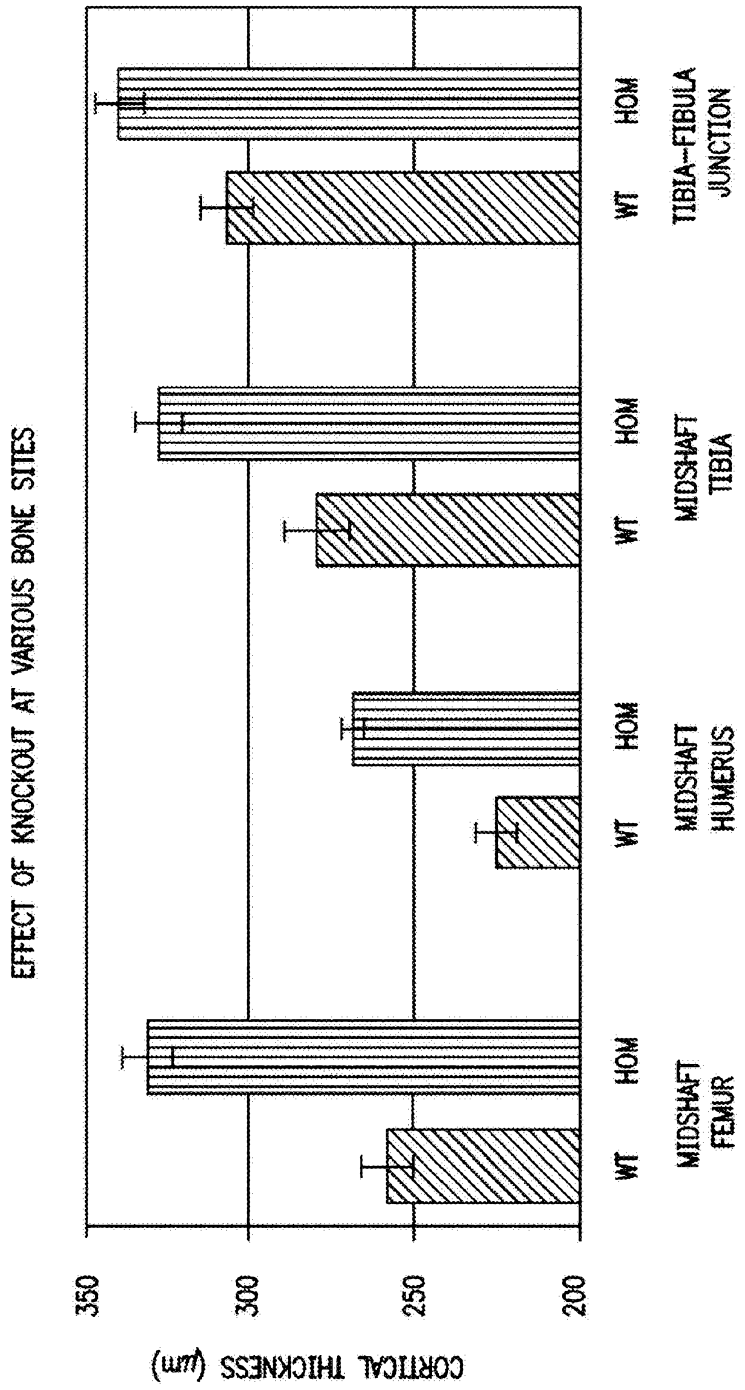

FIG. 1

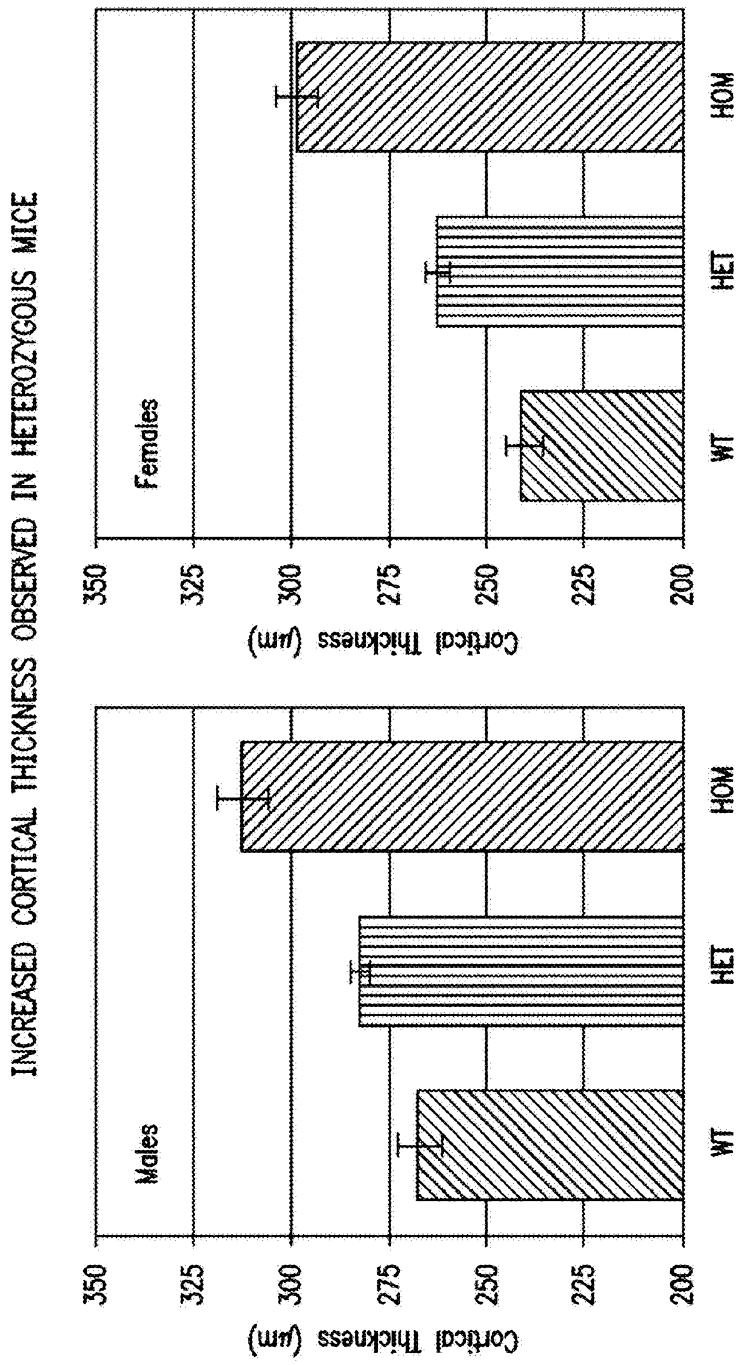
INCREASED CORTICAL THICKNESS OBSERVED IN HETEROZYGOUS MICE
Females
Males
Cortical Thickness (µm)
350
325
300
275
250
225
200
WT
HET
HOM

FIG. 2

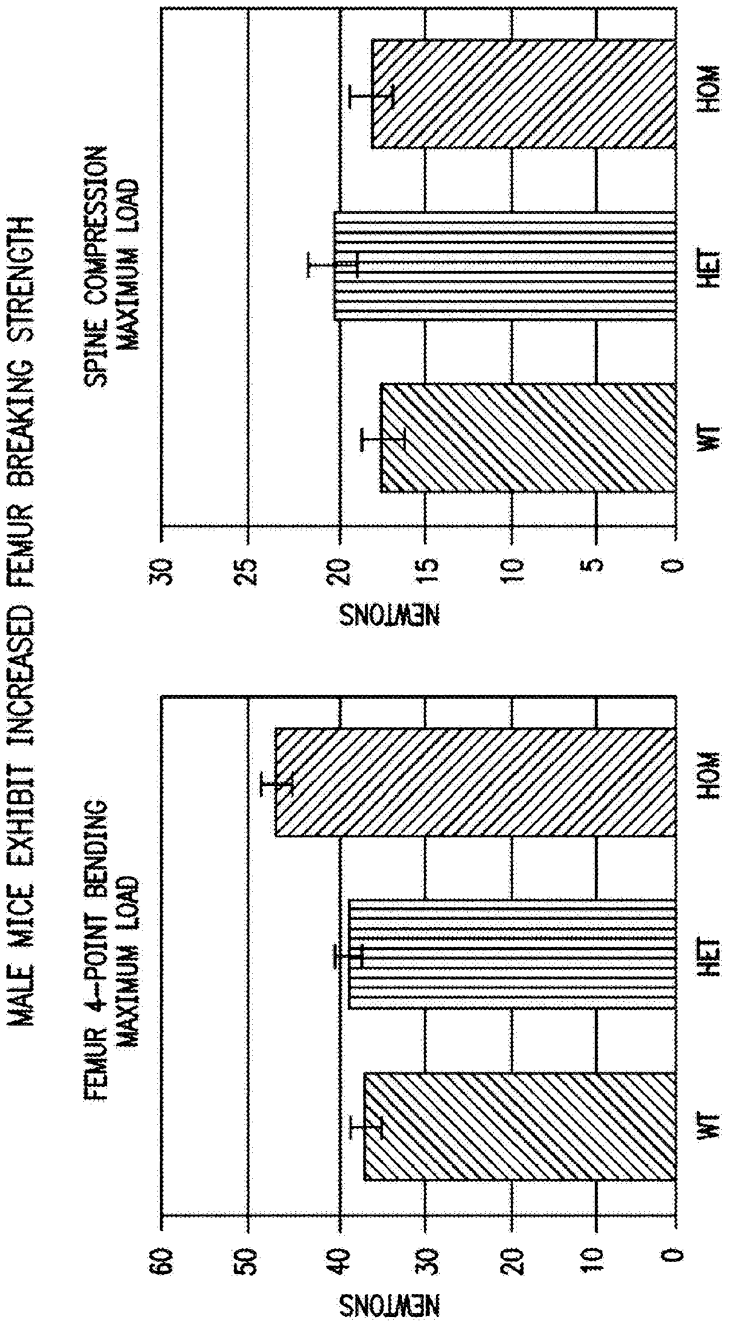
MALE MICE EXHIBIT INCREASED FEMUR BREAKING STRENGTH
FEMUR 4-POINT BENDING
MAXIMUM LOAD
NEWTONS
60
50
40
30
20
10
0
WT
HET
HOM
SPINE COMPRESSION
MAXIMUM LOAD
NEWTONS
30
25
20
15
10
5
0
WT
HET
HOM

FIG. 3

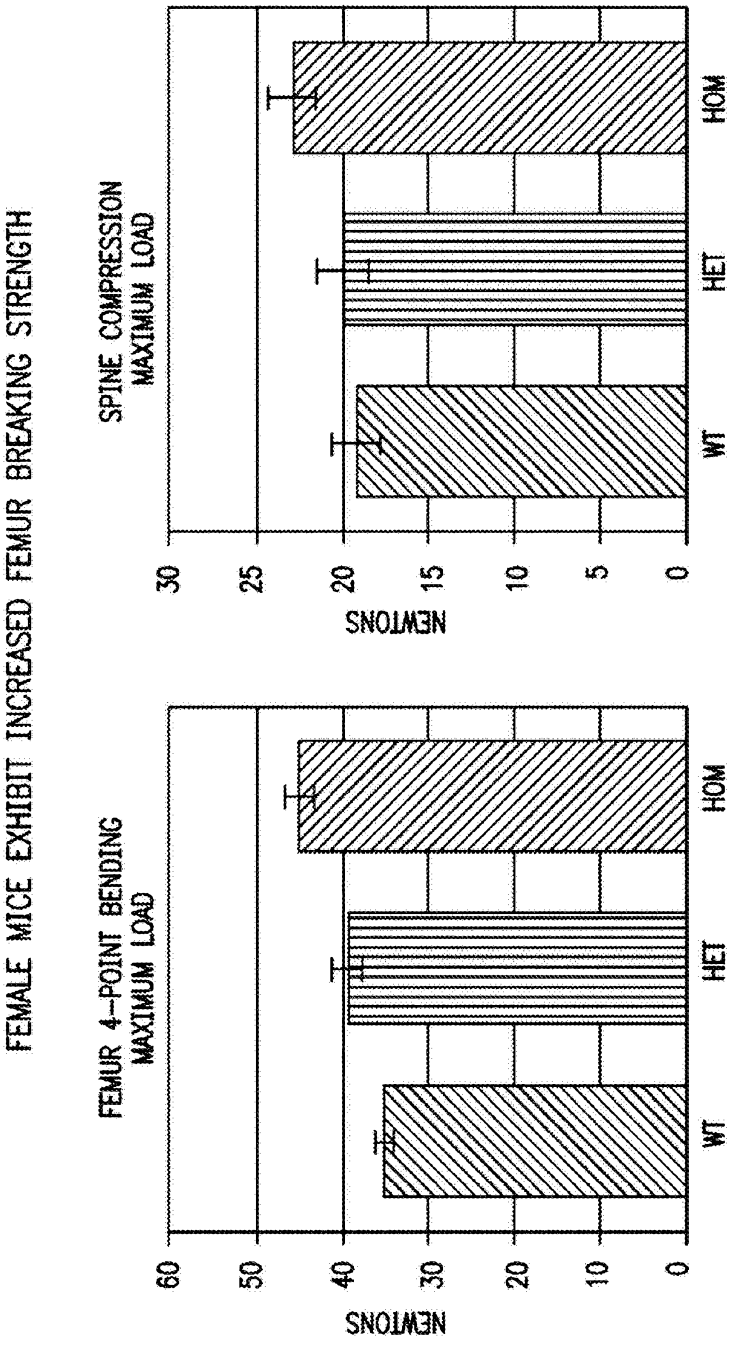
FEMALE MICE EXHIBIT INCREASED FEMUR BREAKING STRENGTH
FEMUR 4-POINT BENDING
MAXIMUM LOAD
NEWTONS
60
50
40
30
20
10
0
WT
HET
HOM
SPINE COMPRESSION
MAXIMUM LOAD
NEWTONS
30
25
20
15
10
5
0
WT
HET
HOM

FIG. 4

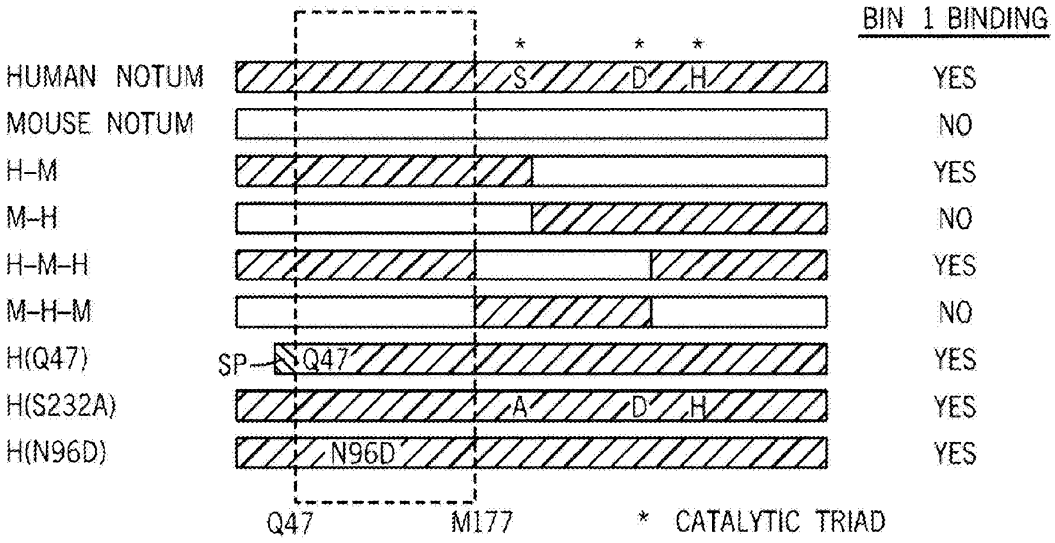

FIG. 5

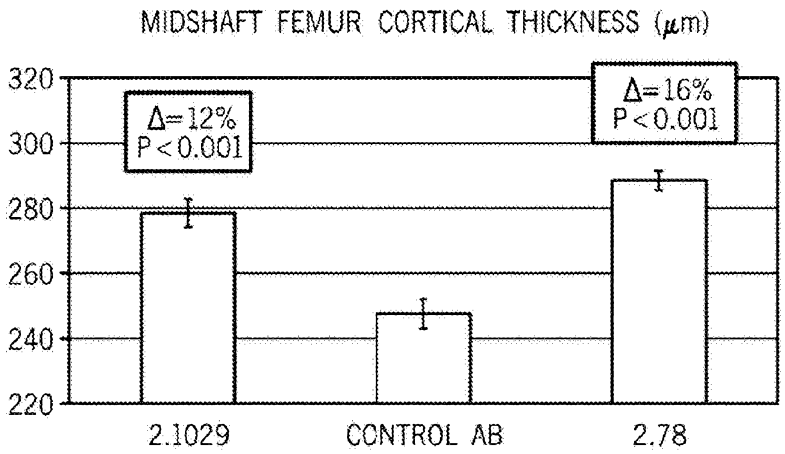
MIDSHAFT FEMUR CORTICAL THICKNESS (μm)
320
300
280
260
240
220
Δ=12%
P<0.001
Δ=16%
P<0.001
2.1029
CONTROL AB
2.78

FIG. 6

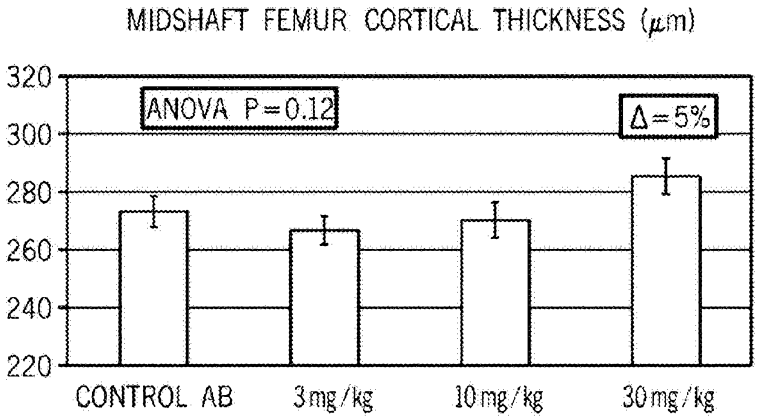
MIDSHAFT FEMUR CORTICAL THICKNESS (μm)
320
300
280
260
240
220
ANOVA P=0.12
Δ=5%
CONTROL AB
3mg/kg
10mg/kg
30mg/kg

FIG. 7

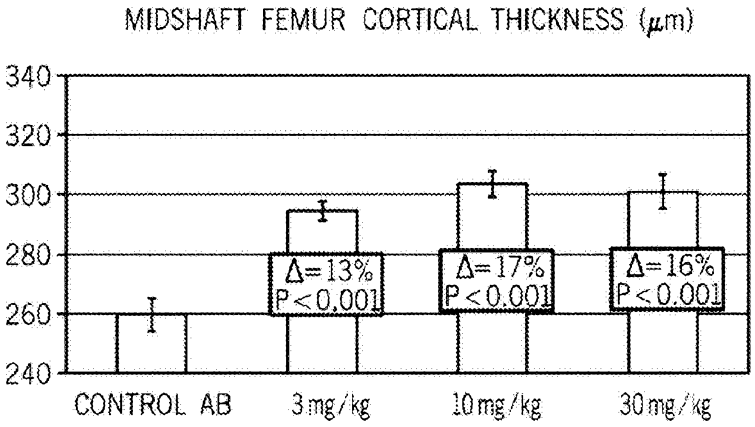
MIDSHAFT FEMUR CORTICAL THICKNESS (μm)
340
320
300
280
260
240
Δ=13%
P<0.001
Δ=17%
P<0.001
Δ=16%
P<0.001
CONTROL AB
3mg/kg
10mg/kg
30mg/kg

FIG. 8A

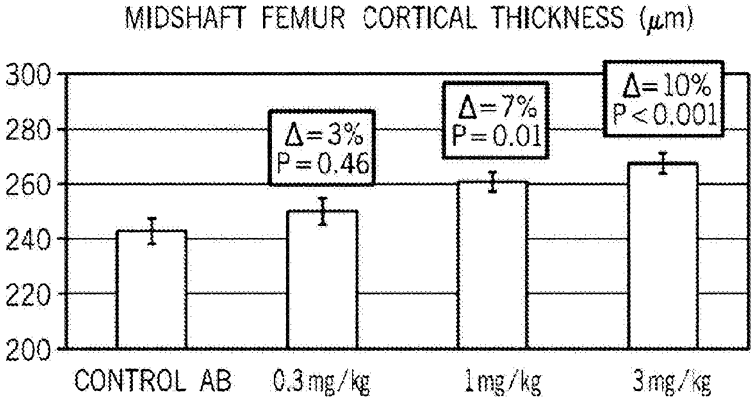
MIDSHAFT FEMUR CORTICAL THICKNESS (μm)
300
280
260
240
220
200
Δ=3%
P=0.46
Δ=7%
P=0.01
Δ=10%
P<0.001
CONTROL AB
0.3mg/kg
1mg/kg
3mg/kg

FIG. 8B

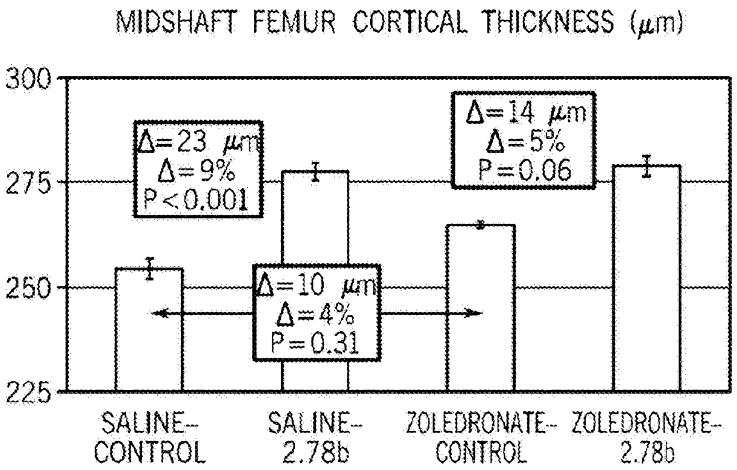
MIDSHAFT FEMUR CORTICAL THICKNESS (μm)
300
275
250
225
Δ=23 μm
Δ=9%
P<0.001
Δ=10 μm
Δ=4%
P=0.31
Δ=14 μm
Δ=5%
P=0.06
SALINE-CONTROL
SALINE-2.78b
ZOLEDRONATE-CONTROL
ZOLEDRONATE-2.78b

FIG. 9A

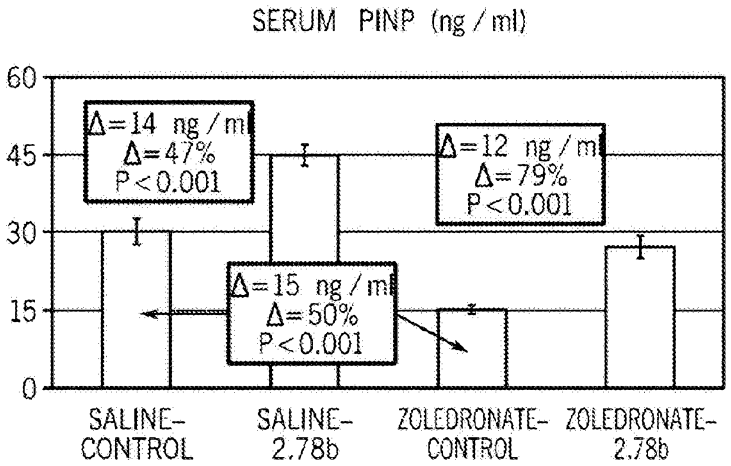
SERUM PINP (ng/ml)
60
45
30
15
0
Δ=14 ng/ml
Δ=47%
P<0.001
Δ=15 ng/ml
Δ=50%
P<0.001
Δ=12 ng/ml
Δ=79%
P<0.001
SALINE-CONTROL
SALINE-2.78b
ZOLEDRONATE-CONTROL
ZOLEDRONATE-2.78b

FIG. 9B

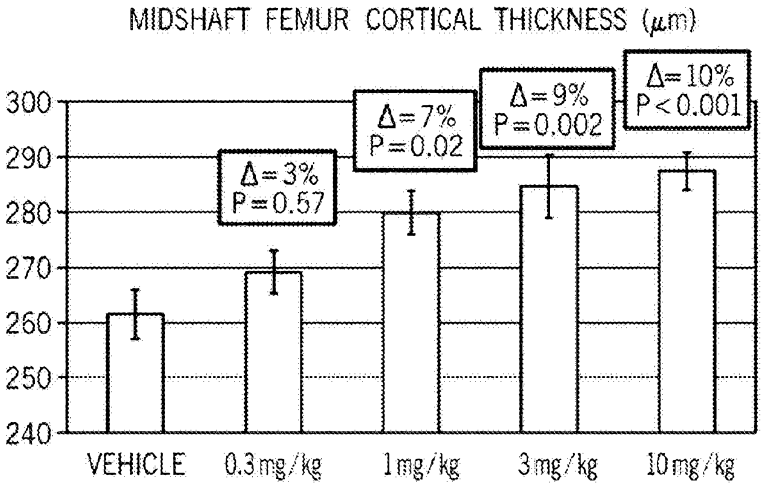

FIG. 10

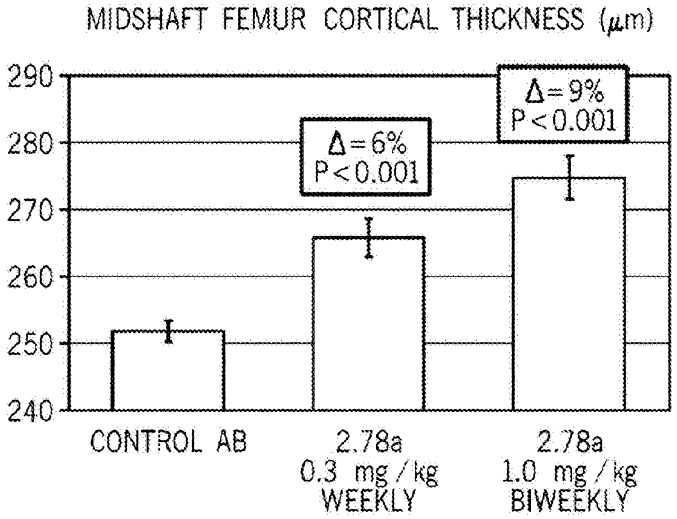

FIG. 11A

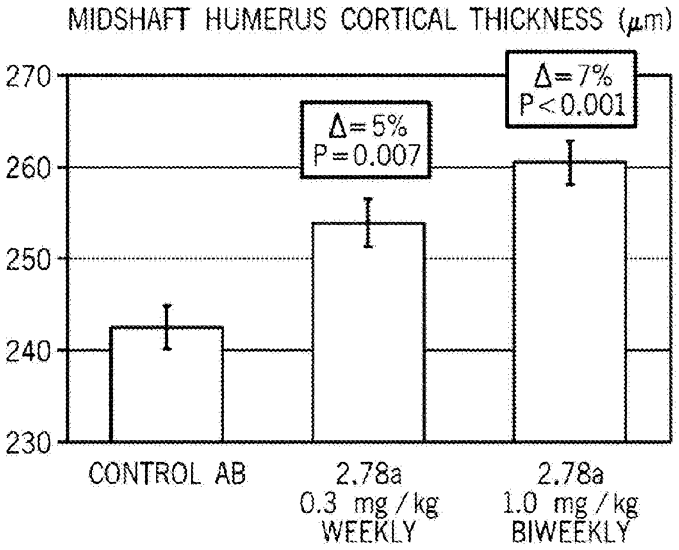

FIG. 11B

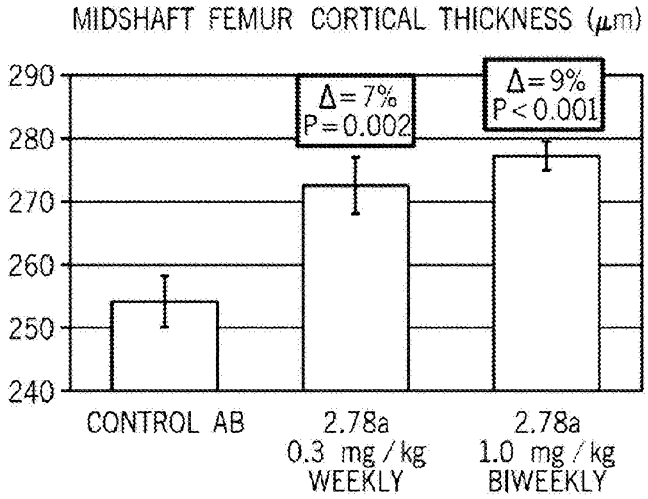

FIG. 12A

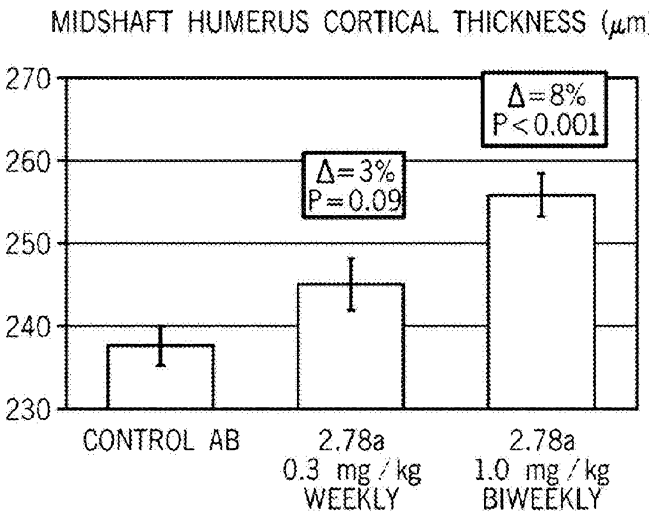

FIG. 12B

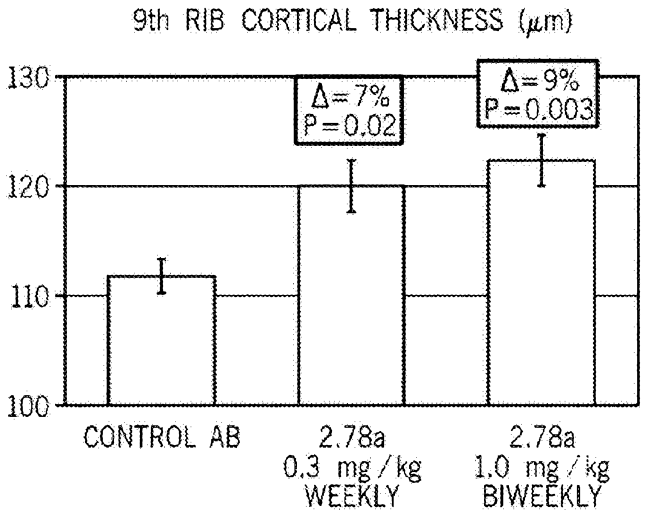

FIG. 12C

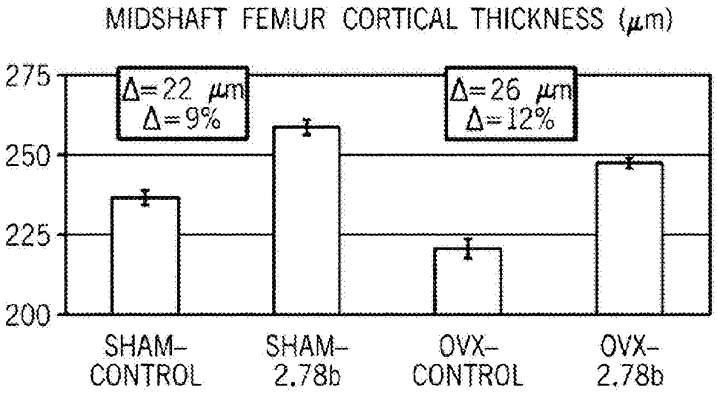

FIG. 13A

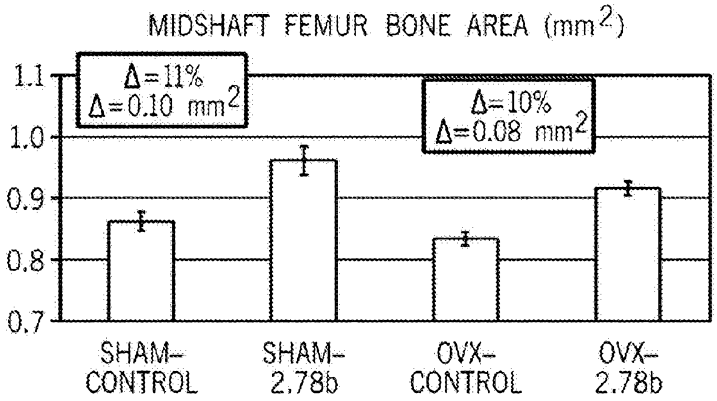

FIG. 13B

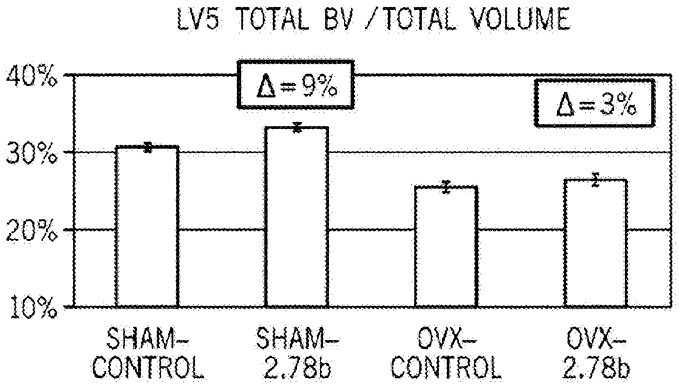

FIG. 14A

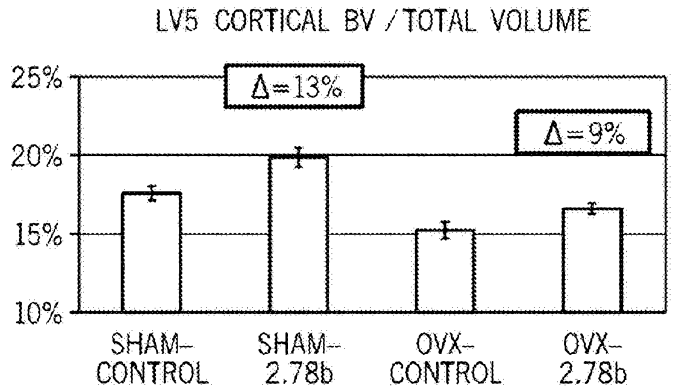

FIG. 14B

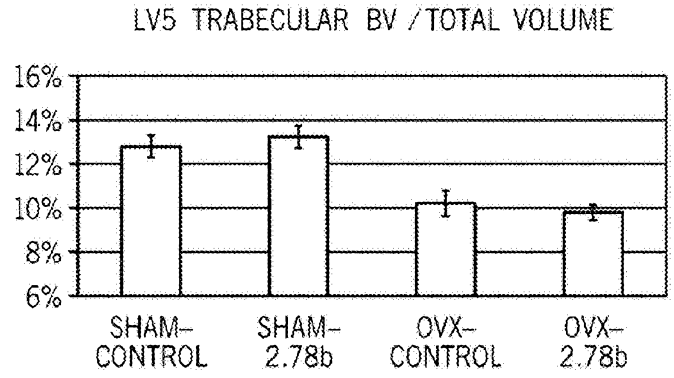

FIG. 14C

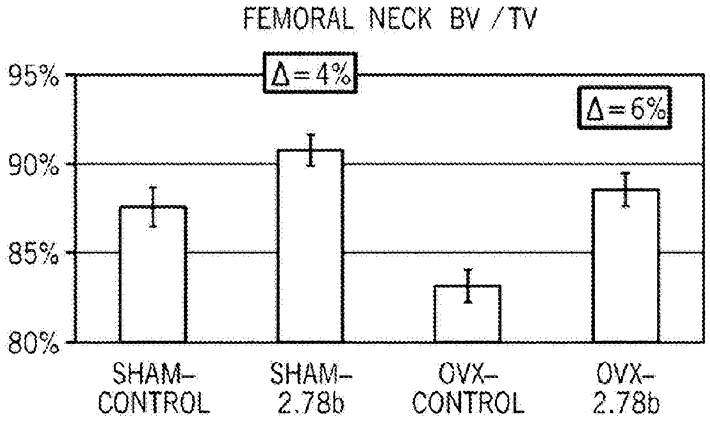
FEMORAL NECK BV / TV
95%
90%
85%
80%
Δ=4%
Δ=6%
SHAM–
CONTROL
SHAM–
2.78b
OVX–
CONTROL
OVX–
2.78b

FIG. 15

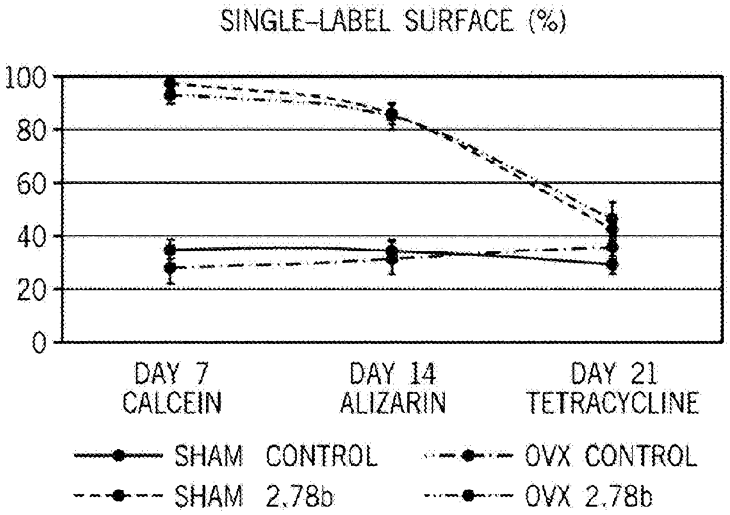
SINGLE-LABEL SURFACE (%)
100
80
60
40
20
0
DAY 7
CALCEIN
DAY 14
ALIZARIN
DAY 21
TETRACYCLINE
SHAM CONTROL
OVX CONTROL
SHAM 2.78b
OVX 2.78b

FIG. 16

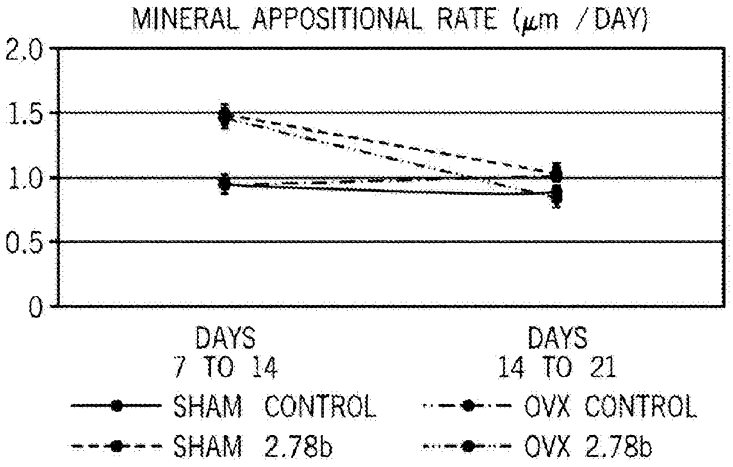

FIG. 17A

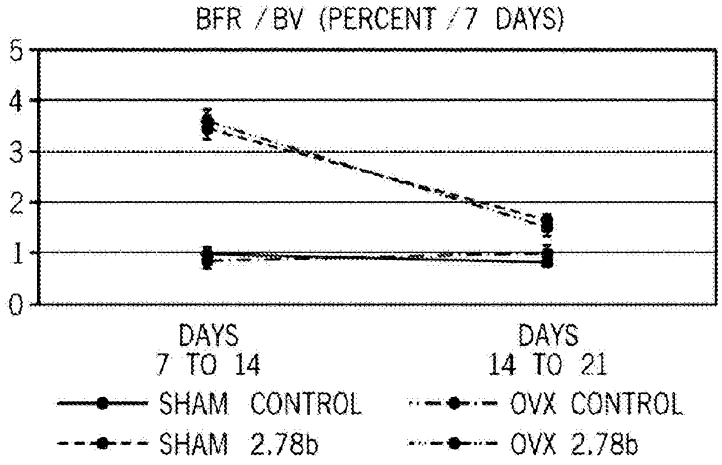

FIG. 17B