Paper No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DAIICHI SANKYO COMPANY, LIMITED
Petitioner

v.

ALETHIA BIOTHERAPEUTICS
Patent Owner

Patent No. 8,168,181
Issue Date: May 1, 2012

Title: METHODS OF IMPAIRING OSTEOCLAST DIFFERENTIATION USING ANTIBODIES THAT BIND SIGLEC-15

Inter Partes Review No. IPR2015-00291

PETITION FOR *INTER PARTES* REVIEW UNDER 35 U.S.C. §§ 311-319 AND 37 C.F.R. § 42.100 *ET. SEQ.*

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EXHIBIT LIST

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1002	WIPO Publication WO 2009/048072
1003	Declaration of Dr. Paul R. Crocker with Curriculum Vitae
1004	Declaration of Dr. Michael R. Clark with Curriculum Vitae
1005	BRITANNICA.COM, Bone Remodeling Definition, http://www.britannica.com/EBchecked/topic/684133/bone-remodeling (last visited Nov. 10, 2014)
1006	M.P. Yavropoulou & J.G. Yovos, *Osteoclastogenesis - Current knowledge and future perspectives*, 8(3) J. MUSCULOSKELET. NEURONAL INTERACT., 204-16 (2008)
1007	N. Ishida-Kitagawa et al., *Siglec-15 Protein Regulates Formation of Functional Osteoclasts in Concert with DNAX-activating Protein of 12 kDa (DAP12)*, 287(21) J. BIOL. CHEM., 17493-17502 (2012)

1008	U.S. Patent Application No. 12/580,943
1009	U.S. Patent Application No. 12/279,054
1010	WIPO Publication WO 2007/093042
1011	K. Henriksen et al., *Generation of Human Osteoclasts from Peripheral Blood*, in METHODS IN MOLECULAR BIOLOGY, VOL. 816: BONE RESEARCH PROTOCOLS, 159-75 (Miep H. Helfrich & Stuart Ralston eds., 2nd ed. 2012)
1012	Amendment filed in U.S. Patent Application No. 12/580,943 on Jan. 3, 2012
1013	Non-final Office action mailed in U.S. Patent Application No. 12/580,943 on Dec. 16, 2011

1014	THE AMERICAN HERITAGE MEDICAL DICTIONARY, Osteoclast Definition, http://dictionary.reference.com/browse/osteoclast (last visited Nov. 14, 2014)
1015	DORLAND'S ILLUSTRATED MEDICAL DICTIONARY, Bone Resorption Definition, 1450 (27th ed. 1988)
1016	U.S. Patent No. 7,989,160
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1018	U.S. Provisional Patent Application No. 60/816,858
1019	U.S. Provisional Patent Application No. 61/248,960
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1021	M. Stuible et al., *Mechanism and Function of Monoclonal Antibodies Targeting Siglec-15 for Therapeutic Inhibition of Osteoclastic Bone Resorption*, J. BIOL. CHEM., published online Jan. 20, 2014, 1-29.
1022	T. Angata et al., *Siglec-15: An Immune System Siglec Conserved Throughout Vertebrate Evolution*, 17(8) GLYCOBIOLOGY, 838–46 (2007)
1023	English Translation of WO 2009/048072
1024	Transmittal Letter showing submission of PCT/CA2007/000210 (WO 2007/093042) to the U.S. Patent and Trademark Office as National Stage for U.S. Patent Application No. 12/279,054
1025	U.S. Patent Publication No. 2010-0209428
1026	T. Miyamoto, *Regulators of Osteoclast Differentiation and Cell–Cell Fusion*, 60(4) KEIO J. MED., 101-5 (2011)

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1028	S. Jones and J.Z. Rappoport, *Interdependent Epidermal Growth Factor Receptor Signalling and Trafficking*, 51(1) INT'L J. OF BIOCHEM. AND CELL BIO., 23-28 (2014)
1029	M.S. Macauley et al., *Siglec-Mediated Regulation of Immune Cell Function in Disease*, 14(1) NAT. REV. IMMUNOL., 653-66 (2014)
1030	A.L. Blasius et al., *Siglec-H is an IPC-Specific Receptor That Modulates Type I IFN Secretion Through DAP12*, 107 BLOOD, 2474-6 (2006)
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1034	H. Hsu et al., *Tumor Necrosis Factor Receptor Family Member RANK Mediates Osteoclast Differentiation and Activation Induced by Osteoprotegerin Ligand*, 96(7) PROC. NAT'L ACAD. SCI., 3540-5 (1999)
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1038	S. Roberts et al., *Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering*, 328 NATURE, 731-734 (1987)
1039	T. Pisitkun et al., *NHLBI-AbDesigner: an online tool for design of peptide-directed antibodies*, 302 AM. J. PHYSIOL. CELL PHYSIOL., C154-64 (2012)

NOTICE OF LEAD AND BACKUP COUNSEL

Lead Counsel: Stephen B. Maebius (Reg. No. 35,264); **Tel:** 202.672.5569

Backup Counsel: Kristel Schorr (Reg. No. 55,600); **Tel:** 202.672.5574

Backup Counsel: Jeffrey N. Costakos (Reg. No. 34,144); **Tel:** 414.297.5782

Address: Foley & Lardner LLP, 3000 K St. NW, Washington, DC. 20008

Fax: 202.672.5399.

NOTICE OF EACH REAL-PARTY-IN-INTEREST

The real-party-in-interest for this Petition is Daiichi Sankyo Company Limited, 3-5-1 Nihonbashi-honcho, Chuo-ku, Tokyo 103-8426, Japan.

NOTICE OF RELATED MATTERS

None.

NOTICE OF SERVICE INFORMATION

Please address all correspondence to the lead counsel at the address shown above. Petitioner also consents to electronic service by email at:

kschorr-IPR@foley.com

GROUNDS FOR STANDING

Petitioner hereby **certifies** that the patent for which review is sought is available for *inter partes* review and that the petitioner is not barred or estopped from requesting an *inter partes* review challenging the patent claims on the grounds identified in the petition.

STATEMENT OF PRECISE RELIEF REQUESTED

Petitioner requests that claims 1-6, 8-11 and 15-23 of U.S. Patent No. 8,168,181 ("the '181 patent") (Ex. 1001) be held unpatentable and, therefore, cancelled.

THRESHOLD REQUIREMENT FOR *INTER PARTES* REVIEW

A petition for *inter partes* review must demonstrate "a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a). The Petition meets this threshold. Each of the elements of claims 1-6, 8-11 and 15-23 of the '181 patent is taught in a single prior art reference, WO 2009/048072 ("'072 Publication") (Ex. 1002), as explained below in the proposed grounds of unpatentability.

STATEMENT OF REASONS FOR RELIEF REQUESTED

A. Technical Introduction

The claims of the '181 patent are directed to methods of impairing osteoclast differentiation or inhibiting bone resorption using an antibody or antigen binding fragment that specifically binds to human or murine Siglec-15. (Ex. 1003, ¶ 6; Ex. 1004, ¶ 13). Osteoclast differentiation and bone resorption are natural processes involving osteoclasts that occur in vivo to maintain normal healthy bone tissue during the process of bone remodeling. (Ex. 1005). In the disease context, a number of bone remodeling disorders would benefit from inhibition of osteoclast

activities, such that osteoclast differentiation and bone resorption are blocked or impaired. (Ex. 1006, p. 204, 213).

Siglec-15 is a member of the sialic-acid-binding immunoglobulin-like lectins and appears to be involved in a pathway signaling osteoclast differentiation and bone resorption. (Ex. 1003, ¶ 5; Ex. 1007 at 14494). Because of this potential involvement in osteoclast differentiation and bone resorption, an antibody or antigen binding fragment that is able to bind Siglec-15 may affect Siglec-15 function in a way that is inhibitory for osteoclastogenesis and bone resorption in vivo. (Ex. 1007 at 17500-1).

B. Construction of the Claims

1. Legal Overview

A claim in *inter partes* review is given its "broadest reasonable construction in light of the specification." 37 C.F.R. § 42.100(b). As stated by the Federal Circuit:

> "[T]he PTO must give claims their broadest reasonable construction consistent with the specification. Therefore, we look to the specification to see if it provides a definition for claim terms, but otherwise apply a broad interpretation."

In re ICON Health and Fitness, Inc., 496 F.3d 1374, 1379 (Fed. Cir. 2007).

2. Claim 1 – "osteoclast differentiation" or "osteoclast differentiation activity"

Claim 1 (as well as claims 2, 3, and 18) recites the term "osteoclast differentiation" or "osteoclast differentiation activity." Petitioner proposes that the

broadest reasonable interpretation of "osteoclast differentiation" and "osteoclast differentiation activity" is "any activity involved in the process of differentiation of an osteoclast precursor cell into a differentiated osteoclast."

The application that issued as the '181 patent, U.S. Pat. Appl. No. 12/580,943 ("'943 Application") (Ex. 1008), does not provide an explicit definition of the term "osteoclast differentiation" or "osteoclast differentiation activity". However, the '943 Application states:

> Antibodies or antigen binding fragments that are encompassed by the present invention include, for example, those that may interfere with (e.g., inhibit) the differentiation of a human osteoclast precursor cell or more specifically, those that may interfere with (e.g., inhibit) the differentiation of a primary human osteoclast precursor cell. Therefore, in accordance with the present invention, the antibody or antigen binding fragment may be capable of inhibiting differentiation of osteoclast precursor cells into differentiated osteoclasts.

Ex. 1008, p. 6, ll. 15-21 (emphasis added). The '943 Application also explains:

> [T]he invention provides a method of modulating (i.e., inhibiting, lowering, impairing) osteoclast differentiation in a mammal in need, the method may comprise administering an antibody or antigen binding fragment that may be capable of modulating the differentiation of an osteoclast precursor cell (e.g., human osteoclast precursor cell, human primary osteoclast precursor cell) into a differentiated osteoclast.

Ex. 1008, p. 9, ll. 4-8 (emphasis added). The '943 Application further provides that the level of differentiation of an osteoclast cell can be determined, for example, by measuring the number of differentiated cells, their rate of differentiation, or a specific marker of differentiation. (Ex. 1008, p. 48, ll. 18-20).

A similar description of osteoclast differentiation is provided in U.S. Pat. Appl. No. 12/279,054 ("Parent '054 Application") (Ex. 1009), which is the national stage application of PCT/CA2007/000210.[1] (Ex. 1003, ¶8). Also, "osteoclast differentiation" and "osteoclast differentiation activity" are synonymous to a person skilled in the art. (Ex. 1003, ¶8).

Further, as described in the art, generation of osteoclasts occurs through osteoclast differentiation, which involves in part cytokine-induced fusion of osteoclast precursor cells, which are myeloid in origin, and is associated with M-CSF and RANKL receptor activation. (Ex. 1011, at 159-60). Thus, consistent with the above proposed construction, osteoclast differentiation refers to the process of differentiating precursor osteoclast cells into a differentiated osteoclast.

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[1] The Parent '054 Application was filed using the WO publication of PCT/CA2007/000210 as its national stage application.

3. Claims 1 and 15 – Construction of "specifically binds"

Claims 1 and 15 recite the phrase "specifically binds" in the context of an antibody or antigen binding fragment which specifically binds to human or murine Siglec-15. Petitioner proposes that, in the context of binding to human Siglec-15, the phrase "specifically binds" should be interpreted as "the ability of an antibody or antigen binding fragment to bind human or mouse Siglec-15 with greater preference over an antigen that is not human or mouse Siglec-15." (Ex. 1004, ¶ 13).

The '943 Application does not provide an explicit definition for the term phrase "specifically binds." The '943 Application does not attribute any particular level of specification of the antibody or antigen binding fragment. (Ex. 1004, ¶ 13). Rather, the '943 Application provides only that the antibodies or antigen binding fragments "*may* be capable of specific binding to SEQ ID NO.:2 or to a variant having at least 80% sequence identity with SEQ ID NO.:2 and of inhibiting a resorptive activity of an osteoclast" (Ex. 1008, p. 10, ll. 23-25), and that "[t]he antibody or antigen binding fragment *may* particularly bind to the extracellular region of SEQ ID NO.:2" (Ex. 1008, p. 6, ll. 10-11) (emphasis added). Also, during prosecution of the '943 Application, the Applicants overcame an indefiniteness rejection under 35 U.S.C. § 112, 2nd paragraph by amending then claim 23, which corresponds to issued claim 1, to replace the phrase "capable of

binding" with the phrase "which specifically binds to". (Ex. 1012, p. 3). This amendment was suggested by the Examiner in the Non-final Office action dated December 16, 2011. (Ex. 1013, p. 6).

The '943 Application further provides that "[s]uitable antibodies may bind to unique antigenic regions or epitopes in the polypeptides, or a portion thereof. Epitopes and antigenic regions useful for generating antibodies may be found within the proteins, polypeptides or peptides by procedures available to one of skill in the art." (Ex. 1008, p. 41, ll. 7-10).

The Parent '054 Application is silent with respect to particular antibodies or antigen binding fragments to any particular antigen, but states generally that "the present invention relates to an antibody (e.g., isolated antibody), or antigen-binding fragment thereof, that may specifically bind to a protein or polypeptide described herein." (Ex. 1009, col. 33, ln. 35 – p. 34, ln 5). The Parent '054 Application further describes the use of such antibodies in detection methods (Ex. 1009, p. 40, ln. 34 – p. 41, ln. 6), but is otherwise silent on the term "specifically binds." (Ex. 1004, ¶ 13).

Moreover, consistent with the construction proposed above, the term "antibody specificity" is generally understood by a skilled artisan to meant the ability of an antibody or fragment thereof to recognize a particular antigen over any other different antigen. (Ex. 1004, ¶ 8). Accordingly, in the context of the '181 patent,

one of skill in the art would interpret the phrase "specifically binds" to mean the ability of an antibody or antigen binding fragment to bind human or mouse Siglec-15 with greater preference over an antigen that is not human or mouse Siglec-15.

4. Claim 15 – Construction of "bone resorption"

Claim 15 recites the term "bone resorption." Petitioner proposes that the broadest reasonable interpretation of the phrase "bone resorption" is "the breakdown of bone by osteoclasts."

The '943 Application does not provide an explicit definition of the term. However, the '943 Application provides that:

> Bone is a dynamic connective tissue comprised of functionally distinct cell populations required to support the structural, mechanical and biochemical integrity of bone and the human body's mineral homeostasis. The principal cell types involved include, osteoblasts responsible for bone formation and maintaining bone mass, and osteoclasts responsible for bone resorption. Osteoblasts and osteoclasts function in a dynamic process termed bone remodeling.

(Ex. 1008, p. 1, ln. 32 – p. 2, ln. 3).

The above passage of the '943 Application is identical to the corresponding paragraph in the Parent '054 Application. (Ex. 1009, p. 1, ln. 28 – p. 2, ln. 1).

Moreover, the dictionary definition of "osteoclast" is "a large multinucleated cell found growing in bone that resorbs bony tissue" (Ex. 1014) and bone resorption is defined in a medical dictionary as "bone loss due to osteoclastic

activity." (Ex. 1015; Ex. 1004, ¶ 8). Furthermore, bone resorption is a process that is part of the bone remodeling process whereby bone mass is diminished. (Ex. 1004, ¶ 8). Thus, "bone resorption" is the breakdown of bone by osteoclasts.

C. Grounds for Unpatentability

Claims 1-6, 8-11 and 15-23 of the '181 patent are unpatentable because they are not entitled to any priority date earlier than April 16, 2009, which is the publication date of WO 2009/048072 (Ex. 1002), and therefore, are anticipated by an intervening prior art reference as discussed in greater detail herein. As shown in the explanation below, claims 1-6, 8-11 and 15-23 of the '181 patent are not adequately described or enabled, as required by 35 U.S.C. § 112, by the parent national stage application (US Application 12/279,054 (Ex. 1009), §371 date of January 13, 2009), the priority PCT application (PCT/CA2007/000210, filed February 13, 2007) or the two provisional applications (US Application Nos. 60/722,585 (Ex. 1017) and US 60/816,858 (Ex. 1018), filed February 13, 2006 and June 28, 2006, respectively) and therefore, do not receive the benefit of a priority date earlier than the actual filing date of the '181 patent (Ex. 1001) or the third provisional application (US 61/248,960 (Ex. 1019)), both filed in October 2009.

Ground 1. Claims 1-6, 8-11 and 15-23 of the '181 Patent are unpatentable under 35 U.S.C. § 102(a) over WO 2009/048072

The '181 patent issued from the '943 Application, which is a continuation-in-part application filed on October 16, 2009, and purports to claim priority to each of:

- U.S. Pat. Appl. No. 12/279,054 ("Parent '054 Application")[2] (Ex. 1009), national stage entry on January 13, 2009, now U.S. 7,989,160 ("'160 Patent") (Ex. 1016), which is a national stage application of PCT/CA2007/000210 filed on February 13, 2007 and published as WO 2007/093042 ("Alethia PCT") (Ex. 1010);
- U.S. Provisional Pat. Appl. No. 60/772,585 (Ex. 1017) filed on February 13, 2006;
- U.S. Provisional Pat. Appl. No. 60/816,858 (Ex. 1018) filed on June 28, 2006; and

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[2] As indicated herein, WO 2007/093042 (Ex. 1010) was used as the national stage application (Ex. 1024) and was assigned U.S. Pat. Appl. No. 12/279,054 (Ex. 1009). Based on our review of Ex. 1009 and corresponding file history, the specification of Ex. 1009 and Ex. 1010 are identical. Claim amendments were introduced in Ex. 1010 during the PCT stage and transmitted with Ex. 1009, as well as a preliminary amendment to the claims, all of which was considered in our priority analysis below.

- U.S. Provisional Pat. Appl. No. 61/248,960 (Ex. 1019) filed on October 6, 2009.

As will be explained in detail below, none of claims 1-6, 8-11, and 15-23 of the '181 patent is entitled to any priority date earlier than April 16, 2009. Although under 35 U.S.C. §§ 120 and 365(c), a claim in a U.S. application or patent is entitled to the benefit of the filing date of an earlier U.S. application or international application, this is only if, among other things, the claimed invention is disclosed in the earlier application in the manner provided by 35 U.S.C. § 112, 1st paragraph. Claims 1-6, 8-11, and 15-23 of the '181 patent, however, are neither adequately described in, nor enabled by, any application filed before the publication date of the prior art reference cited herein.

WO 2009/048072 ("'072 Publication" (Ex. 1002)) (English Transl. Ex. 1023) is prior art against claims 1-6, 8-11 and 15-23 of the '181 Patent. The '072 Publication has a publication date of April 16, 2009, which is earlier than both the October 16, 2009 filing date of the '943 Application and the October 6, 2009 filing date of the third provisional application. The '072 Publication thus qualifies as prior art under 35 U.S.C. § 102(a). Moreover, the '072 Publication teaches every single element of claims 1-6, 8-11, and 15-23 of the '181 Patent, and thus anticipates these claims.

To assist the Board in understanding the applications to which the '181 patent claims priority, demonstrative Ex. 1020 diagrams the relationships.

1. Claims 1–6, 8-11 and 15-23 lack adequate written description in the Parent '054 Application

The Federal Circuit has established that, under 35 U.S.C. § 112(a), the test for sufficiency of written description is whether the disclosure in the patent application relied on reasonably conveys to those skilled in art that the inventor had "possession" of the claimed subject matter as of the application's filing date. *Ariad Pharms. Inc. v. Eli Lilly & Co.*, 598 F.3d 1336 (Fed. Cir. 2010) (en banc). In other words, the specification must demonstrate that the applicant actually invented (*i.e.*, was in possession of) the claimed subject matter. Generic claim language – even appearing in *ipsis verbis* in the original specification – does not satisfy the written description requirement if it fails to support the scope of the genus claimed. *Ariad,* 598 F.3d at 1350. Such situations may be akin to providing no more than an invitation for further research, which is insufficient to meet the written description standard in the U.S.. *Id.*

In addition, the as-filed application must objectively provide descriptive support for each claim limitation within the four corners of the specification. *See Gentry Gallery, Inc. v. Berkline Corp.,* 134 F.3d 1473 (Fed. Cir. 1998); *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1326 (Fed. Cir. 2000).

In the present case, claims 1-6, 8-11, and 15-23 of the '181 patent lack written description in the Parent '054 Application because (1) the Parent '054 Application fails to demonstrate that the applicant was in possession of the claimed subject matter and (2) they lack actual descriptive support of each claim limitation within the four corners of the Parent '054 Application.

(a) The Parent '054 Application fails to establish possession of the claimed subject matter

The Federal Circuit has held that "a patentee of a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from species other than those specifically enumerated." *In re Alonso,* 545 F.3d 1015, 1020 (Fed. Cir. 2008); *Noelle v. Lederman*, 355 F.3d 1343, 1350 (Fed. Cir. 2004). In fact, the Federal Circuit has held a claimed genus of antibodies invalid for lack of written description when the specification describes 300 antibodies that fall only within a portion of the scope of the claimed genus. *AbbVie Deutschland Gmbh v. Janssen Biotech, Inc.*, 2013-1338 (Fed. Cir. 2014).

Further, in *Centocor v. Abbott Labs*, Centocor attempted to claim priority to an earlier application directed to a *mouse* antibody in order to antedate an Abbott patent on a *humanized* antibody specific for the same target. *Centocor v. Abbott Labs*, 636 F.3d 1341 (Fed. Cir. 2011). The Federal Circuit found that Centocor's earlier patent lacked sufficient written description to properly claim priority,

stating "while the patent broadly claims a class of antibodies that contain human variable regions, the specification does not describe a single antibody that satisfies the claim limitations." *Id.* ("The specification at best describes a plan for making fully-human antibodies and then identifying those that satisfy the claim limitations.... At the time the 1994 CIP applications were filed, it was entirely possible that no fully-human antibody existed that satisfied the claims. Because *Centocor* had not invented a fully-human antibody in 1994, a reasonable jury could not conclude that it possessed one."). Also, in cases where functional limitations are present, the specification must disclose "just *which* [compounds] have the desired characteristics. . . . Without such disclosure the claimed methods cannot be said to have been described." *Univ. of Rochester v. G.D. Searle & Co.*, 358 F 3d 916, 927 (Fed. Cir. 2004). This rationale has been extended to antibody technology, and written description was held to be insufficient when the "specification teaches nothing about the structure, epitope characterization, binding affinity, specificity, or pharmacological properties common to the large family of antibodies implicated by the method." *Alonso,* 545 F.3d at 1021-1022.

Here, the Parent '054 Application fails to establish possession of the claimed subject matter. There is no example of a single therapeutic Siglec-15 antibody given in the disclosure, yet the scope of the claims extends to any Siglec-15 antibody. (Ex. 1003, ¶¶ 9, 16; Ex. 1004, ¶ 23). Even though the Parent '054

Application discloses an assay for determining whether "small molecule drugs, peptides or antibodies" inhibit the activity of any of the broad classes of polypeptides described therein (Ex. 1009, p. 85, ln. 32 – p. 86, ln. 3; p. 86, ll. 10-11), it is merely a screening assay for any number of inhibitors of the disclosed sequences and not a disclosure of how to arrive at any therapeutic Siglec-15 antibody. (Ex. 1004, ¶ 27). In fact, it was not even known by February 2006, February 2007, or January 2009 whether a Siglec-15 antibody capable of impairing osteoclast differentiation or inhibiting bone resorption *could* even exist. *See* Ex. 1003, ¶¶ 13, 16. Therefore, without additional disclosure in the Parent '054 Application, the patentee cannot claim to have possessed a Siglec-15 antibody having such an effect.

Further, the Parent '054 Application only discloses the protein sequence for Siglec-15 but does not provide any structural information regarding an antibody that binds this sequence and has the requisite activity set forth in the '181 patent claims. (Ex. 1004, ¶¶ 16, 17, 22, 23, 25). In *Centocor*, the Federal Circuit made clear that merely reciting characteristics of a known protein is insufficient to support a claim to a class of antibodies that has particularly desirable therapeutic properties if "antibodies with those properties have not been adequately described." 636 F.3d at 1352 (emphasis added) ("Claiming antibodies with specific properties ... can result in a claim that does not meet written description

even if the [protein to which the antibodies bind] is disclosed because antibodies with those properties have not been adequately described."). Thus, disclosure of the Siglec-15 polypeptide sequence, the vague statements in the Parent '054 Application regarding polypeptide sequences "involved in the process of bone remodeling" (Ex. 1009, p. 5, ll. 13-22; p. 6, ll. 1-9), and general description of inhibitory compounds that have the desired function of "ameliorating bone remodeling disease or disorder symptoms" or "delaying bone disease or disorder" (Ex. 1009, p. 10, ll. 17-23; p. 10, ln. 31 – p. 6 ln. 2) are insufficient to show possession of the claimed invention as of the priority date of February 13, 2006, February 13, 2007 or January 13, 2009.

Lastly, there is no indication in the Parent '054 Application or confirmation in the literature in 2006, 2007, or 2009, that Siglec-15 is located on the cell surface and accessible to an antibody. (Ex. 1003, ¶ 14). This is an important consideration for anyone of skill in the art seeking to design a therapeutic antibody because such an antibody would be largely ineffective for altering the function of a protein that is inaccessible or intercellular. (Ex. 1004, ¶ 22). As Dr. Crocker explains, the earliest publication characterizing Siglec-15 localization is Angata *et al.*, which is included as Ex. 1022. (Ex. 1003, ¶ 14). Angata describes co-localization with CD-68, a known intracellular protein but Angata is silent on extracellular localization of Siglec-15. (Ex. 1022, p. 840; Ex. 1003, ¶ 14). And while Siglec-15 has a

transmembrane domain, the presence of a transmembrane domain in Siglec-15 alone does not necessarily connote cell surface accessibility of that protein. (Ex. 1003, ¶ 14; Ex. 1004, ¶ 22).

And even if the Parent '054 Application demonstrated that Siglec-15 is a cell surface accessible protein, it is completely unpredictable whether an antibody targeting it would impair osteoclast differentiation or inhibit bone resorption when administered. (Ex. 1003, ¶ 15; Ex. 1004, ¶ 25). Indeed, without having an understanding of how the target behaves *in vivo*, a sense of kinetics and recycling of the target, or having actually made any antibody to the target, the feasibility of the target for antibody therapy is uncertain. (Ex. 1004, ¶ 25). Further, "[t]he lowest POS ["Probability of Success"] is found in Phase II, where nearly half of all therapeutic MAb candidates drop out, mostly due to lack of efficacy." (Ex. 1035, p. 21; *see also* 1004, ¶ 7). Thus, the disclosure in the Parent '054 Application of an assay for determining whether "small molecule drugs, peptides or antibodies" inhibit the activity of any of the polypeptides described therein, and polynucleotides and polypeptides "involved in the process of bone remodeling", cannot be equated with a description of the genus of antibodies with specific functional properties, as claimed.

For all of these reasons, the Parent '054 Application fail to demonstrate possession of a Siglec-15 antibody with the requisite activity and, therefore, fail to

provide adequate written description support for the claims of the '181 patent. Accordingly, the '181 patent cannot properly rely on the benefit of the Parent '054 Application or any claimed priority date earlier than the date of the '072 Publication. Consequently, the '072 Publication (Ex. 1002) is prior art to the '181 Patent.

(b) The Parent '054 Application does not provide adequate descriptive support for impairing osteoclast differentiation or inhibiting bone resorption with (i) "an antibody" out of other therapeutic inhibitors disclosed (ii) that "specifically binds to human Siglec-15 or murine Siglec-15" out of various possible disclosed antigens

Simply identifying a large class of compounds does not satisfy the written description requirement as to particular subset of species. *See, e.g., Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571 (Fed. Cir. 1996); *In re Ruschig*, 379 F.2d 990, 994 (C.C.P.A. 1967); *see also, Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1326 (Fed. Cir. 2000) ("one cannot disclose a forest in the original application, and then later pick a tree out of the forest and say here is my invention."). Rather, where an applicant seeks to claim a particular species, the disclosure must guide a skilled artisan towards choosing that species from among the other possibilities disclosed. *Fujikawa*, 93 F.3d at 1571 (stating that a "laundry list" disclosure of every possible moiety does not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species). Even if the choice of the particular species seems simple and

foreseeable in hindsight, the species is not necessarily described as required by 35 U.S.C. § 112, ¶ 1. *Fujikawa*, 93 F.3d at 1571.

(i) The Parent '054 Application does not specifically identify "an antibody" out of other therapeutic inhibitors disclosed

The Parent '054 Application generally uses the term "inhibitors" but fails to describe an antibody inhibitor that binds to any one of the polypeptides disclosed therein for administration to a mammal or subject in need, as recited in the claims. (Ex. 1003, ¶¶ 7, 17; Ex. 1004, ¶ 21). Independent claims 1 and 15 of the '181 Patent, and claims dependent therefrom, are directed to a method of impairing osteoclast differentiation and a method of inhibiting bone resorption, respectively, comprising administering an antibody or antigen binding fragment that specifically binds to human Siglec-15 (SEQ ID NO.: 2) or murine Siglec-15 (SEQ ID NO.: 108). For the claimed method to work, the antibody or antigen binding fragment recited in all of the claims must have an impairment effect on osteoclast differentiation (claim 1 and claims dependent therefrom) or inhibitory effect on bone resorption (claim 15 and claims dependent therefrom), as it is the only active agent recited in the independent claims. (Ex. 1004, ¶ 13).

The Parent '054 Application, however, only describes certain polynucleotide and polypeptide sequences "involved in the process of bone remodeling" (Ex. 1009, p. 5, ll. 13-22; p. 6, ll. 1-10), and inhibitory compounds in general that have

the desired function of "ameliorating bone remodeling disease or disorder symptoms" or "delaying bone disease or disorder" (Ex. 1009, p. 10, ll. 17-23). But other than the sequences themselves, the Parent '054 Application is devoid of any structural information regarding inhibitory compounds, including antibodies. (Ex. 1003, ¶ 7). Specifically, the Parent '054 Application fails to disclose a single antibody by structure, even partially, that binds to Siglec-15. (Ex. 1004, ¶ 23). Further, the Parent '054 Application fails to describe a single example, either prophetic or actual, of an antibody that binds to Siglec-15 and that has the specific function recited in the claims. (Ex. 1004, ¶ 23). In other words, not even a limited number of species of Siglec-15 antibodies for treatment is described. No species are described. Indeed, the only negative regulator of Siglec-15 that is even disclosed in the Parent '054 Application is shown to be effective at the *genetic* level, through the use of siRNA, and is therefore not an antibody. (Ex. 1003, ¶ 17; Ex. 1004, ¶ 21). This example functions by altering the expression of a target gene, and does not exert its effect at the protein level, as an antibody would. (Ex. 1003, ¶ 17; Ex. 1004, ¶ 21).

While the Parent '054 Application mentions the notion of antibodies binding to the polypeptides described therein (but not Siglec-15 specifically), that disclosure is without any structural guidance and more importantly, is only for the use of such antibodies in *detecting* proteins and diseases, and not for treatment. (Ex. 1003, ¶

18). For example, the Parent '054 Application states that "antibodies obtained by the means described herein may be useful for *detecting* proteins, variant and derivative polypeptides in specific tissues or in body fluids" and that "the present antibodies may be useful for *detecting* diseases associated with protein expression from NSEQs [polynucleotide sequences] disclosed herein." (Ex. 1009, p. 40, ln. 32-p. 41, ln. 1; p. 41, ll. 5-6) (emphasis added).

Moreover, the Parent '054 Application does not teach that making such an antibody with the functional qualities of inhibiting osteoclast differentiation and/or bone resorption is even within the realm of possibility. (Ex. 1004, ¶ 12; *see also id.* ¶¶ 7, 8, 16). Indeed, other than the disclosed polynucleotides and polypeptides themselves for use in treatment, the Parent '054 Application merely discloses inhibitory compounds in general, that have the desired function of "ameliorating bone remodeling disease or disorder symptoms" or "delaying bone disease or disorder" by specifically inhibiting activity or expression of a polynucleotide or a polypeptide described therein. (Ex. 1009, p. 10, ll. 17-23). But the concept of administering an antibody that binds to one of the disclosed polypeptides, much less Siglec-15, to accomplish these effects is not stated in the in the Parent '054 Application with any particularity. (Ex. 1004, ¶ 26; Ex. 1003, ¶¶ 7-8). The Parent '054 Application only makes one broad statement relating to therapy but with no certain antibody in mind: "[n]eutralizing antibodies, such as those that inhibit

dimer formation, are especially preferred for therapeutic use." (Ex. 1009, p. 37, ll. 27-30). But this statement is not tied to an antibody that binds one of the disclosed polypeptides, does not specify that the antibodies can specifically inhibit bone resorption or impair osteoclast differentiation, and is just a generalization. (Ex. 1004, ¶ 27; Ex. 1003, ¶ 9).

Further, inhibition of Siglec signaling would likely not even work with "neutralizing" antibodies "that inhibit dimer formation" as provided in the statement, and actually represents a poor understanding of how Siglecs work in general. (Ex. 1003, ¶¶ 10-11). For example, Stuible *et al.* characterized a Siglec-15 antibody (that appears to have been actually made) as ultimately inducing dimer formation, leading to receptor degradation and inhibition of Siglec-15 receptor function in an indirect manner. (Ex. 1021, at Abstract, p. 1; Ex. 1003, ¶ 11). Therefore, had a skilled person attempted to make a Siglec-15 antibody that impairs osteoclast differentiation or inhibits bone resorption based on the Parent '054 Application, this person would have looked for an antibody that *inhibits* ligand induced dimerization and not for one that *induces* dimerization, as indicated in the current literature for a Siglec-15 antibody. (Ex. 1003, ¶ 11). This underscores the generality of the remark in the specification, the "boiler plate" antibody language, and the lack of teaching a specific Siglec-15 antibody that can be administered for therapy. (Ex. 1004, ¶¶ 16, 26).

(ii) The Parent '054 Application does not identify an antibody that "specifically binds to human Siglec-15 or murine Siglec-15" out of various possible disclosed antigens

The Parent '054 Application does not guide a skilled artisan to Siglec-15 as a target for antibody treatment. The Parent '054 Application discloses about 35 polynucleotides and corresponding polypeptides involved in the process of bone remodeling, including human and mouse AB0326, which encodes human and mouse Siglec-15, respectively (Ex. 1003, ¶ 5). But Siglec-15 is not particularly described in the Parent '054 Application or its priority documents over any other polynucleotide or polypeptide disclosed therein. (Ex. 1003, ¶ 6). In fact, the Parent '054 Application and its priority documents do not describe in any detail the function of Siglec-15 *per se*, the mechanism by which Siglec-15 mediates that function (Ex. 1003, ¶ 8), the extracellular accessibility of Siglec-15 by an antibody (Ex. 1003, ¶ 14), the function of a Siglec-15 antibody (Ex. 1003, ¶¶ 10-14), such that a skilled artisan would have been directed to Siglec-15 as a useful target for antibody therapy.

Additionally, while the Parent '054 Application discloses a screening assay utilizing a cell line in which human Siglec-15 "rescued" the function of cells containing inhibited mouse Siglec-15, the disclosure further states that "[t]his assay is applicable to any gene required for proper osteoclast differentiation" and that "[s]imilar experimentation to those described above are carried out for other

sequences (SEQ ID NO. 3 to SEQ ID NO.: 33 or SEQ ID NO.: 85 or SEQ ID NO.: 86)." (Ex. 1009, p. 86, ll. 4-9). Accordingly, the demonstration that Siglec-15 is required for osteoclastogenesis using RNA interference is diluted by remarks extrapolating the assay to other sequences and genes. (Ex. 1003, ¶ 9).

Therefore, at least because the Parent '054 Application (a) fails to disclose even a single antibody that impairs osteoclast differentiation or inhibits bone resorption, and (b) does not particularly describe Siglec-15 as a target for treatment over any other target disclosed, it follows that the Parent '054 Application does not provide written description support for the claims in the '181 patent.

2. Claims 1–6, 8-11 and 15-23 are not enabled by the Parent '054 Application

In order to fulfill the enablement requirement of 35 U.S.C.§ 112, 1st paragraph, the specification must describe the invention in such terms that one skilled in the art can "make *and* use" the claimed invention. This requirement can be broken down into at least two components: (1) that the claimed invention be enabled so that a person skilled in the art can make and use the invention without "undue experimentation," *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988); and (2) that "the specification disclose as a matter of fact a practical utility for the invention." *In re Cortright*, 165 F.3d 1353, 1356 (Fed. Cir. 1999). In the present case, the Parent '054 Application fails to enable the claims of the '181 Patent in both respects.

(a) The Parent '054 Application does not teach making an antibody that impairs osteoclast differentiation or inhibits bone resorption

Independent claims 1 and 15 of the '181 patent, and claims dependent therefrom, are directed to methods of impairing osteoclast differentiation and methods of inhibiting bone resorption, respectively, comprising administering an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108). The Parent '054 Application, however, does not contain any teachings regarding how to make without undue experimentation an antibody that specifically binds Siglec-15 and impairs osteoclast differentiation or inhibits bone resorption, as required by the claimed methods. (Ex. 1004, ¶¶ 17, 28).

Specifically, the Parent '054 Application does not (i) make even a single antibody that impairs osteoclast differentiation or inhibits bone resorption, either *in vitro* or *in vivo*, (ii) show how to make such an antibody, or (iii) show that making such an antibody would even be within the realm of possibilities. (Ex. 1004, ¶¶ 16, 23, 26). While the Parent '054 Application describes methods for making antibodies against any target in general, the disclosure is not specific for a Siglec-15 antibody with the purpose of impairing osteoclast differentiation or inhibiting bone resorption. (Ex. 1009, p. 33, ln. 5 – p. 41, ln. 6; Ex. 1004, ¶ 16). These

general teachings are not sufficient to inform the skilled artisan how to make a therapeutic Siglec-15 antibody without undue experimentation. (Ex. 1004, ¶ 17).

Further, the generalized teaching regarding "[n]eutralizing antibodies … for therapeutic use" (Ex. 1008, p. 37, ll. 27-28) is a research plan or an invitation for further experimentation at best (*See* Ex. 1004, ¶ 26) and the Federal Circuit has firmly held that such a disclosure is not enabling. For instance, in *Wyeth v. Abbott Laboratories*, the Federal Circuit described the specificity required for enablement in the context of therapeutic compounds as follows:

> [I]n *ALZA Corp. v. Andrax Pharmaceuticals, LLC*, we affirmed a judgment of nonenablement where the specification provided "only a starting point, a direction for further research." 603 F.3d 935, 941 (Fed. Cir. 2010) (internal quotation omitted). We concluded that one of ordinary skill "would have been required to engage in an iterative, trial-and-error process to practice the claimed invention even with the help of the … specification." *Id.* at 943. In *Cephalon*, although we ultimately reversed a finding of nonenablement, we noted that the defendant had not established that required experimentation "would be excessive, e.g., that it would involve testing for an unreasonable length of time." 707 F.3d at 1339 (citing *White Consol. Indus., Inc. v. Vega Servo-Control, Inc.*, 713 F.2d 788, 791 (Fed. Cir. 1983)). Finally, in *In re Vaeck*, we affirmed the PTO's nonenablement rejection of claims reciting heterologous gene expression in as many as 150 genera of cyanobacteria. 947 F.2d 488, 495-96 (Fed. Cir. 1991). The specification disclosed only nine genera, despite

> cyanobacteria being a "diverse and relatively poorly understood group of microorganisms," with unpredictable heterologous gene expression. *Id.* at 496.

Wyeth v. Abbott Laboratories, 720 F.3d 1380, 1386 (Fed. Cir. 2013).

Accordingly, to satisfy the enablement requirement, the specification must contain more than a suggestion that antibodies for treatment would be a good idea. Here, however, the amount of experimentation required to identify such an antibody would be excessive, at least because it is uncertain whether such an antibody could even be made. (Ex. 1003, ¶ 16; Ex. 1004, ¶¶ 17, 28).

More specifically, to make an antibody for use in therapy, a number of steps need to be performed. (Ex. 1004, ¶ 7). An antibody against a target antigen with certain activity may never be created by a one skilled in the art when little, if anything, other than the target protein sequence is understood, and nothing about the structure of such a therapeutic antibody is known. (Ex. 1004, ¶¶ 7, 11, 13). Because Siglec-15 cell surface accessibility, signaling pathway, specific function, recycling kinetics and other relevant information was not known at the time of filing the Parent '054 Application (Ex. 1003, ¶¶ 14, 16.), and no guidance is provided in the Parent '054 Application regarding any structure-function relationship of a Siglec-15 antibody for treatment, a person skilled in the art of therapeutic antibody development would not know how to make, without undue experimentation, a Siglec-15 antibody that impairs osteoclast differentiation or

inhibits bone resorption, as claimed in the '181 patent. (Ex. 1004, ¶ 28). In other words, the Parent '054 Application does not provide any concrete guidance on how to make an antibody that specifically binds Siglec-15 and has the activity as required by the '181 patent claims – there is not even a single example provided of such an antibody. (Ex. 1004, ¶¶ 16, 27).

Additionally, the Parent '054 Application has only one example of a negative regulator of Siglec-15, but it is interfering RNA and not an antibody. (Ex. 1003, ¶ 17; Ex. 1004, ¶ 21). Such a teaching cannot be used to predict the effectiveness of a compound that directly interacts with a target protein. (Ex. 1003, ¶ 17; Ex. 1004, ¶ 21). As such, one skilled in the art cannot assume that simply because siRNA could have an effect on cell function, that an inhibitory antibody could be designed to do the same. (Ex. 1003, ¶ 17; Ex. 1004, ¶ 21). Moreover, if a skilled artisan were to attempt to seek the same effect as siRNA using an antibody, it would certainly require undue experimentation, at least because the disclosure of the Parent '054 Application provides no working examples and no direction regarding the requisite structure of the desired antibody (Ex. 1004, ¶ 23), and the level of unpredictability regarding therapeutic antibody development (Ex. 1004, ¶ 17). The teaching of one example of siRNA surely fails to satisfy the *Wands* factors and would require undue experimentation for one of skill to implement the claims of the '181 Patent. (Ex. 1004, ¶ 21).

And while the Parent '054 Application mentions an assay for identifying inhibitory compounds which may be able to impair the *in vitro* function or expression of the polypeptides described therein (Ex. 1009, p. 85, ln. 4 – p. 86, ln. 11), this is, at best, a screening tool for any number of inhibitors, not necessarily antibodies, of osteoclast differentiation. (Ex. 1004, ¶ 27). And by no means is this assay an indication that a therapeutic Siglec-15 antibody even could be made, much less a recipe for actually making such a therapeutic antibody. *See* Ex. 1004, ¶ 27.

Furthermore, this *in vitro* functional complementation assay for inhibiting activity of osteoclast differentiation may not reflect how the antibody would behave *in vivo*. (Ex. 1004, ¶ 27). In particular, an epitope to which an antibody binds *in vitro* may not be available when the protein is folded into its *in vivo* conformation. (Ex. 1004, ¶¶ 20, 27). As such, the skilled artisan would not know whether an antibody that specifically bound the encoded protein would be able to interact with the Siglec-15 protein or affect its function *in vivo*. (Ex. 1004, ¶ 20). Thus, without ever having made a Siglec-15 antibody, the skilled artisan would not know how to make an antibody that actually contained the claimed activity. (Ex. 1003, ¶ 13).

Also, the cell surface accessibility of Siglec-15 by an antibody was not disclosed in the Parent '054 Application and therefore, the feasibility of Siglec-15

as a target for treatment with an antiboy is not evident from the teachings in the Parent '054 Application or from the relevant literature. (Ex. 1003, ¶¶ 14, 16). As discussed above, Angata is silent on explicit extracellular localization of Siglec-15; it merely describes co-localization with an intracellular protein. (Ex. 1003, ¶ 14). Further, the sequence of Siglec-15 and the lack of sufficient characterization of the protein in the Parent '054 Application and the art also call into question the suitability of Siglec-15 as a target for antibody therapy. (Ex. 1003, ¶ 16).

Therefore, without an indication that Siglec-15 should be pursued for treatment with an antibody, and a description of to how to make, without undue experimentation, a Siglec-15 antibody that impairs osteoclast differentiation or inhibits bone resorption, the *Wands* factors cannot be satisfied and the Parent '054 Application does not enable claims 1-6, 8-11 and 15-22 of the '181 Patent. (Ex. 1004, ¶ 17; Ex. 1003, ¶¶ 8, 13). In other words, the general guidance provided in the specification of the Parent '054 Application for making an antibody for use in treatment is not sufficient to demonstrate to a person in the field of antibody therapeutics how to make an anti-human or anti-mouse Siglec-15 antibody that would be suitable for a therapeutic purpose without conducting undue experimentation. (Ex. 1004, ¶ 17). This is especially true, given the unpredictability in the field of antibody therapy, the lack of disclosure regarding Siglec-15 localization and feasibility of Siglec-15 as a suitable target, the absence

of working examples in the specifications, and the uncertainty as to whether a Siglec-15 antibody with the claimed activity can even be made in view of the lack of disclosure. (Ex. 1004, ¶ 17).

(b) The Parent '054 Application lacks any guidance for a method of treatment with an anti-Siglec-15 antibody

While the claims of the '181 Patent are directed to a method of impairing osteoclast differentiation or inhibiting bone resorption, the Parent '054 Application fails to provide any description regarding the use of antibodies or antigen binding fragments for either of the claimed methods in the '181 Patent, or even the smallest indication that antibodies or antigen binding fragments that bind to Siglec-15 would perform the requisite activity *in vivo*. (Ex. 1004, ¶ 12, 27).

A patent application fails to establish enablement "where there is no indication that one skilled in the art would accept without question statements as to the effects of the claimed drug products and no evidence has been presented to demonstrate that the claimed products do have those effects." *Rasmussen v. SmithKline Beecham Corp.*, 413 F.3d 1318, 1323 (Fed. Cir. 2005) (internal quotations and brackets omitted).

The Federal Circuit has held that a failure to disclose how to use an invention constitutes a failure of enablement "when there is a complete absence of data supporting the statements which set forth the desired results of the claimed invention." *Id.* As the court explained:

> If mere plausibility were the test for enablement under section 112, applicants could obtain patent rights to 'inventions' consisting of little more than respectable guesses as to the likelihood of their success. When one of the guesses later proved true, the 'inventor' would be rewarded the spoils instead of the party who demonstrated that the method actually worked. That scenario is not consistent with the statutory requirement that the inventor enable an invention rather than merely proposing an unproved hypothesis.

Id at 1325.

In the Parent '054 Application, the patentee recited vague contentions regarding antibodies that could bind to one of numerous disclosed peptides and their potential utility as diagnostics. (Ex. 1003, ¶ 18). The patentee never contended that such antibodies could be used as a therapeutic whatsoever, much less a therapeutic that impairs osteoclast differentiation or inhibits bone resorption. (Ex. 1003, ¶¶ 7, 8, 18).

Furthermore, the Parent '054 Application does not describe how to carry out the claimed process. The Parent '054 Application lacks any guidance such as dosage requirements or other direction regarding how to use an antibody that specifically binds Siglec-15 to impair osteoclast differentiation or inhibit bone resorption in a mammal. (Ex. 1004, ¶ 28). This is not surprising because a Siglec-15 antibody that is administered to a mammal or subject, or any antibody that binds the described polypeptides that is administered to a mammal or subject, is not

disclosed in the Parent '054 Application, much less how to use such an antibody in the claimed method. (Ex. 1004, ¶¶ 26, 28; Ex. 1003, ¶ 8). Accordingly, it is not surprising that the Parent '054 Application and its priority documents do not teach how to carry out, without undue experimentation, a method of impairing osteoclast differentiation or inhibiting bone resorption with an antibody that specifically binds to Siglec-15. (Ex. 1003, ¶ 8).

Therefore, the Parent '054 Application lacks the enabling disclosure necessary for the claims of the '181 patent to benefit from the priority date of the Parent '054 Application.

3. The 2006 Provisional Applications and the PCT Application Likewise Fail To Describe or Enable the Claims under § 112, 1st Paragraph

The two provisional applications filed in 2006 (*i.e.*, U.S. Provisional Pat. Appl. No. 60/772,585 and U.S. Provisional Pat. Appl. No. 60/816,858, or Ex. 1017 and 1018, respectively) contain the same or even less disclosure than the later-filed Parent '054 Application. Those provisional applications therefore also necessarily lack descriptive and enabling support for at least the same reasons as the Parent '054 Application set forth above. Likewise, PCT/CA2007/000210 contains the same specification as the Parent '054 Application, as the WO publication corresponding to PCT/CA2007/000210 was submitted for national phase entry and formed the application cited herein as the "Parent '054 Application." Thus,

PCT/CA2007/000210 necessarily fails to describe or enable the claims of the '181 patent for the same reasons set forth above.

4. Claims 1-6, 8-11 and 15-23 are Anticipated by the '072 Publication

(a) Independent Claims 1 and 15

International Application Number PCT/JP2008/068287 to Daiichi Sankyo Co., Ltd., titled "Antibody Targeting Osteoclast-Related Protein Siglec-15" was filed on October 8, 2008, and published in Japanese on April 16, 2009 as WO 2009/048072 ("the '072 publication") (Ex. 1002). The '072 Publication predates the '181 patent effective filing date of October 16, 2009 by six months, and thus qualifies as prior art under 35 U.S.C. § 102(a). Even if the Patent Owner's third provisional application, U.S. Provisional Pat. Appl. 61/248,960, were an effective priority document, the '072 Publication still predates the October 6, 2009 provisional filing by more than 5 months and therefore still qualifies as prior art under section 102(a).

As evidenced by the English translation of Daiichi Sankyo's '072 Publication (Ex. 1023), published as U.S. Pat. Pub. 2010-0209428 (Ex. 1025)[3], the '072

[3] Ex. 1023 and Ex. 1025 and confirm that Ex. 1025 is the USPTO publication Ex. 1023 and therefore contain the same specification, with the exception of a sequence listing in the '428 publication.

Publication describes an antibody or a functional fragment thereof that specifically recognizes human or mouse Siglec-15 (*i.e.*, SEQ ID NOs: 2 and 4 of the English translation of the '072 Publication) and inhibits osteoclast formation and/or impairs bone resorption. (Ex. 1023, p. 5, ll. 1-20; p. 20, ll. 2-14. Ex. 1003, ¶ 19), as recited in claims 1 and 15 of the '181 patent.

More specifically, with regard to claim 1, the '072 Publication describes several Siglec-15 polyclonal and monoclonal antibodies and methods for making them. See, for example, Examples 10 and 11 (anti-mouse Siglec-15 polyclonal antibody), Examples 24 and 25 (anti-mouse Siglec-15 monoclonal antibody), and Examples 33 and 34 (anti-human Siglec-15 polyclonal antibody). (Ex. 1023, p. 93, ln. 16-p. 97, ln. 23; p. 114, ln. 23-p. 117, ln. 16; p. 133, ln. 3-p. 138, ln. 2; Ex. 1003, ¶ 19). The results of testing the Siglec-15 polyclonal and monoclonal antibodies in the '428 publication on osteoclast differentiation is also described. For example, Examples 17, 19 – 26, and 35 of the '428 Publication demonstrate an inhibitory effect of Siglec-15 antibodies on osteoclast differentiation. (Ex. 1023, p. 103, ln. 19-p. 105, ln. 13; 106, ln. 17-p. 119, ln. 4; p. 138, ln. 3-p. 139, ln. 15; Ex. 1003, ¶ 20). The '072 Publication further teaches that "[t]he term 'osteoclast formation' as used therein has the same meaning as 'osteoclast differentiation' or 'osteoclast maturation'." (Ex. 1023, p. 17, ll. 20-21). One skilled in the art would understand that "osteoclast formation", as described in the '072 Publication is

synonymous with "osteoclast differentiation" or "osteoclast differentiating activity", as recited in the '181 patent claims (Ex. 1003, ¶ 20), which connotes differentiation of osteoclast precursor cells into multinucleated osteoclasts. (Ex. 1003, ¶ 20).

Regarding claim 15 of the '181 Patent, the '072 Publication teaches methods of inhibiting bone resorption (Ex. 1023, p. 56, ln. 24-p. 58, ln. 4; claim 33) comprising administering to a subject in need thereof, an antibody or antigen binding fragment which specifically binds to human Siglec-15 or murine Siglec-15 (Ex. 1023, Example 37 (p. 141, ln. 10 – p. 144, ln. 22), p. 11, ll. 3-5; p. 5, ln. 1 - p. 7, ln. 1; p. 17, ll. 5-8, Fig. 36; p. 56, ln. 24-p. 59, ln. 7; claim 33; Ex. 1003, ¶ 23; Ex. 1004, ¶ 31, 33-34).

Further, the '072 Publication teaches administering to a mammal, specifically, a human. (Ex. 1023, p. 36, ll. 11-18 ("an antibody applicable to a human disease can be selected"), p. 65, ln. 16 – p. 66, ln. 2 ("human anti-Siglec-15 antibody is administered to humans")). (Ex. 1004, ¶ 32).

As further shown below, the '072 Publication teaches every limitation of independent claims 1 and 15.

Claim 1 of '181 Patent	**'072 English Translation**
A method of impairing osteoclast differentiation	p. 4, ll. 22-23: "[inventors] found that the **differentiation of osteoclasts is inhibited** by an antibody which specifically binds to Siglec-15, and, thus, the invention has been completed."

	p. 17, ll. 20-21: "The term '**osteoclast formation' as used herein is used in the same meaning as 'osteoclast differentiation'** or 'osteoclast maturation.'" p. 2, ll. 13-20: "[o]**steoclast precursor cells have been found to be differentiated into osteoclasts** by stimulation with RANKL (receptor activator of NF-.kappa.B ligand)...RANKL induces **differentiation of osteoclast precursor cells into multinucleated osteoclasts**, and the like." p. 105, ll. 11-13: "From the above results, it was shown that **the anti-mouse Siglec-15 polyclonal antibody has a potent inhibitory effect on osteoclast formation (osteoclast differentiation and maturation)**." p. 147, ll. 5-8: "**The anti-Siglec-15 antibody of the invention has the ability to inhibit osteoclast differentiation** or bone resorption activity, and a pharmaceutical composition containing the anti-Siglec-15 antibody can be a therapeutic or preventive agent for a disease of abnormal bone metabolism." Claim 33: "A method of treating and/or preventing abnormal bone metabolism characterized by administering at least **one of the antibodies or functional fragments of the antibodies**..."
in a mammal in need thereof,	p. 36, ll. 16-18: "In this case, by examining the cross-reactivity between an antibody binding to the obtained heterologous Siglec-15 and human Siglec-15, **an antibody applicable to a human disease can be selected**." p. 65, ll. 22-24: "**[T]he pharmaceutical composition of the invention for humans** can also be determined based on this result. p. 147, ll. 5-8: "The anti-Siglec-15 antibody of the invention has the ability to inhibit osteoclast

	differentiation or bone resorption activity, and a pharmaceutical composition containing **the anti-Siglec-15 antibody can be a therapeutic or preventive agent for a disease of abnormal bone metabolism.**"
the method comprising administering an antibody or antigen binding fragment	Claim 33 or p. 11, ll. 3-5: "A method of treating and/or preventing abnormal bone metabolism characterized by administering at least **one of the antibodies or functional fragments of the antibodies**..."
which specifically binds to human Siglec-15 (SEQ ID NO.:2)	Claim 1 or p. 5, ll. 1-5: "**An antibody which specifically recognizes one or more polypeptides comprising** an amino acid sequence described in any one of the following (a) to (i) and inhibits osteoclast formation and/or osteoclastic bone resorption, or a functional fragment of the antibody: (a) **an amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing**;" p. 20, ll. 2-5: "The nucleotide sequence of **human Siglec-15** cDNA has been registered in GenBank with an accession number of NM 213602 and is represented by SEQ ID NO: 1 in the Sequence Listing, and **its amino acid sequence is represented by SEQ ID NO: 2 in the Sequence Listing.**"
or murine Siglec-15 (SEQ ID NO.:108)	p. 36, ll. 14-18: "The biological species of Siglec-15 to be used as an antigen is not limited to human, and **an animal can be immunized with Siglec-15 derived from an animal other than human such as mouse**..." Claim 1 or p. 5, ll. 1-4; p. 5, ln. 13: "**An antibody which specifically recognizes one or more polypeptides comprising** an amino acid sequence described in any one of the following (a) to (i) and inhibits osteoclast formation and/or osteoclastic bone resorption, or a functional fragment of the antibody: **...(e) an amino acid sequence represented by SEQ ID NO: 4 in the Sequence Listing;**" p. 20, ll. 5-8: "The nucleotide sequence of **mouse Siglec-15** cDNA has been registered in GenBank with

	an accession number of XM 884636 and is represented by SEQ ID NO: 3 in the Sequence Listing, and **its amino acid sequence is represented by SEQ ID NO: 4 in the Sequence Listing.**"
to said mammal.	p. 36, ll. 11-18: "In this case, by examining the cross-reactivity between an antibody binding to the obtained heterologous Siglec-15 and human Siglec-15, **an antibody applicable to a human disease can be selected**." p. 65, ln. 22-p. 66, ln. 2: "[T]he pharmaceutical composition of the invention for humans can also be determined based on this result. As for the dose, in the case **where a human anti-Siglec-15 antibody is administered to humans**, the antibody may be administered at a dose of from about 0.1 to 100 mg/kg once per one to 180 days."

Claim 15 of '181 Patent	**'072 English Translation**
A method for inhibiting bone resorption	p. 57, ll. 6-9: "The abnormal bone metabolism may be any disorder characterized by net bone loss (osteopenia or osteolysis). **In general, the treatment and/or prevention by the anti-Siglec-15 antibody are/is applied to a case where inhibition of bone resorption is required.**" p. 144, ll. 20-22: "From this result, it was revealed that the **bone resorption activity of human osteoclasts is inhibited by the monoclonal antibody specifically binding to the Siglec-15 protein**".
comprising administering to a subject in need thereof,	p. 57, ll. 6-9: "The abnormal bone metabolism may be any disorder characterized by net bone loss (osteopenia or osteolysis). **In general, the treatment and/or prevention by the anti-Siglec-15 antibody are/is applied to a case where inhibition of bone resorption is required.**"
an antibody or antigen binding fragment which specifically binds to human	Claim 33: "A method of treating and/or preventing abnormal bone metabolism characterized by **administering at least one of the antibodies or**

Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108).	**functional fragments of the antibodies** according to claims 1 to 26." p. 11, ll. 3-5: "A method of treating and/or preventing abnormal bone metabolism characterized by **administering at least one of the antibodies or functional fragments of the antibodies** according to 1 to 26." p. 5, ll. 1-5 and 12: "**An antibody which specifically recognizes one or more polypeptides comprising an amino acid sequence described in any one of the following (a) to (i)** and inhibits osteoclast formation and/or osteoclastic bone resorption, or a functional fragment of the antibody: (a) **an amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing**...(e) **an amino acid sequence represented by SEQ ID NO: 4 in the Sequence Listing**..." p. 20, ll. 2-5: "The nucleotide sequence of **human Siglec-15** cDNA has been registered in GenBank with an accession number of NM 213602 and is represented by SEQ ID NO: 1 in the Sequence Listing, and its **amino acid sequence is represented by SEQ ID NO: 2 in the Sequence Listing.**" p. 20, ll. 5-8: "The nucleotide sequence of **mouse Siglec-15** cDNA has been registered in GenBank with an accession number of XM 884636 and is represented by SEQ ID NO: 3 in the Sequence Listing, and its **amino acid sequence is represented by SEQ ID NO: 4 in the Sequence Listing.**"

(b) Dependent Claims 2-6 and 8-11

Claims 2-6 and 8-11 of the '181 Patent all depend from claim 1, and further limit the claimed method of impairing osteoclast differentiation. Each of these

additional limitations are also anticipated by the '072 Publication and are described in the claim charts and accompanying remarks below.

Dependent claim 2 recites that "the antibody or antigen binding fragment impairs an osteoclast differentiation activity of human or mouse Siglec-15." This limitation is taught in at least p. 5, ll. 1-13 of the English translation of the '072 Publication, which states "[a]n antibody which specifically recognizes one or more polypeptides comprising an amino acid sequence described in any one of the following (a) to (i) and inhibits osteoclast formation and/or osteoclastic bone resorption, or a functional fragment of the antibody: (a) an amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing ... (e) an amino acid sequence represented by SEQ ID NO: 4 in the Sequence Listing."

Moreover, the '072 Publication describes how to make, in working examples, how to make a Siglec-15 antibody, including antigen preparation and monoclonal antibody production. (Ex. 1004, ¶ 30). Several anti-mouse Siglec-15 monoclonal antibodies were made in the '072 Publication from a mammalian cell, including those from hybridomas #1A1, #8A1, #3A1, #24A1, #32A1, #34A1, #39A1, #40A1, #41B1 and #61A1, some of which have been deposited. (Ex. 1004, ¶ 30). Also, the inhibition of both human (*See* Ex. 1023, p. 138, ln. 3-p. 139, ln. 15) and mouse (*See* Ex. 1023, Ex. 1023, p. 103, ln. 19-p. 105, ln. 13; 106, ln. 17-p. 109, ln. 10; p. 116, ln. 10-p. 117, ln. 16) Siglec-15 osteoclast

differentiation activity with an anti-mouse and anti-human Siglec-15 polyclonal antibody, and also an anti-mouse Siglec-15 monoclonal antibody is also disclosed. (Ex. 1003, ¶¶ 20, 22). Indeed, the '072 Publication contains multiple working examples of antibodies that specifically bind Siglec-15 and impair osteoclast differentiation. (Ex. 1003, ¶ 20).

Dependent claim 3 recites "the osteoclast differentiation activity is characterized by differentiation of osteoclast precursor cells into differentiated osteoclasts." This limitation is taught in at least Examples 35 and 37 of the English translation of '072 Publication (Ex. 1023, p. 138, ln. 5-p. 139, ln. 15; p. 141, ln. 10-p. 144, ln. 22), which report that "multinucleation and cell fusion of TRAP-positive osteoclasts from normal human osteoclast precursor cells are inhibited by the antibody specifically binding to Siglec-15." (Ex. 1003, ¶ 24). Figures 31 and 34 also show photomicrographs depicting, by TRAP staining, the inhibition of giant osteoclast formation from normal human osteoclast precursor cells by the addition of an anti-human Siglec-15 polyclonal antibody and rat anti-mouse Siglec-15 monoclonal antibody, respectively. (Ex. 1003, ¶ 24). Additionally, p. 52, ln. 18-p. 53, ln. 7 of the English translation of the '072 Publication discloses antibodies and/or fragments thereof that inhibit the formation of osteoclasts and cell fusion, both of which are known indications of osteoclast differentiation. *See* Ex. 1026 at 101-2; *see also* Ex. 1003, ¶¶ 19, 24.

Dependent claims 4 and 5 require that "the antibody is a polyclonal antibody" and "the antibody or antigen binding fragment is a monoclonal antibody or antigen binding fragment thereof," respectively. Both of these limitations are plainly taught throughout the '072 publication. (Ex. 1023, p. 55, ll. 12-17 ("The antibody of the invention may be a polyclonal antibody"), p. 8, ll. 14-15 ("The antibody or a functional fragment of the antibody according to any one of (1) to (13), characterized in that the antibody is a monoclonal antibody.")). (Ex. 1003, ¶ 19). Furthermore, Examples 8 and 33 describe, in detail, procedures for producing polyclonal anti-Siglec-15 antibodies, and Examples 24 and 38 detail procedures for producing monoclonal anti-Siglec-15 antibodies. (Ex. 1004, ¶ 30) Also, Siglec-15 polyclonal and monoclonal antibodies were actually made. *See* Ex. 1023, Examples 22-23 (p. 110, ln. 23-p. 114, ln. 22); *see also* Ex. 1004, ¶ 31; Ex. 1003, ¶ 19.

Dependent claim 6 further states that "the monoclonal antibody or antigen binding fragment is produced from an isolated mammalian cell." As noted above, Examples 24 and 38 detail procedures for producing monoclonal anti-Siglec-15 antibodies, and both utilize mammalian hybridomas. (Ex. 1004, ¶¶ 30, 31). For instance, the '072 Publication teaches, "Cell fusion was performed according to a common method of fusing mouse (rat) spleen cells with myeloma cells...The collected spleen cells and P3X63Ag8.653 cells (ATCC CRL 1580) which are

mouse myeloma cells were subjected to cell fusion using polyethylene glycol (PEG)." (Ex. 1023, p 113, ln. 18-p. 114, ln. 7).

Dependent claims 8 and 9 require that "the antibody or antigen binding fragment comprises a constant region of a human antibody or a fragment thereof," and "comprises a framework region of a human antibody," respectively. The '072 Publication not only discloses human and humanized antibodies (Ex. 1023, p. 9, ll. 14-15; p. 50, ll. 4-18; p. 50, ln. 19-p. 51, ln. 7; p. 51, ln. 20-p. 52, ln. 14), both of which meet the limitations of claims 8 and 9, it also discloses chimeric antibodies, an exemplary embodiment of which is described as "a chimeric antibody in which a mouse-derived antibody variable region is connected to a human-derived constant region." (Ex. 1023, p. 49, ln. 24-p. 51, ln. 7; Ex. 1004, ¶ 33).

Dependent claim 10 requires that "the antibody or antigen binding fragment is a FV, a Fab, a Fab' or a $(Fab')_2$." This limitation is explicitly recited in p. 53, ll. 8-13 of the English translation of the '072 Publication, which states, "Examples of the fragment of the antibody include Fab, F(ab')2, Fv, single-chain Fv (scFv) in which Fv molecules of the heavy chain and the light chain are ligated via an appropriate linker, a diabody (diabodies), a linear antibody, and a polyspecific antibody composed of the antibody fragment." This same limitation is also taught the English translation of the '072 Publication. (Ex. 1023, p. 10, ll. 14-19; p. 113, ln. 18-p. 114, ln. 7; Ex. 1004, ¶ 33).

Claim 11 depends from claim 3 and further requires that the osteoclast precursor cells are human osteoclast precursor cells. This limitation is anticipated by the same sections of the '072 Publication that anticipated claim 3. (Ex. 1023, p. 52, ln. 18-p. 53, ln. 7; p. 138, ln. 5-p. 139, ln. 15; p. 141, ln. 10-p. 144, ln. 22).

As further shown below, the '072 publication teaches every limitation of dependent claims 2-6 and 8-11.

Claim 2	'072 English Translation
The method of claim 1,	
wherein the antibody or antigen binding fragment impairs an osteoclast differentiation activity	p. 53, ll. 4-6: "**The function of the functional fragment of the antibody according to the invention is preferably an activity of inhibiting the formation of osteoclasts**" p. 17, ll. 20-21: "The term **"osteoclast formation" as used herein is used in the same meaning as "osteoclast differentiation"** or "osteoclast maturation"."
of human Siglec-15	Claim 1 or p. 5, ll. 1-5: "**An antibody which specifically recognizes one or more polypeptides comprising** an amino acid sequence described in any one of the following (a) to (i) **and inhibits osteoclast formation and/or osteoclastic bone resorption,** or a functional fragment of the antibody: **(a) an amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing;"** p. 20, ll. 2-5: "The nucleotide sequence of **human Siglec-15** cDNA has been registered in GenBank with an accession number of NM 213602 and is represented by SEQ ID NO: 1 in the Sequence Listing, and its **amino acid sequence is represented by SEQ ID NO: 2 in the Sequence Listing.**"
or murine Siglec 15.	Claim 1 or p. 5, ll. 1-4 and 12: "**An antibody which specifically recognizes one or more polypeptides**

	comprising an amino acid sequence described in any one of the following (a) to (i) **and inhibits osteoclast formation and/or osteoclastic bone resorption,** or a functional fragment of the antibody: **...(e) an amino acid sequence represented by SEQ ID NO: 4 in the Sequence Listing;"** p. 20, ll. 5-8: "The nucleotide sequence of **mouse Siglec-15** cDNA has been registered in GenBank with an accession number of XM 884636 and is represented by SEQ ID NO: 3 in the Sequence Listing, and its **amino acid sequence is represented by SEQ ID NO: 4 in the Sequence Listing.**"
Claim 3	**'072 English Translation**
The method of claim 2,	
wherein the osteoclast differentiation activity is characterized by differentiation of osteoclast precursor cells into differentiated osteoclasts.	p. 53, ll. 4-6: "**The function of the functional fragment of the antibody according to the invention is preferably an activity of inhibiting the formation of osteoclasts**" p. 17, ll. 20-21: "The term **'osteoclast formation' as used herein is used in the same meaning as** 'osteoclast differentiation' or **'osteoclast maturation'**." p. 138, ln. 5-p. 139, ln. 15: (Example 35, Titled: "Effect of Addition of Rabbit Anti-Human Siglec-15 Polyclonal Antibody on Cell Fusion of Normal Human Osteoclast Precursor Cells (TRAP Staining)"), specifically p. 139, ll. 13-15: "**that multinucleation and cell fusion of TRAP-positive osteoclasts from normal human osteoclast precursor cells are inhibited by the monoclonal antibody specifically binding to the Siglec-15 protein.**"
Claim 4	**'072 English Translation**
The method of claim 2,	
wherein the antibody is a polyclonal antibody.	p. 55, ll. 12-17: "**The antibody of the invention may be a polyclonal antibody which is a mixture of plural types of anti-Siglec-15 antibodies having different amino acid sequences.** As one example of the polyclonal antibody, a mixture of plural types of

	antibodies having different CDR can be exemplified. As such a polyclonal antibody, a mixture of cells which produce different antibodies is cultured, and an antibody purified from the resulting culture can be used (see WO 2004/061104)." p. 105, ll. 11-13: "From the above results, it was shown that the anti-mouse **Siglec-15 polyclonal antibody has a potent inhibitory effect on osteoclast formation** (osteoclast differentiation and maturation)."
Claim 5	**'072 English Translation**
The method of claim 2,	
wherein the antibody or antigen binding fragment is a monoclonal antibody or an antigen binding fragment thereof.	Claim 14: "**The antibody or a functional fragment of the antibody according to any one of claims 1 to 13, characterized in that the antibody is a monoclonal antibody.**" p. 8, ll. 14-15: "**The antibody or a functional fragment of the antibody according to any one of (1) to (13), characterized in that the antibody is a monoclonal antibody.**" p. 36, ll. 19-20: "[A] **monoclonal antibody can be obtained** by fusing antibody-producing cells which produce an antibody against Siglec-15 with myeloma cells to establish a hybridoma"
Claim 6	**'072 English Translation**
The method of claim 5,	
wherein the monoclonal antibody or antigen binding fragment is produced from an isolated mammalian cell.	p. 36, ll. 19-20: "[A] **monoclonal antibody can be obtained by fusing antibody-producing cells which produce an antibody against Siglec-15 with myeloma cells to establish a hybridoma**" p. 40, ln. 23-p. 41, ln. 4: "As the experimental animal, **any animal used in a known hybridoma production method can be used without any trouble. Specifically, for example, mouse, rat, goat, sheep, cattle, horse or the like can be used.** However, from the viewpoint of ease of availability of myeloma cells to be fused with the extracted antibody-producing cells, mouse or rat is

	preferably used as the animal to be immunized."
Claim 8	**'072 English Translation**
The method of claim 6,	
wherein the antibody or antigen binding fragment comprises a constant region of a human antibody or a fragment thereof.	p. 49, ln. 24-p. 50, ln. 3: "As the chimeric antibody, an antibody in which antibody variable and constant regions are derived from different species, for example, **a chimeric antibody in which a mouse-derived antibody variable region is connected to a human-derived constant region can be exemplified**."
Claim 9	**'072 English Translation**
The method of claim 8,	
wherein the antibody or antigen binding fragment comprises a framework region of a human antibody.	p. 50, ll. 9-13: "Further, the antibody of the invention includes a human antibody. **An anti-Siglec-15 human antibody refers to a human antibody having only a gene sequence of an antibody derived from a human chromosome**. The anti-Siglec-15 human antibody can be obtained by a method using a human antibody-producing mouse having a human chromosome fragment containing H-chain and L-chain genes of a human antibody."
Claim 10	**'072 English Translation**
The method of claim 2,	
wherein the antibody or antigen binding fragment is a FV, a Fab, a Fab′ or a $(Fab')_2$.	p. 53, ll. 8-13: "Examples of the fragment of the antibody include **Fab, F(ab')2, Fv,** single-chain Fv (scFv) in which Fv molecules of the heavy chain and the light chain are ligated via an appropriate linker, a diabody (diabodies), a linear antibody, and a polyspecific antibody composed of the antibody fragment. **Further, Fab' which is a monovalent fragment in a variable region of an antibody obtained by treating F(ab')2 under reducing conditions is also included in the fragment of the antibody.**"
Claim 11	**'072 English Translation**
The method of claim 3,	
wherein the osteoclast precursor cells are human osteoclast precursor cells.	p. 138, ln. 5-p. 139, ln. 15: (Example 35, Titled: "Effect of Addition of Rabbit Anti-Human Siglec-15 Polyclonal Antibody on Cell Fusion of Normal Human Osteoclast Precursor Cells (TRAP Staining)"), specifically p. 139,

	ll. 13-15: "**that multinucleation and cell fusion of TRAP-positive osteoclasts from normal human osteoclast precursor cells are inhibited by the monoclonal antibody specifically binding to the Siglec-15 protein**."

(c) Dependent Claims 16-23

Claims 16-23 of the '181 Patent all depend from claim 15, and further limit the claimed method of inhibiting bone resorption. Each of these claims is anticipated in view of the '072 Publication as outlined above, and any additional limitations recited in these claims are likewise found in the '072 Publication.

Dependent claim 16 recites that "the antibody or antigen binding fragment impairs an activity of human Siglec-15 or murine Siglec-15 in osteoclast precursor cells or in osteoclasts." Claim 17 depends from claim 16 and requires that the "activity [of Siglec-15 that is to be impaired] is osteoclastogenesis." The '072 publication repeatedly teaches that anti-Siglec-15 antibodies or fragments thereof impair the activity of human and murine Siglec-15 is osteoclast precursor cells and osteoclasts. (Ex. 1023, Example 37, p. 141, ln. 10-p. 144, ln. 22; p. 6, ll. 20-21; p. 5, ln. 1-p. 7, ln. 1; p. 17, 11. 5-8, Fig 36; p. 56, ln. 24-p. 59, ln. 17; Ex. 1003, ¶¶ 19, 22-25; Ex. 1004, ¶ 30). The '072 Publication is also replete with teachings of impairing osteoclastogenesis even though this particular term was not used. (Ex.

1023, p. 5, ll. 1-13; p. 52, ln. 18-p. 53, ln. 7; Examples 17-20, 25, 35, and 37, Figs. 31 and 34; Ex. 1003, ¶¶ 19, 22-25; Ex. 1004, ¶ 30).

Dependent claim 18 recites that "the antibody or antigen binding fragment inhibits osteoclast differentiation." Similar to claim 17, the limitation recited in this claim is disclosed by the '072 publication. (Ex. 1023, p. 5, ll. 1-13; p. 52, ln. 18-p. 53, ln. 7; Examples 17-20, 25, 35, and 37, Figs. 31 and 34; Ex. 1003, ¶¶ 20, 21-25; Ex. 1004, ¶¶ 30, 31).

Dependent claim 19 recites that the claimed "antibody or antigen binding fragment is administered in combination with a drug or an hormone," and claim 20 further specifies that "the drug is an antiresorptive drug or a drug increasing bone mineral density." The English translation of the '072 Publication disclose methods of treating abnormal bone metabolism by administering Siglec-15 antibodies in combination with hormone preparations, nonsteroidal anti-inflammatory agents, bisphosphonates (*i.e*, drugs that inhibit bone resorption and increase bone mineral density), or other compounds, thus disclosing the limitations of claims 19 and 20. (Ex. 1023, p. 11, ll. 6-17; p. 60, ln. 11-p. 61, ln. 11; p. 63, ll. 6-19; Ex. 1004, ¶ 32).

Claims 21 and 22 require that the claimed method is for use in "a subject suffering from a bone remodeling disorder," and specifically "a bone remodeling disorder [] associated with a decrease in bone mass," respectively. Claim 23

specifies certain bone remodeling disorders. With regard to these limitations, the '072 Publication teaches:

> Such an antibody which neutralizes the biological activity of Siglec-15 ...can be used as a therapeutic and/or preventive agent for abnormal bone metabolism caused by abnormal differentiation and/or maturation of osteoclasts as a medicine. The abnormal bone metabolism may be any disorder characterized by net bone loss (osteopenia or osteolysis).

(Ex. 1023, p. 56, ln. 24-p. 58, ln. 4). Thus, the limitations of claims 21-23 are clearly anticipated by the '072 Publication. (Ex. 1004, ¶ 32).

As further shown below, the '072 Publication teaches every limitation of dependent claims 16-23.

Claim 16	'072 English Translation
The method of claim 15,	
wherein the antibody or antigen binding fragment impairs an activity of human Siglec-15 or murine Siglec-15 in osteoclast precursor cells or in osteoclasts.	p. 56, ln. 24-p. 57, ln. 6: "From the anti-Siglec-15 antibodies obtained by the method described in the above item '4. Production of anti-Siglec-15 antibody', an antibody which neutralizes the biological activity of Siglec-15 can be obtained. **Such an antibody which neutralizes the biological activity of Siglec-15 inhibits the biological activity of Siglec-15 in vivo, i.e., the differentiation and/or maturation of osteoclasts, and therefore can be used as a therapeutic and/or preventive agent for abnormal bone metabolism caused by abnormal differentiation and/or maturation of osteoclasts as a medicine.**" p. 141, ln. 10-p. 144, ln. 22: (Example 37, Titled: "Effect of Addition of Rat Anti-Mouse Siglec-15

	Monoclonal Antibody on Cell Fusion and Bone Resorption Activity of Normal Human Osteoclast Precursor Cells) (Evaluation of In Vitro Biological Activity)), specifically p. 144, ll. 20-22: "From this result, it was revealed **that the bone resorption activity of human osteoclasts is inhibited by the monoclonal antibody specifically binding to the Siglec-15 protein.**"
Claim 17	**'072 English Translation**
The method of claim 16,	
wherein the activity is osteoclastogenesis.	p. 147, ll. 5-6: "**The anti-Siglec-15 antibody of the invention has the ability to inhibit** osteoclast differentiation or **bone resorption activity**" p. 60, ll. 4-10: "As shown in Example 19 of this description, OCIF/OPG which is a decoy receptor for RANKL can inhibit osteoclast formation induced by RANKL but does not inhibit osteoclast formation induced by TNF-α. **On the other hand, the anti-Siglec-15 antibody according to the invention effectively inhibited osteoclast formation induced by both RANKL and TNF- α.** Therefore, it is expected that **the anti-Siglec-15 antibody of the invention can inhibit bone loss and bone destruction** induced by TNF- α in RA or the like **more strongly than** an RANKL blocker (**OCIF**/OPG, an anti-RANKL antibody or the like)." p. 63, ll. 19: "OCIF (osteoclastogenesis inhibitory factor)." p. 105, ll. 11-13: "From the above results, it was shown that **the anti-mouse Siglec-15 polyclonal antibody has a potent inhibitory effect on osteoclast formation (osteoclast differentiation and maturation)**."
Claim 18	**'072 English Translation**
The method of claim 15,	
wherein the antibody or antigen binding fragment	p. 143, ll. 13-14: "As a result, the **formation of TRAP-positive multinucleated osteoclasts was**

inhibits osteoclast differentiation.	**inhibited** in a **#32A1** antibody concentration dependent manner . . . ." p. 144, ll. 18-22: "As a result, the amount of fluorescent collagen fragments increased by the addition of RANKL was reduced by the **#32A1** antibody in a concentration-dependent manner . . . . From this result, it was revealed that the **bone resorption activity of human osteoclasts is inhibited** by the monoclonal antibody specifically binding to the Siglec-15 protein."
Claim 19	**'072 English Translation**
The method of claim 15,	
wherein the antibody or antigen binding fragment is administered in combination with a drug or an hormone.	p. 60, ln. 15-p. 61, ln. 1: "Examples of the therapeutic agent which can be administered along with the anti-Siglec-15 antibody include, but are not limited to, **bisphosphonates, active vitamin D3, calcitonin and derivatives thereof, hormone preparations such as estradiol, SERMs (selective estrogen receptor modulators), ipriflavone, vitamin K2 (menatetrenone), calcium preparations, PTH (parathyroid hormone) preparations, nonsteroidal anti-inflammatory agents, soluble TNF receptor preparations, anti-TNF-α antibodies or functional fragments of the antibodies, anti-PTHrP (parathyroid hormone-related protein) antibodies or functional fragments of the antibodies, IL-1 receptor antagonists, anti-IL-6 receptor antibodies or functional fragments of the antibodies, anti-RANKL antibodies or functional fragments of the antibodies and OCIF (osteoclastogenesis inhibitory factor).**" Claim 29: "A pharmaceutical composition for treating and/or preventing abnormal bone metabolism characterized by comprising at least one of the antibodies or functional fragments of the antibodies according to claims 1 to 26 and **at least one member**

	selected from the group consisting of bisphosphonates, active vitamin D_3, calcitonin and derivatives thereof, hormone preparations such as estradiol, SERMs (selective estrogen receptor modulators), ipriflavone, vitamin K_2 (menatetrenone), calcium preparations, PTH (parathyroid hormone) preparations, nonsteroidal anti-inflammatory agents, soluble TNF receptor preparations, anti-TNF-α antibodies or functional fragments of the antibodies, anti-PTHrP (parathyroid hormone-related protein) antibodies or functional fragments of the antibodies, IL-1 receptor antagonists, anti-IL-6 receptor antibodies or functional fragments of the antibodies, anti-RANKL antibodies or functional fragments of the antibodies and OCIF (osteoclastogenesis inhibitory factor)."
Claim 20	**'072 English Translation**
The method of claim 19,	
wherein the drug is an antiresorptive drug	Claim 29: "A pharmaceutical composition for treating and/or preventing abnormal bone metabolism characterized by comprising at least one of the antibodies or functional fragments of the antibodies according to claims 1 to 26 and **at least one member selected from the group consisting of bisphosphonates**, active vitamin D_3, **calcitonin and derivatives thereof, hormone preparations such as estradiol, SERMs (selective estrogen receptor modulators), ipriflavone, vitamin K_2 (menatetrenone),** calcium preparations, PTH (parathyroid hormone) preparations, nonsteroidal anti-inflammatory agents, soluble TNF receptor preparations, anti-TNF-α antibodies or functional fragments of the antibodies, anti-PTHrP (parathyroid hormone-related protein) antibodies or functional fragments of the antibodies, IL-1 receptor antagonists, anti-IL-6 receptor antibodies or functional fragments of the antibodies, anti-RANKL antibodies or functional fragments of the antibodies and OCIF (osteoclastogenesis inhibitory factor)."

or a drug increasing bone mineral density.	Claim 29: "A pharmaceutical composition for treating and/or preventing abnormal bone metabolism characterized by comprising at least one of the antibodies or functional fragments of the antibodies according to claims 1 to 26 and **at least one member selected from the group consisting of** bisphosphonates, **active vitamin D_3,** calcitonin and derivatives thereof, hormone preparations such as estradiol, SERMs (selective estrogen receptor modulators), ipriflavone, vitamin K_2 (menatetrenone), **calcium preparations, PTH (parathyroid hormone) preparations,** nonsteroidal anti-inflammatory agents, soluble TNF receptor preparations, anti-TNF-α antibodies or functional fragments of the antibodies, anti-PTHrP (parathyroid hormone-related protein) antibodies or functional fragments of the antibodies, IL-1 receptor antagonists, anti-IL-6 receptor antibodies or functional fragments of the antibodies, anti-RANKL antibodies or functional fragments of the antibodies and OCIF (osteoclastogenesis inhibitory factor)."
Claim 21	**'072 English Translation**
The method of claim 15,	
wherein the subject in need thereof, suffers from a bone remodelling disorder.	p. 57, ll. 2-6 : "**Such an antibody which neutralizes the biological activity of Siglec-15 inhibits the biological activity of Siglec-15 in vivo, i.e., the differentiation and/or maturation of osteoclasts, and therefore can be used as a therapeutic and/or preventive agent for abnormal bone metabolism caused by abnormal differentiation and/or maturation of osteoclasts as a medicine.**"
Claim 22	**'072 English Translation**
The method of claim 21,	
wherein the bone remodeling disorder is associated with a decrease in bone mass.	p. 57, ll. 2-7: "Such an antibody which neutralizes the biological activity of Siglec-15 inhibits the biological activity of Siglec-15 in vivo, i.e., the differentiation and/or maturation of osteoclasts, and therefore can be used as a therapeutic and/or preventive agent for abnormal bone metabolism caused by abnormal differentiation and/or maturation of osteoclasts as a

	medicine. **The abnormal bone metabolism may be any disorder characterized by net bone loss (osteopenia or osteolysis).**"
Claim 23	**'072 English Translation**
The method of claim 21,	
wherein the bone remodeling disorder is selected from the group consisting of osteoporosis, osteopenia, osteomalacia, hyperparathyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Turner syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome, Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets, fibrogenesis imperfecta ossium, osteosclerotic disorders, pycnodysostosis, and damage caused by macrophage-mediated inflammatory processes.	p. 57, ll. 9-18: "Examples of the abnormal bone metabolism which can be treated and/or prevented by the anti-Siglec-15 antibody include **osteoporosis** (postmenopausal osteoporosis, senile osteoporosis, secondary osteoporosis due to the use of a therapeutic agent such as a steroid or an immunosuppressant, or osteoporosis accompanying rheumatoid arthritis), bone destruction accompanying rheumatoid **arthritis**, cancerous **hypercalcemia**, bone destruction accompanying **multiple myeloma** or cancer metastasis to bone, giant cell tumor, tooth loss due to **periodontitis**, **osteolysis** around a prosthetic joint, bone destruction in chronic osteomyelitis, **Paget's disease of bone**, renal osteodystrophy and **osteogenesis imperfecta**, however, the abnormal bone metabolism **is not limited thereto as long as it is a disease accompanied by net bone loss caused by osteoclasts**."

Not only is there a written disclosure of every element of claims 1-6, 8-11, and 15-23 in the '181 Patent as shown above, but one of ordinary skill in the art would

have been enabled to practice each of those claims based upon the disclosure in the '072 Publication. (Ex. 1004, ¶¶ 30, 32; Ex. 1003, ¶¶ 22, 23). More specifically, '072 Publication describes several Siglec-15 polyclonal and monoclonal antibodies and methods for making them. (Ex. 1023, Example 10-11, 14-15, 23-24, 33-34, Figs. 26, 33; Ex. 1003, ¶¶ 19, 22, 23). The results of testing these Siglec-15 polyclonal and monoclonal antibodies on osteoclast differentiation are also described. (Ex. 1023, Examples 17, 19-22, Figs. 15-19, 25-26, 32; Ex. 1003, ¶¶ 20, 22, 23). The '072 Publication further describes the effect of an anti-mouse Siglec-15 monoclonal antibody on bone resorption activity. (Ex. 1023, Fig. 36; Ex. 1003, ¶¶ 21-23). Accordingly, the '072 Publication supports the position that the Siglec-15 antibodies disclosed therein have the activity of inhibiting osteoclast differentiation and/or bone resorption. (Ex. 1003, ¶¶ 20-23).

(d) The '072 Publication Was Never Discussed Nor Raised In Any Rejection by the Examiner

Although the '072 Publication was cited in an IDS (Ex. 1027) during prosecution of the '181 Patent, the '072 Publication was never substantively discussed, nor raised in any rejection, by the Examiner. And even if the Examiner had considered the '072 Publication during prosecution (which the Examiner did not), the '072 Publication so plainly anticipates the claims that this is not a case where it would be appropriate for the Board to deny the petition under § 325(d).

See Amneal Pharms. v. Supernus Pharms., IPR2013-00368 (PTAB Dec. 17, 2013); *Synopsys, Inc. v. Mentor Graphics Corp.*, IPR2012-00041 (PTAB Feb. 22, 2013).

STATEMENT OF MATERIAL FACTS

1. The Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), and the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018) do not disclose a single example of an antibody that specifically binds mouse or human Siglec-15 that was actually made.
2. The Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), and the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018) do not disclose any antibody that specifically binds mouse or human Siglec-15 and has the function of impairing osteoclast differentiation and/or inhibiting bone resorption.
3. The Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), and the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018) do not disclose administering an antibody that specifically binds mouse or human Siglec-15 specifically, for impairing osteoclast differentiation.
4. The Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), and the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018) do not disclose administering an antibody that

specifically binds mouse or human Siglec-15 specifically, for inhibiting bone resorption.

5. The only inhibitor demonstrated to impair osteoclast differentiation in the Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), and the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018) is not an antibody.
6. The only inhibitor demonstrated to impair osteoclast differentiation in the Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), and the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018) is not a Siglec-15 antibody.
7. Structural features specific to an antibody that binds human or mouse Siglec-15 and inhibits osteoclast differentiation is not described in the Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), or the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018).
8. Specific structural features of an antibody that binds human or mouse Siglec-15 and impairs osteoclast differentiation is not described in the Parent '054 Application (Ex. 1009), the Alethia PCT (Ex. 1010), or the provisional applications to which these applications claim priority (Ex. 1017 and Ex. 1018).

9. At least Examples 10 and 11 in U.S. Publication No. 2010/0209428 (Ex. 1025) relate to an anti-mouse Siglec-15 polyclonal antibody, and at least Examples 24 and 25 in U.S. Publication No. 2010/0209428 (Ex. 1025) relate to an anti-mouse Siglec-15 monoclonal antibody.

10. At least one Example in U.S. Publication No. 2010/0209428 (Ex. 1025) relates to impairing osteoclast differentiation with an antibody that binds mouse Siglec-15, and at least one Example in U.S. Publication No. 2010/0209428 (Ex. 1025) relates to inhibition of bone formation with an antibody that binds mouse Siglec-15.

CONCLUSION

For the foregoing reasons, the Petitioner respectfully requests that Trial be instituted and the claims 1-6, 8-11 and 15-23 be canceled.

Respectfully submitted,

Dated: November 25, 2014

By: Kristel Schorr Reg 55600
Stephen B. Maebius
Registration No. 35,264

Kristel Schorr
Registration No. 55,600

Foley & Lardner LLP
Counsel for Petitioner

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing petition for inter partes review and all Exhibits and other documents filed together with the petition were served on November 25, 2014, by delivering a copy to FEDERAL EXPRESS directed to the attorneys of record for the patent at the following address:

CHOATE HALL & STEWART

TWO INTERNATIONAL PLACE

BOSTON, MA 02110

By: _____

Stephen B. Maebius
Registration No. 35,264

Kristel Schorr
Registration No. 55,600

Foley & Lardner LLP
Counsel for Petitioner

Trials@uspto.gov
Tel: 571-272-7822

Paper 14
Entered: June 15, 2015

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DAIICHI SANKYO COMPANY, LIMITED,
Petitioner

v.

ALETHIA BIOTHERAPEUTICS, INC.,
Patent Owner

Case IPR2015-00291
Patent 8,168,181

Before MICHAEL P. TIERNEY, ERICA A. FRANKLIN, and SHERIDAN K. SNEDDEN, *Administrative Patent Judges*.

SNEDDEN, *Administrative Patent Judge*.

DECISION
Institution of *Inter Partes* Review
37 C.F.R. § 42.108

I. INTRODUCTION

Daiichi Sankyo Company, Limited ("Petitioner") filed a Petition (Paper 2; "Pet.") to institute an *inter partes* review of claims 1–6, 8–11, and 15–23 of US 8,168,181 B2 (Ex. 1001; "the '181 patent"). Alethia Biotherapeutics, Inc. ("Patent Owner") filed a Patent Owner Preliminary Response. Paper 10 ("Prelim. Resp.").

We have jurisdiction under 35 U.S.C. § 314. The standard for instituting an *inter partes* review is set forth in 35 U.S.C. § 314(a), which states that an *inter partes* review may not be instituted unless "the information presented in the [Petition, taking into account any Preliminary Response,] shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." Upon consideration of the above-mentioned Petition and Preliminary Response we conclude that Petitioner has established that there is a reasonable likelihood that it will prevail with respect to at least one of the challenged claims. We authorize institution of an *inter partes* review as to claims 1–6, 8–11, and 15–23.

A. The '181 Patent (Ex. 1001)

The '181 patent discloses methods of modulating osteoclast differentiation, which may be useful in the treatment of bone loss or bone resorption in patients suffering or susceptible of suffering from a certain conditions such as osteoporosis. Ex. 1001, 7:4–8, 7:41–62.

Independent claims 1 and 15 of the '181 patent provide as follows:

> 1. A method of impairing osteoclast differentiation in a mammal in need thereof, the method comprising administering an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108) to said mammal.
>
> 15. A method for inhibiting bone resorption comprising administering to a subject in need thereof, an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108).

Challenged claims 2–6 and 8–11 depend from claim 1, either directly or indirectly. Challenged claims 16–23 depend from claim 15, either directly or indirectly.

B. Asserted Ground

Petitioner contends that the priority documents of the '181 patent fail to provide adequate written description support and enablement for the subject matter of the challenged claims, and as such, the '181 patent is not entitled to a priority date earlier than April 16, 2009. Pet. 12–33. Petitioner contends that Hiruma[1] (Ex. 1002), thus, qualifies as prior art under 35 U.S.C. § 102(a) and anticipates the subject matter of the claims. *Id.* at 34–58.

[1] Yoshiharu Hiruma et al., WO 2009/048072, published on April 16, 2009. Ex. 1002. An English translation of Ex. 1002 is provided as Ex. 1023.

II. ANALYSIS

A. Claim Interpretation

We interpret claims using the "broadest reasonable construction in light of the specification of the patent in which [they] appear[]." 37 C.F.R. § 42.100(b); *see also* Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,766 (Aug. 14, 2012). Under the broadest reasonable construction standard, claim terms are given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art at the time of the invention. *In re Translogic Tech., Inc*., 504 F.3d 1249, 1257 (Fed. Cir. 2007). "Absent claim language carrying a narrow meaning, the PTO should only limit the claim based on the specification . . . when [it] expressly disclaim[s] the broader definition." *In re Bigio*, 381 F.3d 1320, 1325 (Fed Cir. 2004). "Although an inventor is indeed free to define the specific terms used to describe his or her invention, this must be done with reasonable clarity, deliberateness, and precision." *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

The Petition does not require explicit construction of any claim term at this time. The parties do not dispute on this record that the claim terms should be given their plain and ordinary meaning and that no explicit construction is required at this stage.

B. The '181 Patent Priority Claim

To be entitled to the benefit of a parent application, one requirement is that the invention presently claimed must have been disclosed in the parent application in the manner provided by 35 U.S.C. § 112, first paragraph. *See* 35 U.S.C. § 120; *In re Lukach*, 442 F.2d 967, 968–69 (CCPA 1971). An

ipsis verbis disclosure, however, is not necessary to satisfy the written description requirement. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991). The disclosure need only reasonably convey to persons skilled in the art that the inventor had possession of the subject matter in question, even if every nuance of the claims is not explicitly described in the specification. *Id.*; *see Ariad Pharm., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010).

The test for written description is an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art. Using this test, the invention must be described in a manner sufficient to demonstrate that the inventor actually invented the claimed invention. *Ariad Pharm. Inc. v. Eli Lilly & Co.*, 598 F.3d 1336 (Fed. Cir. 2010). "One shows that one is 'in possession' of the invention by describing the invention, with all its claimed limitations, not that which makes it obvious." *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1571 (Fed. Cir. 1997). Written description is a question of fact judged as of the relevant filing date. *Falko-Gunter Falkner v. Inglis*, 448 F.3d 1357, 1363 (Fed. Cir. 2006).

The '181 patent issued from U.S. Application No. 12/580,943 ("the '943 application") filed on October 16, 2009, which was filed as a continuation-in-part of U.S. Application No. 12/279,054, filed January 13, 2009, now U.S. Patent No. 7,989,160 (the "Parent Application"), which is a national stage application of PCT/CA2007/000210 filed on February 13, 2007.

Petitioner argues that the challenged claims of the '181 patent are entitled to a priority date "no earlier than April 16, 2009," the publication

date of Hiruma, because the challenged claims lack adequate written description support in the Parent Application. Pet. 12–24. Specifically, Petitioner contends that the Parent Application fails to establish possession of the claimed subject matter because:

1) there is no example of a single therapeutic Siglec-15 antibody given in the specification of the Parent Application, yet the scope of the claims extends to any Siglec-15 antibody, including inhibitory antibodies. *Id.* at 14 (citing Ex. 1003[2] ¶¶ 9, 16; Ex. 1004 ¶ 23).
2) while the Parent Application discloses the protein sequence for Siglec-15, there is no disclosure of "any structural information regarding an antibody that binds this sequence and has the requisite activity set forth in the '181 patent claims." *Id.* at 15 (citing Ex. 1004 ¶¶ 16, 17, 22, 23, 25).
3) as of the filing date of the '943 application, Siglec-15 was not known as an extracellular protein and was not sufficiently characterized such that an antibody targeting an extracellular domain and having the necessary therapeutic activity could be predictably made. *Id.* at 16–19 (citing Ex. 1003 ¶ 14; Ex. 1004 ¶ 22).
4) the Parent Application provides a general disclosure regarding inhibitory compounds, but lacks any specific structural guidance necessary to show possession of antibodies that can specifically inhibit bone resorption or impair osteoclast differentiation. *Id.* at

[2] Declaration of Dr. Paul R. Crocker.

19–21 (citing Ex. 1003 ¶¶ 7, 17, 18; Ex. 1004 ¶¶ 7, 8, 12, 13, 16, 21, 23).

5) the Parent Application fails to disclose an antibody that specifically binds to human Siglec-15 or murine Siglec-15. *Id.* at 23–24.

Petitioner further contends that the Parent Application fails to enable the claimed subject matter because it "does not contain any teachings regarding how to make, without undue experimentation, an antibody that specifically binds Siglec-15 and impairs osteoclast differentiation or inhibits bone resorption, as required by the claimed methods." *Id.* at 25 (citing Ex. 1004 ¶¶ 17, 28). Petitioner also contends that the Parent Application lacks any guidance for a method of treatment using anti-Siglec-15 antibody. *Id.* at 31–33.

In response, Patent Owner requests that we use our discretion under 35 U.S.C. § 325(d) to deny institution of an *inter partes* review because the issues raised in the Petition have been previously presented to the Patent Office. First, Patent Owner contends that the Office determined that U.S. Application No. 13/152,205 ("the '205 application"), a divisional of the Parent Application, fully satisfies the written description requirement. Prelim. Resp. 18–26, 34–35. The '205 application was filed as a divisional application of the Parent Application and issued with claims directed to antibodies or antigen binding fragments that bind to Siglec-15 and inhibit osteoclast differentiation or bone resorption activity of osteoclasts. Ex. 2020. We note, however, that the scope of the claims in the '205 application differs significantly from the scope of the challenged claims. As such, we decline to use our discretion under 35 U.S.C. § 325(d) to deny

institution of an *inter partes* review based on issues considered in the '205 application.

Second, Patent Owner contends that the same § 112 written description and enablement arguments have been previously presented to the Office multiple times during the prosecution of patent applications represented as owned by Petitioner that also disclose Siglec-15 antibodies. Prelim. Resp. 13–16, 35–36. We are not persuaded that the Office's consideration of § 112 written description and enablement issues in an unrelated application (i.e., having a different disclosure) is relevant to the issues in this case.

After careful review of both party's arguments, Petitioner has presented sufficient evidence, on the present record, to persuade us that the challenged claims of the '181 patent are entitled to a priority date no earlier than April 16, 2009, on the basis of lack of adequate written description support and/or enablement of the claim subject matter in the Parent Application.

C. Asserted Grounds of Unpatentability

1. Anticipation of Claims 1–6, 8–11, and 15–23 by Hiruma

The Court of Appeals for the Federal Circuit summarized the analytical framework for determining whether prior art anticipates a claim as follows:

> If the claimed invention was "described in a printed publication" either before the date of invention, 35 U.S.C. § 102(a), or more than one year before the U.S. patent application was filed, 35 U.S.C. § 102(b), then that prior art anticipates the patent. Although § 102 refers to "the invention" generally, the anticipation inquiry proceeds on a claim-by-claim

> basis. *See Hakim v. Cannon Avent Group, PLC*, 479 F.3d 1313, 1319 (Fed.Cir.2007). To anticipate a claim, a single prior art reference must expressly or inherently disclose each claim limitation. *Celeritas Techs., Ltd. v. Rockwell Int'l Corp.*, 150 F.3d 1354, 1361 (Fed.Cir.1998). But disclosure of each element is not quite enough—this court has long held that "[a]nticipation requires the presence in a single prior art disclosure of all elements of a claimed invention *arranged as in the claim*." *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1548 (Fed.Cir.1983) (citing *Soundscriber Corp. v. United States*, 175 Ct.Cl. 644, 360 F.2d 954, 960 (1966) (emphasis added)).

Finisar Corp. v. DirecTV Grp., Inc., 523 F.3d 1323, 1334–35 (Fed. Cir. 2008). We must analyze prior art references as a skilled artisan would. *See Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 1576 (Fed. Cir. 1991) (to anticipate, "[t]here must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention").

Petitioner contends that claims 1–6, 8–11 and 15–23 of the '181 patent are anticipated by Hiruma. Pet. 34–56. Hiruma discloses the amino acid sequence of human Siglec-15 (SEQ ID NO: 2) and mouse Siglec-15 (SEQ ID NO: 4). Ex. 1023, 20:2–14. Hiruma discloses antibodies that specifically recognize human or mouse Siglec-15 and inhibit osteoclast formation and/or osteoclastic bone resorption. *Id.* at 5:1–20, 56:24–58:4, claim 33; Ex. 1003 ¶ 19. Examples 17, 19–26, and 35 of Hiruma disclose the results of experiments showing the inhibitory effect of Siglec-15 antibodies on osteoclast differentiation. Ex. 1023, 103:19–105:13, 106:17–119:4, 138:3–139:15; Ex. 1003 ¶¶ 19–20. Example 37 of Hiruma discloses the results of an experiment showing the use of a Siglec-15

antibody for inhibiting bone resorption. Ex.1023, 141:10–144:22. Hiruma further discloses administering a Siglec-15 antibody for the purposes of inhibiting or neutralizing the biological activity of Siglec-15 (i.e., the differentiation and/or maturation of osteoclasts). *Id.* at 56:24–59:7, 11:3–5, 5:1–7:1, 17:5–8, Fig. 36, claim 33; Ex. 1003 ¶ 23; Ex. 1004 ¶¶ 31, 33–34.

In support of its assertion that Hiruma teaches each element of claims 1–6, 8–11, and 15–23, Petitioner sets forth the foregoing teachings of Hiruma and provides a detailed claim chart explaining how each claim limitation is disclosed. Pet. 36–40. Petitioner argues additionally that Hiruma was never substantively discussed, nor raised in any rejection, by the Examiner during the prosecution of the '181 patent. *Id.* at 57.

Patent Owner does not dispute at this time that Hiruma discloses the limitations recited in the challenged claims.

Upon review of Petitioner's analysis and supporting evidence, we determine that there is a reasonable likelihood that Petitioner would prevail in demonstrating the unpatentability of claims 1–6, 8–11, and 15–23 as anticipated by Hiruma.

III. CONCLUSION

For the foregoing reasons, we determine that the information presented in the Petition demonstrates a reasonable likelihood that Petitioner would prevail in challenging claims 1–6, 8–11, and 15–23 are unpatentable under 35 U.S.C. § 102(a) over Hiruma.

IV. ORDER

For the reasons given, it is

ORDERED that an *inter partes* review is hereby instituted with regard

to the following asserted ground:

Claims 1–6, 8–11, and 15–23 of the '181 patent under 35 U.S.C. § 102(a) as anticipated by Hiruma;

FURTHER ORDERED that pursuant to 35 U.S.C. § 314(a), *inter partes* review of the '181 patent is hereby instituted commencing on the entry date of this Order, and pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4, notice is hereby given of the institution of a trial.

FURTHER ORDERED that the trial is limited to the ground listed in the Order. No other grounds are authorized.

PETITIONER:

Stephen B. Maebius
Kristel Schorr
Jeffrey N. Costakos
FOLEY & LARDNER LLP
smaebius@foley.com
kschorr-IPR@foley.com
jcostakos@foley.com

PATENT OWNER:

Fangli Chen
Stephanie L. Schonewald
Robert N. Sahr
CHOATE HALL & STEWART LLP
fchen@choate.com
sschonewald@choate.com
rsahr@choate.com

Janique Forget
Alethia Biotherapeutics Inc.
janique.forget@alethiabio.com

PROTECTIVE ORDER MATERIAL

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

DAIICHI SANKYO COMPANY, LIMITED

Petitioner

v.

ALETHIA BIOTHERAPEUTICS INC.

Patent Owner

Case IPR2015-00291

Patent 8,168,181

CORRECTED PATENT OWNER'S RESPONSE

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EXHIBIT LIST

EXHIBIT NUMBER	DESCRIPTION
2001	Alethia pipeline downloaded from http://alethiabio.com/research-and-development/products/ab-25e9-anti-siglec-15/ on March 16, 2015
2002	Daiichi pipeline downloaded from http://www.daiichisankyo.com/rd/pipeline/pdf/Pipeline_EN.pdf on March 16, 2015
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2100	Declaration of Dr. Mario Filion
2101	Declaration of Dr. Gilles Tremblay
2102	Declaration of Dr. Janique Forget
2103	Declaration of Mr. Yves Cornellier Concerning Alethia Business Records
2104	Declaration of Mr. Jean-Nicolas Delage Concerning Fasken Martineau DuMoulin Business Records
2105	Patent Owner's Diligence Chart
2106	Email from A. Fortin to G. Tremblay, 07/17/2009, 19:00:47
2107	Email from M. Sasseville to G. Tremblay, 07/27/2009, 18:31:08
2108	Email from G. Tremblay to J. Forget, 08/18/2009, 07:10 PM
2109	Email from J. Forget to G. Tremblay, 09/17/2009, 12:06 PM
2110	Email chain between G. Tremblay, J. Forget and M. Sasseville, ending with email from G. Tremblay to M. Sasseville, 09/18/2009, 10:25 AM
2111	Email from J. Forget to G. Tremblay, 09/18/2009, 03:30 PM
2112	Email from G. Tremblay to J. Forget, 09/18/2009, 03:46 PM
2113	Email from M. Sasseville to J. Forget, 09/18/2009, 03:57 PM
2114	Excel file attached to email from M. Sasseville to J. Forget, 09/18/2009, 03:57 PM
2115	Email chain between G. Tremblay and J. Forget, ending with email from G. Tremblay to J. Forget, 09/21/2009, 11:31 AM
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2119	Email chain between G. Tremblay, J. Forget, C. Daoust, ending with email from J. Forget to C. Daoust, 09/25/2009, 01:00 PM
2120	Email chain between G. Tremblay and J. Forget, ending with email from G. Tremblay to J. Forget, 09/25/2009, 02:01 PM
2121	Email chain between G. Tremblay and J. Forget, ending with email from G. Tremblay to J. Forget, 10/02/2009, 08:59 AM
2122	Email chain between G. Tremblay and J. Forget, ending with email from J. Forget to G. Tremblay, 10/02/2009, 09:03 AM
2123	Email from J. Forget to G. Tremblay, 10/02/2009, 01:57 PM
2124	Email from G. Tremblay to J. Forget, 10/05/2009, 08:44 AM
2125	Email chain between G. Tremblay and J. Forget, ending with email from J. Forget to G. Tremblay, 10/05/2009, 08:48 AM
2126	Email chain between G. Tremblay and J. Forget, ending with email from G. Tremblay to J. Forget, 10/05/2009, 08:52 AM
2127	Email chain between G. Tremblay and J. Forget, ending with email from G. Tremblay to J. Forget, 10/05/2009, 09:24 AM
2128	Email chain between G. Tremblay and J. Forget, ending with email from J. Forget to G. Tremblay, 10/05/2009, 09:56 AM
2129	Email chain between G. Tremblay and J. Forget, ending with email from G. Tremblay to J. Forget, 10/05/2009, 12:23 PM
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2131	Email chain between G. Tremblay and J. Forget, ending with email from J. Forget to C. Daoust, 10/09/2009, 02:10 PM
2132	Time entries for J. Forget from Fasken Martineau, Matter #: 280317.00008, Invoice #: 457300, Re: US (CIP)-Sooknanan – Polynucleotides and Polypeptide Sequences Involved in the Process of Bone Remodeling, for the period of September 3, 2009 to October 5, 2009.
2133	A copy of a draft of Siglec-15 antibody patent figures sent to J. Forget from G. Tremblay on or about September 3, 2009, and that

	was time stamped as last modified on September 3, 2009.
2134	A copy of a draft claim set drafted by J. Forget and that was time stamped as last modified on September 14, 2009.
2135	A copy of a draft claim set specific to Siglec-15 drafted by J. Forget and that was time stamped as last modified on September 18, 2009.
2136	A copy of a Clustal analysis sent to J. Forget by G. Tremblay on or about September 18, 2009 and that was time stamped as last modified on September 18, 2009.
2137	A copy of a draft of the continuation-in-part U.S. Patent Application No. 12/279,054 ("CIP") as modified from the Alethia PCT Application that was time stamped as last modified on September 22, 2009.
2138	A draft of figures for inclusion in the draft CIP application drafted by J. Forget and that was time stamped as last modified on September 24, 2009.
2139	A draft of the CIP application drafted by J. Forget and that was time stamped as last modified on September 24, 2009.
2140	A draft of the CIP application drafted by J. Forget and that was time stamped as last modified on September 28, 2009.
2141	A draft of figures for inclusion in the draft CIP application drafted by J. Forget and that was time stamped as last modified on September 28, 2009.
2142	A draft of figures for inclusion in the draft provisional application drafted by J. Forget and that was stamped as last modified on September 30, 2009 and attached to Exhibit 2123.
2143	A draft of figures for inclusion in the CIP application drafted by J. Forget and that was stamped as last modified on October 5, 2009.
2144	A copy of a draft of the CIP application drafted by J. Forget and that was time stamped as last modified on October 5, 2009.
2145	A copy of the October 6, 2009 transmittal to the USPTO of the provisional application.
2146	USPTO filing receipt for U.S. Provisional Application Ser. No. 61/248,960, reflecting a filing date of October 6, 2009.

2147	A copy of a draft of the CIP application drafted by J. Forget and that was time stamped as last modified on October 9, 2009.
2148	A copy of a draft of the CIP application time drafted by J. Forget and that was time stamped as last modified on October 13, 2009.
2149	A copy of a draft of the CIP application drafted by J. Forget and reviewed by the inventor and that was time stamped as last modified on October 13, 2009.
2150	A letter from Dr. Forget to Dr. Fangli Chen, dated October 16, 2009, instructing Dr. Chen to file a new CIP 12/279,054 for the '181 patent and attaching the final text and figures of U.S. Patent Application No. 12/580,943.
2151	USPTO filing receipt for U.S. Application No. 12/580,943, reflecting a filing date of October 16, 2009.
2152	Alethia Laboratory Notebook 110, for the time period of November 26, 2007 to January 4, 2010.
2153	Pages 52 through 64 of Alethia Laboratory Notebook 0117, for the time period of September 30, 2009, to November 24, 2009.
2154	McGill University Genome Center (Centre d'Innovation Génome Québec et Université McGill, hereinafter the "Genome Center") Sequence Submission No. 85533 of SEQ090602, accepted on June 2, 2009 and sequenced on June 8, 2009.
2155	Genome Center Sequence Submission No. 85378 of SEQ090528, accepted on May 28, 2009 and sequenced on June 3, 2009.
2156	Genome Center Sequence Submission No. 83656 of SEQ090417, accepted on April 17, 2009 and sequenced on April 23, 2009.
2157	Genome Center Sequence Submission No. 83346 of SEQ090409, accepted on April 9, 2009 and sequenced on April 16, 2009.
2158	Genome Center Sequence Submission No. 83845 of SEQ090423, accepted on April 23, 2009 and sequenced on April 29, 2009.
2159	Email correspondence between Dr. Tremblay, and Aida Kalbakji of Alethia and Dr. Nicholas Bertos and Dongmei Zuo of the McGill University Breast Cancer Functional Genomics Group, dated June 18, 2009 through July 27, 2009, regarding slide scanning service for 19 immunohistochemistry slides.

2160	A copy of an alignment of the different chimeric anti-Siglec-15 antibody HC and LC protein sequences (the "mAb a.a._align file") created by G. Tremblay and that was time stamped on July 15, 2009.
2161	A copy of a preliminary draft of a patent application directed to anti-Siglec-15 antibodies (the "AB-0326 mAbs patent 090715 file"), created by G. Tremblay on July 17, 2009 and that was time stamped as last modified on July 24, 2009.
2162	A document created by G. Tremblay and that was time stamped as last modified on July 20, 2009, listing the Siglec-15 mRNA and protein sequences from mouse and human tissues (the "0326 cDNA sequence file").
2163	Invoices for Alethia's Purchase Order Nos. A-2437 (May 21, 2009), A-2515 (July 17, 2009), and A-2574 (September 23, 2009) from Pantomics.
2164	A document created by G. Tremblay and that was time stamped as last modified on July 21, 2009, reflecting the cDNA sequence of AB-0326 monoclonal antibodies (the "AB-0326 mAB seqs 090721 file").
2165	Email correspondence between M. Sasseville and G. Tremblay attaching a document with CDR Clustal analysis of AB-0326, dated August 28, 2009.
2166	Pages from the laboratory notebook of M. Sasseville reflecting entries dated July 28, 2009 and August 26, 2009.
2167	A Progress Report prepared by Alethia personnel for Biosite Incorporated for the period between February 1, 2009, and July 31, 2009 (the "Biosite Progress Report").
2168	Pages from the laboratory notebook of S. Roy, dated August 27, 2009.
2169	Pages from the laboratory notebook of A. Kalbakji, dated August 31, September 2, and September 11, 2009.
2170	Pages from the laboratory notebook of A. Fortin, dated September 10-11 and September 15, 2009.
2171	International Searching Authority, PCT/CA2007/000210 (WO 2007/093042), May 30, 2007, Written Opinion.

2172	A document created by M. Sasseville and that was time stamped on August 11, 2009 providing a computer algorithm script which he wrote to perform sequence alignments of antibody heavy chains sequences that are presented to the program in FASTA-style text format (the "Bionf 2009 – fasta_H_test file").
2173	A document created by M. Sasseville and that was time stamped on August 11, 2009 providing a computer algorithm script which he wrote to perform sequence alignments of antibody heavy chains sequences that are presented to the program in FASTA-style text format (the "Bionf 2009 – fasta_L_test file").
2174	A document created by M. Sasseville and that was time stamped on August 12, 2009 providing a computer algorithm script which he wrote to perform sequence alignments of antibody heavy chains sequences that are presented to the program in Gap-tab-style text format (the "Bionf 2009 – Gap-tab_H_vmAbs_format").
2175	A document created by M. Sasseville and that was time stamped on August 12, 2009 providing a computer algorithm script which he wrote to perform sequence alignments of antibody heavy chains sequences that are presented to the program in Gap-tab-style text format (the "Bionf 2009 – Gap-tab_L_vmAbs_format").
2176	Time stamped microscopy images of TRAP stained cells from the osteoclast differentiation experiment performed on July 14, 2009 and recorded in Exhibit 2152 (Laboratory Notebook 0110), pp. 75-79
2177	Time stamped microscopy images of plates of TRAP stained cells from the osteoclast differentiation experiment performed on July 9, 2009 and recorded in Exhibit 2152 (Laboratory Notebook 0110), p. 76.
2178	A copy of an Excel file containing the results of ELISA assays comparing the binding affinity of the different anti-Siglec-15 Fabs with the corresponding anti-Siglec-15 chimeric antibodies created by Alethia personnel that was time stamped as last modified on September 21, 2009.
2179	A copy of the Qiagen Purification Handbook published by the manufacturer in August 2003 (the "Qiagen Handbook").
2180	Canadian Patent Application No. CA02750836, June 17, 2014,

	Office Action.
2181	Genome Center Sequence Submission No. 91463 of SEQ091016, accepted on October 16, 2009 and sequenced on October 20, 2009.
2182	Invoice No. 502 from McGill University Breast Cancer Functional Genomics Group for slide scanning services, dated July 24, 2009.
2183	Genome Center Sequence Submission No. 84571 of SEQ090511, accepted on May 11, 2009 and sequenced on May 12, 2009.
2184	U.S. Patent Application No. 12/279,054, January 28, 2010, Restriction Requirement.
2185	European Patent Application No. EP07 710 624.3, July 7, 2009, Communication.

I. INTRODUCTION

Patent Owner Alethia Biotherapeutics Inc. ("Alethia") respectfully submits this Response to the Petition for *Inter Partes* Review ("IPR") filed on behalf of Petitioner Daiichi Sankyo Company, Limited ("Daiichi"). This filing is timely under 35 U.S.C. §§ 311-19 and 37 C.F.R. § 42.120.

Daiichi's Petition is premised on its assertion that claims 1-6, 8-11, and 15-23 of Alethia's U.S. Patent No. 8,168,181 ("the '181 patent") (Ex. 1001) are not adequately described or enabled under 35 U.S.C. § 112 by Alethia's parent application, PCT/CA2007/000210, filed on February 13, 2007 and published as WO 2007/093042 (the "Alethia PCT")[1]. Daiichi therefore alleges that the claims are not entitled to the priority date and thus are anticipated by Daiichi's own intervening filing, WO 2009/048072 ("the '072 Publication").

Daiichi's Petition should be denied. As described in this Response, Alethia's inventors were the first to discover that Siglec-15 is required for

[1] The filing date of the Alethia PCT precedes the alleged intervening prior art proffered by Daiichi. Because a determination of priority of the '181 patent's claims to the Alethia PCT is sufficient to defeat Daiichi's challenge, Alethia's Response is specifically directed to showing entitlement for priority to the Alethia PCT. However, Alethia reserves its right to establish priority to one or more applications filed prior to the Alethia PCT. *See, e.g.*, Exs. 1017-1019.

osteoclast differentiation and bone resorption using *in vitro* assays reliably predictive of *in vivo* activity. At the time of the invention, it was well known that Siglec-15 and Siglec proteins generally are cell surface glycoproteins, and that antibodies against Siglec-15 had already been made and shown to bind surface-expressed Siglec-15 in a cell-based assay. The Alethia PCT discloses Alethia's novel discovery of Siglec-15's new osteoclast-specific function and its desirability as a therapeutic antibody target for regulating bone remodeling processes involved in bone disease. This discovery forms the basis of the invention described in the Alethia PCT.

Daiichi's arguments rest on a series of mischaracterizations about the state of the art concerning bone biology, the Siglec-15 protein, antibody technology, and the groundbreaking teachings of the Alethia PCT. For example, in arguing that it would be "unpredictable" that antibodies to Siglec-15 could be made, Daiichi purposefully ignores prior art evidence (particularly the Nakamura publication in 2004 -- Ex. 2065) showing that Siglec-15 had been shown to be a cell-surface protein and that antibodies binding to it already had been made. Daiichi went so far as to intentionally omit from its Petition this critical prior art reference and withhold it from its own experts, notwithstanding that it contradicted its experts' opinions and was necessary to accurately reflect the state of the art. Likewise, Daiichi ignored the state of the art concerning the ease with which antibodies could

be made to a known antigen using standard, well-established technology that is expressly identified in the Alethia PCT. Daiichi also omitted from its Petition the fact that as of 2007, a number of well-characterized assays had been developed -- including those expressly described in the Alethia PCT -- to reliably test whether antibodies inhibit osteoclast differentiation. Only on the basis of these misstatements and omissions about the state of the art, most of which were later discredited by Daiichi's own experts on cross-examination, could Daiichi purport to argue that making antibodies to Siglec-15 to impair osteoclast differentiation and inhibit bone resorption would be unpredictable in light of the teachings of the Alethia PCT.

Additionally, Daiichi in its own asserted '072 Publication merely followed the teaching of the Alethia PCT by using routine antibody production methods and known tests for evaluating inhibition of osteoclast differentiation to carry out the methods claimed in the '181 patent. In fact, Daiichi omits from its Petition that it was the Alethia inventors who first taught Daiichi about the therapeutic potential of making antibodies to Siglec-15 for bone diseases. In June 2007 one of the Alethia inventors met with Daiichi and presented details of Alethia's ongoing Siglec-15 antibody development program for bone disease. Just four months later, on October 11, 2007, Daiichi filed its Japanese provisional application on the same subject matter, *i.e.*, the use of anti-Siglec-15 antibodies to treat bone diseases by

inhibiting Siglec-15 activity. Thereafter, Daiichi generated polyclonal and monoclonal antibodies and *in vitro* data using standard methods known in the art and as described in the Alethia PCT, and included that data in its own PCT application filed October 8, 2008 and published as the '072 Publication (the only alleged intervening prior art proffered by Daiichi in this IPR). One year later, Alethia filed a continuation-in-part (CIP) in the United States to include its own monoclonal antibodies and *in vitro* data, again generated using standard methods known in the art and described in the Alethia PCT. The CIP issued as the '181 patent. Thus, Daiichi's own '072 Publication, as well as Alethia's '181 patent, merely further showed that the invention described in and enabled by the Alethia PCT works as the inventors conceived. Daiichi did not invent anything new.

"In an *inter partes* review…, the petitioner shall have the burden of proving a proposition of unpatentability by a preponderance of the evidence." 35 U.S.C. § 316(e). In sum, rather than take on this burden directly, Daiichi attempts to distract the Board by pretending, contrary to the state of the art and well-established scientific principles, that the properties of Siglec-15 as an antibody target were not well understood. The actual evidence belies Daiichi's assertions. Alethia's pioneering invention is properly described and enabled by its PCT, and Alethia's discovery carries the potential to benefit millions of patients who suffer from bone diseases. It should be upheld.

II. TECHNOLOGY BACKGROUND AND ALETHIA'S INVENTION

The claims of the '181 patent are directed to methods of impairing osteoclast differentiation or inhibiting bone resorption by using an antibody or antigen binding fragment that specifically binds to human or murine Siglec-15. Therefore, whether the claims are adequately supported and enabled under 35 U.S.C. § 112 as of the filing date of the Alethia PCT (February 13, 2007) should be determined in view of the state of art in the field of bone biology, antibody technology, and the Siglec proteins -- in particular Siglec-15.

A. State of the Art

1. *Bone Biology*. As of 2007, the field of bone biology was well developed. Ex. 2074, ¶ 8. For example, it was known that bone mass in mammals is regulated by the activities of bone forming cells called *osteoblasts* and bone-resorbing/degrading cells called *osteoclasts*. *Id.* *See also* Ex. 1010, pp. 1-2. These cells normally work together in a process called *bone remodeling* whereby osteoclasts remove worn out or damaged bone and osteoblasts lay down new bone to restore the bone surface. Ex. 2074, ¶ 8; Ex. 1010, pp. 1-5. Disruption of this process occurs during aging and from various bone diseases. Ex. 2074, ¶ 8.

Osteoclast differentiation refers to the formation of mature osteoclasts from osteoclast precursor cells. Ex. 2074, ¶ 9. Impairing osteoclast differentiation reduces the formation of mature osteoclasts, resulting in inhibition of "bone

resorption" (breakdown of bone by those osteoclasts). Ex. 2074, ¶¶ 8-9, 37. It was well known by 2007 that impairing osteoclast differentiation or inhibiting bone resorption can have certain therapeutic benefits, particularly in preventing bone destruction caused by such conditions as osteoporosis, Paget's disease, metastatic bone disease, and inflammatory bone diseases including rheumatoid arthritis and periodontal disease. Ex. 2074, ¶¶ 8-9; Ex. 1010, pp. 1-5.

In 2007, those of ordinary skill in the bone field were particularly focused on the development of therapeutic antibodies for treating bone disease, in light of the success of denosumab, a monoclonal antibody that targets Receptor Activator of Nuclear Factor Kappa-B Ligand ("RANKL"), an essential regulator of osteoclastogenesis and bone resorption. Ex. 2074, ¶¶ 9-11, 28. As of 2007, denosumab was already being tested in phase III clinical trials and had been shown to be "a potent, long-acting, well-tolerated anti-resorptive agent with the potential for broad application in the treatment of bone disorders." *Id.* ¶ 9. Accordingly, in 2007, the use of antibodies to regulate the bone remodeling process was both known and promising. *See id.* ¶¶ 9-11, 16-17.

2. *Antibody Technology*. By 2007, there was also a high level of knowledge and skill in the field of antibodies both generally and in particular for therapeutic use. Ex. 2076, ¶¶ 15-23. Antibodies evolved as a natural defense mechanism to protect a mammalian body and are a class of proteins produced by

plasma cells of the immune system to neutralize pathogens such as bacteria and viruses that invade the body. *Id.* ¶ 15. An antibody functions by binding to a target molecule, called an antigen, with a high degree of specificity. *Id.*; Ex. 2079, pp. 3, 7. The complementarity of antibody-antigen relationships -- compared in the art to the fitting of a key in a lock -- is unique. Ex. 2079, pp. 1, 7; Ex. 2076, ¶ 15. By early in the 20th century, Paul Ehrlich had already envisioned the use of antibodies as therapeutics. Ex. 2076, ¶ 15. But the iconic Y-shaped antibody structure was not determined until the 1960's by Edelman, Porter and Hilschman. *Id.* As Dr. Stein, a well-known expert in the antibody field explained, "the origin and developmental nature of antibodies tells us that **antibodies, unlike certain other therapeutic molecules, can be made and used for desired benefits without the knowledge of structure and mechanism**." *Id.* (emphasis added).

It was well-known by the early 20th century, long before any antibody structural information was known, that animals could be immunized against a target antigen and resulting polyclonal antibodies collected from the animal serum. Ex. 2076, ¶¶ 15-16. Monoclonal antibodies (mAbs) have been efficiently made since the 1970's using "hybridoma" technology, which typically involves fusion of B cells from an immunized animal with a myeloma cell to create an immortal monoclonal antibody-producing cell line. *Id.* ¶ 16. Since the 1980's, the development of recombinant techniques further advanced the antibody field,

including the use of various library selection assays such as phage and yeast display for developing monoclonal antibodies. *Id.* *See also* Ex. 2086, pp. 58, 62.

By 2007, the use of functional assays to obtain antibodies with a desired function -- such as to inhibit a particular protein function *in vivo* -- also was conventional. Ex. 2076, ¶¶ 17-19. Various methods for screening for antibody function, in addition to antigen binding, were promulgated in the 1980's. *Id.* ¶ 17; Ex. 2087. It was also conventional by 2007 to select and use *in vitro* functional assays reliably predictive of *in vivo* activity. Ex. 2076, ¶¶ 17-18; Ex. 2088.

The most critical step for making a functional antibody against a target protein was to identify and characterize the target protein itself. Once the target protein and its function were characterized, the development of antibodies could be accomplished routinely, often by outsourcing the work to any of a number of standard contract laboratories. Ex. 2076, ¶ 18. As Dr. Stein stated, "by 2007 (and well before) one could expect to be able to develop an antibody to inhibit a particular function of a target antigen *in vivo* using conventional methods with reasonable certainty." *Id.* Dr. Clark, Daiichi's own antibody expert, agrees. *See, e.g.*, Ex. 2075, at 43:10-13, 145:20-146:3.

By 2007, it also was well understood that the precise mechanism of action of an antibody or antigen, the specific epitope target, and the amino acid sequence would not need to be determined to develop an antibody with desired activity. Ex.

2076, ¶ 19. Indeed, by 2007, many therapeutic antibodies selected by *in vitro* functional assays had been successfully approved by the FDA or were in the process of pre-clinical or clinical development for various disease areas. *Id.* ¶¶ 21-23. Also, antibody-drug conjugates (ADCs) have been in development for therapeutic use since the 1980's. *Id.* ¶ 22. ADCs work particularly well for target proteins that undergo endocytosis (like Siglec-15), and therefore are able to bring the drug conjugate, such as cytotoxin, into the target cell and kill it based on antibody-triggered endocytosis. *Id.* ¶¶ 22, 33; Ex. 2074, ¶ 30. Significantly, "several antibody drugs have been approved without the knowledge of their precise mechanism of action. Rituximab (anti-CD20) is one of them." Ex. 2076, ¶ 23. *See* Ex. 2090; Ex. 2058, at 113:6-114:3.

3. *Therapeutic antibodies for bone disease*. Osteoclastogenesis assays have been used in the bone field since the late 1990's to successfully identify regulators (*e.g.*, inhibitors or stimulators) of osteoclast differentiation and bone resorption, and to correlate and reliably predict *in vivo* activity. Ex. 2074, ¶¶ 10, 11, 28. For example, the particular osteoclastogenesis assay disclosed by the Alethia PCT was commonly accepted in the bone field in 2007 as being reliably predictive of *in vivo* osteoclast differentiation and/or bone resorption inhibitory function. *Id.* ¶¶ 28-29. *See also* Ex. 2057, Example 5. In fact, it was the same assay previously used to develop denosumab, an antibody now marketed by

Amgen that inhibits osteoclast differentiation by binding to RANKL. Ex. 2074, ¶ 10. Other well-known functional assays specific to bone biology, such as the collagen release assay, also were available in 2007. *See id.* ¶¶ 11, 37.

Thus, as described above, a person of ordinary skill in the art in 2007 was well equipped to use only conventional methods to make antibodies to bind to a target antigen and perform a particular function. Ex. 2076, ¶ 20; Ex. 2074, ¶¶ 27-29. And, those in the field of bone biology were particularly aware that an antibody could be used against a target antigen to impair osteoclast differentiation or inhibit bone resorption, and that such an antibody would be useful to treat bone diseases. Ex. 2074, ¶¶ 9-11, 16-17. Against this backdrop of what was known and standard in antibody technology and bone biology, as more fully explained below, Alethia's invention was the discovery of a novel use of an antibody to Siglec-15 to impair osteoclast differentiation and inhibit bone resorption.

4. *Siglec-15.* Siglec-15 is in a protein family known as the sialic acid-binding immunoglobulin-type lectins ("Siglecs"). Ex. 2074, ¶ 18. By 2007 Siglecs were known as single pass type-I membrane proteins with an extracellular region containing a homologous V-set Ig-like domain and a varying number of C2-set Ig-like domains at the N-terminus, a transmembrane domain, and a cytoplasmic tail. *Id.*; Ex. 2058, at 11:3-12:1. Persons of skill in the art knew that the primary

function of Siglecs is to bind glycans containing sialic acid, which are commonly found at cell surfaces and in the extracellular environment. Ex. 2074, ¶ 18.

As early as 2004, Siglec-15 itself had been sequenced and characterized in great detail at the molecular and cellular level, despite its then unknown biological function. See Ex. 2065; Ex. 2074, ¶¶ 19-21; Ex. 2076, ¶¶ 29, 34-42. By 2004 it was known that: (1) Siglec-15 is a cellular adhesion molecule having a robust extracellular region (corresponding to amino acids 1-254), which contains two immunoglobulin domains and a sialic acid binding motif, and has sequence similarity with CD33; (2) antibodies (both polyclonal and monoclonal) against Siglec-15 could be and in fact already had been made, including antibodies that bound the full length Siglec-15 protein; (3) Siglec-15 is expressed on the surface of the cell; and (4) anti-Siglec-15 antibodies can bind to Siglec-15 recombinantly or endogenously expressed on a cell surface. See Ex. 2065; Ex. 2074, ¶¶ 17-21; Ex. 2076, ¶¶ 29, 34-42. Therefore, by 2007, it already was clear to a skilled artisan that Siglec-15 is normally a cell surface protein and readily accessible to antibodies, despite its then unknown function. Ex. 2074, ¶¶ 17-21.

B. Alethia's Invention

Alethia's inventors were the first to discover that Siglec-15 is a key regulator of osteoclast differentiation and bone resorption and to envision the use of

antibodies that specifically bind to Siglec-15 for the treatment of diseases in which normal bone remodeling is disturbed. *See* Ex. 1010, pp. 1-5; Ex. 2074, ¶ 15.

Alethia inventors used a systematic approach to identify Siglec-15 (referred to as "AB0326" in the Alethia PCT) as a protein with specifically upregulated expression in osteoclasts. Ex. 2074, ¶¶ 12-15. Subsequently, Alethia inventors validated its function in osteoclast differentiation by demonstrating that blocking expression of Siglec-15 using a short hairpin RNA (shRNA) knockdown assay (a well-accepted *in vitro* genetic approach in 2007) significantly impaired formation of osteoclasts from precursor cells. *Id.* ¶¶ 14-15, 26, 41; Ex. 1010, pp. 81-84. These experiments proved AB0326 plays an essential role in osteoclast differentiation. The Alethia inventors further confirmed the role of Siglec-15 in osteoclast differentiation by rescuing the mouse Siglec-15 knockdown phenotype using human Siglec-15. Ex. 1010, Example L. As Dr. Boyce explained, "the Alethia inventors convincingly demonstrated the essential role of AB0326 (*i.e.*, Siglec-15) in osteoclastogenesis" and "the Alethia inventors made an important contribution to the field by discovering this new regulator of osteoclast differentiation, AB0326 (*i.e.*, Siglec-15)." Ex. 2074, ¶¶ 14, 15.

The Alethia PCT was filed on February 13, 2007. Ex. 1010. The Alethia PCT disclosed Alethia's groundbreaking discovery of this new function of Siglec-15 in osteoclast differentiation and bone resorption and clearly envisioned using

antibodies that specifically bind Siglec-15 to treat bone remodeling diseases or disorders. *See* Ex. 1010, pp. 1-5, p. 10, ll. 17-23, p. 10, l. 31-p. 11, l. 2, p. 32, ll. 26-31; Ex. 2074, ¶¶ 12-18. For example, the Alethia PCT teaches using various methods known in the art to make antibodies against a target protein. *See, e.g.*, Ex. 1010, p. 33, l. 33-p. 36, l. 6. It also teaches the use of robust and well-recognized functional assays, including osteoclastogenesis assays, to identify anti-Siglec-15 antibodies that inhibit the differentiation of osteoclast precursor cells. *See*, *e.g.*, *id.* at p. 61, l. 28-p. 62, l. 23, Example L; Ex. 2074, ¶ 27.

In sum, in view of the high levels of skill in the field relating to bone biology, antibodies, and Siglec-15 in 2007, a person of ordinary skill in the art would understand the Alethia PCT to describe the use of an antibody or antigen-binding fragment that specifically binds to human or murine Siglec-15 to impair osteoclast differentiation in a mammal and/or to inhibit bone resorption, and would be able to generate such anti-Siglec-15 antibodies to practice the claimed invention without undue experimentation. Ex. 2074, ¶¶ 8-31, 42-43; Ex. 2076, ¶¶ 15-42.

C. The Alethia-Daiichi Meeting on June 19, 2007

On June 19, 2007, Alethia inventor Mario Filion presented details of Alethia's scientific programs, including its lead program AB0326 (*i.e.*, Siglec-15), to Daiichi for the purpose of a potential strategic partnership. Ex. 2100, ¶¶ 2-3. Dr. Filion presented to Dr. Akira Yoshimoto (an executive in Daiichi's R&D

Department) Alethia's convincing data demonstrating the essential role of AB0326 in osteoclast formation/differentiation and bone resorption. *Id.* ¶¶ 2, 4. Dr. Filion also informed Dr. Yoshimoto that AB0326 is an excellent therapeutic antibody target and that Alethia then was working on developing antibodies targeting AB0326 as its lead clinical program. *Id.* ¶ 5; Ex. 2080. Dr. Filion further disclosed that AB0326 is a "cell surface glycoprotein with two immunoglobulin domains." Ex. 2100, ¶ 6. A copy of Dr. Filion's presentation was sent to Dr. Yoshimoto on June 27, 2007. *Id.* ¶ 7; Ex. 2180; Ex. 2181. Two months later, on August 23, 2007, the Alethia PCT published, specifically linking (by sequence) "AB0326" to "Siglec-15." Ex. 2100, ¶ 8; Ex. 1010.

On October 11, 2007, four months after Alethia's presentation to Daiichi, Daiichi filed its Japanese provisional application on the same subject matter, *i.e.*, the use of anti-Siglec-15 antibodies to impair osteoclast differentiation and inhibiting bone resorption. Ex. 1023. Daiichi then generated monoclonal and polyclonal antibodies and *in vitro* data using the same standard methods known in the art and described in the Alethia PCT, and ultimately included that data in its PCT application, which published as the '072 Publication, the only alleged intervening prior art proffered by Daiichi in this proceeding. Ex. 2074, ¶¶ 34-38.

At the same time, Alethia continuously worked on its AB0326 anti-Siglec-15 monoclonal antibody program. On October 16, 2009, Alethia filed a

continuation-in-part (CIP) application to include its own monoclonal antibodies and *in vitro* data, generated using standard methods known in the art and described in the Alethia PCT. Ex. 1008. The CIP issued as the '181 patent. Ex. 1001.

III. CLAIM CONSTRUCTION

Claims 1-6, 8-11 and 15-23 of the '181 patent are at issue in this proceeding. Independent claims 1 and 15 of the '181 patent are:

> 1. A method of impairing osteoclast differentiation in a mammal in need thereof, the method comprising administering an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.: 2[2]) or murine Siglec-15 (SEQ ID NO.: 108) to said mammal.
>
> 15. A method for inhibiting bone resorption comprising administering to a subject in need thereof, an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO. 2) or murine Siglec-15 (SEQ ID NO.: 108).

In this *inter partes* review proceeding, the claims are given their "broadest reasonable interpretation" consistent with the specification. *In re Cuozzo Speed Techs.*, 2015 U.S. App. LEXIS 11714 (Fed. Cir. July 8, 2015). *See* 37 C.F.R. § 42.100(b). "A construction that is unreasonably broad and which does not reasonably reflect the plain language and disclosure will not pass muster."

[2] These sequences correspond to the Alethia PCT's designations of human Siglec-15 protein (SEQ ID NO.: 48) and murine Siglec-15 protein (SEQ ID NO.: 82).

Microsoft Corp. v. Proxyconn, 2015 U.S. App. LEXIS 10081, at *7 (Fed. Cir. June 16, 2015) (quotations omitted).

A. "osteoclast differentiation"/"osteoclast differentiation activity"

Daiichi's Proposed Construction	Alethia's Proposed Construction
Both terms: any activity involved in the process of differentiation of an osteoclast precursor cell into a differentiated osteoclast	**"osteoclast differentiation":** the formation of mature osteoclasts from osteoclast precursor cells. **"osteoclast differentiation activity":** any activity required for the formation of mature osteoclasts from osteoclast precursor cells.

Alethia disputes Daiichi's construction for a number of reasons. First, Daiichi improperly and confusingly uses part of the term to be construed -- "differentiation" -- in its proposed construction. Second, Daiichi's insertion of "any activity involved in the process of differentiation" is overly broad and nonsensical in context. Claim 1 recites a method of "*impairing* osteoclast differentiation." Inserting Daiichi's construction would make the claim cover a method of *impairing* "any activity involved in the process of differentiation of an osteoclast precursor cell into a differentiated osteoclast," *regardless of whether impairing such process actually impairs osteoclast differentiation*. It is evident from the Alethia PCT that the invention is directed to impairment of osteoclast differentiation itself -- that is, reducing or impairing the formation of mature osteoclasts from osteoclast precursor cells -- and not to impairment of any specific

process "involved in" osteoclast differentiation. *See* Ex. 1001, 8:18-23 ("*A reduced osteoclast differentiation* … may thus positively identify an antibody or antigen binding fragment which may be capable of inhibiting differentiation of an osteoclast precursor cell into an osteoclast.") (emphasis added). *See also id.*, 35:56-67; Ex. 1010, p. 43, ll. 21-27.

Alethia's construction is consistent with the specification's focus on the formation of mature osteoclast cells, as opposed to the any number of activities "involved in" osteoclast differentiation that may or may not impact the formation of mature osteoclasts from osteoclast precursor cells. Further, it is consistent with the understanding of those of skill in the art of bone biology and with the claims and specification of the '181 patent. *See* Ex. 2074, ¶¶ 8-9.

As to "osteoclast differentiation *activity*," the broadest reasonable construction is "any activity required for the formation of mature osteoclasts from osteoclast precursor cells." This construction avoids re-using part of the term in its own construction and is consistent with the proper construction of "osteoclast differentiation" and with the claims and specification of the '181 patent.

B. "specifically binds" and "bone resorption"

Alethia accepts Daiichi's constructions of these terms for this proceeding.

IV. THE CLAIMS OF THE '181 PATENT ARE SUPPORTED BY THE WRITTEN DESCRIPTION OF THE EARLIER ALETHIA PCT

Daiichi's challenge to claims 1-6, 8-11 and 15-23 of the '181 patent should

be rejected because the claims are properly supported by the written description of the Alethia PCT. As set forth below, the Alethia PCT clearly describes and establishes Alethia's possession of anti-Siglec-15 antibodies and using those antibodies to impair osteoclast differentiation and inhibit bone resorption.

A. Legal Standard

35 U.S.C. § 112 requires that a patent's specification "shall contain a written description of the invention." "The invention is, for purposes of the written description inquiry, whatever is now claimed." *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1564 (Fed. Cir. 1991). To determine whether claims receive the benefit of an earlier filed application, the test is "whether a person of ordinary skill in the art would *recognize* that the applicant *possessed* what is claimed in the later filed application as of the filing date of the earlier application." *Noelle v. Lederman*, 355 F.3d 1343, 1348 (Fed. Cir. 2004) (emphasis added). The earlier application "does not, however, have to provide *in haec verba* support for the claimed subject matter at issue." *Cordis Corp. v. Medtronic Ave*, *Inc.*, 339 F.3d 1352, 1364 (Fed. Cir. 2003). Instead, "the hallmark of written description is disclosure." *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010).

It is well established that "the level of detail required to satisfy the written description requirement varies depending on the nature and scope of the claims and on the complexity and predictability of the relevant technology." *Id.* at 1351. The

"written description requirement must be applied in the context of *the particular invention* and the *state of the knowledge*" in the relevant art. *Capon v. Eshhar*, 418 F.3d 1349, 1358 (Fed. Cir. 2005) (emphasis added).

Both the USPTO and Federal Circuit have recognized that, in the written description context, inventions concerning antibodies are unique. Because of the long-standing, conventional state of antibody technology, claims to a basic targeting antibody are adequately supported by a written description that does not disclose the *antibody* itself, as long as the *target antigen* is adequately described. This antibody rule has been incorporated into the USPTO's examiner training materials on written description since at least 2001. *See* Ex. 2077 (Example 13); Ex. 2078 (Example 16). Example 13 of the 2008 Manual describes a claim that is directed to "an isolated antibody capable of binding to antigen X." Ex. 2077. The exemplary specification (1) discloses the amino acid sequence of an antigen X that is useful for detection of HIV infections, (2) provides a general discussion of antibodies that might specifically bind to antigen X, and (3) asserts that such antibodies can be used in immunoassays to detect HIV, but (4) does *not* identify or provide a working example of an antibody that binds to antigen X. *Id.* at 45. The Manual instructs examiners that "the level of skill and knowledge in the art of antibodies at the time of filing was such that production of antibodies against a well-characterized antigen was conventional." *Id.* The Manual further states:

> Considering the facts, including the routine art-recognized method of making antigen-specific antibodies, the adequate description of antigen X, the well-defined structural characteristics for the classes, subclasses and isotypes of antibody, the functional characteristics of antibody binding, and the fact that antibody technology was well developed and mature, one of skill in the art would have recognized that the disclosure of the adequately described antigen X put the applicant in possession of antibodies which bind to antigen X.

Id. at 46. *See also* Ex. 2078, Example 16. Thus, the USPTO Manual concludes that a claim to a targeting antibody is supported by a written description of the target antigen, without requiring any disclosure of the specific physical or chemical properties of the claimed antibody, its structure, or any methods of making it. *Id.*

Similarly, the Federal Circuit has established that "[a]s long as an applicant has disclosed *a fully characterized antigen*, either by its structure, formula, chemical name, or physical properties…, *the applicant can then claim an antibody* by its binding affinity to that described antigen." *Noelle*, 355 F.3d at 1349 (emphasis added). *See also Enzo Biochem*, 323 F.3d at 963 (endorsing the USPTO antibody example and "adopt[ing] the PTO's applicable standard for determining compliance with the written description requirement").

The rationale behind the USPTO antibody written description rule is appropriately rooted in the state of the art. The relationship between an antibody and its target antigen is so readily discernable and well understood that disclosure

of a specific antigen necessarily allows a person of ordinary skill in the art to recognize that the applicant also possesses the corresponding, targeting antibody. *See* Ex. 2079, pp. 1, 3, 7, 17; Ex. 2076, ¶¶ 15-20. As Dr. Stein explained, "one could obtain an antibody to specifically bind to any particular target antigen through routine use of those well-developed methods long before 2007." Ex. 2076, ¶ 16. Daiichi's experts agree that, given a protein, there is a "99.9 percent probability of developing some antibody that at least specifically bound to the protein." Ex. 2075, at 43:10-13. *See also* Ex. 2058, at 102:22-103:4.

B. The Alethia PCT Discloses Possession of Anti-Siglec-15 Antibodies

As set forth below, the Alethia PCT clearly establishes possession of the claimed subject matter, *i.e.*, the use of an antibody or antigen binding fragment which specifically binds to human or murine Siglec-15 for impairing osteoclast differentiation or inhibiting bone resorption.

The independent claims of the '181 patent simply require an antibody that specifically binds to human or murine Siglec-15. Thus, the antibody written description rule squarely applies. "As long as an applicant has disclosed *a fully characterized antigen*, either by its structure, formula, chemical name, or physical properties," the applicant can claim a targeting antibody without disclosure of its physical or chemical properties. *Noelle*, 355 F.3d at 1349 (emphasis added); *Enzo Biochem*, 323 F.3d at 963 (citing USPTO Manual (Ex. 2077)).

The Alethia PCT provides extensive characterization of Siglec-15 (AB0326). It discloses the Siglec-15 gene and protein sequences (SEQ ID NOS.: 1 and 48). It includes the available information about Siglec-15 in the public GenBank database. Ex. 2058, at 51:7-19. It lists Siglec-15 in its Table 1 with the NCBI gene symbol "CD33L3." Ex. 2074, ¶ 17; Ex. 1010, Table 1. This well-known "cluster of differentiation" (CD) designation, developed to identify and study cell surface molecules and monoclonal antibodies, suggests to a person of skill in the art either that (1) antibodies against AB0326 are already available (despite its previously unknown function); or (2) AB0326 belongs to a family of proteins initially identified by antibody recognition. Ex. 2074 ¶ 17; Ex. 2076 ¶¶ 27-28. *See also* Ex. 2075, at 156:25-157:3. Therefore, based on this description alone, a person of skill would have recognized immediately that Siglec-15 (AB0326) was likely a cell surface protein and a promising antibody target.

The Alethia PCT also discloses details of the function of Siglec-15 to establish, for the first time, that Siglec-15 plays an essential role in osteoclast differentiation and bone resorption. For example, the Alethia PCT teaches that the Siglec-15 gene (SEQ ID NO.:1) "is markedly upregulated in intermediate and mature osteoclast compared to precursor cells," and thus "this gene may be required for osteoclastogenesis and/or bone remodeling." Ex. 1010, p. 70, ll. 26-29. The Alethia PCT then validates Siglec-15's function in osteoclast

differentiation using a shRNA knockdown assay, showing that knockdown of human Siglec-15 (Example J) and mouse Siglec-15 (Example K) significantly impaired the formation of human and mouse osteoclasts from precursor cells. *Id.*, pp. 82-84 (Examples J and K); Ex. 2074 ¶¶ 14-15, 26. The Alethia PCT then further confirms Siglec-15's role in osteoclast differentiation by rescuing the mouse Siglec-15 knockdown phenotype using human Siglec-15. Ex. 1010, p. 85 (Example L). As Dr. Boyce explained, "[t]his so-called complementation or add back experiment was generally considered a powerful and reliable method for validating a biological function in 2007." Ex. 2074, ¶ 14. Thus, to a person of skill, the Alethia PCT thoroughly characterized Siglec-15 and its novel function and convincingly demonstrated its essential role in osteoclastogenesis. Both Dr. Boyce and Daiichi's expert, Dr. Crocker, agree on this point. *See id.* ¶¶ 14-15, 26; Ex. 2058, at 86:2-17; 87:16-88:10.

The Alethia PCT also clearly discloses using antibodies or antigen binding fragments (such as Fv, Fab, Fab' or $(Fab')_2$) that specifically bind Siglec-15 to impair osteoclast differentiation or inhibit bone resorption.[3] *See, e.g.*, Ex. 1010, p. 34, ll. 14-16. The Alethia PCT first provides, as Daiichi's own expert, Dr. Crocker, acknowledges, "a very elaborate section giving standard procedures for

[3] In this Response, "antibody" or "antibodies" is intended to include "antigen-binding fragments," consistent with Dr. Stein's explanation. *See* Ex. 2076, ¶ 20.

antibodies" for generating anti-Siglec-15 antibodies. Ex. 2058, at 182:7-9. *See also id.* at 95:6-11; Ex. 1010, pp. 33-42. Indeed, the Alethia PCT describes in great detail procedures for generating antibodies, such as hybridoma technology, phage display technology and mammal immunization techniques, all of which were well-known. Ex. 2058, at 95:18-22; Ex. 2075, at 25:2-10, 28:22-29:4; Ex. 2076 ¶ 31; Ex. 2074 ¶ 27. The Alethia PCT also clearly describes using such techniques with well-known osteoclastogenesis assays to generate and identify antibodies that specifically inhibit Siglec-15. *See* Ex. 1010, p. 86, ll. 1-3 (specifically disclosing applying a library to a RAW 264.7 cell line expressing Siglec-15 "to identify molecules (small molecule drugs, peptides, or *antibodies*) capable of *inhibiting AB0326*.") (emphasis added).

In sum, the Alethia PCT disclosed "*a fully characterized antigen"* by both its structure (*e.g.*, sequence) and function in osteoclastogenesis. Applying the antibody written description rule embraced by the USPTO and the Federal Circuit, the Alethia PCT clearly establishes possession of anti-Siglec-15 antibodies, including particularly those that impair osteoclast differentiation and bone resorption, given its extensive disclosures as set forth above.

C. Daiichi's arguments are baseless and factually wrong

Daiichi's purported written description challenge to Alethia's PCT rests on a series of mischaracterizations about the state of the art and the applicable law.

1. *Antibodies can be made without knowledge of structure and mechanism of action*

Daiichi asserts the Alethia PCT is insufficient because it does not describe the structure of an antibody that binds to Siglec-15. Petition at 15. Daiichi simply ignores the well-settled antibody written description rule. *See supra* pp. 18-21. As described above, that rule is rooted in the well-established science concerning antibodies. In particular, the function of antibodies was well understood since long before antibody structure or detailed mechanism of action was appreciated. *See* Ex. 2076, ¶¶ 15-23, 52. Therefore, the antibody rule recognizes that, unlike other compounds such as small molecules, it has been well known that "the origin and developmental nature of antibodies tells us that antibodies…can be made and used for desired benefits without the knowledge of structure and mechanism." *Id.* ¶ 15.

In fact, and not surprisingly given the antibody rule, the amino acid sequence and other structural features of an antibody are unnecessary to characterize an antibody's ability to bind to and inhibit the function of a target antigen like Siglec-15. For example, none of the routine methods for making antibodies (hybridoma technology, phage display, etc.) requires knowledge of antibody structure. As Dr. Stein pointed out, "the primary amino acid sequence will not tell you the antigen or epitope to which the antibody binds. Nor will the hypervariable region sequences, which provide an antibody its specificity, tell you the antigen or epitope to which the antibody binds or provide any information

about whether the antibody has a desired activity." Ex. 2076, ¶ 19. Dr. Boyce also explained, "anti-RANKL antibodies that led to denosumab were obtained without knowing the amino acid sequence of the antibody, the epitope on RANKL to which they bound, or its precise mechanism of action. Indeed, the process of developing an antibody with desired osteoclast inhibitory activity typically starts with a functional test." Ex. 2074, ¶ 11. *See* Ex. 2075, at 124:9-125:3, 127:7-13, 226:2-12; Ex. 2058, at 101:6-103:10, 113:6-114:12, 130:22-131:4.

2. *Anti-Siglec-15 antibodies could be routinely made and already existed at the time of Alethia's filing*

One of Daiichi's primary assertions is that, allegedly, it was not known as of the filing of the Alethia PCT whether an antibody that binds Siglec-15 was even possible, or whether Siglec-15 was expressed on the cell surface and accessible.

Daiichi's argument is demonstrably false. In fact, it was known at the time of Alethia's PCT that: (i) antibodies to Siglec-15 had been created and reported; and (ii) Siglec-15 was expressed on the cell surface and accessible to an antibody. *See* Ex. 2065. Moreover, Daiichi not only was aware when it filed its Petition of the critical art that directly contradicts its assertions, but it intentionally chose not to disclose that art either to the Board or even its own experts. *See* Ex. 2073 (Daiichi application rejections citing Nakamura). *Compare* Petition at 16 ("there is no indication in the Parent '054 Application or confirmation in the literature in 2006, 2007, or 2009 that Siglec-15 is located on the cell surface and accessible to

an antibody"); Ex. 2058, at 161:15-16 ("Q: Have you ever seen this [Nakamura] patent application? A: No."), 161:21-162:1; Ex. 2075, at 162:14-19 ("Q: Okay. Have you ever reviewed this [Nakamura patent] document? A: No, I haven't. Q: Have you discussed this document with counsel? A: No, I haven't.").

On the basis of Daiichi's misrepresentations about this material fact alone, Daiichi's arguments should be ignored in their entirety.[4] Furthermore, Daiichi's intentional failure to inform its own experts about such critical prior art entirely discredits Daiichi's experts' testimony.[5] Their testimony should be given no

[4] *See* 37 C.F.R. § 42.11 ("Parties and individuals involved in the proceeding have a duty of candor and good faith to the Office…."); 37 C.F.R. § 42.12(a)(3) ("The Board may impose a sanction against a party for misconduct, including: Misrepresentation of a fact."); 37 C.F.R. § 42.51(b)(1)(iii) ("Unless previously served, a party must serve relevant information that is inconsistent with a position advanced by the party during the proceeding concurrent with the filing of the documents or things that contains the inconsistency.").

[5] Daiichi's experts also confirmed that a person of ordinary skill in the art would have run a search using the sequence of Siglec-15 (disclosed in the Alethia PCT) as part of routine practice in 2007, and that such a search (which they also admitted they did not perform) would have led to references like Nakamura. *See* Ex. 2058, at 193:17-194:11; Ex. 2075, at 39:5-40:10, 147:25-148:6, 230:2-231:11.

weight at all.

As set forth below, it is clear that as of 2004 -- three years before the Alethia PCT -- Nakamura *et al.* had disclosed that Siglec-15 was expressed on the cell surface and that antibodies could be made -- and indeed, had been made -- to Siglec-15. *See* Ex. 2065; Ex. 2074, ¶¶ 19-21; Ex. 2076, ¶¶ 28-29, 34-42. Nakamura describes as its SEQ ID NO.:2 a polypeptide, HRC12337, with identical sequence to Siglec-15. Ex. 2076, ¶ 28. HRC12337 is described as a novel "cellular adhesion molecule" having an extracellular region (corresponding to its amino acids 1-254) containing immunoglobulin domains and a transmembrane domain. Ex. 2065, ¶ [0003]. It was understood in the art that proteins that mediate "cell adhesion" are expressed on the cell surface. Ex. 2058, at 162:13-18. Nakamura describes numerous conventional methods of generating antibodies to bind to the HRC12337 protein, and indeed discloses the making of such antibodies using routine methods. Ex. 2076, ¶¶ 29, 39. Moreover, Nakamura provides data explicitly confirming expression of HRC12337 on the cell surface. Ex. 2065, Example 9 (using fluorescence activated cell sorting (FACS) analysis to demonstrate cell surface expression); Ex. 2076, ¶ 40; Ex. 2074, ¶ 20; Ex. 2075, at 151:23-152:25, 176:1-4, 192:21-193:4; Ex. 1004, ¶ 22 ("the presence of a protein on the cell membrane is usually confirmed by FACS analysis"). Thus, as of 2004, it was known that Siglec-15 was both accessible on the cell surface to a targeting

antibody, and in fact that such antibodies had been made.

Notably, Daiichi's expert, Dr. Crocker, formulated his opinion that Siglec-15 *appears* to be an *intracellular* protein solely based on an immunostaining result shown in a *post-filing* publication, Angata, T., *et al.*, Glycobiology ("Angata"). Angata reports the apparent co-localization of Siglec-15 with CD68 (*see, e.g.*, Ex. 1022, p. 840, Fig. 4), a known intracellular protein according to Dr. Crocker. Ex. 1003, ¶ 14. As pointed out by Dr. Boyce, however, the immunostaining reported by Angata was done "using a polyclonal antibody on formalin-fixed, paraffin-embedded samples of human lymph node and spleen." Ex. 2074, ¶ 22. Polyclonal antibodies tend to bind non-specifically to fragments of proteins in tissue sections *other than* the target protein, and thus may lead to inaccurate false positive staining results. *See id.*; Ex. 2058, at 139:12-22. Thus, "monoclonal antibodies are used in order to achieve more specific and accurate immunostaining results. None of Angata's staining was done using monoclonal antibodies." Ex. 2074, ¶ 22; Ex. 1022, p. 840, 844.

Moreover, even if Angata were correct that "the localization of Siglec-15 overlapped with that of CD68," CD68 was known to shuttle between the cell surface and subcellular compartments. Thus, Siglec-15 would do the same. Ex. 2074, ¶ 23. Thus, Angata does not suggest that Siglec-15 is *exclusively* intracellular and inaccessible by antibodies. On the contrary, Angata recognizes

that Siglec-15 may "translocate[] to the cell surface on some cue." *Id.*; Ex. 1022, p. 842. *See also* Ex. 2058, at 34:3-37:22.

Moreover, as grounds for his opinion, Dr. Crocker stated he believed "Angata is the earliest publication characterizing Siglec-15 localization." Ex. 1003, ¶ 14. Similarly, Dr. Crocker emphasized at deposition his reliance on the fact that Angata's data "were the *only* data showing the localization" of Siglec-15. Ex. 2058, at 159:1-4 (emphasis added). His assumptions are simply untrue. As discussed above, Nakamura had demonstrated in 2004 that Siglec-15 was expressed on the cell surface. Daiichi was fully aware of that fact, because Nakamura had been cited against Daiichi's related filings in the U.S. *See* Ex. 2073. Yet Daiichi intentionally chose not to disclose this critical prior art to Dr. Crocker. *See* Ex. 2058, at 161:15-162:1. As Dr. Boyce stated, "[i]f Dr. Crocker had been aware of Nakamura's finding, I believe he would have come to a different conclusion." Ex. 2074, ¶ 24; *see* Ex. 2058, at 193:2-7.

Daiichi also argues that, "without having an understanding of how the target behaves *in vivo*, a sense of kinetics and recycling of the target, or having actually made any antibody to the target, the feasibility of the target for antibody is uncertain." Petition at 17. Again, Daiichi disregards the actual facts. First, as discussed above, anti-Siglec-15 antibodies had already been made at the time of the Alethia PCT. *See supra* pp. 26-30. Second, the Alethia PCT discloses well-

accepted osteoclastogenesis assays that are predictive of inhibitory activity *in vivo*. *See* Ex. 2074, ¶¶ 10-11, 28-29, 33. Further, it was well known by 2007 that a person of skill would *not* need to understand the mechanism of action of either the target protein or antibody to make antibodies or even to get an antibody drug approved by FDA. Ex. 2076, ¶¶ 19, 52; Ex. 2071, p. 21; Ex. 2096; Ex. 2097; Ex. 2058, at 113:6-114:12. *See also supra* pp. 8-9 (discussing the development of antibody therapeutics, such as Rituximab, without knowing precise mechanism of action). Thus, as explained by Dr. Stein, who served as FDA Director of the Division of Monoclonal Antibodies and was responsible for writing the FDA Guidance Document on therapeutic antibodies, the FDA position is that "[a] complete biochemical characterization *may not be possible or necessary* in all cases." Ex. 2076, ¶ 23 (emphasis added).

3. *The Alethia PCT clearly describes the use of anti-Siglec-15 antibodies for impairing osteoclast differentiation and inhibiting bone resorption*

Daiichi argues that the Alethia PCT does not provide adequate descriptive support for impairing osteoclast differentiation or inhibiting bone resorption with (i) "an antibody" (as opposed to other disclosed compounds), (ii) or one that specifically binds to "Siglec-15" (as opposed to other disclosed antigens). Petition at 18. Daiichi's argument fails for several reasons. In fact, if a person of skill in the art had read the Alethia PCT in 2007, she would have immediately recognized

that Alethia's inventors envisioned using anti-Siglec-15 antibodies for impairing osteoclast differentiation or inhibiting bone resorption. *See* Ex. 2076, ¶ 24.

i. Daiichi applies the wrong legal standard.

The law is clear that the "written description requirement must be applied in the context of *the particular invention*." *Capon*, 418 F.3d at 1357 (emphasis added); *Vas-Cath Inc.*, 935 F.2d at 1564 ("[t]he invention is, for purposes of the 'written description' inquiry, whatever is now *claimed*") (emphasis added). The '181 patent claims are directed specifically to Siglec-15. Ex. 1001; Ex. 2075, at 233:23-234:2. Thus, the question for a person of ordinary skill in the art is whether the Alethia PCT shows the inventors were in possession of *the claimed method* of using antibodies to *Siglec-15* to impair osteoclast differentiation or inhibit bone resorption. The written description inquiry does not require persons of ordinary skill to evaluate the inventions of the Alethia PCT in a vacuum and guess what the claim at issue is. *See X2Y Attenuators, LLC v. ITC*, 757 F.3d 1358, 1365 (Fed. Cir. 2014) (failure to construe the claim and evaluate the claim scope "render[s] baseless any determination of written support in an earlier patent").

Siglec-15 is not a species among a broader genus; rather it is one of multiple independent inventions disclosed in the Alethia PCT. The Alethia PCT describes 35 nucleotide and protein sequences differentially expressed in mature osteoclasts. Ex. 1010, Table 1. As Dr. Boyce pointed out, "[b]ased on my review, these 35

sequences do not appear to share any sequence or structural similarity and are independent sequences, which I understand is normally the case for sequences identified using differential expression techniques." Ex. 2074, ¶ 13. Each of the 35 nucleotides and proteins are independent inventions. In fact, the U.S., Europe, and the International Bureau have all treated each sequence as an independent invention. *See* Ex. 2171; Ex. 2184; Ex. 2185. Contrary to Daiichi's suggestion, this is not a case where an applicant discloses a broad genus and attempts to claim a specific species without *expressly* identifying it or directing a person of skill to it. *Compare In re Ruschig*, 379 F.2d 990, 993 (C.C.P.A. 1967) (where a claim is to a single compound, and the specification encompasses "something like half a million possible compounds," a sufficient disclosure is one which sets out "blaze marks which single out particular trees") *and Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571 (Fed. Cir. 1996) (disclosure of a "laundry list" of possible moieties for a compound claim insufficiently described the particular compound subgenus claimed); *with In re Driscoll*, 562 F.2d 1245, 1249 (C.C.P.A. 1977) (finding sufficient written description where the claimed compound was expressly disclosed as one of fourteen possible compounds). Daiichi's reliance on *Purdue* is also unfounded, as that case involved a claim to a specific concentration ratio and the specification provided no indication that the ratio was "an important defining quality of the formulation" or would "motivate one to calculate the ratio." *Purdue*

Pharma L.P. v. Faulding Inc., 230 F.3d 1320, 1327 (Fed. Cir. 2000). The Alethia PCT simply describes multiple inventions in addition to Siglec-15; this does not render its disclosure of Siglec-15 any less meaningful. Ex. 2074, ¶ 15.

ii. Siglec-15 stands out in the Alethia PCT as a particularly promising therapeutic antibody target.

Also, contrary to Daiichi's claims, Siglec-15 stands out in the Alethia PCT as a particularly promising therapeutic antibody target. Siglec-15 (AB0326) was identified as SEQ ID NO.:1, listed on the top of Table 1, and, more importantly, had the most robust and convincing functional validation data. It is one of two targets validated by the shRNA knockdown experiments to demonstrate its osteoclast inhibitory function, and the *only* target further confirmed in the functional complementation assay. *See* Ex. 2074, ¶¶ 14-15; Ex. 2076, ¶¶ 25-27; Ex. 2075, at 233:23-234:25; Ex. 1010, Examples J-L. Daiichi and its expert, Dr. Crocker, allege that "[t]he demonstration that Siglec-15 is required for osteoclastogenesis using RNA interference is *diluted* by remarks extrapolating the use of the assay to other sequences and genes." Ex. 1003, ¶ 9 (emphasis added). Yet as Dr. Boyce explained, "any biologist would understand that a function of a particular protein is a scientific fact that cannot be changed or 'diluted' simply because the method used to discover this function can also be used to assay other proteins. … Applying Dr. Crocker's logic, all new discoveries made by such techniques would be diluted simply because they can be used to test other

sequences or genes." Ex. 2074, ¶ 15.

In addition, Siglec-15 (AB0326) is designated as "CD33L3" in Table 1 and is the only sequence with a "CD" designation. This further distinguishes Siglec-15 because a CD designation suggests either that (1) antibodies against AB0326 have already been made; or (2) AB0326 belongs to a family of proteins initially identified by antibody recognition (*i.e.*, Siglecs). *See supra* pp. 10-11 (describing common features of Siglecs). Daiichi's antibody expert agrees. *See* Ex. 2075, at 155:19-21, 156:25-157:7, 158:13-20. Therefore, one of ordinary skill in the art, upon reviewing the Alethia PCT, would have recognized that Siglec-15 stands out as a particularly promising therapeutic target for antibodies. *See* Ex. 2074 ¶¶ 17-18; Ex. 2076, ¶¶ 26-28.

Finally, Daiichi's argument that Siglec-15 was not highlighted in the Alethia PCT is ironic and disingenuous, because Daiichi itself expressly noted in its own '072 Publication that the Alethia PCT disclosed that "the differentiation of osteoclast is inhibited by decreasing the expression of Siglec-15 by RNA interference (WO 2007/093042)." Ex. 1023, p. 3, l. 24-p. 4, l. 4. Thus, contrary to the position Daiichi now takes in its Petition, Siglec-15 clearly stood out in the Alethia PCT as a particularly promising therapeutic antibody target in 2007 and enabled Daiichi to generate anti-Siglec-15 antibodies to impair osteoclast differentiation or bone resorption using the routine methods in the Alethia PCT.

iii. The Alethia PCT specifically describes the use of functional assays to identify anti-Siglec-15 antibodies that inhibit the differentiation of osteoclast precursor cells.

Finally, Daiichi argues that the Alethia PCT uses the term "inhibitors" but fails to describe an antibody inhibitor. Petition at 19. This argument is unfounded.

A person of skill would have immediately recognized the Alethia PCT was largely focused on antibody therapy, especially as to AB0326 (Siglec-15). The Alethia PCT devoted at least seven (7) entire pages to describing techniques for generating antibodies. *See* Ex. 2076, ¶¶ 30-31; Ex. 1010, pp. 33-40. Daiichi claims these antibody disclosures are "boiler plate." Petition at 22. But as Dr. Stein stated, "to the extent the language appears to be 'boiler plate,' it is merely a reflection of the fact that the technology was so standard and was universally applicable to the development of most antibodies at the time." Ex. 2076, ¶ 31.

The Alethia PCT further describes using a functional osteoclastogenesis assay to identify anti-Siglec-15 antibodies that inhibit the differentiation of osteoclasts. *See, e.g.*, Ex. 1010, p. 61, l. 28-p. 62, l., 23, Example L. Specifically, it teaches to "identify molecules (small molecule drugs, peptides, *or antibodies*) capable of inhibiting *AB0326*." *Id.*, p. 86, ll. 1-3 (emphasis added). As discussed above, osteoclastogenesis assays described in the Alethia PCT were well-recognized functional assays routinely used to identify inhibitors, such as antibodies, of osteoclast differentiation and to correlate and predict *in vivo* activity.

See supra pp. 8-10.

Daiichi asserts that the Alethia PCT does not teach that making anti-Siglec-15 antibodies with the functional qualities of inhibiting osteoclast differentiation and/or bone resorption is even within the realm of possibility. Petition at 21. Here again, Daiichi ignores the facts. As Dr. Boyce explained: "[t]he Alethia PCT specifically describes the use of such functional assays including, in particular osteoclastogenesis assays, to identify those anti-Siglec-15 antibodies that inhibit the differentiation of osteoclast precursor cells." Ex. 2074, ¶ 29. And, because osteoclastogenesis assays were so well-developed in the bone field in 2007, "[b]ased on my experience, once the antibodies were obtained, the test results of the osteoclastogenesis assays could have been obtained routinely in a short period of time." *Id.* As discussed above, anti-Siglec-15 antibodies were indeed available as early as 2004. *See supra* pp. 26-30. Thus, a skilled artisan could make and select anti-Siglec-15 antibodies that inhibit the differentiation of osteoclast precursor cells using routine techniques and osteoclastogenesis assays known in the art and described in the Alethia PCT.

Daiichi also alleges that Alethia's Example L "functions by altering the expression of a target gene, and does not exert its effect at the protein level, as antibody would." Petition at 20. Daiichi relies on its expert, Dr. Crocker's, assertion that such shRNA knockdown data "are not reliably correlative with the

effect of an antibody that inhibits the function of the protein *per se*." Ex. 1003, ¶ 17. His conclusion rests principally on his erroneous assumption (relying solely on Angata) that Siglec-15 was intracellular and there was no guarantee that antibodies against Siglec-15 could be made. *See* Ex. 1003, ¶ 14; Ex. 2058, at 159:1-4. When asked at his deposition if one could find an antibody that would bind to a protein and inhibit its function assuming the protein was accessible on the cell surface, Dr. Crocker stated "there is a reasonable chance that could happen, but there is also possibility that it may be actually very difficult to make antibody in the first place. … Indeed it is now known that you can make antibodies to Siglec-15. But at the time this was written, there was no indication of that." Ex. 2058, at 110:4-12, 111:5-7. Again, he fatally misunderstands the facts, as he was unaware that Nakamura had already made anti-Siglec-15 antibodies as early as 2004 and had shown Siglec-15 is a cell-surface protein accessible to antibodies on the cell surface. *See id.*, at 161:15-162:1. As Dr. Boyce again observed, "if Dr. Crocker had been informed of Nakamura's finding, I believe he would have come to a different conclusion." Ex. 2074, ¶ 26; Ex. 2058, at 193:2-7.

In a further attempt to attack Alethia's disclosure of therapeutic antibodies, Daiichi appears to equate "neutralizing" antibodies disclosed in the Alethia PCT with antibodies that "inhibit[] ligand induced dimerization." Daiichi cites the Stuible reference in an attempt to show that an anti-Siglec-15 antibody induces

dimer formation. Here Daiichi mischaracterizes the teachings of the Alethia PCT, which states only that "[n]eutralizing antibodies, *such as those* that inhibit dimer formation, are especially preferred for therapeutic use." Ex. 1010, p. 37, ll. 27-28 (emphasis added). A person of skill in the art, upon reading this sentence, would readily understand inhibiting dimer formation is only a potential exemplary mechanism for neutralizing antibodies. As Dr. Boyce explains, "[n]o skilled artisan would read this sentence in the Alethia PCT as to mean that neutralizing antibodies must inhibit dimerization." Ex. 2076, ¶ 40. Even Dr. Crocker agreed on cross-examination that "inhibiting dimer formation is an example of a neutralizing antibody described here," in sharp contrast to his statement in his declaration. *Compare* Ex. 2058, at 123:10-18 *and* Ex. 1003, ¶¶ 9-12.

Indeed, in 2007, it was well accepted that "neutralizing antibodies" refers to those antibodies that neutralize the biological activity of a target. As Dr. Stein explained, "the phrase 'neutralizing antibodies' is a term of art in immunology. It originated as a term to describe antibodies in the body that were able to neutralize and clear infectious agents, particularly viruses. In the context of a target protein, however, the phrase 'neutralizing antibodies' is used to indicate that the antibody inhibits the activity of the target protein. The method by which the antibody inhibits the activity of the target protein can occur in any number of ways (*e.g.*, blocking a binding site, preventing a conformational change, preventing binding to

a ligand, or preventing multimerization)." Ex. 2076, ¶ 53. *See also* Ex. 2074, ¶ 40. Moreover, Dr. Crocker conceded that it is unnecessary to know the precise mechanism of action to generate neutralizing antibodies. *See* Ex. 2058, at 130:22-131:4 ("when you generate antibodies, you have no idea generally of what mechanism they would require in order to mediate the effects that you're interested in."). Instead, it was well understood that a robust functional assay (like the osteoclastogenesis assays described in the Alethia PCT) and a known correlation between that assay and a therapeutic benefit (like the known correlation between inhibiting osteoclast differentiation and the therapeutic benefit of impeding bone resorption) is all that is necessary to identify therapeutic antibodies. *See* Ex. 2074, ¶¶ 10-11, 28-29, 37; Ex. 2058, at 93:20-95:3, 181:4-15.

Finally, Daiichi alleges that the Alethia PCT discloses antibodies "in *detecting* proteins and diseases, and not for treatment." Petition at 20-21 (emphasis in original). This is simply false. The Alethia PCT specifically identifies, as an object of the invention, the idea of specifically inhibiting a particular protein described in the specification, such as Siglec-15, to ameliorate the symptoms of the bone remodeling diseases and disorders. *See, e.g.*, Ex. 1010, p. 10, ll. 17-23. Contradicting his declaration again, Daiichi's expert Dr. Crocker acknowledged, during his deposition, that the concept of "identify[ing] compounds which inhibited the function of the proteins [including Siglec-15] that are disclosed

in the [Alethia] application" was an "object of the invention." Ex. 2058, at 72:13-73:4. *See* Ex. 2074, ¶ 31 ("there is no doubt that the Alethia PCT contemplates the therapeutic use of anti-Siglec-15 antibodies for inhibiting osteoclast differentiation/formation and/or bone resorption"); Ex. 2075, at 268:6-11.

Indeed, the Alethia PCT even teaches an alternative approach to use Siglec-15 as a therapeutic target for inhibiting osteoclast formation and bone resorption by linking antibodies that specifically bind Siglec-15 with a toxin using the standard antibody drug conjugation (ADC) technology. *See, e.g.*, Ex. 1010, p. 40, ll. 26-31. This ADC therapeutic approach (*see supra* p. 9) had been known in the art since the 1980's. Ex. 2076, ¶ 22. Daiichi asserts in the petition that "[f]or the claimed method to work, the antibody or antigen binding fragment recited in the claims must have an impairment effect on osteoclast differentiation…or inhibitory effect on bone resorption…." Petition at 19. That is not necessarily the case. Using an ADC, as contemplated by the Alethia PCT, antibodies can bind to surface-expressed Siglec-15 in osteoclasts and deliver a toxin to the cell, killing it via antibody-triggered endocytosis to achieve the claimed effect. Ex. 2074, ¶ 30.

D. Daiichi's Reliance on *Alonso*, *Rochester*, *Centocor* and *AbbVie* is Misplaced

Daiichi cites a number of cases, in particular *Alonso*, *Rochester*, *Centocor*, and *AbbVie*, to attempt to support its assertion that the claims of the '181 patent are not supported by the written description of the earlier Alethia PCT. *See* Petition at

13-14. None of the cases cited by Daiichi are applicable.

In *Alonso*, the claims involved the use of an unknown monoclonal antibody "idiotypic" to the neurofibrosarcoma of a human. *In re Alonso*, 545 F.3d 1015, 1017 (Fed. Cir. 2008). A primary issue in *Alonso* was that the specification did not sufficiently characterize the antigen to which the required antibodies must bind. *Id.* at 1021. Indeed, in *Alonso*, the antigen was described only by its molecular weight. *Id.* Thus, because there was no specific description of the target antigen, Alonso was required to provide some description of the antibodies themselves in order to meet the written description requirement. *See id.* at 1021-22. *Alonso* does not apply here because, as discussed above, the Alethia PCT provides extensive structural and functional characterization of antigen Siglec-15.

Similarly, in *Rochester*, the claims involved the use of a "non-steroidal compound" that "selectively inhibits" activity of the PGHS-2 gene. *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 917 (Fed. Cir. 2004). Notably, the *Rochester* invention was directed to small molecules, not antibodies, and thus there was no applicable written description antibody rule based upon well-understood antibody-target relationships or a predictable art. *See id.* at 925 (also distinguishing claims in "the chemical arts" from DNA cases where a DNA sequence supports claims to the complementary molecules that can hybridize to it).

Daiichi's reliance on the *Centocor* and *AbbVie* cases is similarly misplaced.

In *Centocor*, the patents claimed anti-TNF-a antibodies with specific structural or structurally dependent features: a human constant region, a human variable region, neutralizing activity, and the ability to bind to an antigen in the same place as a known mouse antibody. *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1346-47 (Fed. Cir. 2011). Thus, the claimed invention was the specific improvement of anti-TNF-a antibodies based on specific structurally dependent and mechanism of action dependent features. Therefore, the disclosure of a well-known antigen (TNF-a) alone was not sufficient under the rationale of the antibody rule because the claimed invention was based on undisclosed specific structural and mechanistic features of the antibody. *See id.* at 1350-51 (citing the USPTO Manual (Ex. 2077) and *Noelle,* 355 F.3d 1343).

Likewise, in *AbbVie*, the claims were directed to improved human antibodies to a known antigen, IL-12, that were neutralizing and had a specific, required binding affinity (k_{off}) rate. *AbbVie Deutschland GmbH & Co. v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1290 (Fed. Cir. 2014). The required affinity (k_{off}) rate of the claimed antibodies was also "dependent on the structure of the antibody." *Id.* at 1298. Yet there was no correlation established between certain structural features and the claimed specific binding affinity, and no evidence that skilled artisans could have made predictable changes to the structures of the disclosed antibodies to arrive at the other antibodies included in the claimed genus. *Id.* at 1301. In this

case, in contrast, the claims of the '181 patent do not require any structural or structurally dependent antibody features, and, more importantly, as discussed above, the function of the claimed methods is not tied to any such structural features. In fact, as discussed above, a person of ordinary skill in the art knows that the structure of the antibody used in the claimed method is irrelevant to practicing the method. Ex. 2076, ¶¶ 15-23, 52; Ex. 2074, ¶¶ 11, 39-40; Ex. 2058, at 26:18-29:12, 100:4-10, 102:7-21 (Q: "you don't need structural information concerning an antibody in order to make an antibody with a particular, with a particular binding affinity for your target protein, right?" A: No, you have no idea what the structure will be."); Ex. 2075, at 30:24-31:8, 124:9-125:3, 125:18-126:1, 226:2-12. Thus, unlike in *AbbVie* or *Centocor*, the function that is claimed in the '181 patent is not based upon an understanding of structure such that representative species with specific structures need be disclosed.

V. THE CLAIMS OF THE '181 PATENT ARE ENABLED BY THE ALETHIA PCT

For many of the same reasons, Daiichi's enablement arguments also fail. The evidence shows the '181 patent claims were sufficiently enabled by the Alethia PCT. Moreover, post-filing evidence, both from Alethia and Daiichi itself, confirms the Alethia PCT was sufficiently enabling.

A. Legal Standard

To be enabling, the specification must describe "the manner and process of

making and using [the invention] in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same" without "undue experimentation." 35 U.S.C. § 112; *Invitrogen Corp. v. Clontech Labs., Inc.*, 429 F.3d 1052, 1070 (Fed. Cir. 2005). "That is not to say that the specification itself must necessarily describe how to make and use every possible variant of the claimed invention, for the artisan's knowledge of the prior art and routine experimentation can often fill gaps." *AK Steel Corp. v. Sollac*, 344 F.3d 1234, 1244 (Fed. Cir. 2003). "[A] patent need not teach, and preferably omits, what is well known in the art." *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986).

In considering whether experimentation is "undue," the Federal Circuit has held that "[e]nablement is not precluded by the necessity for some experimentation such as *routine screening*." *In re Wands*, 858 F.2d 731, 736 (Fed. Cir. 1988) (emphasis added). The "key word is 'undue,' not 'experimentation.'" *Id.* The enablement analysis "requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art." *Id.* This "is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." *Id.* In *Wands*, the Federal Circuit considered the following factors to decide whether a person of ordinary skill in the art could make and use claimed antibodies without undue experimentation: (1) the

quality of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (5) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *Id.* at 737. Applying these factors in view of the well-developed nature of antibody technology, and expert testimony that "there is a *very high likelihood* that [the claimed] high affinity … antibodies will be found," the *Wands* court held that the antibody claims at issue were properly enabled. *Id.* at 738 (emphasis added).

B. Analysis

1. *The Alethia PCT enables a person of ordinary skill in the art to make an antibody that impairs osteoclast differentiation or inhibits bone resorption without undue experimentation*

Daiichi alleges that the Alethia PCT does not teach how to make, without undue experimentation, an antibody that specifically binds Siglec-15 and impairs osteoclast differentiation or inhibits bone resorption. Daiichi's allegation is baseless.[6] First, well before the Alethia PCT was filed, anti-Siglec-15 antibodies

[6] Daiichi's position is also disingenuous. Notably, Daiichi expressly represented to the Canadian Patent Office, in connection with prosecution of its own '072 Publication, that a skilled person in 2007 would be able to make an anti-Siglec-15 antibody that impairs osteoclast differentiation or inhibits bone resorption using standard methods without undue experimentation. *See* Ex. 2180, pp. 3-4.

already had been made using routine methods and had been shown to be able to bind surface-expressed Siglec-15 in a cell-based assay. *See* Ex. 2065; Ex. 2074, ¶¶ 19-21; Ex. 2076, ¶¶ 28-29, 34-42. Therefore, there is no doubt that anti-Siglec-15 antibodies can be made without undue experimentation. The Alethia PCT also includes "a very elaborate section giving standard procedures" for generating anti-Siglec-15 antibodies, including hybridoma technology, phage display techniques, and mammal immunization methods, all of which were also well-known in the art. Ex. 2058, at 182:7-9, 95:6-22; Ex. 2075, at 25:2-10, 28:22-29:4, 43:10-13; Ex. 2076, ¶¶ 16-23,30-31; Ex. 2074, ¶ 27; Ex. 1010, pp. 33-42.

The Alethia PCT also teaches the new inhibitory function of Siglec-15 in osteoclast differentiation, and the use of known and reliable osteoclastogenesis assays to select anti-Siglec-15 antibodies that can inhibit osteoclast differentiation or bone resorption. *See* Ex. 2074, ¶¶ 10-11, 14-15, 28-29; Ex. 2076, ¶¶ 26, 30-32; Ex. 2058, at 93:20-95:3, 181:4-9; Ex. 2075, at 100:10-18, 101:17-102:1. In 2007, using such functional assays to identify antibodies with a particular function was a standard practice and did not require "undue" experimentation. Ex. 2058, at 181:4-9; Ex. 2075, at 100:10-18, 101:17-102:1. *See also Wands*, 858 F.2d at 740. The osteoclastogenesis assay disclosed by the Alethia PCT was a well-known and robust assay in 2007 to demonstrate osteoclast differentiation function, to identify regulators (e.g., inhibitors) of osteoclast differentiation and bone resorption, and to

correlate and reliably predict *in vivo* osteoclast and bone resorptive activity. Ex. 2074, ¶¶ 10-11, 28-29; Ex. 2058, at 93:20-95:3, 181:4-9; Ex. 2075, at 100:10-18, 101:17-102:1.

Furthermore, as described previously, there was "a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known." *Wands*, 858 F.2d at 740. A person of ordinary skill in the relevant field would have at least a Ph.D. in the field of bone biology, immunology, molecular biology or related field and have at least 2 years of experience making or using antibodies. Ex. 2074, ¶ 7; Ex. 2076, ¶ 6. *Cf.* Ex. 1003, ¶ 7; Ex. 1004, ¶ 13. As set forth above, following the teachings of the Alethia PCT, such a person of skill would have been able to utilize the standard methods described above to create antibodies to Siglec-15 and to demonstrate its function without undue experimentation.

Moreover, in 2007, persons of skill in the art, upon reading the Alethia PCT, readily would have used the disclosed sequence of Siglec-15 to search for and find the relevant, pre-existing information demonstrating Siglec-15 was a cell-surface protein and that antibodies specific to Siglec-15 already existed. *See* Ex. 2076, ¶ 28; Ex. 2074, ¶ 18; Ex. 2058, at 193:17-194:11; Ex. 2075, at 39:5-40:10, 147:25-148:6, 230:2-231:11; *In re Howarth*, 654 F.2d 103, 106 (C.C.P.A. 1981) ("part of the skills of such persons includes not only basic knowledge of the particular art to

which the invention pertains but also the knowledge of where to search out information"). And, with the sequence of Siglec-15 and its known cell-surface accessibility and antibody history, persons of skill would have considered antibody-based therapies against Siglec-15 as both promising and achievable. *See* Ex. 2074, ¶¶ 9-11, 16-17; Ex. 2076, ¶¶ 27-29. Persons of skill would then have used the various methods described in the Alethia PCT and known in the antibody art to generate and select anti-Siglec-15 antibodies that impair osteoclast differentiation and inhibit bone resorption.

In fact, as discussed below, both Alethia and Daiichi did exactly what persons of skill would have done in view of the Alethia PCT -- used its disclosed invention and methods to generate anti-Siglec-15 antibodies and demonstrate the efficacy of those antibodies, as set forth in their '181 patent and '072 Publication. *See* Ex. 1001; Ex. 1023; Ex. 2074, ¶¶ 33, 34-38; Ex. 2058, at 178:6-181:15.

Thus, the evidence indicates that no "undue" experimentation was necessary for a person of ordinary skill to practice the claimed methods of the '181 patent using what was known in the art in conjunction with the disclosure of the Alethia PCT. *See Wands*, 858 F.2d at 740 ("The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics.")

In an attempt to challenge enablement, Daiichi relies on Dr. Clark's

declaration regarding asserted unpredictability in therapeutic antibody development. *See* Petition at 28-29; Ex. 1004, ¶¶ 7-11, 17, 20, 25. However, as Dr. Stein pointed out, "Dr. Clark appears to confuse the inventive process of therapeutic antibody development with the commercialization or FDA regulatory approval process." Ex. 2076, ¶ 14. Indeed, the risk and unpredictability Dr. Clark discussed is the risk and unpredictability of commercialization and the FDA approval process. *See* Ex. 1004, ¶¶ 7-11. As Dr. Stein explained, "[b]ased on my many years of experience in FDA, I can say that those risks and unpredictability normally have nothing to do with the invention itself." Ex. 2076, ¶ 14. *See also In re Brana*, 51 F.3d 1560, 1568 (Fed. Cir. 1995). In fact, at his deposition, Dr. Clark acknowledged that the unpredictability he refers to relates to clinical trial, FDA approval, and commercialization. Ex. 2075, at 71:23-73:10. He particularly clarified the statement he made in his declaration that "there are many steps involved in the process of therapeutic antibody development. While many of these processes are established, it is quite feasible that a therapeutic antibody against a particular target, even one that is accessible from the cell surface, will never be created." Ex. 1004, ¶ 7. Specifically, he admitted that his opinion referred to aspects of antibody commercialization and approval that are not relevant to the '181 patent claims (Ex. 2075, at 122:13-123:14) and acknowledged that "there is 99.9% chance to create an antibody against a target protein." *Id.*, at 42:21-43:13.

The independent claims of the '181 patent simply require, with respect to the antibody itself, an antibody that specifically binds to human or murine Siglec-15. These claims cover anti-Siglec-15 antibodies generated for therapeutic purposes, including those that have or are reasonably likely to have a measurable effect on osteoclast differentiation *in vivo* as measured in a correlative *in vitro* bioassay. Some of these antibodies may eventually be approved by FDA and commercialized, but specific FDA approval or commercialization process is not required by the claims of the '181 patent. Thus, all of Dr. Clark's statements and core opinions on risk and unpredictability in his declaration are inapplicable to the '181 patent claims. *See* Ex. 2076, ¶ 14; Ex. 2075, at 122:13-123:14.

Daiichi's reliance on the *Wyeth* case is also improper. The *Wyeth* case dealt with using delivery mechanisms for thousands of heterologous small molecules. *Wyeth v. Abbott Labs.*, 720 F.3d 1380, 1384 (Fed. Cir. 2013). The claims in *Wyeth* encompassed "tens of thousands of candidates," with potentially heterologous structure, with no disclosure of how to modify those structures of the claim limitations. *Id.* That is obviously not the case here. As discussed above, the antibody-antigen relationship, unlike the variation in small molecules, is unique and far more predictable. Ex. 2079, pp. 1, 3, 7, 17. Moreover, the generation of antibodies to Siglec-15 was routine and straightforward, and the disclosed osteoclastogenesis assays were standard and highly predictive of antibody function

in osteoclastogenesis *in vivo*. Ex. 2076, ¶¶ 16-23, 30-31, 34-42; Ex. 2074, ¶¶ 9-11, 16-21, 27-29. Therefore, unlike in *Wyeth*, a skilled artisan would not have to engage in a prolonged "iterative trial-and error process" and experimentation that "would involve testing for an unreasonable length of time" to generate anti-Siglec-15 antibodies that impair osteoclast differentiation or inhibit bone resorption. By contrast, as Dr. Boyce indicated, and as Alethia's and Daiichi's own experiences (discussed below) showed, anti-Siglec-15 antibodies could be readily selected using the osteoclastogenesis assays disclosed in the Alethia PCT. *See also* Ex. 2074, ¶ 29.

Daiichi's argument that Alethia was required to disclose a working example of an antibody that binds to Siglec-15 that impairs osteoclast differentiation and inhibits bone resorption also misses the mark. First, Daiichi again relies on its repeated (and intentional) mischaracterization of fact -- that as of 2007, Siglec-15 was not known to be expressed on the cell surface or accessible to a targeting antibody. *See* Petition at 27-28. As discussed previously, this assertion is simply false. *See supra* pp. 26-30. Second, because Siglec-15 was in fact known to be a cell surface protein and accessible to a targeting antibody, a person of ordinary skill in the art would have expected, based upon the disclosed shRNA knockdown data that use of an antibody to inhibit Siglec-15 would be "highly likely" to yield the same results. *See* Ex. 2076, ¶¶ 16-42; Ex. 2074, ¶¶ 9-31; *Wands*, 858 F.2d at

738. Moreover, the Alethia PCT expressly described the use of antibodies to Siglec-15 to impair osteoclast differentiation and inhibit bone resorption. *See* Ex. 1001, Example L; Ex. 2058, at 181:13-14 ("It established the principal that this assay could be used for antibodies…."). Thus, Alethia was "not required to provide actual working examples" of an antibody to enable the claimed invention, as the Federal Circuit has "rejected enablement challenges based on the theory that there can be no guarantee that prophetic examples actually work." *Allergan, Inc. v. Sandoz Inc.*, 2015 U.S. App. LEXIS 13616, at**34-35 (Fed. Cir. Aug. 4, 2015) ("A patent does not need to guarantee that the invention works for a claim to be enabled. And efficacy data are generally not required in a patent application. Only a sufficient description enabling a person of ordinary skill in the art to carry out an invention is needed.") (internal quotations omitted).

Given the combination of the shRNA examples using Siglec-15, the express teaching of the use of antibodies to inhibit Siglec-15, the extensive disclosures of known methods to generate antibodies, the knowledge (and fact) that Siglec-15 was cell surface accessible and that anti-Siglec-15 antibodies had been made, and the fact that *in vivo* osteoclast and bone resorptive inhibitory activity could be reliably predicted using osteoclastogenesis assays described in the Alethia PCT, the Alethia PCT enables a person of ordinary skill in the art to make and use an antibody to impair osteoclast differentiation or inhibit bone resorption without

undue experimentation. Daiichi has failed to prove otherwise by a preponderance of the evidence.

2. *The Alethia PCT provides sufficient guidance for the use of anti-Siglec-15 to impair osteoclast differentiation in a mammal and to inhibit bone resorption in a subject in need*

Daiichi alleges that the Alethia PCT fails to provide any description regarding the use of antibodies or antigen-binding fragments for either of the claimed methods in the '181 patent, or "even the smallest indication" that anti-Siglec-15 antibodies would perform the requisite activity *in vivo*. Petition at 31. Daiichi's arguments fail for at least the reasons below. First, Alethia's shRNA knockdown examples, in the context of osteoclastogenesis, are proofs of concept and a strong indicator of the results *in vivo* of using antibodies to interfere with Siglec-15 in osteoclast differentiation/formation. *See* Ex. 2074, ¶¶ 14-15, 26; Ex. 2076, ¶ 26. *See also Ex Parte Rodriguez-LaFrasse*, 2014 Pat. App. LEXIS 533, at **3-4, 6-7 (specification was sufficiently enabling to cover any inhibitors of hsp27 in cancer cells, including antibodies, where it teaches generally using both antisense oligonucleotides as well as antibodies, and there were several working examples using the oligonucleotides in cancer cells). Siglec-15 was a known cell surface protein and antibody target at the time of Alethia's PCT, and the PCT describes the use of such osteoclastogenesis assays to identify anti-Siglec-15 antibodies that inhibit osteoclast differentiation/formation/bone resorption. *See*

supra pp. 8-10, 26-30.

There was also a well-known, reliable correlation between such *in vitro* osteoclastogenesis assay and *in vivo* results for osteoclast formation/differentiation and bone resorption by 2007, including the previous use of such *in vitro* assays to predict *in vivo* activity for and develop therapeutic antibodies such as denosumab. *See supra* pp. 8-10. *See also* Ex. 2074, ¶¶ 10-11, 14, 28-29; Ex. 2076, ¶¶ 26, 30-32. *See also Edwards Lifesciences AG v. CoreValve, Inc.*, 699 F.3d 1305, 1310 (Fed. Cir. 2012) ("An *in vitro* or *in vivo* animal model example in the specification, in effect, constitutes a working example if that example correlates with a disclosed or claimed method invention.") (quoting M.P.E.P §2164.02). Daiichi's own expert concedes that *in vitro* results on osteoclast formation/differentiation are enabling for a method of impairing osteoclast differentiation or inhibiting bone resorption *in vivo*. Ex. 1003, ¶¶ 22, 23; Ex. 2058, at 188:14-17. *See also* Ex. 2075, at 263:20-264:5 (unable to opine on the issue).

Therefore, this is not a case like *Rasmusson*[7], cited by Daiichi, where "there

[7] The claims of *Rasmusson* required proof that the invention could be *effective* in *treating* cancer. *Rasmusson v. SmithKline Beecham Corp.*, 413 F.3d 1318, 1324 (Fed. Cir. 2005). Alethia's invention requires only inhibition of pharmacological activity *in vivo*, *e.g.*, impairing osteoclast differentiation and inhibiting bone resorption. *See* Ex. 1001; *Cross v. Iizuka*, 753 F.2d 1040, 1050 (Fed. Cir. 1985).

is no indication that one skilled in the art would accept without question statements as to the effects of the claimed drug products and no evidence has been presented to demonstrate that the claimed products do have those effects" and "there is a complete absence of data supporting the statements which set forth the desired results of the claimed invention." *See* Petition at 31. To the contrary, the Alethia PCT provides ample indication and supporting data that correlate the osteoclast and bone resorptive inhibitory effects of anti-Siglec-15 antibody *in vivo*, that one skilled in the art would recognize and accept. *See supra* pp. 11-13, 21-24.

Finally, for completeness, the Alethia PCT also satisfies the practical utility requirement in view of the above. The law is clear that all that is required for satisfying the practical utility requirement is that the "tests be *reasonably* indicative of the desired pharmacological response." *Fujikawa*, 93 F.3d at 1564 (emphasis in original) (citing *Nelson v. Bowler*, 626 F.2d 853, 856 (C.C.P.A. 1980)). As set forth above, the Alethia PCT clearly provides sufficient data and a correlation between that data and the asserted biologic activity so as to convince those of skill in the art, "to a reasonable probability," that anti-Siglec-15 antibodies will exhibit the asserted function *in vivo*. *Id.*

In light of the foregoing, application of the *Wands* factors and other applicable case law indicate that the Alethia PCT provides sufficient disclosure to enable a person of ordinary skill in the art to practice the claims of the '181 patent

without undue experimentation.

3. *Post-filing data confirms that the Alethia PCT was in fact enabling*

While the question of enablement is determined as of the application filing date, post-filing evidence "can be used to substantiate any doubts as to the asserted utility since this pertains to the accuracy of a statement already in the specification." *Brana*, 51 F.3d at 1566 n.19. While post-filing evidence "does not render an insufficient disclosure enabling," it can "prove that the disclosure was in fact enabling when filed." *Id.* *Cf. In re Horton*, 439 F.2d 220, 222 and n.4 (C.C.P.A. 1971) (references used to substantiate enablement are "[n]ot necessarily prior art…since the question would be regarding the accuracy of a statement in the specification, not whether that statement had been made before").

In this case, both Alethia's '181 patent and Daiichi's own '072 Publication confirm the accuracy of the statements in the specification of the Alethia PCT. Alethia's '181 patent describes generating basic targeting antibodies to Siglec-15 using the same routine methods disclosed in the Alethia PCT, and selecting functional anti-Siglec-15 antibodies using the same osteoclastogenesis assay disclosed in the Alethia PCT. Ex. 2074, ¶¶ 32-33; Ex. 2076, ¶ 43. Inhibition of osteoclast differentiation and bone resorption was observed with every exemplary Siglec-15 antibody that was tested in the osteoclastogenesis assay. Ex. 1001, 59:25-29 ("This result is in complete agreement with the experiments disclosed by

Sooknanan (Sooknanan et al., 2007) that showed that knockdown of Siglec-15 expression by RNA interference caused inhibition of human osteoclast differentiation.").

Similarly, Daiichi's own '072 Publication demonstrates that Daiichi used the standard immunization and hybridoma technology described in the Alethia PCT to generate polyclonal and monoclonal antibodies, and using the same osteoclastogenesis assay described in the Alethia PCT to test the activity of those antibodies. *See* Ex. 2074, ¶¶ 34-38; Ex. 2076, ¶¶ 44-48; Ex. 2075, at 216:4-22, 218:16-24, 220:3-11, 285:3-25. In fact, contrary to Daiichi's position in the Petition, the '072 Publication shows that Daiichi created antibodies to Siglec-15 that inhibited osteoclast differentiation without knowing the mechanism of action of Siglec-15. Ex. 2075, at 126:13-20. Daiichi's '072 Publication also does not include any *in vivo* testing, signaling pathway, or recycling kinetics; instead, Daiichi used the same RAW 264.7 and human osteoclast cells as disclosed in the Alethia PCT in its experiments to test the effect of its antibodies on osteoclast differentiation. Ex. 2074, ¶¶ 34-38; Ex. 2076, ¶¶ 44-48. Indeed, Daiichi's experts confirm that Daiichi successfully made antibodies to Siglec-15 using the same, conventional methods disclosed in the Alethia PCT, and tested those antibodies using the same *in vitro* osteoclastogenesis assay disclosed in the Alethia PCT. *See* Ex. 2058, at 98:1-99:3, 104:4-9, 131:14-133:19, 178:6-181:15, 182:4-13; Ex. 2075,

at 103:12-104:10, 216:4-220:11, 285:3-25. As Dr. Boyce pointed out, "a person of skill in the bone field would not have felt that he/she learned anything new after reading the '072 Publication." Ex. 2074, ¶ 35.

Thus, because Daiichi '072 Publication simply follows the teachings of the Alethia PCT and does not disclose anything new[8], Daiichi cannot claim that its own '072 Publication is sufficiently enabled and yet also argue that the Alethia PCT is non-enabling. Rather, its own proffered intervening "prior" art in fact merely further confirms that Alethia's invention is fully enabled. *See Ex Parte Li*, 2010 Pat. App. LEXIS 14138 (P.T.A.B. Mar. 18, 2010) (enablement demonstrated by post-filing reference confirming SEQ ID NO.:2 is a G-protein chemokine receptor for three ligands and that those ligands, interacting with CXCR3, resulted in the chemotaxis); *Ex Parte Latta*, 2007 Pat. App. LEXIS 4901 (P.T.A.B. Feb. 28, 2007) (post-filing declarations showing additional mouse data and that mouse data

[8] Daiichi appears to suggest that the only new disclosure in the '072 Publication is that they made and tested anti-Siglec-15 antibodies. *See* Ex. 1023, p. 3, l. 8-p. 4, l. 5. As Dr. Stein pointed out, "[c]onsidering the very high level of skill of the antibody field in 2007 and the fact that anti-Siglec-15 antibodies had already been made previously, it is hard to image that simply by making antibodies using the same routine methods described in the Alethia PCT, and testing them again using the same functional assays, would be inventive in 2007." Ex. 2076 ¶ 48.

was the favored disease model were sufficient to prove enablement when filed).

VI. THE '072 PUBLICATION IS NOT PRIOR ART

As described above, the Alethia PCT fully describes and enables the invention as claimed in the '181 patent and any epitope mapping, CDR sequencing or other clinical development lead optimization activities are not required by or to enable the claims of the '181 patent. However, to the extent the Board believes such activities are relevant to the reduction to practice of the claimed invention, Alethia presents evidence below to demonstrate that Alethia's inventors were diligently working on obtaining such information, among other things, during the legally relevant period to reduce the invention to practice. Accordingly, the alternative grounds stated here demonstrate that the lone prior art reference cited by Daiichi in its Petition, Daiichi's own '072 Publication", purporting to cover the same subject matter as the '181 patent, does not actually constitute intervening prior art under 35 U.S.C. § 102(a).

In the Petition, Daiichi asserts that the '181 patent is invalid under Section 102(a) in light of its '072 Publication, published on April 16, 2009. Petition at 34-58. Yet a reference is prior art under Section 102(a) only if published before the date the patent owner invented the subject of the patent. *Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1576 (Fed. Cir. 1996). A patent owner may antedate and overcome an alleged Section 102(a) reference by showing he (i) conceived of the

invention prior to publication of the reference and (ii) thereafter diligently reduced it to practice during the legally relevant time period (a moment just prior to the publication of the reference until the invention was constructively reduced to practice). *Id.* at 1577-78; *Teva Pharm. Indus. v. AstraZeneca Pharms.*, 661 F.3d 1378, 1383 (Fed. Cir. 2011); Paper 56, IPR2014-00233 at 14-17.

As demonstrated in the Diligence Chart and the supporting evidence submitted herewith, the inventors of the '181 patent conceived of their entire invention at least by February 13, 2007 -- over two years before the '072 Publication was published. In fact, in June 2007, one of the co-inventors of the '181 patent presented the very invention in the '181 patent -- in the form of Alethia's monoclonal antibody programs to develop antibodies to AB0326 (*i.e.*, Siglec-15), to impair osteoclast differentiation and bone resorption for its clinical pipeline -- to Daiichi for the purpose of a potential strategic partnership.[9] Ex. 2080. The inventors also thereafter diligently reduced their invention to practice, including between April 9, 2009 and October 16, 2009, on which date they constructively reduced it to practice at the latest by filing U.S. Patent App. 12/580,943 (the "'943 application"), which issued as the '181 patent. Accordingly,

[9] Daiichi filed its Japanese provisional application for the '072 Publication in October 2007, four months after meeting with Alethia's inventors.

the '072 Publication does not constitute prior art and cannot invalidate the '181 patent.

A. Alethia's Inventors Conceived the Invention Claimed in the '181 Patent Before April 16, 2009

1. *Legal standard*

"[Conception] is the formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice." *Burroughs Wellcome Co. v. Barr Labs.,* 40 F.3d 1223, 1228 (Fed. Cir. 1994) (citations and internal quotations omitted). An idea is sufficiently definite "when the inventor has a specific, settled idea, a particular solution to the problem at hand, not just a general goal or research plan he hopes to pursue." *Id.*

Inventor testimony regarding conception and reduction to practice must be corroborated, but "[t]here is no particular formula that an inventor must follow in providing corroboration of his testimony." *Chen v. Bouchard*, 347 F.3d 1299, 1309-10 (Fed. Cir. 2003); *Brown v. Barbacid*, 436 F.3d 1376, 1380 (Fed. Cir. 2006).[10] The rule of reason is used to evaluate all pertinent evidence to determine the credibility of the inventor's story. *Chen*, 347 F.3d at 1309-10. Circumstantial evidence of an independent nature can satisfy the corroboration requirement.

[10] No corroboration of the technical content in documentary evidence is required. *Mahurkar*, 79 F.3d at 1577; Paper 59, IPR 2012-00001, at 22.

Thus, testimony from co-workers, lab notebooks, and test results of inventors are all routinely used to corroborate inventor testimony regarding conception and reduction to practice. *Cooper v. Goldfarb*, 154 F.3d 1321, 1330 (Fed. Cir. 1998).

2. *Conception of the invention of the '181 patent*

The claims of the '181 patent define the invention, which is the use of an antibody or antigen-binding fragment that specifically binds to human or murine Siglec-15 to impair osteoclast differentiation in a mammal and/or to inhibit bone resorption. Alethia's inventors conceived the invention of the '181 patent at least as of February 13, 2007 when they filed the Alethia PCT, because the Alethia PCT disclosed a definite, permanent, complete and operative idea of Alethia's entire invention, as shown in the claim chart below.

The Alethia PCT alone is sufficient evidence of conception. *See In re Costello*, 717 F.2d 1346, 1350 (Fed. Cir. 1983); *Burroughs*, 40 F.3d at 1229-30 (draft British patent application sufficient to corroborate conception); *Krantz v. Olin*, 356 F.2d 1016, 1019-20 (CCPA 1966). The chart below demonstrates that the Alethia PCT teaches each and every element of the challenged claims of the '181 patent:

Claim Language	Exemplary Description in Alethia PCT
1. A method of impairing osteoclast differentiation in a mammal in need thereof, the method	"The present invention also relates to ***a method of ameliorating bone remodeling disease or disorder symptoms, or for inhibiting or delaying bone disease or disorder***, the method may comprise: contacting a

comprising administering an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108) to said mammal.	compound capable of ***specifically inhibiting activity*** . . . ***a polypeptide described herein, in osteoclasts so that symptoms of the bone remodeling disease or disorder may be ameliorated, or the disease or disorder may be prevented, delayed or lowered***." (PCT, p. 10, lines 17-23). The polypeptide of human Siglec-15 is disclosed as SEQ ID NO:48 in the Alethia PCT. The polypeptide of murine Siglec-15 is disclosed as SEQ ID NO:82 in the Alethia PCT. "The present invention also relates to a compound and ***the use of a compound able to inhibit (e.g., in an osteoclast precursor cell) the activity*** or expression of a polypeptide which may be selected, for example, from the group consisting of ***SEQ ID NO.: 48 [human siglec-15]*** to 80 or a polypeptide encoded by SEQ ID NO.:85 or SEQ ID NO.:86, in the preparation of ***a medicament for the treatment of a bone disease in an individual in need thereof***." (PCT p. 32, line 26-31). "This particular type of cell-based assay can now serve as the basis for screening ***compounds capable of binding to and inhibiting the function of human AB0326***. A compound library could be applied to this 'rescued' cell line in order to identify molecules (small molecule drugs, peptides, or ***antibodies) capable of inhibiting AB0326***. Any reduction in osteoclast differentiation measured by a reduction in the expression of TRAP would be indicative of a decrease in human AB0326 activity." (PCT p. 85, line 32 to page 86, line 4). "In a further aspect, the present invention relates to ***an antibody*** (e.g., isolated antibody), ***or antigen-binding fragment thereof***, that may ***specifically bind*** to a protein or polypeptide described herein." *Id*. at p.

	33, l. 33-p. 34-l. 5.
2. The method of claim 1, wherein the antibody or antigen binding fragment impairs an osteoclast differentiation activity of human Siglec-15 or murine Siglec 15.	*See* Claim 1 above. "This particular type of cell-based assay can now serve as the basis for screening compounds capable of binding to and ***inhibiting the function of human AB0326***. A compound library could be applied to this 'rescued' cell line in order to identify molecules (small molecule drugs, peptides, or ***antibodies) capable of inhibiting AB0326***. Any ***reduction in osteoclast differentiation*** measured by a reduction in the expression of TRAP would be indicative of ***a decrease in human AB0326 activity***." *Id*. at p. 85, l. 32-p. 86, l. 4; *see also id*. at p. 83, l. 9-13; p. 84, ll. 30-33.
3. The method of claim 2, wherein the osteoclast differentiation activity is characterized by differentiation of osteoclast precursor cells into differentiated osteoclasts.	*See* Claim 2 above. "After 24h, the infected cells were treated with same medium containing 100 ng/ml RANK ligand for 5-8 days to allow for ***differentiation of osteoclast from precursor cells***." *Id*. at p. 82, ll. 30-32.
4. The method of claim 2, wherein the antibody is a polyclonal antibody.	*See* Claim 2 above. "The antibody may be, for example, a monoclonal antibody, a ***polyclonal antibody*** an antibody generated using recombinant DNA technologies." *Id*. at p. 34, ll. 2-4; *see also id*. at p. 34, ll. 21-30; p. 38, ll. 7-11.
5. The method of claim 2, wherein the antibody or antigen binding fragment is a monoclonal antibody or an antigen binding fragment thereof.	*See* Claim 2 above. "The antibody may be, for example, ***a monoclonal antibody***, a polyclonal antibody an antibody generated using recombinant DNA technologies." *Id*. at p. 34, ll. 2-4; *see also id*. at p. 35, l. 1-p. 36, l. 3; p. 37, ll. 28-32; p. 38, ll. 12-18. "Suitable antibodies may also include, for example, ***an antigen-binding fragment***, an Fab fragment; an $F(ab')_2$ fragment, and Fv fragment; or a single-chain

	antibody comprising an antigen-binding fragment (e.g., a single chain Fv)." *Id*. at p. 34, ll. 14-16.
6. The method of claim 5, wherein the monoclonal antibody or antigen binding fragment is produced from an isolated mammalian cell.	*See* Claim 5 above. "***Monoclonal antibodies (MAbs)*** may be made by one of several procedures available to one of skill in the art, for example, by fusing ***antibody producing cells*** with immortalized cells and thereby making a hybridoma. … Another example is the ***generation of MAbs*** from mRNA extracted from ***bone marrow and spleen cells of immunized animals*** using combinatorial antibody library technology." *Id*. at p. 38, ll. 12-18; *see also id*. at p. 35, ll. 1-12; p. 35, 23-p. 36, l. 3.
8. The method of claim 6, wherein the antibody or antigen binding fragment comprises a constant region of a human antibody or a fragment thereof.	*See* Claim 6 above. "The antibody may also be a ***chimeric antibody*** which may comprise, for example, ***variable domains of a non-human antibody and constant domains of a human antibody***." *Id*. at p. 34, ll. 11-13; *see also id*. at p. 39, ll. 20-21.
9. The method of claim 8, wherein the antibody or antigen binding fragment comprises a framework region of a human antibody.	*See* Claim 8 above. "The antibody may also…comprise a surface residue of a human antibody and/or ***framework regions of a human antibody***." *Id*. at p. 34, ll. 8-11.
10. The method of claim 2, wherein the antibody or antigen binding fragment is a F_V, a Fab, a Fab' or a (Fab')2.	*See* Claim 2 above. "Suitable antibodies may also include, for example, an antigen-binding fragment, ***an Fab fragment; an F(ab')$_2$ fragment, and Fv fragment***; or a single-chain antibody comprising an antigen-binding fragment (e.g., ***a single chain Fv***)." *Id*. at p. 34, ll. 14-16; *see also id*. at p. 36, ll. 10-22; p. 38, 23-27.
11. The method of claim 3, wherein the osteoclast precursor cells are human osteoclast precursor cells.	*See* Claim 3 above. "***Human osteoclast precursors*** purchased from Cambrex (East Rutherford. NJ) … After 24h, the infected cells were treated with same medium

	containing 100 ng/ml RANK ligand for 5-8 days to allow for ***differentiation of osteoclast from precursor cells***." *Id*. at p. 82, ll. 25-32.
15. A method for inhibiting bone resorption comprising administering to a subject in need thereof, an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108).	*See* Claim 1 above.
16. The method of claim 15, wherein the antibody or antigen binding fragment impairs an activity of human Siglec-15 or murine Siglec-15 in osteoclast precursor cells or in osteoclasts.	*See* Claim 2 above.
17. The method of claim 16, wherein the activity is osteoclastogenesis.	*See* Claim 3 above.
18. The method of claim 15, wherein the antibody or antigen binding fragment inhibits osteoclast differentiation.	*See* Claim 2 above.
19. The method of claim 15, wherein the antibody or antigen binding fragment is administered in combination with a drug or an hormone.	*See* Claim 15 above. "***Antibodies of the invention may include complete anti-polypeptide antibodies as well as antibody fragments and derivatives*** that comprise a binding site for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof. ***Derivatives are macromolecules that comprise a binding site linked to a functional domain***. Functional domains may

	include, but are not limited to signalling domains, ***toxins, enzymes and cytokines***." *Id*. at p. 40, ll. 26-31; *see also id*. at p. 3, ll. 19-26.
20. The method of claim 19, wherein the drug is an antiresorptive drug or a drug increasing bone mineral density.	*See* Claim 19 above. "Another example is osteoporosis where the only current medications approved by the FDA for use in the United States are ***the anti-resorptive agents*** that prevent bone breakdown. Estrogen replacement therapy is one example of an anti-resorptive agent. Others include alendronate [list of anti-resorptive agents omitted]. . .)." *Id*. at p. 3, ll. 19-26.
21. The method of claim 15, wherein the subject in need thereof, suffers from a bone remodelling disorder.	*See* Claim 15 above. "In accordance with the present invention, ***the mammal may suffer, for example, from a condition selected from the group consisting of osteoporosis, osteopenia, [additional diseases omitted]***, etc." *Id*. at p. 32, ll. 8-22; *see also id.* at claims 25-26.
22. The method of claim 21, wherein the bone remodelling disorder is associated with a decrease in bone mass.	*See* Claim 21 above. "A primary cause of ***this reduction in bone mass*** is an increase in osteoclast number and/or activity. The most common of such disease, and perhaps the best known, is osteoporosis occurring particularly in women after the onset of menopause." p. 2, ll. 11-14.
23. The method of claim 21, wherein the bone remodelling disorder is selected from the group consisting of osteoporosis, … and damage caused by macrophage-mediated inflammatory processes.	*See* Claims 20 and 21 above.

Additionally or alternatively, the conception of Alethia's invention may be further established by the presentation that Alethia inventor Mario Filion made to

Daiichi on June 19, 2007, prior to the publication of the '072 Publication. Ex. 2080. Dr. Filion presented details of Alethia's AB0326 (*i.e.*, Siglec-15) program, including convincing data demonstrating the essential role of AB0326 in osteoclast formation/differentiation and bone resorption, Ex. 2080, pp. 21-22, and identification of antibodies as therapeutic drug candidates to target AB0326. Ex. 2080, p. 37. Thus, the June 19, 2007 presentation shows a definite, permanent, complete and operative idea of Alethia's invention as claimed in the '181 patent.

B. Alethia Used Reasonable Diligence to Reduce Its Invention to Practice

Reasonable diligence in reducing an invention to practice is required throughout the relevant time period. "The basic inquiry is whether, on all of the evidence, there was reasonably continuing activity to reduce the invention to practice." *Brown*, 436 F.3d at 1380. In this case, the relevant time period begins just prior to April 16, 2009, the '072 Publication date, and ends on October 16, 2009, the filing date of Alethia's '943 application. *See Bey v. Kollonitsch*, 806 F.2d 1024, 1026 (Fed. Cir. 1986).

Whether an inventor has shown diligence in reduction to practice is a case-specific inquiry. *Monsanto Co. v. Mycogen Plant Sci.*, 261 F.3d 1356, 1369 (Fed. Cir. 2001). An inventor's diligence also includes his attorney's efforts to file a patent application to achieve a constructive reduction to practice. *Kollonitsch*, 806

F.2d at 1026.[11] To make the required showing of reasonable diligence, "there need not necessarily be evidence of activity on every single day if a satisfactory explanation is evidenced." *Id.* at 1369; *see also Brown*, 436 F.3d at 1380-81. Indeed, "courts may consider the reasonable everyday problems and limitations encountered by an inventor." *Griffith v. Kanamaru*, 816 F.2d 624, 626 (Fed. Cir. 1987). For example, people may be sick or take vacations (thereby creating gaps in activity) while still being diligent. *See Reed v. Tornqvist*, 436 F.2d 501, 504-05 (CCPA 1971).

Accordingly, the Federal Circuit has found that inventors have exercised reasonable diligence in reducing an invention to practice despite significant evidentiary gaps in activity ranging from days to months. *See Monsanto Co.*, 261 F.3d at 1369 (finding diligence despite various gaps in recorded activity, some spanning up to three weeks, in view of documents suggesting ongoing activity); *Tyco Healthcare Grp. v. Ethicon Endo-Surgery*, 774 F.3d 968, 975 (Fed. Cir. 2014) (five-month gap in weekly records during sixteen-month period excusable based on periodic reports showing lab results, due dates, milestones, and similar evidence of ongoing activity); *Brown*, 436 F.3d at 1381 (reasonable diligence

[11] Reduction to practice may be either actual or constructive. Constructive reduction to practice may be accomplished by filing a patent application. *See, e.g.*, *In re Costello*, 717 F.2d at 1350; MPEP § 2138.05.

found despite numerous short gaps of inactivity); *Rey-Bellet v. Engelhardt*, 493 F.2d 1380, 1388-89 (CCPA 1974) (three-month delay due to shortage of test subjects was excusable); *Jones v. Evans*, 18 CCPA 866, 874-75 (1931) (one to two-month gap excusable based on evidence of ongoing activity despite lack of affirmative evidence that "steps were being taken").

1. *The inventors diligently reduced their invention to practice from just prior to April 16, 2009 until October 16, 2009*

The following chronological account and supporting daily diligence chart (filed herewith as Exhibit 2105 ("Diligence Chart")) demonstrate that the inventors and their attorneys worked continuously throughout the relevant time period, beginning just prior to publication of the '072 Publication on April 16, 2009, and ending on October 16, 2009, to reduce the invention to practice by preparing antibodies that specifically bind to Siglec-15 and by selecting and characterizing lead candidates, as more fully described below, until constructively reducing the invention to practice by filing the '943 application on October 16, 2009.

In sum, during the relevant time period, inventor Dr. Tremblay and his team at Alethia, including Anna Moraitis ("Moraitis"), Martine Pagé ("Pagé"), Aida Kalbakji ("Kalbakji"), Annie Fortin ("Fortin"), Marc Sasseville ("Sasseville") and Sophie Roy ("Roy"), engaged in a consistent and intense effort to prepare and perform experiments to carry out the invention as previously conceived. Ex. 2101, ¶¶ 4-8. Many of these experiments included unavoidable or inherent time lags and

limitations, such as rates of reactions, times for culturing of cells, and the need to await analyses performed by contracted third parties. Often the team at Alethia worked to overcome these limitations by concurrently running multiple experiments and preparations over the same time frame. The Alethia team also met every two weeks to discuss results and establish priorities and next steps, which were not recorded in their laboratory notebooks. In September 2009, the inventors provided the results of this work to their patent attorney, Dr. Janique Forget, so that she could prepare a patent application. From September 2009 to October 16, 2009, Dr. Forget worked diligently to prepare and file the provisional and the '943 application that issued as the '181 patent. Ex. 2102, ¶¶ 5-9.

The chronology is as follows:

As of April 9, 2009, the Alethia inventors had identified multiple (46 in total) antigen-binding fragments ("Fab") that bound to Siglec-15 and were screened using the teachings of the Alethia PCT for inhibition of osteoclast differentiation. From April 9-16, 2009, the inventors amplified the DNA fragments encoding the 46 candidate Fab fragments and sent the fragments to the Genome Center, an independent laboratory that performs DNA sequencing, for sequencing and awaited for the sequence results. The sequence results were necessary for cloning the corresponding variable regions of the desired candidate

Fab fragments into appropriate vector constructs for antibody production for further functional testing. *See* Diligence Chart, Entries 001-006.

The sequence results arrived on April 16, 2009, and were immediately analyzed. 10 candidates were selected for generating chimeric antibodies containing human constant regions for further testing in human cells. Specifically, the heavy chain ("HC") and light chain ("LC") variable regions of individual candidates were amplified and sent out for sequencing. These Fab candidates included Alethia's lead antibody ([redacted]) and other antibodies shown to bind to Siglec-15 ([redacted]), all of which were ultimately disclosed in the '181 patent. *See* Diligence Chart, Entries 006-007.

The period of April 17, 2009 to May 14, 2009 was a period of intense activity. *See* Diligence Chart, Entries 007-026. As soon as the Fab sequences became available, Sasseville analyzed the sequences and chose 10 candidates ([redacted]) to use in generating chimeric antibodies and to further test the ability to bind to Siglec-15. Ex. 2152, pp. 33-35; Ex. 2156. The chimeric antibodies were designed as chimeric IgG2 antibodies that contain mouse variable regions and human constant regions, such that they could be administered to humans. Sasseville and Fortin first successfully cloned 7 of the candidate Fab regions into HC and LC expression vectors. Ex. 2152, pp. 35-37, 46-47; Ex. 2158. Even before the DNA sequences

of the candidate chimeric antibodies were confirmed, Pagé also began to express and purify the chimeric anti-Siglec-15 antibodies from human 2936E cell cultures. Leading up to May 14, 2009, Pagé successfully expressed and purified 7 separate chimeric anti-Siglec-15 antibodies ([redacted]) from human 2936E cells and confirmed that they maintained binding activity by ELISA. Ex. 2152, pp. 40, 49-50. Concurrently, Sasseville performed large-scale DNA preps of the Siglec-15 HC and LC chimeric expression vectors to have sufficient material for future experiments. Ex. 2152, p. 47. Pagé also performed several rounds of binding studies of the anti-Siglec-15 monoclonal antibody using synthetic Siglec-15 peptides as part of routine characterization of the antibody-antigen binding, specificity and epitope mapping. Ex. 2152, pp. 41-45, 47.

Beginning May 12, 2009, and continuing through June 8, 2009 (*see* Diligence Chart, Entries 024-042), Fortin successfully cloned several recombinant mouse Siglec-15 constructs and confirmed their sequences. Ex. 2152, pp. 55-61; Ex. 2154. During the same time, Kalbakji cultured RAW 264.7, mouse and human bone marrow cells and performed the first rounds of osteoclastogenesis assays to test the function of the chimeric anti-Siglec-15 antibodies *in vitro*. Ex. 2152, pp. 62-71. Pagé also successfully cloned Siglec-15 fused to the human Fc domain of an IgG into a mammalian expression vector. Ex. 2152, pp. 51-53; Ex. 2155.

From June 9, 2009 to July 24, 2009, the Alethia team continued to develop materials and methods and perform binding and functional characterizations of anti-Siglec-15 antibodies, in particular, those chimeric antibodies. *See* Diligence Chart, Entries 043-074. The activities included: expressing mouse Siglec-15 from 2936E cells and testing the binding affinity of an anti-human Siglec-15 monoclonal antibody (Ex. 2152, pp. 72-74); expressing the Siglec-15-Fc fusion protein to be used in Siglec-15 binding assays (Ex. 2152, p. 53); designing an immunohistochemistry ("IHC") protocol based on standard methods to visualize the specific binding between anti-Siglec-15 antibodies and Siglec-15 in various human and mouse tissues with assistance from a third party contractor at McGill University (Ex. 2159; Ex. 2152, pp. 83-85); and performing osteoclast differentiation assays testing the anti-Siglec-15 antibodies on both human and mouse bone marrow precursor cells and analyzing the results using TRAP staining (Ex. 2152, pp. 75-79; Ex. 2177; Ex. 2176).

From July 20, 2009 to August 28, 2009, Sasseville performed bioinformatic analysis of various anti-Siglec-15 antibodies to fine tune the selection of the candidate antibodies and allow further characterization of the Siglec-15 binding ability in order to select leads for further testing in animal studies. *See* Diligence Chart, Entries 70-99. At this time, Pagé performed binding affinity comparisons of the anti-Siglec-15 Fab regions compared to the whole chimeric antibodies to assess

the extent to which the chimeric antibodies retained binding affinity for Siglec-15. The Siglec-15-Fc fusion protein described above was used in these experiments. Ex. 2152, pp. 80-82, 86-89. Roy and Pagé also expressed and purified more chimeric anti-Siglec-15 antibodies for continued experiments. Ex. 2152, p. 53; Ex. 2168.

Experiments characterizing anti-Siglec-15 antibodies continued from August 31, 2009 until October 16, 2009. *See* Diligence Chart, Entries 100-132. Kalbakji performed another round of osteoclastogenesis assays testing chimeric anti-Siglec-15 antibodies on mouse bone marrow cell cultures as well as an IHC staining of Siglec-15 expressed in bone tissue slices using anti-Siglec-15 antibodies. Ex. 2169; Ex. 2152, p. 94. Sasseville performed binding studies with several chimeric anti-Siglec-15 antibodies. Ex. 2152, pp. 91-93. Pagé expressed and purified another batch of the anti-Siglec-15 [redacted] chimeric antibodies. Ex. 2152, pp. 95-97. Fortin performed Western analysis using the anti-Siglec-15 [redacted] chimeric antibodies and anti-Siglec-15 omniclonal antibody, and also cloned RANK ligand into different expression vectors for future functional analyses. Ex. 2170; Ex. 2153 pp. 52-57.

In early September 2009, Dr. Tremblay delivered the results of the functional assays of the anti-Siglec-15 antibodies to Alethia's outside intellectual property attorney, Dr. Janique Forget, so that she could begin drafting the '943

patent application in collaboration with the inventors. Diligence Chart, Entries 103-132. From September 3 through 29, 2009, Dr. Forget worked continuously on drafting the provisional application (eventually filed as U.S. Provisional Application Ser. No. 61/248,960 on October 6, 2009) and the '943 patent application. Dr. Forget's patent drafting and preparation efforts continued through the filing of the provisional application on October 6, 2009 and then through the filing of the '943 application on October 16, 2009. Ex. 2102, ¶¶ 5-9.

The records showing the activities of the inventors and their team were maintained regularly and continuously in the course of business and have been authenticated by Alethia's records custodian. Ex. 2103. Dr. Forget's work in preparing the patent applications is corroborated by her billing records, e-mail communications with Dr. Tremblay and members of his team, and drafts of patent applications relating to the '181 patent. Dr. Forget's records were maintained regularly and continuously in the ordinary course of business by the Fasken Martineau DuMoulin law firm ("Fasken"), and have been authenticated by Fasken's records custodian. Ex. 2104. The dates patent applications were filed with the USPTO are corroborated by filing receipts that have been downloaded from the USPTO website, Public Pair.

C. The '943 Application Constructively Reduced to Practice the Invention Claimed in the '181 Patent

The '181 patent issued directly from the '943 application. The '943 application constitutes a constructive reduction to practice because it sufficiently describes and enables the invention of the '181 patent in accordance with Section 112. *Frazer v. Shlegel*, 498 F.3d 1283, 1287-88 (Fed. Cir. 2007). Daiichi has not challenged whether the '943 application adequately describes and enables the claims of the '181 patent, and is statutorily *precluded* from doing so in this proceeding. 35 U.S.C. § 311(b). Nonetheless, as discussed more fully below (including in the claim chart *infra* at 20-24), there can be no serious dispute that the '943 application fully complies with Section 112. Indeed, the '943 application discloses antibodies to Siglec-15 that inhibit osteoclast differentiation or bone resorption to the same extent as Daiichi's own '072 Publication, which at minimum gives the '181 patent priority "with respect to so much of the claimed invention as the reference happens to show." *In re Stempel*, 241 F.2d 755, 759 (CCPA 1957). In any event, the '181 specification is fully descriptive and enabling as set forth below:

Claim Language	Exemplary Support in '943 Application
1. A method of impairing osteoclast differentiation in a mammal in need thereof, the method comprising administering an antibody or antigen binding fragment which	"In yet an additional aspect, the present invention relates to ***a method of modulating*** (i.e., inhibiting, lowering, ***impairing***) ***osteoclast differentiation in a mammal in need, the method may comprise administering the antibody or antigen binding fragment of the present invention***." U.S. Patent App. No. 12/580,943 (" '943 application"), p. 9, ll. 1-3; *see also id*. at p. 9, ll. 4-24.

specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108) to said mammal.	"The present invention relates in another aspect thereof to an isolated ***antibody or antigen binding fragment capable of binding to a polypeptide*** …One such particular polypeptide may be, for example, ***SEQ ID NO.:2 or a variant*** having at least 80% sequence identity with SEQ ID NO.:2." *Id*. at p. 6, ll. 6-10. "…***SEQ ID NO.:2 or a SEQ ID NO.:2 variant (including*** SEQ ID NO.:4 and ***SEQ ID NO.: 108***)." *Id*. at p. 12, ll. 29-32. *See also id.* at Examples 8-15 and claims 23-24, as filed.
2. The method of claim 1, wherein the antibody or antigen binding fragment impairs an osteoclast differentiation activity of human Siglec-15 or murine Siglec 15.	*See* Claim 1 above. "The present invention relates in another aspect thereof to an isolated antibody or antigen binding fragment capable of binding to a polypeptide able to promote osteoclast differentiation and of ***interfering with (e.g., inhibiting) an osteoclast differentiation activity of the polypeptide***. One such particular polypeptide may be, for example, ***SEQ ID NO.:2 or a variant*** having at least 80% sequence identity with SEQ ID NO.:2." *Id*. at p. 6, ll. 6-10; *see also id.* at p. 12, ll. 29-32; Examples 14 and 15; and claims 23-24, as filed.
3. The method of claim 2, wherein the osteoclast differentiation activity is characterized by differentiation of osteoclast precursor cells into differentiated osteoclasts.	*See* Claim 2 above. "the method may ***comprise administering an antibody or antigen binding fragment that may be capable of modulating the differentiation of an osteoclast precursor cell (e.g., human osteoclast precursor cell, human primary osteoclast precursor cell) into a differentiated osteoclast*** and that is produced in mammalian cells (e.g., human cell)." *Id*. at p. 9, ll. 9-14; s*ee also id.* at Example 14.
4. The method of claim 2, wherein the antibody is a polyclonal antibody.	*See* Claim 2 above. "In an embodiment of the invention, the antibody may be, for example, ***a polyclonal antibody***." *Id*. at p. 6, ll. 22-23; *see also id*. at p. 8, ll. 13-15; p. 39, ll. 5-6; p. 41, ll. 16-18; and claim 4, as originally filed.
5. The method of claim	*See* Claim 2 above.

2, wherein the antibody or antigen binding fragment is a monoclonal antibody or an antigen binding fragment thereof.	"In another embodiment of the invention, the antibody or antigen binding fragment may be, for example, ***a monoclonal antibody or a fragment thereof***." *Id*. at p. 6, ll. 23-24; *see also id*. at p. 7, ll. 11-17; p. 8, ll. 13-15; Examples 12 and 13; and claim 5, as originally filed.
6. The method of claim 5, wherein the monoclonal antibody or antigen binding fragment is produced from an isolated mammalian cell.	*See* Claim 5 above. "The antibody or antigen binding fragment of the present invention may be ***produced from an isolated mammalian cell*** or by a hybridoma cell. …The isolated mammalian cell may be, for instance, a human cell." *Id*. at p. 6, ll. 28-32; *see also id*. at p. 8, ll. 16-18; Example 13; and claim 6, as originally filed.
8. The method of claim 6, wherein the antibody or antigen binding fragment comprises a constant region of a human antibody or a fragment thereof.	*See* Claim 6 above. "An exemplary embodiment of an antibody or antigen binding fragment of the present invention is one that may ***comprise (amino acids of) a constant region of a human antibody or a fragment thereof***." *Id*. at p. 7, ll. 1-3; *see also id*. at p. 7, ll. 7-10; Example 13; and claim 8, as originally filed.
9. The method of claim 8, wherein the antibody or antigen binding fragment comprises a framework region of a human antibody.	*See* Claim 8 above. "Another exemplary embodiment of an antibody or antigen binding fragment of the present invention is one that may ***comprise (amino acids of) a framework region of a human antibody***." *Id*. at p. 7, ll. 4-10; *see also id*. at claim 9, as originally filed.
10. The method of claim 2, wherein the antibody or antigen binding fragment is a F_V, a Fab, a Fab' or a (Fab')2.	*See* Claim 2 above. "Exemplary embodiments of antigen binding fragments include, for example, ***a FV (e.g., scFv), a Fab, a Fab' or a(Fab')2***." *Id*. at p. 7, ll. 26-27; *see also id*. at p. 39, ll. 15-17; Examples 12 and 13; and claim 12, as originally filed.
11. The method of claim 3, wherein the osteoclast precursor cells are	*See* Claim 3 above. "the method may comprise administering an antibody or antigen binding fragment that may be

human osteoclast precursor cells.	capable of modulating the differentiation of an osteoclast precursor cell (e.g., ***human osteoclast precursor cell, human primary osteoclast precursor cell***) into a differentiated osteoclast and that is produced in mammalian cells (e.g., human cell)." *Id*. at p. 9, ll. 9-14; *see also id*. at Example 14; and claim 13, as originally filed.
15. A method for inhibiting bone resorption comprising administering to a subject in need thereof, an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108).	"The present invention relates to the use of ***anti-Siglec-15 antibodies or antigen binding fragments*** as blockers of osteoclast differentiation and ***which may be used for impairing bone loss or bone resorption*** in bone-related diseases, such as cancer-induced severe bone loss." *Id*. at p. 5, ll. 27-29. "The present invention also relates to an isolated antibody or antigen binding fragment which may be capable of ***specific binding to SEQ ID NO.:2 or to a variant*** having at least 80% sequence identity with SEQ ID NO.:2 and of ***inhibiting a resorptive activity of an osteoclast***." *Id*. at p. 10, ll. 23-25. "…***SEQ ID NO.:2 or a SEQ ID NO.:2 variant*** (including SEQ ID NO.:4 and ***SEQ ID NO.: 108***)." *Id*. at p. 12, ll. 29-32. *See also id*. at Examples 8-15.
16. The method of claim 15, wherein the antibody or antigen binding fragment impairs an activity of human Siglec-15 or murine Siglec-15 in osteoclast precursor cells or in osteoclasts.	*See* Claim 15 above. "The present invention relates in another aspect thereof to an isolated antibody or antigen binding fragment capable of binding to a polypeptide able to promote osteoclast differentiation and of ***interfering with (e.g., inhibiting) an osteoclast differentiation activity of the polypeptide***. One such particular polypeptide may be, for example, ***SEQ ID NO.:2 or a variant*** having at least 80% sequence identity with SEQ ID NO.:2." *Id*. at p. 6, ll. 6-10; *see also id.* at p. 9, ll. 9-14; p. 12, ll. 29-32; Examples 14 and 15; and claim 24, as filed.
17. The method of claim 16, wherein the activity is osteoclastogenesis.	*See* Claim 16 above. "the method may comprise administering ***an antibody or antigen binding fragment that may be***

	capable of modulating the differentiation of an osteoclast precursor cell (e.g., human osteoclast precursor cell, human primary osteoclast precursor cell) into a differentiated osteoclast and that is produced in mammalian cells (e.g., human cell)." *Id*. at p. 9, ll. 9-14; *see also id*. at Examples 14 and 15; and claim 24, as filed.
18. The method of claim 15, wherein the antibody or antigen binding fragment inhibits osteoclast differentiation.	*See* Claim 15 above. "The present invention relates in another aspect thereof to an isolated ***antibody or antigen binding fragment capable of binding to a polypeptide able to promote osteoclast differentiation and of interfering with (e.g., inhibiting) an osteoclast differentiation activity*** of the polypeptide. One such particular polypeptide may be, for example, SEQ ID NO.:2 or a variant having at least 80% sequence identity with SEQ ID NO.:2." *Id*. at p. 6, ll. 6-10; *see also id*. at Examples 14 and 15.
19. The method of claim 15, wherein the antibody or antigen binding fragment is administered in combination with a drug or an hormone.	*See* Claim 15 above. "Another example is osteoporosis where the only current medications approved by the FDA for use in the United States are the anti-resorptive agents that prevent bone breakdown. ***Estrogen replacement therapy*** is one example of an ***anti-resorptive agent***. Others include ***alendronate (Fosamaxia biphosphonate anti-resorptive),[additional drugs or hormones omitted]***." *Id*. at p. 3, ll. 18-24; *see also id*. at p. 3, l. 25-p. 4, l. 2.
20. The method of claim 19, wherein the drug is an antiresorptive drug or a drug increasing bone mineral density.	*See* Claim 19 above.
21. The method of claim 15, wherein the subject in need thereof, suffers from a bone remodelling disorder.	*See* Claim 15 above. "The antibody or antigen binding fragment may thus be particularly useful to treat ***bone loss*** or ***bone resorption*** in patients suffering or susceptible of

	suffering from a condition selected from the group consisting of ***osteoporosis, osteopenia***, … ." *Id*. at p. 9, l. 27-p. 10, l. 4; *see also id*. at p. 1, ll. 10-22; p. 36, ll. 6-19.
22. The method of claim 21, wherein the bone remodelling disorder is associated with a decrease in bone mass.	*See* Claim 21 above.
23. The method of claim 21, wherein the bone remodelling disorder is selected from the group consisting of osteoporosis, osteopenia, … and damage caused by macrophage-mediated inflammatory processes.	*See* Claim 21 above.

VII. CONCLUSION

Daiichi's attempt to invalidate Alethia's patents through this *inter partes review* proceeding should be rejected. As demonstrated by Alethia's disclosures and the well-established methods and knowledge in the art, the '181 patent is both sufficiently described and properly enabled by the Alethia PCT.

Alternatively, for all of the reasons above, the '072 Publication is not prior art. As demonstrated by the evidence submitted herewith, Alethia conceived of each claim limitation in the '181 patent by February 2007, months before Daiichi filed the '072 Publication. During the entire relevant time period, the Alethia team and its prosecution counsel worked continuously to reduce the invention to practice

and file the '943 application on October 16, 2009. Finally, the '943 application undisputedly complies with the requirements of Section 112.

The Patent Owner here is a biotechnology company that made a pioneer invention and is working hard to develop a therapeutic antibody drug based on the invention that will have a real impact in patients' lives. Alethia has now also again confirmed that anti-Siglec-15 antibodies can indeed effectively inhibit osteoclast differentiation and bone resorption in animal models including primates, as envisioned by Alethia's inventors in the Alethia PCT. *See* Ex. 2100, ¶¶ 9-11. Alethia's lead antibody, based on its invention, is ready to be tested in humans in clinical trials. This therapeutic antibody can improve the bone health of millions of patients who are suffering from debilitating bone diseases. The challenged '181 patent plays a vital role in protecting Alethia's invention and its efforts to develop therapeutic antibody products from larger competitors like Daiichi.

For all of these reasons, Alethia respectfully requests that the Board confirm the patentability of claims 1-6, 8-11, and 15-23 of Alethia's '181 patent.

RESPONSE TO PETITIONER'S STATEMENT OF MATERIAL FACTS

Response to Statement 1: Deny.

Response to Statement 2: Deny.

Response to Statement 3: Deny.

Response to Statement 4: Deny.

Response to Statement 5: Deny.

Response to Statement 6: Deny.

Response to Statement 7: Deny.

Response to Statement 8: Deny.

Response to Statement 9: Admit.

Response to Statement 10: Admit.

September 28, 2015

Respectfully submitted,

CHOATE HALL & STEWART, LLP

/Fangli Chen/

Fangli Chen (Reg. No. 51,551)
Eric J. Marandett (admitted *pro hac vice*)
Daniel C. Winston (admitted *pro hac vice*)
Stephanie L. Schonewald (Reg. No. 72,452)
Two International Place
Boston, MA 02110
(617) 248-5000
(617) 248-4907

Janique Forget, Ph.D. (Reg. No. 58,995)
Alethia Biotherapeutics Inc.
141 Président-Kennedy Ave.
Suite SB-5100, 5th floor
Montréal, Québec
Canada, H2X 1Y4
(514) 858-7666 x202
(514) 858-5333

Attorneys for Patent Owner -- Alethia Biotherapeutics, Inc.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that the foregoing **CORRECTED PATENT OWNER'S RESPONSE** was served electronically in its entirety on Attorneys for Petitioner by filing this document through the Patent Review Processing System and via e-mail on September 28, 2015, to kschorr-IPR@foley.com, as consented to on page 1 of the Petition.

Respectfully submitted,

CHOATE HALL & STEWART, LLP

/Fangli Chen/

Fangli Chen (Registration No. 51,551)
Two International Place
Boston, MA 02110
(617) 248-5000
(617) 248-4907

Date: September 28, 2015

Attorney for Patent Owner -- Alethia Biotherapeutics Inc.

Paper No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

_____

DAIICHI SANKYO COMPANY, LIMITED
Petitioner
v.
ALETHIA BIOTHERAPEUTICS INC.
Patent Owner

_____

Case No. IPR2015-00291
U.S. Patent No. 8,168,181

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PETITIONER'S REPLY TO CORRECTED PATENT OWNER'S RESPONSE

Table of Contents

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1053	A. Scott et al., *Antibody therapy of cancer*, 12 NATURE 278-87 (2012)

I. Introduction

The Board, in instituting this *inter partes* review, found that Petitioner established a reasonable likelihood that challenged claims 1-6, 8-11, and 15-23 of U.S. Patent 8,168,181 ("the '181 Patent," Ex. 1001) are invalid under 35 U.S.C. 102(a), as anticipated by Daiichi Sankyo's WIPO Publication WO 2009/048072 ("the '072 Publication," Ex. 1002). Because the challenged claims are not adequately described or enabled in any document filed in 2006 or 2007 to which the '181 patent claims priority, these challenged claims of the '181 patent are not entitled to any such priority dates, and the instituted claims should be found unpatentable.

In response to the Board's institution decision, Patent Owner did not make any substantive arguments against the anticipatory effect of the '072 Publication but instead, focused entirely on the alleged sufficiency of disclosure in its 2007 priority document (the Alethia PCT, Ex. 1010) and also a misguided attempt to antedate the publication date. A preponderance of the evidence, however, proves that the '181 Patent is not entitled to a priority date earlier than April 16, 2009, and Patent Owner's attempt to antedate the '072 Publication fails to prove conception and continuous diligence during the critical period with corroboration, as required by *Price v. Symsek*, 988 F.2d 1187, 1190 (Fed. Cir. 1993). After the Board's

institution decision, Patent Owner has not met its burden to overcome the invalidity issues raised by the ’072 Publication based on antedating.

II. Claim Construction

Petitioner acknowledges Patent Owner’s acceptance of the interpretations of “specifically binds” and “bone resorption” that were advanced in the Petition for IPR (Paper 2 at 3-5). *See* Paper 39 at 17. Concerning the terms “osteoclast differentiation” and “osteoclast differentiation activity”, the outcome of this IPR would not change if the Board construes these terms according to Patent Owner or Petitioner because Patent Owner has not adequately described or enabled in its 2006 and 2007 priority documents a Siglec-15 antibody with any therapeutic activity.

III. The Alethia PCT and Its Priority Documents Fail to Provide Adequate Written Description of the Challenged Claims

As longstanding case law explains, “the ‘essential goal’ of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed.” *In re Barker,* 559 F.2d 588, 592 n.4, (CCPA 1977) (emphasis added).

But the Alethia PCT never once even mentions a Siglec-15 antibody specifically for use in a therapeutic context, as recited in the claims. Ex. 1045 at 90:25 to 91:6 (In response to whether the PCT publication discloses any antibody that specifically binds to mouse Siglec-15 and impairs osteoclast differentiation,

Dr. Filion said "No.") and 96:19-97:9 (In response to whether the Alethia PCT discloses administering Siglec-15 antibodies to inhibit bone resorption, Dr. Filion stated "Yes, in broad terms" (emphasis added) and failed to identify any specific example.).

Moreover, Patent Owner was not in actual possession of any antibody capable of binding Siglec-15 until well after its 2006 and 2007 priority dates (Ex. 1045 at 49:7-10), and was even further from finding Siglec-15 antibodies that possess the claimed therapeutic function at the time of those priority dates. Paper 39 at 72; Ex. 2105 at 7, Entry 027 (showing actual inhibition assays in osteoclasts with a Siglec-15 antibody did not begin until at least May 15, 2009); Ex. 1045 at 81:21-24 (In response to when Alethia had a Siglec-15 antibody in hand that inhibited osteoclast differentiation, Dr. Filion stated "That was around 2009, I believe.").

A. Therapeutic Properties of an Antibody, if Any, Are Not Predictable

Patent Owner contends in its Response that "by 2007, it already was clear to a skilled artisan that Siglec-15 is normally a cell surface protein and readily accessible to antibodies." Paper 39 at 11, citing Ex. 2074 at ¶ 17-21. But simply knowing an antibody binds a target protein exposed on a cell surface is not sufficient to reasonably predict that an antibody will have a therapeutic effect. Ex.

1046 at 39:5-9, 85:5-87 ("[S]ome will be inhibitory because they bind specifically to critical parts of the polypeptide and others may not and will not be inhibitory."); *see also* Ex. 1047 at 68:23-24 ("[T]here could be antibodies that don't inhibit.") and 94:12-17 (In response to whether it is correct that an antibody that binds a cell surface protein is necessarily inhibitory, Dr. Stein responded "It would have to be tested."); Ex. 1044 at ¶ 10-11(Cell surface expression alone is insufficient to determine whether an antibody binding to Siglec-15 would impair osteoclast differentiation and inhibit bone resorption, or promote both or do neither).

Of course, Patent Owner is well aware of the difficulties and predictability associated with obtaining an antibody with a specific therapeutic function, even for antibodies with targets expressed on the cell surface. Ex. 2167 at 1, 3; *see also* Ex. 1046 at 39:5-9, 85:5-7; Ex. 1048 at 1. Indeed, Patent Owner's June 2007 presentation (Ex. 2080) characterizes AB-0440, which is a cell surface protein now known as Tsp50, as "[o]ne of the most promising targets identified by Alethia." Ex. 2080 at 38. This optimistic characterization presumably was based on data in the presentation reporting decreased osteoclast activity with AB-0440 shRNA and inhibition of osteoclast differentiation with a polyclonal antibody that binds AB-0440. (Ex. 2080 at 17, 18; Ex. 1045 at 31:9-32:4). In fact, it appears that AB-0440 was a higher priority target than Siglec-15, as Patent Owner lists AB-0440 as one of three targets in its "Therapeutic product pipeline" (Ex. 2080 at 7), while AB-

0326 (Siglec-15) is only listed as a "key prospect" in its "Drug discovery pipeline." *Id*. at 38. By Patent Owner's own account, Tsp50 had therapeutic potential for treating bone loss with antibodies (*id*.), and as of 2007, Patent Owner had actually tested antibodies targeting Tsp50. *Id*. at 17; Ex. 1045 at 31:25-32:5. Indeed, even in April 2009, Tsp50 was considered "the Company's prioritized target in its severe bone loss program" when Patent Owner announced that "[l]ead candidate [Tsp50] monoclonal antibodies are currently under evaluation for animal studies that will commence soon." Ex. 1049. But, these early hopes for the success of Tsp50 were not borne out.

At least as of July 31, 2009, Patent Owner in its progress report for Biosite (Ex. 2167) stated that "[w]ork on anti-Tsp50 antibodies also progressed but the lack of cross-reactivity of the antibodies between the mouse and human Tsp50 coupled with a relatively low efficacy in cell-based osteoclast differentiation assays led to some important strategic changes in the severe bone loss program." Ex. 2167 at 1 (emphasis added). The same progress report explained that "[i]n subsequent experiments, it was difficult to reproduce the results described above [with the chimeric monoclonal Tsp50 antibodies] . . . ." *Id*. at 3 (emphasis added). So, while Tsp50 had been validated using the same shRNA methods taught in the Alethia PCT (Ex. 2080 at 17), and had been additionally validated with polyclonal antibody data (Ex. 2080 at 18), Patent Owner found after further

experimentation that Tsp50 antibodies did not perform as predicted (Ex. 2167 at 1, 3) and the target was deprioritized (Ex. 1045 at 32:6-10, 31:25-32:10).[1] Thus what once was a "most promising target" lost its luster when monoclonal antibodies to that target were evaluated. *See* Ex. 2167 at 1.

The unpredictability surrounding whether a given antibody will produce clinically beneficial effects is well understood by those of ordinary skill in the art, as not every protein target studied *in vitro* translates into an *in vivo* method of treatment. Ex. 1046 at 34:2-6 ("[I]n vivo findings may be different from in vitro findings."), and 39:5-9 ("In my understanding when antibodies are being generated, some will be inhibitory because they bind specifically to critical parts of the polypeptide and others may not and will not be inhibitory."); *see also* Ex. 1048 at 1; Ex. 1003 at ¶ 13, 15. This underscores the unpredictability inherent in developing antibodies for a specific therapeutic purpose, and is consistent with Petitioner's position regarding the inadequacy of Patent Owner's priority documents. *See* Paper 1 at 17; *see also* Ex. 1003 at ¶ 15; Ex. 1004 at ¶ 25.

[1] Not surprisingly, Patent Owner failed to pay the maintenance fees on its first patent relating to methods of identifying compounds that bind Tsp50 and inhibit osteoclast differentiation. Ex. 1050.

B. Patent Owner's Reliance on the "Antibody Rule" is Misplaced

Patent Owner relies heavily on the MPEP and the "antibody rule" for rebutting the lack of written description in the Alethia PCT. Paper 39 at 21-31. Specifically, Patent Owner alleges that "'As long as an applicant has disclosed a *fully characterized antigen*, either by its structure, formula, chemical name, or physical properties,' the applicant can claim a targeting antibody without disclosure of its physical or chemical properties." Paper 39 at 21 (*citing Noelle v. Lederman*, 355 F.3d 1343, 1349 (Fed. Cir. 2004)). But Patent Owner is not claiming an antibody. The '181 Patent claims a method of impairing osteoclast differentiation and inhibiting bone resorption. Thus, the "antibody rule" is *per se* irrelevant to the claims at issue.

Nevertheless, Patent Owner argues that the "fully characterized antigen" allegedly disclosed in the Alethia PCT is sufficient to describe a therapeutic Siglec-15 antibody because "procedures for generating antibodies" and "using such techniques with well-known osteoclastogenesis assays to generate and identify antibodies that specifically inhibit Siglec-15" are included in the specification. Paper 39 at 24. Patent Owner supports this position in part with expert testimony from Dr. Boyce, stating that "the discovery of the essential role of Siglec-15 in osteoclast differentiation made it an obvious target for the development and use of therapeutic antibodies to impair osteoclast differentiation or inhibit bone resorption

by inhibiting Siglec-15 activity." Ex. 2074 at ¶ 16. But a disclosure that makes something obvious may not be adequate to establish possession, as the Federal Circuit has explained:

> [W]hile the description requirement does not demand any particular form of disclosure, *Carnegie Mellon Univ. v. Hoffmann-La Roche Inc.*, 541 F.3d 1115, 1122 (Fed. Cir. 2008), or that the specification recite the claimed invention *in haec verba*, a description that merely renders the invention obvious does not satisfy the requirement, *Lockwood v. Am. Airlines*, 107 F.3d 1565, 1571-72 (Fed. Cir. 1997).

Ariad Pharmaceuticals, Inc. v. Eli Lilly & Co., 598 F.3d 1336, 1352 (Fed. Cir. 2010) (*en banc*).

Moreover, even if the Alethia PCT made Siglec-15 an "obvious target" for development of a therapeutic antibody, such an invitation to experiment fails to provide written description support for the instituted claims. *Ralston Purina Co. v. Far-Mar-Co.*, 772 F.2d 1570, 1575 (Fed. Cir. 1985). Accordingly, merely identifying a target for a yet to be developed antibody with a yet to be disclosed structural feature, having a yet to be confirmed function, is not sufficient to satisfy written description of a method of using such an antibody to elicit a specific therapeutic effect. *See Ariad*, 598 F.3d at 1352.

C. Patent Owner Improperly Dismisses Federal Circuit Written Description Case Law

Patent Owner identifies insignificant differences in the present facts from written description case law in an attempt to distinguish recent Federal Circuit decisions that contradict its position. Paper 39 at 41-44. For example, Patent Owner inaccurately asserts that *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916 (Fed. Cir. 2004) is not applicable here because the claims in *Rochester* were directed to methods of eliciting a biological effect by administering a small molecule drug instead of an antibody. Paper 39 at 42. Patent Owner's argument is contradicted in *Rochester* itself, where the court noted that the law of written description applies to chemical and biological claims alike. *Rochester* 358 F.3d at 925.

In fact, this case is similar to *Rochester* because like the disclosure at issue in that case, the Alethia PCT fails to disclose any actual examples of anti-Siglec-15 antibodies with the recited therapeutic function, and provides nothing more than a means of performing trial-and-error research to find an antibody that could be used in the method claims of the '181 Patent. The court in *Rochester* found such a disclosure was insufficient to satisfy written description, explaining "[i]t is not a question whether one skilled in the art might be able to construct the patentee's device from the teachings of the disclosure of the application. Rather, it is a

question whether the application necessarily discloses that particular device." *Rochester* 358 F.3d at 923 (quoting *Jepson v. Coleman*, 314 F.2d 533, 536 (CCPA 1963)).

Further, Patent Owner alleges that in *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341 (Fed. Cir. 2011), the disclosure of a well-known antigen only was held to be insufficient because the claimed invention was the specific improvement of anti-TNFα antibodies that was "based on undisclosed specific structural and mechanistic features of the antibody." Paper 39 at 43. But *Centocor* is more similar to the '181 patent claims than Patent Owner admits; the Alethia PCT also fails to provide *any* structural information about *any* antibody, much less a Siglec-15 antibody, that would function in the claimed methods. *See Centocor*, 636 F.3d at 1246-47. Because not all Siglec-15 antibodies will be inhibitory (*see* Ex. 1045 at 91:7-10; Ex. 1046 at 39:5-9; Ex. 1047 at 68:23-24), and perhaps not even any will be (*see* Ex. 1044 at ¶ 10-11), the antibodies recited in the instituted claims embody nothing more than "a wish list of properties" for which the specification "at best describes a plan for making….and then identifying." *Centocor*, 636 F.3d at 1251. Such a disclosure does not satisfy the written description requirement. *Id*.

While Patent Owner agrees that the antibodies in the claims at issue in *AbbVie Deutschland GmbH & Co. v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1290

(Fed. Cir. 2014), require a particular function (an affinity rate (k_{off})), and therefore, should have included further characterization of the antibodies in order to satisfy the written description requirement, it disputes that it should be required to do the same. Paper 39 at 43. In particular, where the claims at issue in *AbbVie* were directed to a neutralizing antibody that bound to IL-12 with a specific dissociation constant (*Abbvie*, 759 F.3d at 1292), Patent Owner argues that its claims are different because the function of its claimed methods are not tied to any such structural features. Paper 39 at 44.

Of course this rationale is flawed. Because the instituted claims encompass a genus of antibodies, the specification must disclose a "representative number of species" in order to satisfy the written description requirement. *Abbvie*, 759 F.3d at 1300. The antibodies recited in the instituted claims have the function of "impairing osteoclast differentiation" or "inhibiting bone resorption." Like the binding constant property in *AbbVie*, these functions are tied to the structure to the antibody. Ex. 1051 at Abstract, 1; *see also* Ex. 2167 at 3. Accordingly, as in *AbbVie*, "structural features common to the members of the claimed genus" needed to have been disclosed, and the Alethia PCT needed to have done more than identify a putative general binding target in order to satisfy the written description requirement. *See AbbVie*, 759 F.3d at 1290, 1299; *see also Centocor*, 636 F.3d at 1350-51.

IV. The Alethia PCT and Its Priority Documents Fail to Enable the Challenged Claims

To be enabling under section 112, the specification of a patent must teach those skilled in the art "how to make and how to use the invention as broadly as it is claimed" without undue experimentation. *See In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991) Furthermore, the scope of the enablement must be commensurate with the scope of the claims. *See Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1216-17 (Fed. Cir. 1991). In other words, where a range of options are claimed, there must be enablement of the full scope of the range. *See Liebel-Flarsheim Co. v. Medrad, Inc.*, 481 F.3d 1371, 1380 (Fed. Cir. 2007).

A. A Siglec-15 Antibody With Therapeutic Properties is Not Enabled by the Alethia PCT

It is undisputed that, by February 13, 2007, a number of antibody therapeutics had been developed for certain indications (Ex. 2089 at Abstract), and that general methods of producing both monoclonal and polyclonal antibodies were known in the art. Ex. 2086. But the '181 Patent does not claim just any antibody that binds a particular target or a method of making such an antibody; it claims a method of *impairing* osteoclast differentiation or *inhibiting* bone resorption by administering an antibody or antigen binding fragment that specifically binds human or mouse Siglec-15. Ex. 1001 at 181:36-41, 182:44-48. Accordingly, the claim requires that the antibody have a precise therapeutic

function. Paper 39 at 51. Thus, the issue is not whether the Alethia PCT enables methods for creating *any* antibody at the time of filing the PCT; the issue is whether the Alethia PCT enables an antibody for use in the claimed method of treatment without undue experimentation at the time of filing the Alethia PCT. This is a burden that the Alethia PCT cannot satisfy. The Alethia PCT does not teach how to make an antibody inhibitor of Siglec-15 that functions as required by the claims.

In fact, because not every antibody will have a therapeutic function (Ex. 1045 at 91:7-10; Ex. 1046 at 39:5-9; Ex. 1047 at 68:23-24), the Alethia PCT only provides a starting point for further research. The Federal Circuit has repeatedly held such disclosures are non-enabling. *See Wyeth v. Abbott Laboratories*, 720 F.3d 1380, 1386 (Fed. Cir. 2013); *ALZA Corp. v. Andrax Pharmaceuticals, LLC*, 603 F.3d 935, 941 (Fed. Cir. 2010). Although Patent Owner would have the Board believe that one of skill in the art would need nothing more than the osteoclastogenesis assay disclosed in the Alethia PCT (*see* Paper 39 at 55), Patent Owner actually used several different types of assays and experiments to later find an antibody capable of performing the functions recited in the challenged claims, including epitope mapping, functional characterization of lead sequences, and bioinformatics analysis. Paper 39 at 73-76; Ex. 2105 at 3, 21-23, 28-29. Neither these methodologies nor the information they revealed are disclosed in the Alethia

PCT (Paper 39 at 74-76), yet this kind of additional characterization and experimentation is essential to successful development of antibodies with a therapeutic function. *See* Ex. 1053 at 3; Ex. 1048 at 1. Accordingly, the methods claimed in the '181 Patent required considerable amount of time, labor, and undue experimentation beyond what was disclosed in the Alethia PCT. *See ALZA Corp.*, 603 F.3d at 941.

Further, Patent Owner misrepresents the testimony of Petitioner's experts and misapplies statements made about antibodies in general (*i.e.* those without therapeutic functions) to the <u>therapeutic</u> antibodies recited in the claims. Paper 39 at 50. For instance, Patent Owner cites to a section of Dr. Clark's testimony regarding the 99% certainty of being able to create an antibody that binds a target, but ignores the remainder of his testimony that explains this was without regard to function. Ex. 2075 at 273:15-274:24 (on redirect, Dr. Clark clarified that he meant there was a 99% chance of developing an antibody with "no additional functions of that antibody specified"). Accordingly, Patent Owner attempts to improperly support its position by focusing only on enablement of an antibody binding its target, and not on an antibody that would function therapeutically as required by the claims.

B. Nakamura Does Not Sufficiently Supplement the Disclosure in Alethia's PCT to Satisfy Enablement

1. Nakamura does not teach that Siglec-15 is accessible on the cell surface of osteoclasts

Patent Owner asserts that "[a]s early as 2004, Siglec-15 itself had been sequenced and characterized in great detail at the molecular and cellular level." Paper 39 at 11. Patent Owner relies almost exclusively on Nakamura (Ex. 2065) to show that "it already was clear to a skilled artisan that Siglec-15 is normally a cell surface protein and readily accessible to antibodies, despite its then unknown function." Paper 39 at 11, *citing* Ex. 2074 at ¶ 17-21; *see also* Ex. 2074 at ¶ 19-21; Ex 2076 at 29, 32-34. This proposition is plainly untrue; a skilled artisan would not know from Nakamura that Siglec-15 would be on the cell surface of osteoclasts. Ex. 1044 at ¶ 4-9. In this regard, paragraph [0154] of Nakamura states:

> [T]he expression of HRC12337 [Siglec-15] in peripheral blood monocytes was hardly confirmed when any stimulation was not applied, but it was increased when the stimulation by PMA+Ionomycin or PHA-L was applied… Thus, it is thought that HRC12337 is expressed on activated T-cells.

Ex. 2065 at 31 (emphasis added).

Although Nakamura demonstrates that Siglec-15 can be expressed on the cell surface of T cells (and not, as improperly understood by Dr. Boyce, monocytes from which osteoclasts are derived (Ex. 1046 at 110:16-23)), Nakamura is

completely silent with regard to osteoclasts. Ex. 1044 at ¶ 4. Further, T cells are not precursors of osteoclasts and a determination as to whether Siglec-15 is expressed on the surface of osteoclasts cannot be made one way or another based on Nakamura. Ex. 1044 at ¶ 4-5, 8-9.

Additionally, cell surface Siglec-15 expression was at low levels or absent on resting T cells and was only appreciably expressed on the cell surface when the T cells were artificially stimulated with pharmacological agents (phorbol 12-myristate 13-acetate ("PMA") and ionomycin, or leucoagglutinin ("PHA-L")). Ex. 2065 at 12, Figure 9; Ex. 1044 at ¶ 5-6; *see also* Ex. 2065 at 31. Nakamura similarly teaches that COS cells do not express Siglec-15 until transformed with a Siglec-15-expressing construct, which results in approximately a ten-fold increase in expression. Ex. 2065 at 31, and 11, Figure 8; Ex. 1044 at ¶ 6. These data would indicate that Siglec-15 was only substantially expressed in an artificial system, which may not correlate with the *in vivo* situation, and stimulation is needed to activate transcription of the Siglec-15 gene. Ex. 1044 at ¶ 5-7, 10. Moreover, cell surface expression of Siglec-15 in one cell type is not confirmatory of cell surface expression of that protein in a different cell type. Ex. 1044 at ¶ 8. Therefore, contrary to Patent Owner's position, Nakamura is not the "critical prior art" reference that establishes cell surface accessibility of Siglec-15 and supports enablement. *See, e.g.*, Paper 39 at 2, 26, 27, 30.

Patent Owner also relies on expert testimony from Dr. Boyce to state that had Dr. Crocker been aware of Nakamura, he would have come to a different conclusion regarding the understanding of Siglec-15 in 2006-2007. (Paper 39 at 30, 38 *citing* Boyce Declaration Ex 2074 at ¶ 24, 26). But Dr. Crocker believes otherwise. After careful consideration of Nakamura, the reference did not dissuade him from his position that nothing in the art provided convincing evidence that Siglec-15 would be expressed on the cell surface of osteoclasts. Ex. 1044 at ¶ 9. Moreover, Patent Owner's other expert, Dr. Stein, is not an expert in Siglecs (Ex. 2076 at 40-51) and neither is Dr. Boyce ("[I first learned of Siglecs] when I was asked to give expert witness *[sic]* in this case [in July 2015]" (Ex. 1046 at 18:11-16)). Neither is Dr Boyce an antibody expert ("Well, I don't make antibodies. If I wanted to make an antibody, I would have a company or someone make it for me." (Ex. 1046 at 80:15-17)). Thus, the value of their "expert" testimony with regard to the relevance of Nakamura's teachings concerning Siglec-15 cell surface expression is questionable.

2. <u>One of skill in the art would not have found the Nakamura reference at least as of the Alethia PCT filing date</u>

Contrary to testimony from Patent Owner's own expert, Patent Owner incorrectly argues that one of ordinary skill in the art would have found Nakamura as of the filing date of the Alethia PCT and its priority documents. *See, e.g.*, Paper 39 at 27; Ex. 2058, at 193:17-194:11; Ex. 2075, at 39:5-40:10. But as Dr. Filion

stated in his deposition with regard to Nakamura and HRC12237, "if that sequence was in the public domain, we would have found that" (Ex. 1045 at 70:22-23) and "it was probably not in databases in the public domain" (*id*. at 71:5-6) and ". . . probably at the time where this patent was published[,] that was not common practice to publish sequences from patent applications in databases such as GenBank." *Id*. at 69:8-14. Dr. Filion also stated that he did not become aware of Nakamura until "after we filed our patent application." *Id*. at 68:24-25.

The testimony from Drs. Stein and Boyce on this point should not be given any weight, as neither had conducted sequence searches during the relevant timeframe, neither is competent to speak to the availability of certain sequences in 2006 or 2007, and neither knew whether the sequences even were publicly available during that time. Ex. 1047 at 44:9-45:10, 45:24-47:7; Ex. 1046 at 58:4-6, 59:19-23, 60:9-13.

Other than arguing that HRC12237 (Siglec-15) could have been found during the relevant timeframe only because it can be found now (Ex. 1047 at 44:9-23, 46:5-7), Patent Owner has not provided any evidence that searching for the amino acid sequence of Siglec-15 at the time of the filing date of the Alethia PCT, would have led one of skill in the art to Nakamura.

V. Patent Owner Has Not Met Its Burden of Antedating the Prior Art '072 Publication

Patent Owner seeks to establish that the '072 Publication is not prior art and therefore bears the burden of producing evidence supporting a date of invention before the '072 Publication date. *Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1576-77 (Fed. Cir. 1996). As the Board has recognized, "[a]n inventor may antedate a reference if the inventor was the first to conceive of a patentable invention, and then connects the conception of the invention with its constructive reduction to practice by reasonable diligence on the inventor's part, such that conception and diligence are substantially one continuous act." *Olympus American, Inc. v. Perfect Surgical Techniques, Inc.*, IPR2014-00233, Paper 56 at 15 (*citing Mahurkar*, 79 F.3d at 1577).

Patent Owner has not met its burden of producing evidence supporting a date of invention before the publication date of the '072 Publication. In particular, Patent Owner has failed to establish conception prior to the '072 Publication and failed to establish continued, reasonable diligence through its asserted reduction to practice with corroboration.

A. The Alethia PCT and Ex. 2080 Are Insufficient To Meet Patent Owner's Burden Of Establishing A Conception Date

Patent Owner alleges that the inventors conceived the invention of the '181 patent at least as of February 13, 2007, when they filed the Alethia PCT or at least

as of June 19, 2007, the date of Alethia's presentation to Daiichi Sankyo. Paper 39 at 13. But the Alethia PCT and the 2007 presentation (Ex. 2080) do not meet the Patent Owner's evidentiary burden for establishing conception.

First, as discussed above with regard to the lack of written description and enablement of the Alethia PCT, there is no disclosure of antibodies that could function in the claimed methods. Second, the claimed subject matter is recognized as unpredictable and therefore could not have been conceived until it was determined that the antibodies recited in the claims actually worked for their intended purpose as claimed in the methods. *See Mycogen Plant Sci., Inc. v. Monsanto Co.*, 243 F.3d 1316, 1330 (Fed. Cir. 2001). Further, the June 2007 presentation contains an additional deficiency, in that it is a pitch by Alethia in pursuit of a joint collaboration (Ex. 2080 at 1) and does not constitute probative evidence of conception, as described in more detail below. *See Kridl v. McCormick*, 105 F.3d 1446, 1449 (Fed. Cir. 1997).

1. <u>The claimed therapeutic methods are sufficiently unpredictable that patent owner could not have conceived of the invention without significant experimentation</u>

It is well established that, in the unpredictable arts such as chemistry and biology, conception often occurs simultaneously with reduction to practice. *Mycogen*, 243 F.3d at 1330. In *Mycogen*, the Federal Circuit analyzed the requirements for establishing conception of claims related to transgenic plants that

were modified to express a pesticidal toxin. *Id*. at 1322-24. The court noted that "[i]t seems plausible to find that the type of invention embodied in these claims might not have been conceived until it was determined that the process claimed actually did [produce the claimed function]." *Id*. at 1331. Indeed, when results at each step do not follow as anticipated, but are achieved empirically by what amounts to "trial and error," a patentee will have greater difficulty proving conception prior to reduction to practice. *Alpert v. Slatin*, 305 F.2d 891, 894 (CCPA 1962).

Even after the Alethia PCT was filed, Patent Owner was still conducting "trial and error" experimentation that the *Alpert* court characterized as evidencing a lack of conception. *See Alpert*, 305 F.2d at 894. For example, according to Patent Owner's own account, it was still screening and sequencing fragments of antigen-binding sequences as of April 17, 2009. *See* Ex. 2105, Diligence Chart, pp. 1-2 (screening of 46 candidate fragment antigen-binding (Fab) sequences); *see also* Paper 39 at 72-73. Further, Patent Owner's records indicate that it was not until at least May 15, 2009 when any of their candidate antibodies were actually tested *in vitro* to determine their effect on isolated osteoclasts. Ex. 2105 at 7; Paper 39 at 74. Because critical research activity was still necessary before identifying a Siglec-15 antibody with therapeutic efficacy (*see generally* Ex. 2105), "the mental embodiment of that [claimed conception] date [embodied in the Alethia PCT] was

a mere hope or expectation, a statement of a problem, but not an inventive conception." *See Alpert*, 305 F.2d at 894.

2. The Alethia PCT is not a definite and permanent idea of the complete and operative invention as claimed

Patent Owner shoehorns disparate portions of the Alethia PCT specification into a claim chart in an attempt to show conception. Paper 39 at 63-68. But even this exercise pieces together little more than a general goal or plan that Patent Owner may have hoped to achieve, and fails to establish "a definite and permanent idea of the complete and operative invention" as required to establish conception. *See Burroughs Wellcome Co. v. Barr Labs.*, 40 F.3d 1223, 1228 (Fed. Cir. 1994); *see also Hitzeman v. Rutter*, 243 F.3d 1345, 1356-57 (Fed. Cir. 2001) (holding that inventor's "hope" that a genetically altered yeast would produce antigen particles having the particle size and sedimentation rates recited in the claims did not establish conception.); *Coleman v. Dines*, 754 F.2d 353 (Fed. Cir. 1985) ("It is settled that, in establishing conception, a party must show possession of every feature recited in the count.").

Hitzeman provides relevant insight, in its explanation of the *Burroughs* holding:

> *Burroughs* concerned six patents directed toward administering a drug, AZT, to AIDS patients. It was undisputed that the inventors had already synthesized the AZT. The claims of the first five patents

> recited various permutations of administering the AZT to patients, without reciting details of how the human body would react to the drug. As to the claims of these five patents, we held… that the developers of AZT had sufficiently established conception of the limitations of the claims (*i.e.*, the drug itself and the intention to administer it to humans), and that it was immaterial that the inventors lacked a "reasonable expectation" as to how non-claimed aspects of the drug would work (*i.e.*, the particular effect of the drug on the body). However, as to the claims of the sixth patent, which recited details of an anticipated immune response to the drug (*i.e.*, "a method of increasing the number of T-lymphocytes in a human infected with the [HIV] virus …."), we held that this claim was not conceived in advance of further studies because of uncertainty as to whether administering AZT actually would promote T-lymphoctye production, *i.e.*, the claimed intended use. Thus, the inventors in *Burroughs* lacked a "definite and permanent idea" as to whether this recited claim limitation of the sixth patent would be met by administering the drug. In the present case, like the claims of the sixth patent discussed in *Burroughs*, Hitzeman claimed the specific result of a biological process. Because Hitzeman failed to show that he had a reasonable expectation that the claimed result of the biological process would occur, his conception argument cannot prevail.

Hitzeman, 243 F.3d at 1358 (*citing Burroughs*, 40 F.3d at 1225-32) (internal citations omitted).

Like the sixth patent at issue in *Burroughs* and that patent at issue in *Hitzeman*, the challenged claims of the ’181 Patent require specific results of biological processes. Thus, as for those patents, conception requires evidence of a reasonable expectation that the claimed results would occur. Patent Owner has produced no such evidence. Given the complete lack of experimentation or mention of any exemplary therapeutic antibodies, Patent Owner relies on the mere hope that it might one day be able to make antibodies with the requisite function necessitated by the claims, but that is insufficient to show conception. *See Hitzeman*, 243 F.3d at 1356-57. Thus, just as in *Burroughs* and *Hitzeman*, the Alethia PCT at best suggests a desire to produce the claimed subject matter, rather than “a ‘definite and permanent idea’ as to whether this recited claim limitation …would be met by administering the drug.” *See Hitzeman*, 243 F.3d at 1356-58.

3. Alethia’s Presentation of June 19, 2007 does not evidence conception

Patent Owner alleges that Alethia’s Presentation of June 19, 2007 (Ex. 2080) (“Alethia’s Presentation”) includes “convincing data demonstrating the essential role of AB0326 in osteoclast formation/differentiation and bone resorption… and identification of antibodies as therapeutic drug candidates to target AB0326.” Paper 39 at 69. However, to establish conception of claims that require a biological response, the evidence must show that the inventors had a “definite and permanent idea” that the claimed response would actually occur. *Hitzeman*, 243

F.3d at 1358 (*citing Burroughs*, 40 F.3d at 1225-32). Alethia's Presentation does not constitute such evidence.

Patent Owner has provided little more than conclusory statements about the "convincing data" in Alethia's Presentation (Paper 39 at 69; Ex. 2080 at 21), which do not address the issue of conception. Alethia's Presentation only describes Siglec-15 as an "attractive target" for antibody development (*see e.g.*, Ex. 2080 at 38). But a plan to target Siglec-15 for development is not sufficient to establish conception. *Burroughs*, 40 F.3d at 1228; *Amgen*, 927 F.2d at 1206 (finding no conception of a nucleic acid based solely on its proposed biological activity). Accordingly, Alethia's Presentation, alone or in combination with Alethia's PCT, is insufficient to establish conception.

B. Patent Owner Fails To Meet Its Evidentiary Burden For Antedating Due To Lack of Corroboration and, Even Ignoring Corroboration, Due To Failure to Show Reasonable Diligence

Patent Owner also must meet the evidentiary burden of demonstrating reasonable diligence in reducing the invention to practice during the critical period. *Mahurkar*, 79 F.3d 1572 at 1577. A party alleging diligence must provide corroboration with evidence that is specific both as to facts and dates. *Gould v. Schawlow*, 363 F.2d 908, 920 (CCPA 1966). The rule of reason does not dispense with the need for corroboration of diligence that is specific as to dates and facts.

Gould, 363 F.2d at 920; *Kendall v. Searles*, 173 F.2d 986, 993 (CCPA 1949); *see also Coleman*, 754 F.2d at 360.

Evidence in the form of a notebook may be weighed for whatever it is worth. *See Hahn v. Wong*, 892 F.2d 1028, 1033 (Fed. Cir. 1989). However, little weight should be afforded to an unwitnessed notebook, or a notebook witnessed well after the fact. *Id*. (stating that "affiants' statements that by a certain date they had 'read and understood' specified pages of [] laboratory notebooks did not corroborate a reduction to practice … because they established only that those pages existed on a certain date … [and] did not independently corroborate the statements made on those pages"). Furthermore, the testimony of an interested party is not sufficient to authenticate a document offered for purposes of corroboration in a diligence inquiry. *See Micorosoft Corp. v. Surfcast, Inc.*, IPR2013-00292, Paper 93 at 20 (*citing Kridl*, 105 F.3d at 1449 (Fed. Cir. 1997)). Patent Owner has failed to demonstrate reasonable diligence under these governing legal principles.

1. Alethia Laboratory Notebook 110 is of little to no probative value

Patent Owner relies on Exhibit 2152, Alethia Laboratory Notebook 110, as evidence of diligence on numerous dates throughout the critical period. *See generally*, Ex. 2105. But the Alethia Laboratory Notebook 110 suffers from several fatal flaws, including that it was not maintained in accordance with good laboratory practices, at least according to Ex. 2103.

First, Alethia Laboratory Notebook 110 was countersigned well after the days on which the experiments were allegedly performed. Specifically, the witness—allegedly Dr. Matthew Stuible (*see* Ex. 1045 at 78:14-16)—signed almost every page of the notebook on April 15 or 16, 2010, more than a full year after the alleged dates of the earliest pages on which Patent Owner relies. Thus, Dr. Stuible's signature indicating that he "read and understood" specified pages of the laboratory notebook cannot attest to anything more than the fact that the pages of the laboratory notebook physically existed on April 15 or 16, 2010. *See Hahn*, 892 F.2d at 1033. Furthermore, Dr. Stuible was not even employed at Alethia during the time frame when the experiments reported in the cited notebook pages were allegedly performed (*see* Ex. 1052), further showing that he is not competent to corroborate them. Moreover, Patent Owner has not provided any declaratory evidence or other testimony from Dr. Stuible authenticating his signature.[2] Thus, the contents of Alethia Laboratory Notebook 110 relied upon by Patent Owner are not corroborated, and so cannot support Patent Owners' assertions that certain research activity identified in the Diligence Chart was performed at all, much less that it was performed on the alleged dates during the critical period.

[2] Petitioner's request on November 4, 2015 to depose Dr. Stuible was denied by counsel for Patent Owner because, according to Patent Owner's Counsel, direct testimony from Dr. Stuible had not been submitted.

Second, the entries in Alethia Laboratory Notebook 110 appear to have been made by multiple researchers, including Annie Fortin, Aida Kalbakji, Martine Pagé, and Marc Sasseville, none of which attested to the authenticity of the notebook or their signatures. The Federal Circuit has addressed the minimal value of a laboratory notebook in a similar situation:

> Where a laboratory notebook authored by a non-inventor is offered into evidence pursuant to authentication by an inventor, where the author of the notebook has not testified at trial or otherwise attested to its authenticity, and where the notebook has not been signed or witnessed and has not been maintained in reasonable accordance with good laboratory practices sufficient to reasonably ensure its genuineness under the circumstances, then the corroborative value of the notebook is minimal.

Medichem S.A. v. Rolabo S.L., 437 F.3d 1157, 1173 (Fed. Cir. 2006).

Further, Yves Cornellier acknowledged that "[i]t is a 'best practice' at Alethia for another laboratory researcher to countersign a laboratory notebook soon after information has been entered" (Ex. 2103 at ¶ 3). Yet, all of the pages in Alethia Laboratory Notebook 110 were signed anywhere from *one to three years later*. Ex. 2152 at 33-85. Thus, the notebook was not maintained in accordance with Patent Owner's own standard, let alone in reasonable accordance with good laboratory practices. Accordingly, Exhibit 2152, is of little to no probative value for the purposes of Patent Owner's motion to antedate.

2. The sequence submissions are not properly authenticated so as to provide corroborative value

The sequence submissions provided by Patent Owner (Exs. 2154-2158) are purportedly authenticated by Gilles Tremblay (Ex. 2101) and Yves Cornellier (Ex. 2103). Dr. Tremblay is a co-inventor of the '181 patent and Vice-President of Research at Alethia, and Mr. Cornellier is President and Chief Executive Officer at Alethia, and each party has an "interest" in the outcome of this proceeding. However, testimony of an interested party is not sufficient to authenticate a document offered for purposes of corroboration in a diligence inquiry. *See Microsoft Corp. v. Surfcast, Inc.*, IPR2013-00292, Paper 93 at 20 (*citing Kridl v. McCormick*, 105 F.3d 1446, 1449 (Fed. Cir. 1997)). Because Patent Owner has not provided evidence from an uninterested party to authenticate the alleged sequence submissions provided by Patent Owner (Exs. 2154-2158), the documents are of little probative weight.

3. Patent Owner has failed to establish reasonable diligence

Patent Owner provided a Diligence Chart purporting to show evidence that is specific both as to facts and dates during the critical period. Ex. 2105. However, when the independent, corroborated evidence is considered, the Diligence Chart contains multiple days on which there is no corroborated evidence of activity to support reasonable diligence or the relevance of the alleged support is entirely unclear. *See, e.g.*, Ex. 2105 at 1-2.

For example, Patent Owner's Response attempts to establish a diligence chronology starting at page 72. For the first set of dates in Patent Owner's Response, April 9-16, which are key to Patent Owner's ability to establish diligence began before the April 16th prior art publication date, the Response cites solely to Patent Owner's diligence chart. Paper 39 at 72-73, *citing* Ex. 2105. The diligence chart, in turn, cites to four other exhibits: Ex. 2152, Ex. 2157, Ex. 2101, and Ex. 2103. But the cited portions of Ex. 2152 are in French, and Patent Owner failed to provide a translation of the supposedly relevant pages. Ex. 2157, a list of undefined products that Patent Owner allegedly sent to the Genome Center for sequencing, makes no reference to Siglec-15 or AB-0326. The paragraphs cited in the diligence chart for Ex. 2101, which is Dr. Tremblay's Declaration, fail to make any reference to Siglec-15 or AB-0326 and do not relate the sequence submission in Ex. 2157 to Siglec-15 or Alethia Laboratory Notebook 110 (Ex. 2152) in any way. Ex. 2103 (Mr. Cornellier's declaration), which was cited in its entirety, does nothing more than attest that the submitted references are Patent Owner's records, and fails to indicate what they are records of, or why or how they might be supportive of diligence. Thus, Patent Owner has not established or explained how the cited evidence shows the activity alleged to have been performed before the critical period. Ex. 2105 at 1.

Patent Owner has left open gaps throughout its purported diligence period after the '072 prior art publication date as well. For example, Entry 007, referencing April 17, 2009, cites laboratory notebook pages 33-35, which compared to April 9, 2009 and April 20, 2009, respectively. But there are no pages in the laboratory notebook dated April 17, 2005. Additionally, Entries 007 and 008 in the diligence chart cite to laboratory notebook pages that are in French (Ex. 2152 at 2), and another sequence submission (Ex. 2156) that does not state its relationship to Siglec-15. Patent Owner provides no further explanation of the relevance of the cited pages. Patent Owner's naked assertions without substantive explanation of what it is citing is insufficient to fulfill its burden of showing diligence. *Kendall*, 173 F.2d at 993 (CCPA 1949) (diligence requires that applicants must be specific as to dates and facts). *See also Oracle Corp. v. Click-To-Call Tech. LP*, IPR2103-00312, Paper 52 at 19, 24-25 (a moving part must be "specific as to facts and dates for the entire critical period during which diligence is required") and *In re Mulder*, 716 F.2d 1542, 1542-46 (Fed. Cir. 1983) (a determination of lack of reasonable diligence, where the evidence of record was lacking for even a two-day critical period).

It is not the responsibility of the Board (or Petitioner), to scour the record in search of evidence relevant to a particular issue in order to make Patent Owner's case for it, and the Board should not have to strain to fit evidence together into a

coherent explanation to supports Patent Owner's argument. *Corning Inc. v. DSM IP Assets B.V.*, IPR2013-00049, slip op. at 14 (PTAB May 9, 2014). Because Patent Owner has not explained the evidence it cites, it has not met its burden to establish diligence.

VI. Patent Owner's Derivation Assertions Are Not Only False, But Also Are Irrelevant to the Patentability Questions in This IPR

Although completely irrelevant to the issues in this IPR, Patent Owner, throughout its Corrected Patent Owner Response, repeatedly insinuates that Petitioner stole or otherwise derived the subject matter of the '072 Publication from Patent Owner. Nevertheless, because the issues raised by Patent Owner were clearly intended to denigrate Petitioner's reputation, Petitioner addresses them here.

Patent Owner states that Petitioner filed its Japanese provisional application to which the '072 Publication claims priority, just four months [114 days] after meeting with Alethia in June 2007. Paper 39 at 61. But the first identification of AB0326 as Siglec-15 was not until Alethia's PCT Publication on August 23, 2007 (Ex. 1045 at 35:10-12), and Petitioner's provisional filing was only 49 days thereafter. Thus, Petitioner can only conclude that these allegations were made in bad faith as anyone reading the specification of the '072 Publication or any of its priority documents would immediately see that the individual experiments alone described therein took longer than 114 days.

For instance, Example 10 of the ’072 Publication (and the Japanese provisional application), disclose the production of rabbit anti-mouse Siglec-15 polyclonal antibodies, which takes at least 106 days (1 day for the first immunization + 14 days x 7 for the subsequent immunizations + 7 days for blood collection). Ex. 1023 at 95:8-9. And this does not even consider the amount of time that would have been required to produce the antigen used in Example 10, as discussed in Example 5 to 9 (Ex. 1023 at 82:6-94:14), or conduct the additional experiments in Examples 11-15 with the antibodies produced in Example 10. Ex. 1023 at 95:15-102:25. Hence, Petitioner necessarily had to have been working on this invention prior to the meeting on June 19, 2007, and certainly prior to the PCT publication on August 23, 2007.

Accordingly, any assertion that Petitioner derived the subject matter of the ’072 Publication from Patent Owner is knowingly and demonstrably false.

VII. Conclusion

The ’181 patent claims challenged in this IPR are not adequately described or enabled by its 2006 and 2007 priority documents and therefore, are not entitled to a priority date earlier than April 16, 2009. Accordingly, Petitioner’s ’072 Publication is 102(a) prior art and Patent Owner has failed to meet its burden of proof in showing prior conception and reasonably-diligent reduction to practice of the claimed subject matter to successfully antedate the reference. Thus, the

challenged claims of the ’181 Patent are invalid for lack of novelty over the ’072 Publication.

Respectfully submitted,

Dated: November 30, 2015

By: /Kristel Schorr/_____
Stephen B. Maebius
Registration No. 35,264

Kristel Schorr
Registration No. 55,600

Foley & Lardner LLP
Counsel for Petitioner

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing Petitioner's Reply to Patent Owner's Response was served on November 30, 2015, by email directed to the attorneys of record for the Patent Owner at the following addresses:

fchen@choate.com
emarandett@choate.com
dwinston@choate.com
sschonewald@choate.com
janique.forget@alethiabio.com
patentdocket@choate.com

Dated: November 30, 2015

By: /Kristel Schorr/

Stephen B. Maebius
Registration No. 35,264

Kristel Schorr
Registration No. 55,600

Foley & Lardner LLP
Counsel for Petitioner

US008168181B2

(12) **United States Patent**
Sooknanan et al.

(10) **Patent No.: US 8,168,181 B2**
(45) **Date of Patent: May 1, 2012**

(54) **METHODS OF IMPAIRING OSTEOCLAST DIFFERENTIATION USING ANTIBODIES THAT BIND SIGLEC-15**

(75) Inventors: **Roy Rabindranauth Sooknanan**, Beaconsfield (CA); **Gilles Bernard Tremblay**, La Prairie (CA); **Mario Filion**, Longueuil (CA)

(73) Assignee: **Alethia Biotherapeutics, Inc.**, Montreal (CA)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **12/580,943**

(22) Filed: **Oct. 16, 2009**

(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation-in-part of application No. 12/279,054, filed as application No. PCT/CA2007/000210 on Feb. 13, 2007, now Pat. No. 7,989,160.

(60) Provisional application No. 60/772,585, filed on Feb. 13, 2006, provisional application No. 60/816,858, filed on Jun. 28, 2006, provisional application No. 61/248,960, filed on Oct. 6, 2009.

(51) **Int. Cl.**
A61K 39/00 (2006.01)
A61K 39/395 (2006.01)

(52) **U.S. Cl.** **424/130.1**; 424/133.1; 424/134.1; 424/135.1; 424/139.1; 424/141.1; 424/142.1; 424/152.1; 514/16.7; 514/16.8; 514/16.9; 514/17.1

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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Primary Examiner — Elizabeth C Kemmerer

(74) *Attorney, Agent, or Firm* — Choate, Hall & Stewart, LLP; Fangli Chen; Robert N. Sahr

(57) **ABSTRACT**

This invention relates, in part, to unique and newly identified genetic polynucleotides involved in the process of bone remodeling; variants and derivatives of the polynucleotides and corresponding polypeptides; uses of the polynucleotides, polypeptides, variants and derivatives; and methods and compositions for the amelioration of symptoms caused by bone remodeling disorders. Disclosed in particular are, the isolation and identification of polynucleotides, polypeptides, variants and derivatives involved in osteoclast activity, validation of the identified polynucleotides for their potential as therapeutic targets and use of the polynucleotides, polypeptides, variants and derivatives for the amelioration of disease states and research purposes.

25 Claims, 11 Drawing Sheets

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WO	WO2009008727	1/2009
WO	WO2009023125	2/2009
WO	WO2009039854	2/2009
WO	WO2009031835	3/2009
WO	WO2009031836	3/2009
WO	WO2009032158	3/2009
WO	WO2009038756	3/2009
WO	WO2009146179	3/2009
WO	WO-2009048072	4/2009
WO	WO2009050453	4/2009
WO	WO2009059379	5/2009
WO	WO2009059972	5/2009
WO	WO2009061130	5/2009
WO	WO2009061890	5/2009
WO	WO2009132876	5/2009
WO	WO2009090651	7/2009
WO	WO2009106715	9/2009
WO	WO2009108261	9/2009
WO	WO2009112645	9/2009
WO	WO2009139599	11/2009
WO	WO2009020101	12/2009
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ENSEMBL Protein ID: ENSECAP00000015632; Jul. 19, 2010.
ENSEMBL Protein ID: ENSFCAP00000009910; Jul. 19, 2010.
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ENSEMBL Protein ID: ENSMLUP00000004457; Jul. 19, 2010.
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FIGURE 1

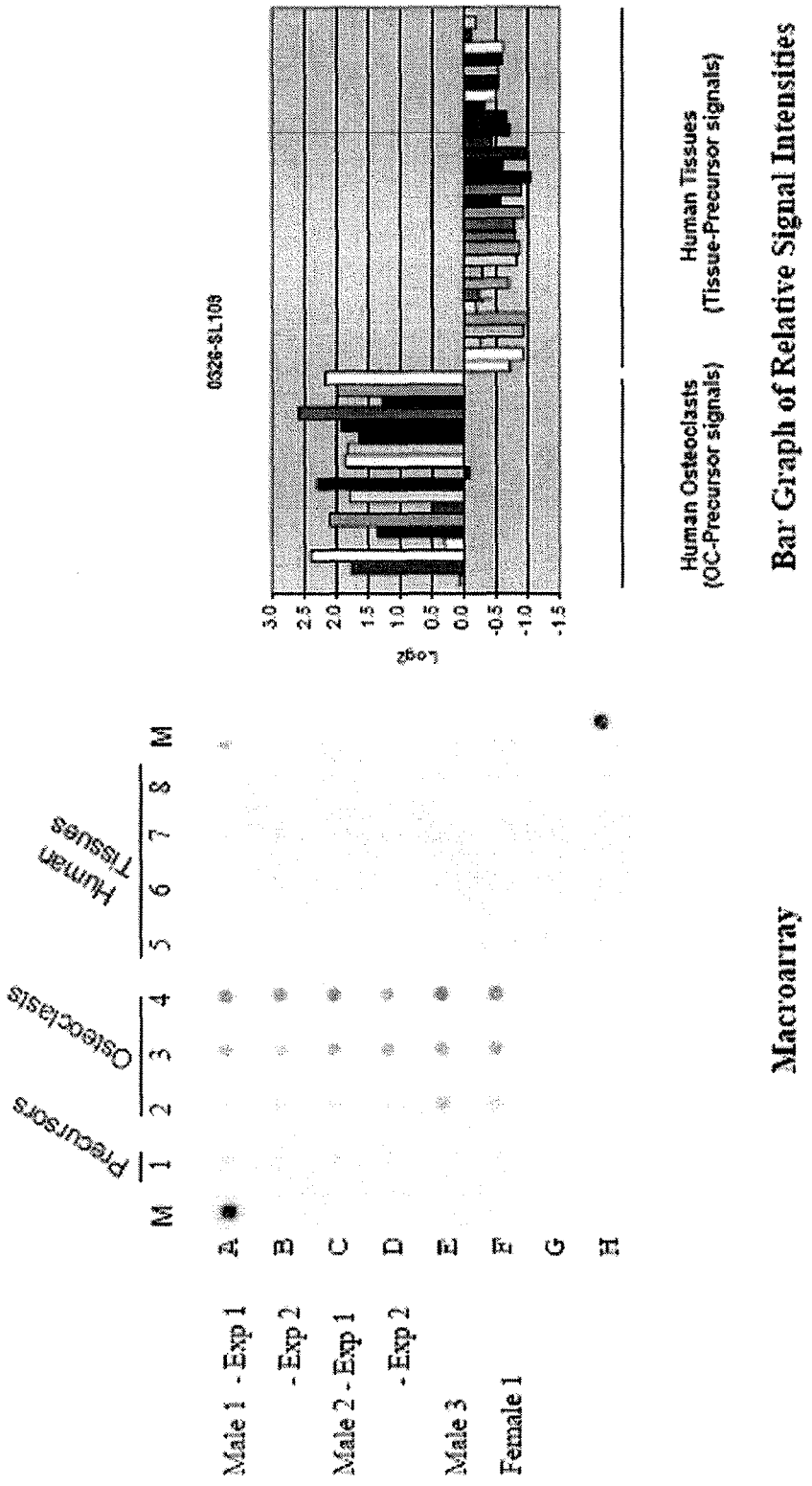

Macroarray
Bar Graph of Relative Signal Intensities
Human Osteoclasts (OC-Precursor signals)
Human Tissues (Tissue-Precursor signals)
0326-SL109
log2
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Precursors
Osteoclasts
Human Tissues
M 1 2 3 4 5 6 7 8 M
A
B
C
D
E
F
G
H
Male 1 - Exp 1
- Exp 2
Male 2 - Exp 1
- Exp 2
Male 3
Female 1

FIGURE 2

AB0326 and AB0369 are required for differentiation of human osteoclasts

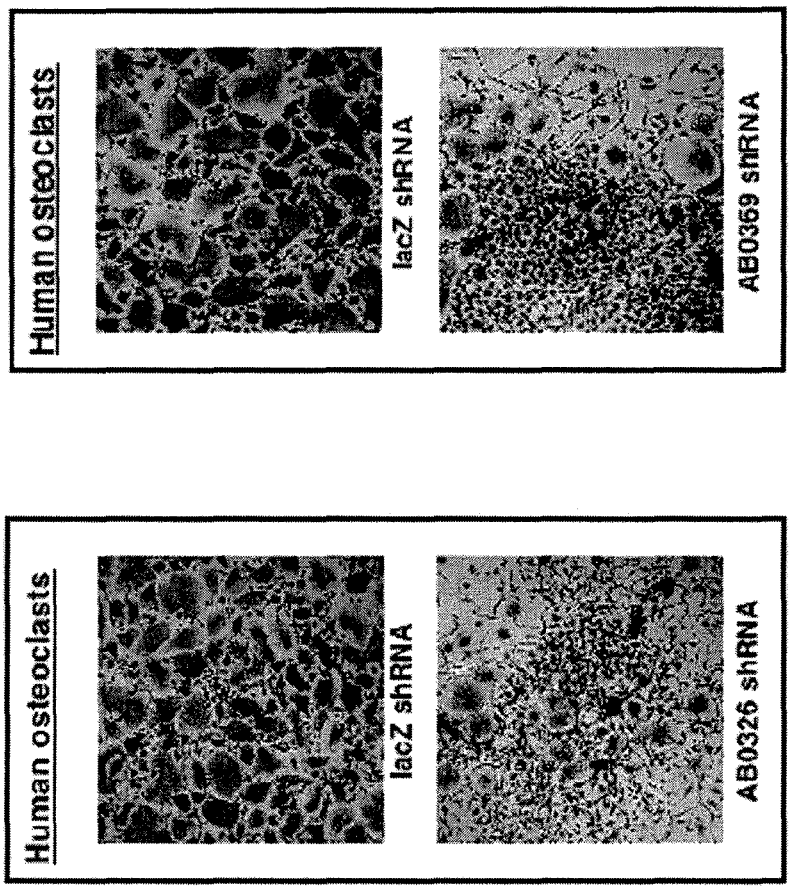


FIGURE 3

The knockdown effects on osteoclastogenesis of the mouse orthologue for AB0326 (SEQ. ID. NO. 35) in the RAW 264.7 model

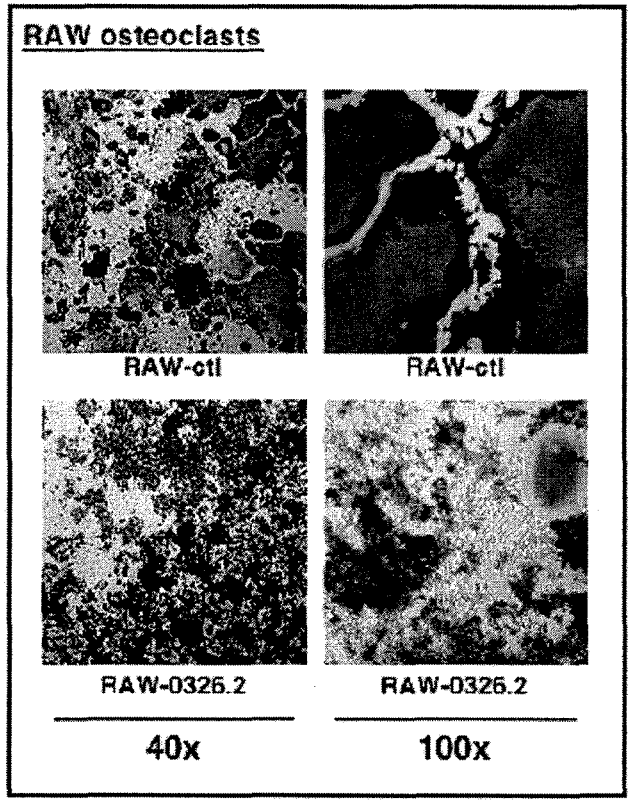
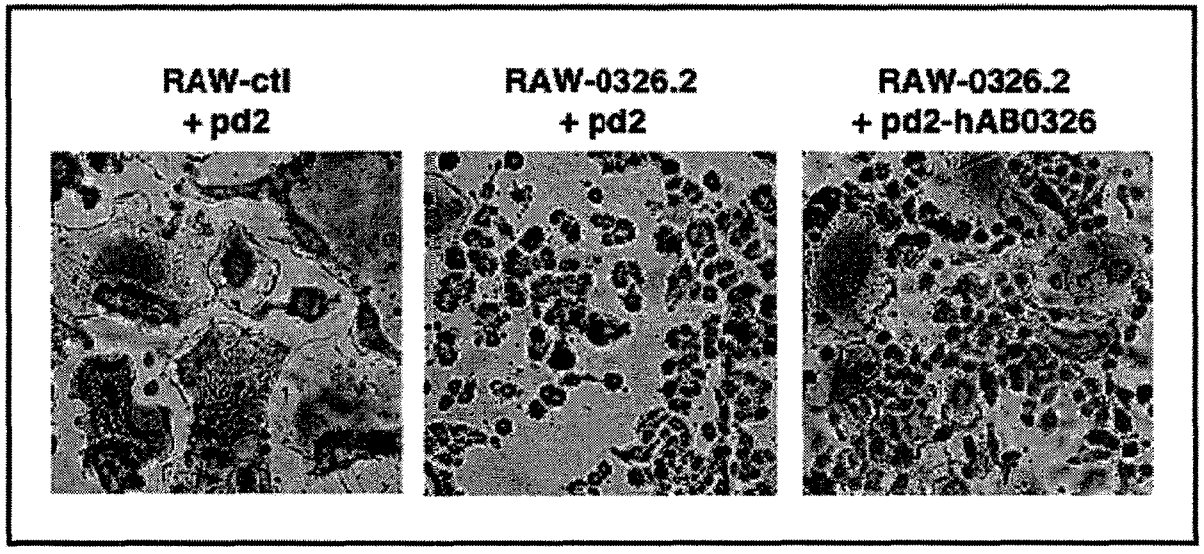


FIGURE 4

A functional complementation assay for SEQ. ID. NO. 1 (AB0326) in RAW-0326.2 cells to screen for inhibitors of osteoclastogenesis



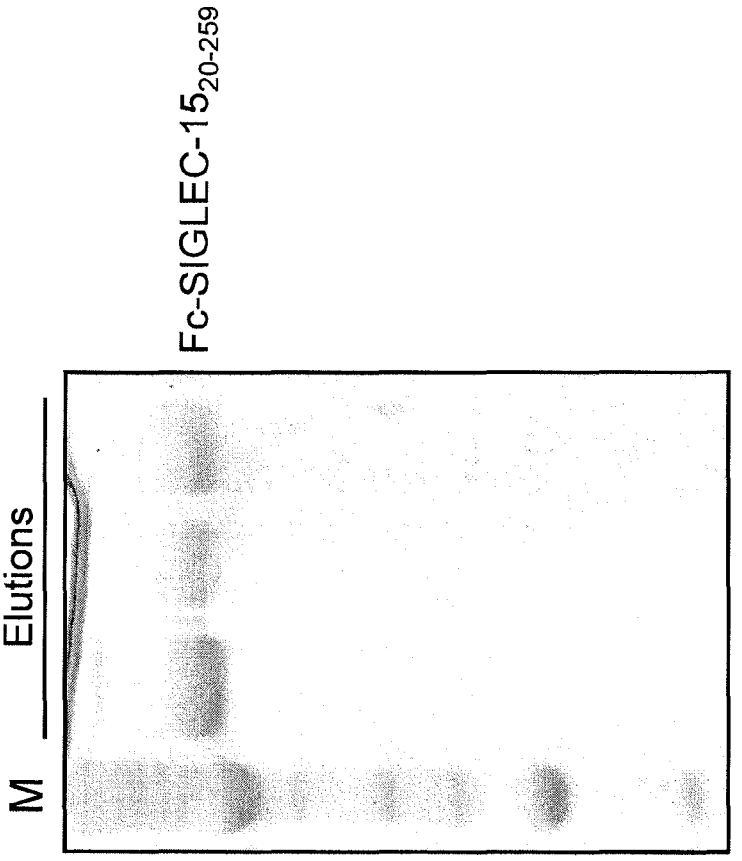


FIGURE 5

FIGURE 6

A

ELISA with biotinylated Fc-SIGLEC-15_{20-259}

	1	2	3	4	5	6	7	8	9	10	11	12
A	**0.793**	0.828	1.079	0.151	0.98	0.125	0.133	0.133	0.136	0.15	0.782	0.384
B	0.603	0.158	0.147	**1.001**	0.143	0.313	0.141	**0.613**	0.716	0.156	0.457	1.052
C	**0.473**	0.155	0.443	0.134	0.118	1.005	0.163	0.517	0.966	0.93	1.059	0.151
D	0.152	0.17	1.319	1.118	1.07	1.094	0.161	**0.909**	0.155	0.979	0.158	0.148
E	0.354	0.167	0.952	0.169	**0.312**	**0.436**	0.518	0.968	**0.491**	0.13	0.169	1.018
F	0.142	1.131	1.111	1.027	0.573	0.751	0.818	0.15	0.845	0.512	0.888	0.997
G	0.153	0.162	1.106	0.854	0.509	0.246	0.732	0.869	0.39	0.847	0.356	0.221
H	0.916	1.254	0.18	0.81	1.192	1.219	0.905	0.868	0.24	0.518	0.479	1.115

B

ELISA with biotinylated Fc

	1	2	3	4	5	6	7	8	9	10	11	12
A	**0.118**	1.879	0.112	0.119	0.119	0.113	0.102	1.002	0.123	0.101	0.133	1.603
B	1.811	0.129	0.123	**0.12**	0.124	0.134	0.231	**0.151**	1.872	0.185	0.124	0.152
C	**0.168**	0.185	1.585	0.13	0.161	0.122	0.138	1.771	0.167	0.16	1.946	0.261
D	0.117	0.173	0.134	0.12	0.133	0.128	0.133	**0.137**	0.152	0.209	0.219	0.255
E	1.284	0.126	1.883	0.138	**0.132**	**0.135**	0.135	0.12	**0.143**	0.151	0.139	0.148
F	0.116	0.146	0.14	1.805	0.197	0.145	0.144	0.132	0.158	0.152	0.13	0.14
G	0.128	0.13	0.138	0.128	0.137	0.134	0.126	0.125	0.135	0.134	0.132	0.146
H	0.128	0.139	0.13	0.124	0.141	0.147	0.136	0.138	0.131	0.127	0.134	1.982

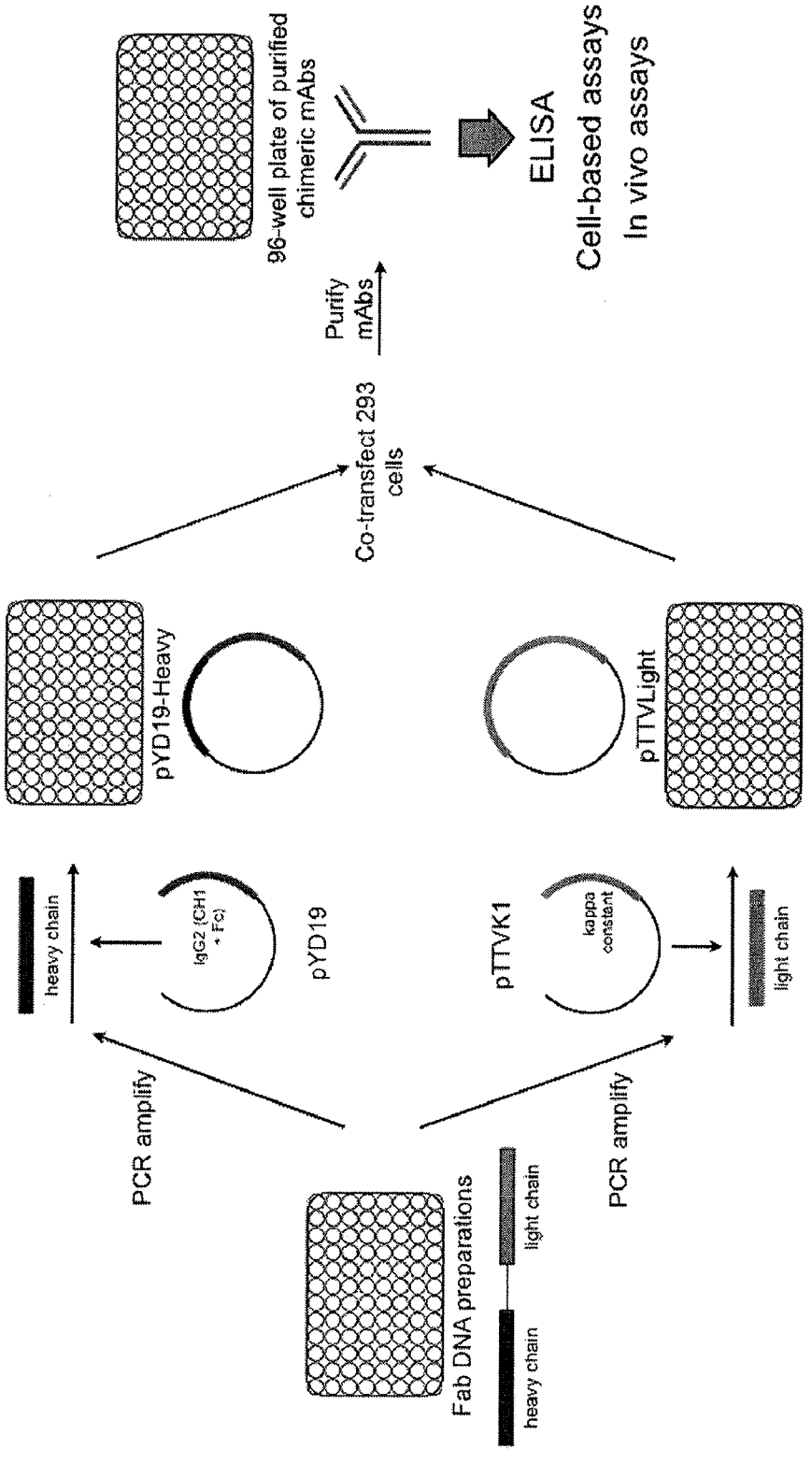
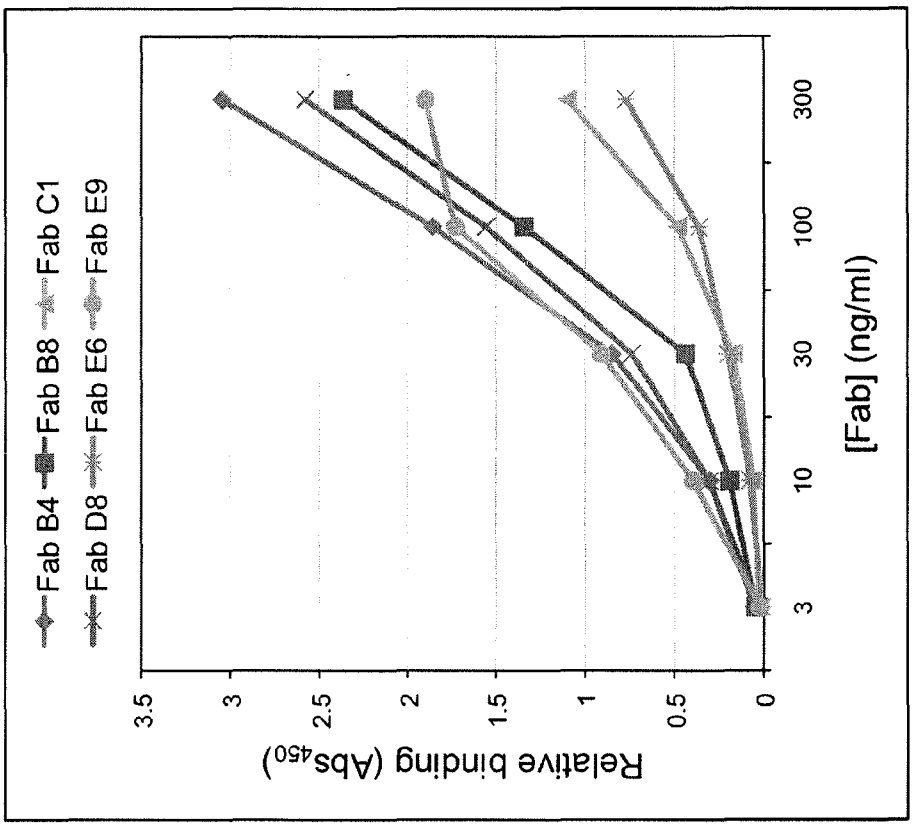

96-well plate of purified chimeric mAbs
ELISA
Cell-based assays
In vivo assays
Purify mAbs
Co-transfect 293 cells
pYD19-Heavy
pTTVLight
heavy chain
IgG2 (CH1 + Fc)
pYD19
pTTVK1
kappa constant
light chain
PCR amplify
Fab DNA preparations
heavy chain
light chain
PCR amplify

FIGURE 7

FIGURE 8


Fab B4
Fab B8
Fab C1
Fab D8
Fab E6
Fab E9
Relative binding (Abs450)
[Fab] (ng/ml)
0
0.5
1
1.5
2
2.5
3
3.5
3
10
30
100
300

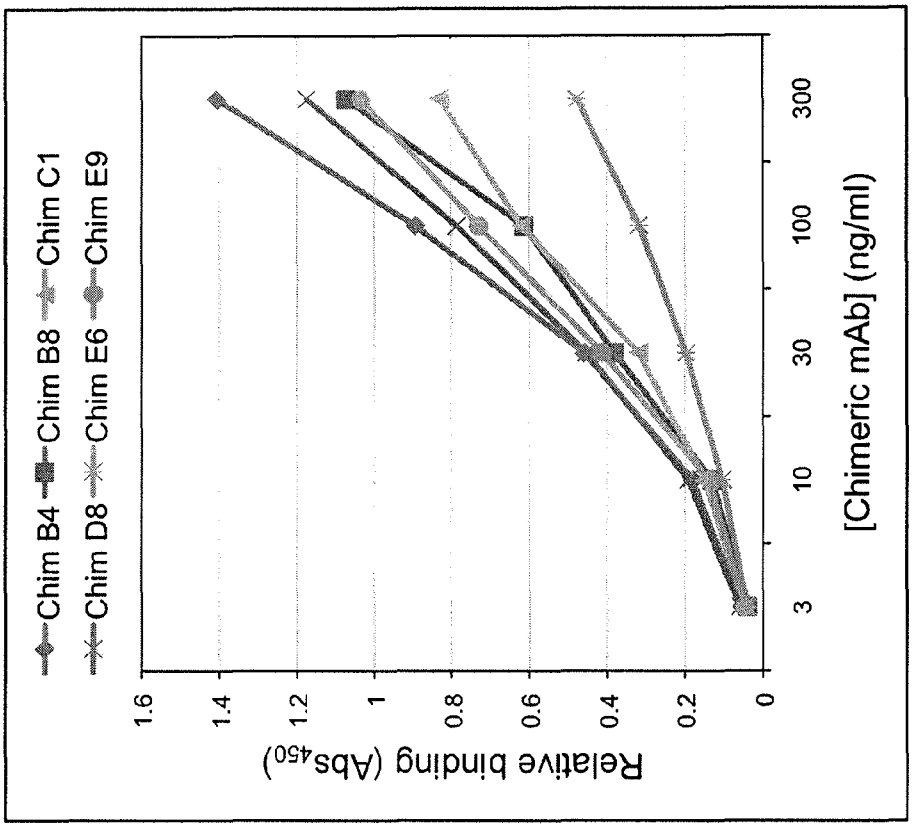

Chim B4
Chim B8
Chim C1
Chim D8
Chim E6
Chim E9
Relative binding (Abs450)
[Chimeric mAb] (ng/ml)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
3
10
30
100
300

FIGURE 9

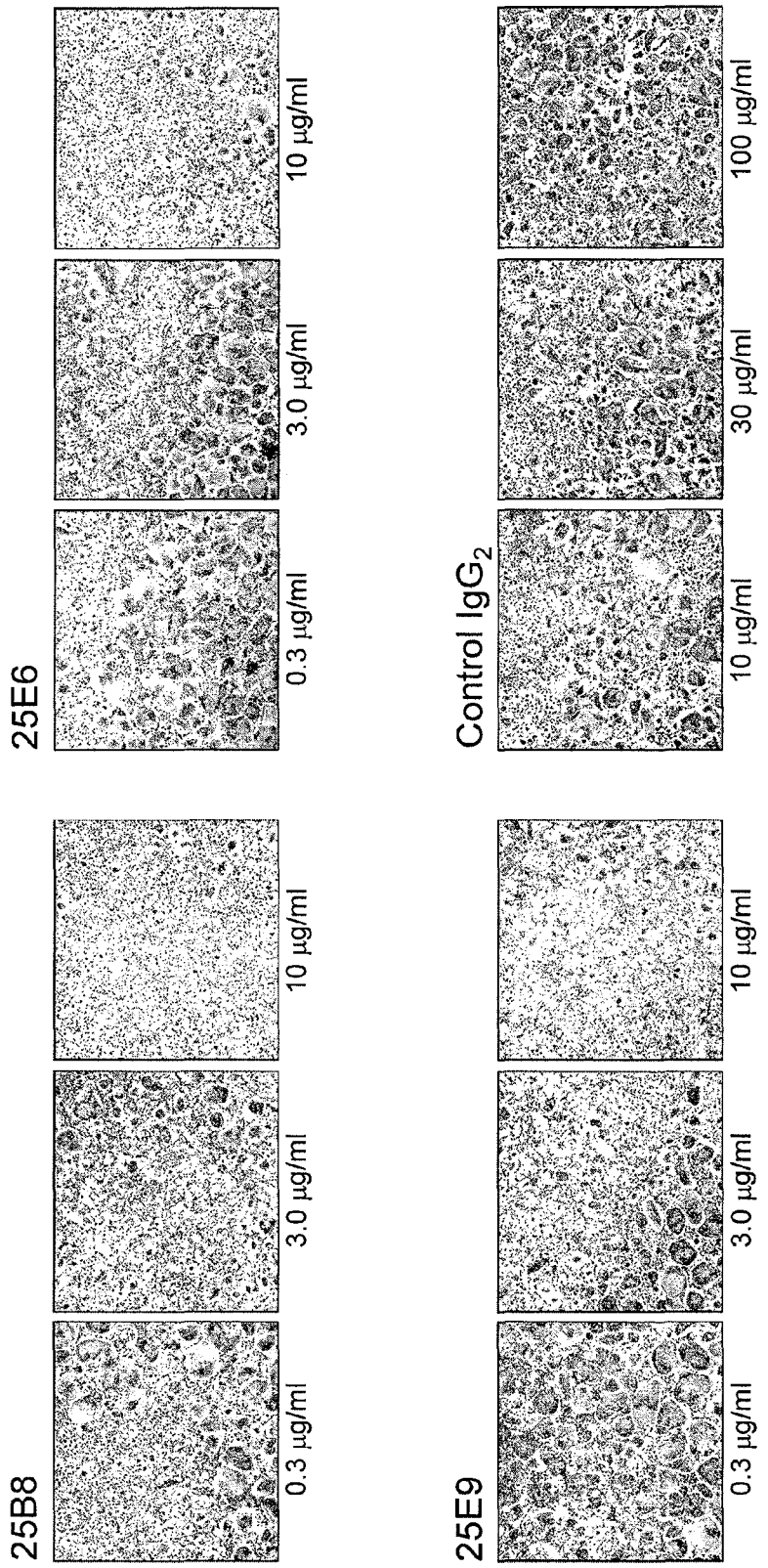
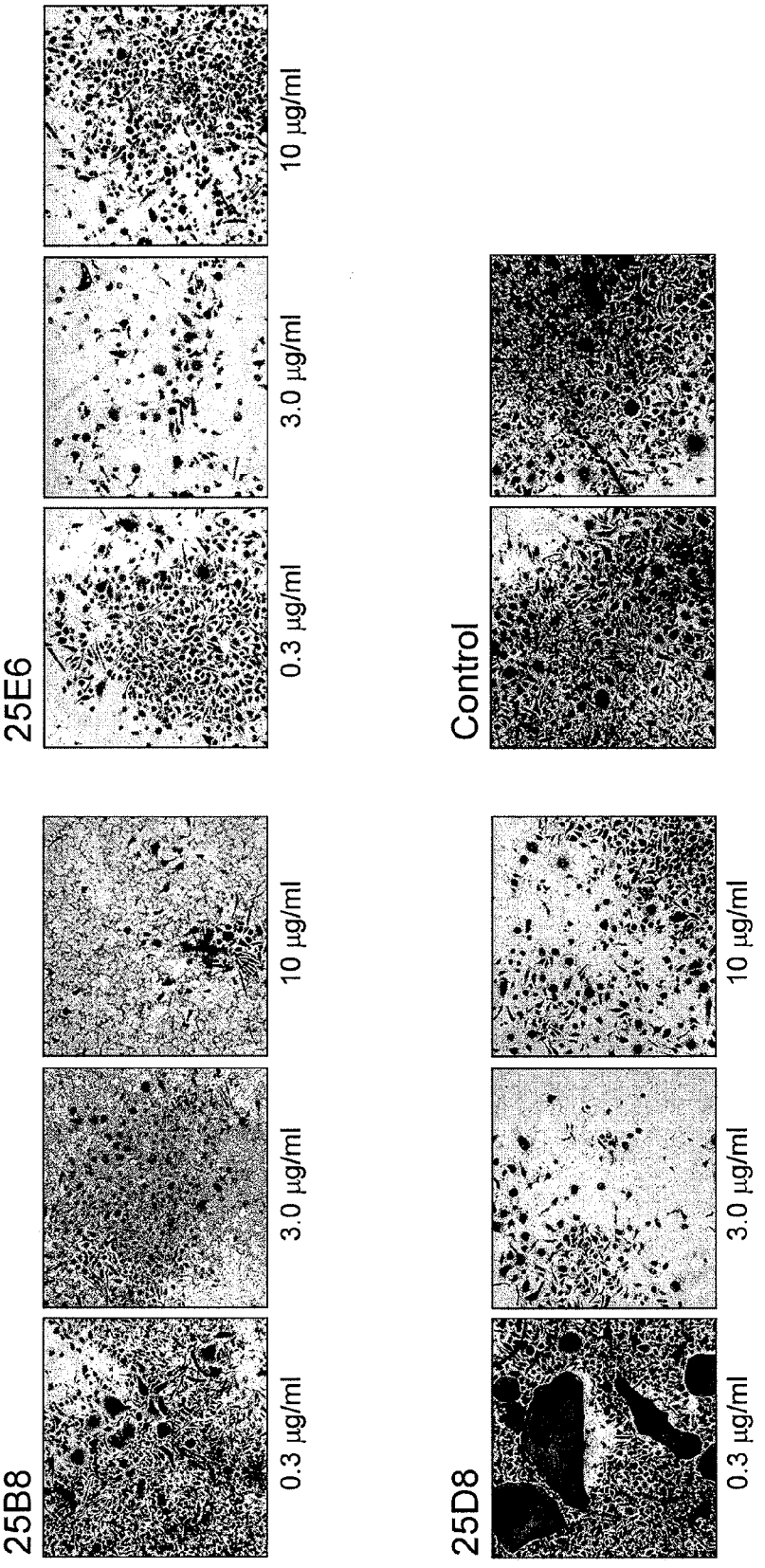

25E6
0.3 µg/ml
3.0 µg/ml
10 µg/ml
Control IgG2
10 µg/ml
30 µg/ml
100 µg/ml
25B8
0.3 µg/ml
3.0 µg/ml
10 µg/ml
25E9
0.3 µg/ml
3.0 µg/ml
10 µg/ml

FIGURE 10


25E6
0.3 µg/ml
3.0 µg/ml
10 µg/ml
Control
25B8
0.3 µg/ml
3.0 µg/ml
10 µg/ml
25D8
0.3 µg/ml
3.0 µg/ml
10 µg/ml

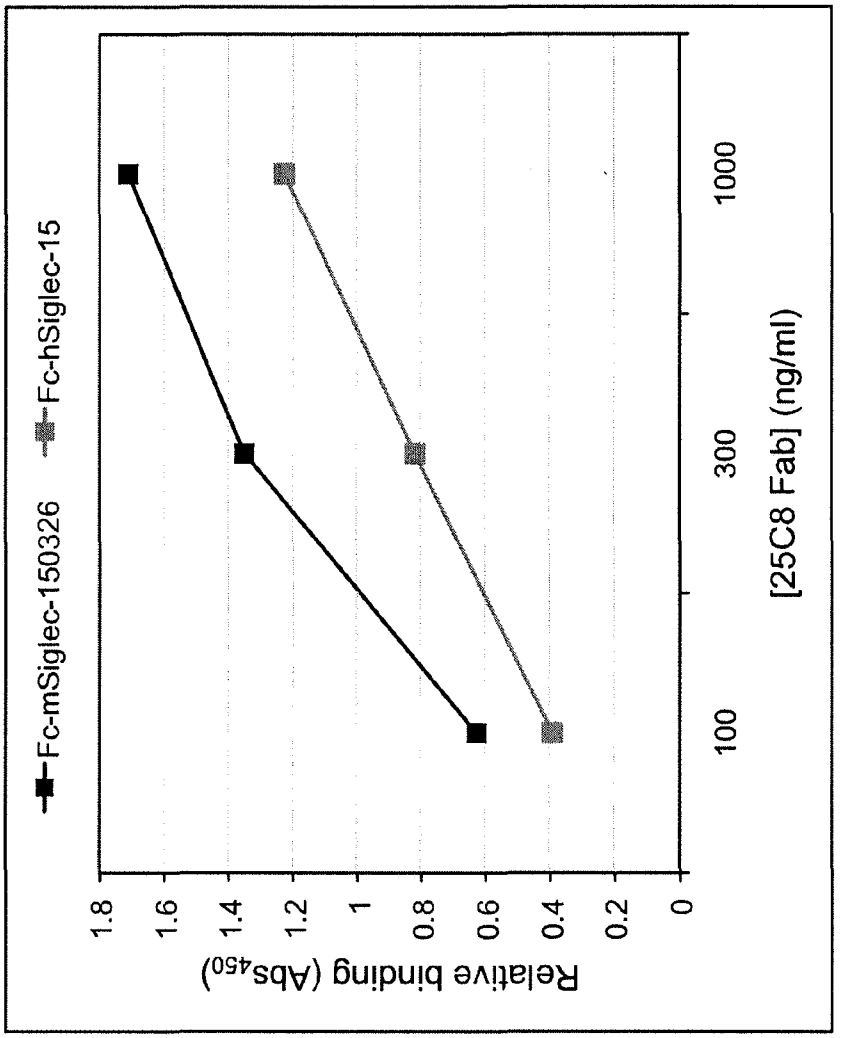

Fc-mSiglec-150326
Fc-hSiglec-15
Relative binding (Abs450)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
100
300
1000
[25C8 Fab] (ng/ml)

FIGURE 11

METHODS OF IMPAIRING OSTEOCLAST DIFFERENTIATION USING ANTIBODIES THAT BIND SIGLEC-15

This application is a continuation-in-part of U.S. Ser. No. 12/279,054, filed Jan. 13, 2009, now U.S. Pat. No. 7,989,160, which is a national stage application of PCT/CA2007/000210 filed on Feb. 13, 2007, the entire content of which is incorporated herein by reference, which application claims the benefit of U.S. Provisional Application Ser. No. 60/772,585 filed on Feb. 13, 2006 and U.S. Provisional Application Ser. No. 60/816,858 filed on Jun. 28, 2006 the entire content of which is incorporated herein by reference. This application claims the benefit of U.S. Provisional Application Ser. No. 61/248,960 filed Oct. 6, 2009.

In accordance with 37 CFR 1.52(e)(5), a Sequence Listing in the form of a text file (entitled "Sequence listing.txt," created on Dec. 28, 2009, and 160 kilobytes) is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

This invention relates, in part, to unique and newly identified genetic polynucleotides involved in the process of bone remodeling; variants and derivatives of the polynucleotides and corresponding polypeptides; uses of the polynucleotides, polypeptides, variants and derivatives; methods and compositions for the amelioration of symptoms caused by bone remodeling disorders, including but not limited to osteoporosis, osteopenia, osteomalacia, hyperparathyroidism, hypothyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Turner syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome, Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets (including vitamin D dependent, type I and II, and x-linked hypophosphatemic rickets), fibrogenesis imperfecta ossium, osteosclerotic disorders such as pycnodysostosis and damage caused by macrophage-mediated inflammatory processes.

In particular, this invention relates to antibodies and antigen binding fragments, polynucleotide expression profiles of active osteoclasts, the isolation and identification of polynucleotides, polypeptides, variants and derivatives involved in osteoclast activity, validation of the identified polynucleotides for their potential as therapeutic targets and use of the polynucleotides, polypeptides, variants and derivatives for the amelioration of disease states and research purposes, as well as in diagnosis of disease states or in the predisposition to develop same.

BACKGROUND OF THE INVENTION

Bone is a dynamic connective tissue comprised of functionally distinct cell populations required to support the structural, mechanical and biochemical integrity of bone and the human body's mineral homeostasis. The principal cell types involved include, osteoblasts responsible for bone formation and maintaining bone mass, and osteoclasts responsible for bone resorption. Osteoblasts and osteoclasts function in a dynamic process termed bone remodeling. The development and proliferation of these cells from their progenitors is governed by networks of growth factors and cytokines produced in the bone microenvironment as well as by systemic hormones. Bone remodeling is ongoing throughout the lifetime of the individual and is necessary for the maintenance of healthy bone tissue and mineral homeostasis. The process remains largely in equilibrium and is governed by a complex interplay of systemic hormones, peptides and downstream signalling pathway proteins, local transcription factors, cytokines, growth factors and matrix remodeling genes.

Any interference or imbalance arising in the bone remodeling process can produce skeletal disease, with the most common skeletal disorders characterized by a net decrease in bone mass. A primary cause of this reduction in bone mass is an increase in osteoclast number and/or activity. The most common of such disease, and perhaps the best known, is osteoporosis occurring particularly in women after the onset of menopause. In fact osteoporosis is the most significant underlying cause of skeletal fractures in late middle-aged and elderly women. While estrogen deficiency has been strongly implicated as a factor in postmenopausal osteoporosis, there is longstanding evidence that remodeling is a locally controlled process being that it takes place in discrete packets throughout the skeleton as first described by Frost over forty years ago (Frost H. M. 1964).

Since bone remodeling takes place in discrete packets, locally produced hormones and enzymes may be more important than systemic hormones for the initiation of bone resorption and the normal remodeling process. Such local control is mediated by osteoblasts and osteoclasts in the microenvironment in which they operate. For example, osteoclasts attach to the bone matrix and form a separate compartment between themselves and the bone surface delimited by a sealing zone formed by a ring of actin surrounding the ruffled border. Multiple small vesicles transport enzymes toward the bone matrix and internalize partially digested bone matrix. The microenvironment within the sealing zone is rich with the presence of lysosomal enzymes and is highly acidic compared to the normal physiological pH of the body. The ruffled border membrane also expresses RANK, the receptor for RANKL, and macrophage-colony stimulating factor (M-CSF) receptor, both of which are responsible for osteoclast differentiation, as well as the calcitonin receptor capable of rapidly inactivating the osteoclast (Baron, R. 2003).

In a complex pattern of inhibition and stimulation, growth hormone, insulin-like growth factor-1, the sex steroids, thyroid hormone, calciotrophic hormones such as PTH and prostaglandin E2, various cytokines, such as interleukin-1 beta, interleukin-6, and tumour necrosis factor-alpha, and 1,25-dihydroxyvitamin D (calcitriol) act co-ordinately in the bone remodeling process (Jilka et al. 1992; Poli et al. 1994; Srivastava et al. 1998; de Vemejoul 1996).

Thus, it stands to reason that the unique local environments created by these specialized cells is due to the expression of either unique genetic sequences not expressed in other tissues and/or splice variants of polynucleotides and polypeptides expressed in other tissues. The isolation and identification of polynucleotides, polypeptides and their variants and derivatives specific to osteoclast activity will permit a clearer understanding of the remodeling process and offer tissue specific therapeutic targets for the treatment of disease states related to bone remodeling.

Many diseases linked to bone remodeling are poorly understood, generally untreatable or treatable only to a limited extent. For example, osteoarthritis is difficult to treat as there is no cure and treatment focuses on relieving pain and preventing the affected joint from becoming deformed. Non-steroidal anti-inflammatory drugs (NSAIDs) are generally used to relieve pain.

Another example is osteoporosis where the only current medications approved by the FDA for use in the United States

are the anti-resorptive agents that prevent bone breakdown. Estrogen replacement therapy is one example of an anti-resorptive agent. Others include alendronate (Fosamax—a biphosphonate anti-resorptive), risedronate (Actonel—a bisphosphonate anti-resorptive), raloxifene (Evista—selective estrogen receptor modulator (SERM)), calcitonin (Calcimar—a hormone), and parathyroid hormone/teriparatide (Forteo—a synthetic version of the human hormone, parathyroid hormone, which helps to regulate calcium metabolism).

Bisphosphonates such as alendronate and risedronate bind permanently to the surface of bone and interfere with osteoclast activity. This allows the osteoblasts to outpace the rate of resorption. The most common side effects are nausea, abdominal pain and loose bowel movements. However, alendronate is reported to also cause irritation and inflammation of the esophagus, and in some cases, ulcers of the esophagus. Risedronate is chemically different from alendronate and has less likelihood of causing esophagus irritation. However, certain foods, calcium, iron supplements, vitamins and minerals, or antacids containing calcium, magnesium, or aluminum can reduce the absorption of risedronate, thereby resulting in loss of effectiveness.

The most common side effect of Raloxifen and other SERMS (such as Tamoxifen) are hot flashes. However, Raloxifene and other hormone replacement therapies have been shown to increase the risk of blood clots, including deep vein thrombosis and pulmonary embolism, cardiovascular disease and cancer.

Calcitonin is not as effective in increasing bone density and strengthening bone as estrogen and the other anti-resorptive agents. Common side effects of either injected or nasal spray calcitonin are nausea and flushing. Patients can develop nasal irritations, a runny nose, or nosebleeds. Injectable calcitonin can cause local skin redness at the site of injection, skin rash, and flushing.

A situation demonstrative of the link between several disorders or disease states involving bone remodeling is that of the use of etidronate (Didronel) first approved by the FDA to treat Paget's disease. Paget's disease is a bone disease characterized by a disorderly and accelerated remodeling of the bone, leading to bone weakness and pain. Didronel has been used 'off-label' and in some studies shown to increase bone density in postmenopausal women with established osteoporosis. It has also been found effective in preventing bone loss in patients requiring long-term steroid medications (such as Prednisone or Cortisone). However, high dose or continuous use of Didronel can cause another bone disease called osteomalacia. Like osteoporosis, osteomalacia can lead to weak bones with increased risk of fractures. Because of osteomalacia concerns and lack of enough studies yet regarding reduction in the rate of bone fractures, the United States FDA has not approved Didronel for the treatment of osteoporosis.

Osteoporosis therapy has been largely focused on antiresorptive drugs that reduce the rate of bone loss but emerging therapies show promise in increasing bone mineral density instead of merely maintaining it or slowing its deterioration. The osteoporosis early stage pipeline consists largely of drug candidates in new therapeutic classes, in particular cathepsin K inhibitors, osteoprotegerin and calcilytics as well as novel bisphosphonates. Some of these are examples where novel drugs exploiting genomics programs are being developed based on a deeper understanding of bone biology and have the potential to change the face of treatment of bone disorders in the long term.

There thus remains a need to better understand the bone remodeling process and to provide new compositions that are useful for the diagnosis, prognosis, treatment, prevention and evaluation of therapies for bone remodeling and associated disorders. A method for analysing polynucleotide expression patterns has been developed and applied to identify polynucleotides, polypeptides, variants and derivatives specifically involved in bone remodeling. Methods of identifying compounds for modulating osteoclast differentiation were developed and therapeutic antibodies and antigen binding fragments against SIGLEC-15 (SEQ ID NO.:2) and against SIGLEC-15 variants were obtained.

Sialic-acid-binding immunoglobulin-like lectins (Siglecs) are members of the immunoglobulin (Ig) superfamily that have the ability to interact with sialic acids (McMillan and Crocker, 2008; Crocker et al., 2007). There are several Siglec family members that all share specific structural features, in particular, displaying an amino-terminal V-set Ig domain that binds to sialic acid and a variable number of C2-set Ig domains. These membrane receptors are generally expressed in highly specific manners and many of the family members are expressed in hematopoietic cells (McMillan and Crocker, 2008). These proteins are thought to promote cell-cell interactions, mediate signaling, and regulate immune functions through the recognition of glycans (Crocker et al., 2007). Sialic acids are nine-carbon sugars typically located at the ends of complex glycoconjugates on the surface of cells. They can be attached to a wide variety of proteins and lipids (McMillan and Crocker, 2008).

Siglec-15 is one of the most recently described Siglec family members that has a high homology to Siglec-14 (Angata et al., 2007). These authors reported that it preferentially binds to sialyl Tn structure and that it interacts with DAP12 and DAP10. The functional significance of these interactions is not known but it was proposed that Siglec-15 probably harbors an activating function (Angata et al., 2007). A recent publication showed that the presence of sialic acid at the end of surface glycoconjugates was required for proper osteoclast differentiation and were probably important for the fusion of osteoclast precursor cells (Takahata et al., 2007). This last observation creates a direct functional link between sialic acid binding and the expression of Siglec-15 in differentiating osteoclasts and strongly suggested that Siglec-15 plays a role in the early differentiation program of osteoclast precursors.

Thus, the expression profile of Siglec-15, its strong inducibility during osteoclast differentiation, its localization at the surface of the membrane, and its structural features all contribute to the feasibility of targeting this protein at the cell surface with monoclonal antibodies. The only other example of monoclonal antibody-based therapy that target osteoclasts is denosumab, a human monoclonal antibody that is specific for RANKL (Ellis et al. 2008). The present invention relates to the use of anti-Siglec-15 antibodies or antigen binding fragments as blockers of osteoclast differentiation and which may be used for impairing bone loss or bone resorption in bone-related diseases, such as cancer-induced severe bone loss.

The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

The present invention relates in one aspect to a therapeutic antibody and antigen binding fragments thereof which targets SIGLEC-15 or SIGLEC-15 analogues. These antibodies or antigen binding fragments may be advantageously recombinantly expressed in a mammalian cell system.

The present invention relates in another aspect thereof to an isolated antibody or antigen binding fragment capable of binding to a polypeptide able to promote osteoclast differentiation and of interfering with (e.g., inhibiting) an osteoclast differentiation activity of the polypeptide. One such particular polypeptide may be, for example, SEQ ID NO.:2 or a variant having at least 80% sequence identity with SEQ ID NO.:2. The antibody or antigen binding fragment may particularly bind to the extracellular region of SEQ ID NO.:2 or of the SEQ ID NO.:2 variant. The antibody or antigen binding fragment may thus modulate the differentiation of osteoclast precursor cells into differentiated osteoclasts that occurs through the SEQ ID NO.:2 or its variant.

Antibodies or antigen binding fragments that are encompassed by the present invention include, for example, those that may interfere with (e.g., inhibit) the differentiation of a human osteoclast precursor cell or more specifically, those that may interfere with (e.g., inhibit) the differentiation of a primary human osteoclast precursor cell.

Therefore, in accordance with the present invention, the antibody or antigen binding fragment may be capable of inhibiting differentiation of osteoclast precursor cells into differentiated osteoclasts.

In an embodiment of the invention, the antibody may be, for example, a polyclonal antibody. In another embodiment of the invention, the antibody or antigen binding fragment may be, for example, a monoclonal antibody or a fragment thereof. In yet another embodiment, the antibody or antigen binding fragment may be, for example, a chimeric antibody or a fragment thereof. In a further embodiment, the antibody or antigen binding fragment may be, for example, an isolated human antibody or a fragment thereof.

The antibody or antigen binding fragment of the present invention may be produced from an isolated mammalian cell or by a hybridoma cell. Although hybridoma cells are encompassed by the present invention, the antibody or antigen binding fragment may preferably be produced in a cell other than an hybridoma cell. The isolated mammalian cell may be, for instance, a human cell.

An exemplary embodiment of an antibody or antigen binding fragment of the present invention is one that may comprise (amino acids of) a constant region of a human antibody or a fragment thereof.

Another exemplary embodiment of an antibody or antigen binding fragment of the present invention is one that may comprise (amino acids of) a framework region of a human antibody.

Antibodies or antigen binding fragments that are especially encompassed by the present invention include those that comprises (amino acids of) a constant region of a human antibody or a fragment thereof and/or those that comprises (amino acids of) a framework region of a human antibody and that are produced in mammalian cells, or more particularly in human cells.

Yet other antibodies or antigen binding fragments that are especially encompassed by the present invention include monoclonal antibodies or those that comprises (amino acids of) a constant region of a human antibody or a fragment thereof and/or those that comprises (amino acids of) a framework region of a human antibody and that may interfere with (e.g., inhibit) the differentiation of human osteoclast precursor cells into differentiated human osteoclast, or more particularly those that may interfere with (e.g., inhibit) the differentiation of primary human osteoclast precursor cells into differentiated human osteoclast.

Yet further antibodies or antigen binding fragments that are especially encompassed by the present invention include monoclonal antibodies or those that comprises (amino acids of) a constant region of a human antibody or a fragment thereof and/or those that comprises (amino acids of) a framework region of a human antibody and that may interfere with (e.g., inhibit) the differentiation of human osteoclast precursor cells into differentiated human osteoclast, or more particularly those that may interfere with (e.g., inhibit) the differentiation of primary human osteoclast precursor cells into differentiated human osteoclast and that are produced in mammalian cells, or more particularly in human cells.

Exemplary embodiments of antigen binding fragments include, for example, a FV (e.g., scFv), a Fab, a Fab' or a $(Fab')_2$.

In accordance with the present invention, the antibody or antigen binding fragment may comprise (amino acids of) constant region from an IgG1, IgG2, IgG3, or IgG4. More particularly, the (amino acids of) the constant region may be from an IgG2.

The present invention also provides in a further aspect, a pharmaceutical composition which may comprise an antibody or antigen binding fragment of the present invention and a pharmaceutically acceptable carrier.

More specifically, the present invention provides a pharmaceutical composition which may comprise:

a. an isolated antibody or antigen binding fragment that may be capable of binding to a polypeptide able to promote osteoclast differentiation and of interfering with (e.g., inhibiting, impairing) an osteoclast differentiation activity of the polypeptide such as a polypeptide which may be selected from the group consisting of SEQ ID NO.:2 and a variant having at least 80% sequence identity with SEQ ID NO.:2, and;

b. a pharmaceutically acceptable carrier.

The pharmaceutical composition may thus comprise an antibody or antigen binding fragment that may impair (interfere with) the differentiation of osteoclast precursor cells into differentiated osteoclasts promoted by SEQ ID NO.:2 or its variant.

Exemplary embodiments of antibodies or antigen binding fragments that are encompassed by the present invention, include for example, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a human antibody or a fragment thereof.

Exemplary embodiments of pharmaceutical compositions are those which comprises an antibody or antigen binding fragment that is produced from an isolated mammalian cell such as a human cell.

Exemplary embodiments of pharmaceutical compositions are those which comprises an antibody or antigen binding fragment that may interfere with the differentiation of human osteoclast precursor cells into differentiated osteoclasts.

Other exemplary embodiments of pharmaceutical compositions are those which comprises an antibody or antigen binding fragment that may interfere with the differentiation of primary human osteoclast precursor cells into differentiated osteoclasts.

Yet other exemplary embodiments of pharmaceutical compositions are those which comprises an antibody or antigen binding fragment that interfere with the differentiation of human osteoclast precursor cells (e.g., primary human osteoclast precursors cells) into differentiated osteoclasts and that are produced in mammalian cells (e.g., human cells).

In an additional aspect, the present invention provides an isolated cell which may comprise (e.g., that has been injected or transformed or else), that is capable of expressing or that may express an antibody or antigen binding fragment of the present invention. In accordance with the present invention,

the isolated cell may be, for instance a mammalian cell. In a more specific embodiment, the isolated cell may be, for example, a human cell.

In yet an additional aspect, the present invention relates to a method of modulating (i.e., inhibiting, lowering, impairing) osteoclast differentiation in a mammal in need, the method may comprise administering the antibody or antigen binding fragment of the present invention.

In an exemplary embodiment, the invention provides a method of modulating (i.e., inhibiting, lowering, impairing) osteoclast differentiation in a mammal in need, the method may comprise administering an antibody or antigen binding fragment that may be capable of modulating the differentiation of an osteoclast precursor cell (e.g., human osteoclast precursor cell, human primary osteoclast precursor cell) into a differentiated osteoclast.

In another exemplary embodiment, the invention provides a method of modulating (i.e., inhibiting, lowering, impairing) osteoclast differentiation in a mammal in need, the method may comprise administering an antibody or antigen binding fragment that may be capable of modulating the differentiation of an osteoclast precursor cell (e.g., human osteoclast precursor cell, human primary osteoclast precursor cell) into a differentiated osteoclast and that is produced in mammalian cells (e.g., human cell).

In yet another exemplary embodiment, the invention provides a method of modulating (i.e., inhibiting, lowering, impairing) osteoclast differentiation in a mammal in need, the method may comprise administering an antibody or antigen binding fragment that is capable of modulating (i.e., inhibiting, lowering, impairing) the differentiation of an osteoclast precursor cell (e.g., human osteoclast precursor cell, human primary osteoclast precursor cell) into a differentiated osteoclast, where the antibody or antigen binding fragment may comprise, for example, a monoclonal antibody or a fragment thereof or that may comprise (amino acids) of a human constant region or a fragment thereof, and/or amino acids of a framework region of a human antibody. Such antibodies or antigen binding fragments include those that are produced in mammalian cells (e.g., human cell).

The antibody or antigen binding fragment of the present invention may thus be administered to a mammal (e.g., human) which may suffer from undesirable (e.g., excessive) bone loss or bone resorption. The antibody or antigen binding fragment may thus be particularly useful to treat bone loss or bone resorption in patients suffering or susceptible of suffering from a condition selected from the group consisting of osteoporosis, osteopenia, osteomalacia, hyperparathyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Tumer syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome, Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets (including vitamin D dependent, type I and II, and x-linked hypophosphatemic rickets) or other form of vitamin D deficiency such as vitamin D deficiency associated with chronic kidney disease or kidney failure, fibrogenesis imperfecta ossium, osteosclerotic disorders such as pycnodysostosis and damage caused by macrophage-mediated inflammatory processes.

The present invention also provides in a further aspect, a method of identifying an therapeutic antibody or antigen binding fragment able to impair an osteoclast differentiation activity of a polypeptide such as, for example, SEQ ID NO.:2 or a variant having at least 80% sequence identity with SEQ ID NO.:2. The method may comprise contacting the polypeptide or a cell expressing the polypeptide with a candidate antibody or antigen binding fragment and measuring the activity of the polypeptide. A reduction in the osteoclast differentiation activity (in the presence of antibody or antibody fragment in comparison with the absence of antibody or antibody fragment) may thus positively identify an inhibitory antibody or antigen binding fragment.

The present invention also relates in a further aspect to an antibody or antigen binding fragment which may be capable of inhibiting differentiation of an osteoclast precursor cell into an osteoclast and which may be obtained by the method of providing an antibody or antigen binding fragment able to bind to the polypeptide described herein (SEQ ID NO.:2 or to a variant having at least 80% sequence identity with SEQ ID NO.:2) to an osteoclast precursor cell and inducing differentiation. A reduced osteoclast differentiation (in the presence of antibody or antibody fragment in comparison with the absence of antibody or antibody fragment) may thus positively identify an antibody or antigen binding fragment which may be capable of inhibiting differentiation of an osteoclast precursor cell into an osteoclast.

The present invention also relates to an isolated antibody or antigen binding fragment which may be capable of specific binding to SEQ ID NO.:2 or to a variant having at least 80% sequence identity with SEQ ID NO.:2 and of inhibiting a resorptive activity of an osteoclast.

The invention also provides a method of generating an antibody or antigen binding fragment which may be capable of inhibiting differentiation of an osteoclast precursor cell (into an osteoclast) or of inhibiting a resorptive activity of an osteoclast. The method may comprise administering SEQ ID NO.:2, a variant having at least 80% identity with SEQ ID NO.:2 or a fragment of at least 10 amino acids thereof, to a mammal (e.g., especially an animal) under conditions allowing for the production of antibodies (under conditions which induces humoral immunity). The method may also comprise isolating or purifying the antibody or antigen binding fragment from the mammal.

The invention additionally provides an antibody or antigen binding fragment that comprises at least one CDRL1, CDRL2, CDRL3, CDRH1, CDRH2 and/or CDRH3 described herein. Identification of CDRs in a light chain or heavy chain may be made in accordance with the Kabat or Chotia method or by other methods known in the art

In an exemplary embodiment, the antibody or antigen binding fragment may comprise any individual CDR or a combination of CDR1, CDR2 and/or CDR3 of the light chain variable region. The CDR3 may more particularly be selected. Combination may include for example, CDRL1 and CDRL3; CDRL1 and CDRL2; CDRL2 and CDRL3 and; CDRL1, CDRL2 and CDRL3.

In another exemplary embodiment, the antibody or antigen binding fragment may comprise any individual CDR or a combination of CDR1, CDR2 and/or CDR3 of the heavy chain variable region. The CDR3 may more particularly be selected. Combination may include for example, CDRH1 and CDRH3; CDRH1 and CDRH2; CDRH2 and CDRH3 and; CDRH1, CDRH2 and CDRH3.

In accordance with the present invention, the antibody or antigen binding fragment may comprise at least two CDRs of a CDRL1, a CDRL2 or a CDRL3.

Also in accordance with the present invention, the antibody or antigen binding fragment may comprise one CDRL1, one CDRL2 and one CDRL3.

In accordance with the present invention, the antibody or antigen binding fragment may comprise at least two CDRs of a CDRH1, a CDRH2 or a CDRH3.

Also in accordance with the present invention, the antibody or antigen binding fragment may comprise one CDRH1, one CDRH2 and one CDRH3.

Further in accordance with the present invention, the antibody or antigen binding fragment may comprise:

a. At least two CDRs of a CDRL1, CDRL2 or CDRL3 and;

b. At least two CDRs of a CDRH1, one CDRH2 or one CDRH3.

The antibody or antigen binding fragment may more preferably comprise one CDRL1, one CDRL2 and one CDRL3.

The antibody or antigen binding fragment may also more preferably comprise one CDRH1, one CDRH2 and one CDRH3.

The invention further provides antibody or antigen binding fragment that comprises amino acids of the light chain variable region and/or of the heavy chain variable region described herein.

The present invention relates to polynucleotides comprising sequences involved in the process of bone remodeling, the open reading frame of such sequences, substantially identical sequences (e.g., variants (e.g., allelic variant), non human orthologs), substantially complementary sequences and fragments of any one of the above thereof.

The present invention relates to polypeptide comprising sequences involved in the process of bone remodeling including biologically active analogs and biologically active fragments thereof. The present invention also relates to compositions that are useful for the diagnosis, prognosis, treatment, prevention and/or evaluation of therapies for bone remodeling and associated disorders.

In addition, the present invention relates to a method for analyzing polynucleotide expression patterns, and applied in the identification of polynucleotides, polypeptides, variants and derivatives specifically involved in bone remodeling.

The present invention relates to polynucleotide expression profiles of osteoclasts, the isolation and identification of polynucleotides, their corresponding polypeptides, variants and derivatives involved in osteoclast activity, validation of these identified elements for their potential as therapeutic targets and use of said polynucleotides, polypeptides, variants and derivatives for the amelioration of disease states.

It is an object of the present invention to provide polynucleotides and/or related polypeptides that have been isolated and identified. More specifically, the invention provides (isolated or substantially purified) polynucleotides comprising or consisting of any one of SEQ ID NO.:1, its coding sequence (open reading frame) substantially identical sequence (e.g., variants, orthologs (e.g., SEQ ID NO.:3; SEQ ID NO.:107)), substantially complementary sequences and related polypeptides comprising any one of SEQ ID NO.:2, SEQ ID NO.:4 or SEQ ID NO.:108 which have been shown to be upregulated in a highly specific fashion in osteoclasts.

NSEQ refers generally to polynucleotide sequences of the present invention and includes for example, SEQ. ID. NO.:1, SEQ ID NO.:3 and SEQ ID NO.:107 whereas PSEQ refers generally to polypeptide sequences of the present invention and includes, for example, SEQ ID NO.:2 or a SEQ ID NO.:2 variant (including SEQ ID NO.:4 and SEQ ID NO.:108). Of course it will be understood that NSEQ also encompasses polynucleotide sequences which are designed or derived from SEQ. ID. NO.:1, SEQ ID NO.:3 or and SEQ ID NO.:107 including for example, their coding sequence, complementary sequences etc.

As used herein the term "NSEQ" refers generally to polynucleotides sequences comprising or consisting of any one of SEQ. ID. NO.:1, SEQ ID NO.:3, or SEQ ID NO.:107 (e.g., an isolated form) or comprising or consisting of a fragment of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107. The term "NSEQ" more particularly refers to a polynucleotide sequence comprising or consisting of a transcribed portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, which may be, for example, free of untranslated or untranslatable portion(s) (i.e., a coding portion of any one of SEQ ID No.:1, SEQ ID NO.:3 or SEQ ID NO.:107). The term "NSEQ" additionally refers to a sequence substantially identical to any one of the above and more particularly substantially identical to polynucleotide sequence comprising or consisting of a transcribed portion of any one of SEQ. ID. Nos.: 1 or 3, which may be, for example, free of untranslated or untranslatable portion(s). The term "NSEQ" additionally refers to a polynucleotide sequence region of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 which encodes or is able to encode a polypeptide. The term "NSEQ" also refers to a polynucleotide sequence able of encoding any one of the polypeptides described herein or a polypeptide fragment of any one of the above. Finally, the term "NSEQ" also comprise a sequence substantially complementary to any one of the above.

The term "inhibitory NSEQ" generally refers to a sequence substantially complementary to any one of SEQ. ID. NO.:1, SEQ ID NO.: 3 or SEQ ID NO.:107, substantially complementary to a fragment of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, substantially complementary to a sequence substantially identical to SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 and more particularly, substantially complementary to a transcribed portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 (e.g., which may be free of unstranslated or untranslatable portion) and which may have attenuating or even inhibitory action against the transcription of a mRNA or against expression of a polypeptide encoded by a corresponding SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107. Suitable "inhibitory NSEQ" may have for example and without limitation from about 10 to about 30 nucleotides, from about 10 to about 25 nucleotides or from about 15 to about 20 nucleotides. As used herein the term "nucleotide" means deoxyribonucleotide or ribonucleotide. In an exemplary embodiment, the use of nucleotide analogues is also encompassed in the present invention.

The present invention relates in one aspect thereof to an isolated polynucleotide sequence having at least from about 80% to about 100% (e.g., 80%, 90%, 95%, etc.) sequence identity to a polynucleotide sequence selected from the group consisting of polynucleotides comprising (a) any one of a SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107; (b) an open reading frame of (a); (c) a full complement of (a) or (b), and; (d) a fragment of any one of (a) to (c).

As used herein the term "unstranscribable region" may include for example, a promoter region (or portion thereof), silencer region, enhancer region etc. of a polynucleotide sequence.

As used herein the term "unstranslatable region" may include for example, an initiator portion of a polynucleotide sequence (upstream of an initiator codon, e.g., AUG), intronic regions, stop codon and/or region downstream of a stop codon (including polyA tail, etc.).

Complements of the isolated polynucleotide sequence encompassed by the present invention may be those, for example, which hybridize under high stringency conditions to any of the nucleotide sequences in (a), or (b). The high

stringency conditions may comprise, for example, a hybridization reaction at 65° C. in 5×SSC, 5×Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA.

In accordance with the present invention, the polynucleotide sequence may be used, for example, in the treatment of diseases or disorders involving bone remodeling.

Fragments of polynucleotides may be used, for example, as probes for determining the presence of the isolated polynucleotide (or its complement or fragments thereof) in a sample, cell, tissue, etc. for experimental purposes or for the purpose of diagnostic of a diseases or disorders involving bone remodeling.

The present invention also relates to a combination comprising a plurality of polynucleotides (substantially purified and/or isolated). The polynucleotides may be co-expressed with one or more genes known to be involved in bone remodeling. Furthermore, the plurality of polynucleotides may be selected, for example, from the group consisting of a polynucleotide comprising (a) any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107; (b) an open reading frame of (a); (c) a polynucleotide sequence comprising or consisting of a transcribed portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, which may be, for example, free of untranslated or untranslatable portion(s) (d) a complementary sequence of any one of (a) to (c); (e) a sequence that hybridizes under high stringency conditions to any one of the nucleotide sequences of (a) to (d) and; (f) fragments of any one of (a) to (e).

The present invention further relates to a polynucleotide encoding any one of the polypeptides described herein. In accordance with the present invention, the polynucleotide (RNA, DNA, etc.) may encode a polypeptide which may be selected from the group consisting of any one of SEQ ID NO.:2 or a SEQ ID NO.:2 analogue such as, for example, SEQ ID NO.:4 or SEQ ID NO.:108, or fragments thereof (e.g., biologically active fragments, immunologically active fragments, etc.).

The present invention also relates to an isolated nucleic acid molecule comprising the polynucleotides of the present invention, operatively linked to a nucleotide sequence encoding a heterologous polypeptide thereby encoding a fusion polypeptide.

The invention further relates to a polypeptide encoded by a polynucleotide of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 or more particularly from the open reading frame of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, or a portion thereof. The invention also comprises the product of a gene that is co-expressed with one or more genes known to be involved in bone remodeling.

Isolated naturally occurring allelic variant are also encompassed by the present invention as well as synthetic variants (e.g., made by recombinant DNA technology or by chemical synthesis, etc.) such as biologically active variant which may comprise one or more amino acid substitutions (compared to a naturally occurring polypeptide), such as conservative or non conservative amino acid substitution.

The present invention, further provides a vector (mammalian, bacterial, viral, etc.) comprising the polynucleotides described herein or fragments thereof, such as an expression vector. The vector may further comprise a nucleic acid sequence which may help in the regulation of expression of the polynucleotide and/or a nucleotide sequence encoding a tag (e.g., affinity tag; HA, GST, His etc.).

In accordance with the present invention, an expression vector may comprise, for example, the following operatively linked elements:

a) a transcription promoter;

b) a polynucleotide segment (which may comprise an open reading frame of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107); and

c) a transcription terminator.

The invention also relates to an expression vector comprising a polynucleotide described herein, a host cell transformed with the expression vector and a method for producing a polypeptide of the present invention.

The invention further relates to a vector comprising a polynucleotide or polynucleotide fragment. Vectors which may comprise a sequence substantially complementary to the polynucleotides of the present invention (e.g., siRNA, shRNA) are thus encompassed by the present invention. The vector may comprise sequences enabling transcription of the polynucleotide or polynucleotide fragment.

More particularly, the present invention therefore provides a cell which may be genetically engineered to contain and/or to express the polynucleotide (including complements and fragments) and/or polypeptides of the present invention. The cell may be, for example, a mammalian cell, an insect cell, a bacteria cell, etc.

The present invention therefore provides a host cell which may comprise a vector as described herein. The cell may be, for example, a mammalian cell, an insect cell, a bacteria, etc. The cell may be able to express or expresses a polypeptide encoded by the polynucleotide described herein.

Methods of producing the polypeptides of the present invention encompassed herewith includes for example, culturing the cell in conditions allowing the transcription of a gene or expression of the polypeptide. The polypeptide may be recovered, for example, from cell lysate or from the cell supernatant.

The invention relates to the use of at least one polynucleotide comprising any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 their coding sequence, substantially identical sequences, substantially complementary sequences or fragments thereof on an array. The array may be used in a method for diagnosing a bone remodeling disease or disorder by hybridizing the array with a patient sample under conditions to allow complex formation, detecting complex formation, and comparing the amount of complex formation in the patient sample to that of standards for normal and diseased tissues wherein the complex formation in the patient sample indicates the presence of a bone remodeling disease or disorder. Of course, the use of a polynucleotide of the present invention in a diagnosis method is not dependent exclusively by way of a specific assay. The sequence or sequences may be used in conventionally used diagnosis methods known in the art.

The present invention also relates to a method of ameliorating bone remodeling disease or disorder symptoms, or for inhibiting or delaying bone disease or disorder, the method may comprise: contacting a compound capable of specifically inhibiting activity or expression of a polynucleotide sequence described herein or a polypeptide described herein, in osteoclasts so that symptoms of the bone remodeling disease or disorder may be ameliorated, or the disease or disorder may be prevented, delayed or lowered.

The present invention further relates to a method for ameliorating bone remodeling disease or disorder symptoms, or for inhibiting or delaying bone disease or disorder, the method may comprise: contacting a compound capable of specifically promoting activity or expression of a polynucleotide sequence described herein or a polypeptide described herein, in osteoclasts so that symptoms of the bone remodel-

ing disease or disorder may be ameliorated, or the disease or disorder may be prevented, delayed or lowered.

The present invention also relates to a method of treating a condition in a mammal characterized by a deficiency in, or need for, bone growth or replacement and/or an undesirable level of bone resorption, which method may comprise administering to a mammalian subject in need of such treatment an effective amount of a suitable compound described herein.

The present invention further relates to a method of using a polynucleotide sequence described herein, a polypeptide described herein on an array and for the use of the array in a method for diagnosing a bone remodeling disease or disorder by hybridizing the array with a patient sample under conditions to allow complex formation, detecting complex formation, and comparing the amount of complex formation in the patient sample to that of standards for normal and diseased tissues wherein the complex formation in the patient sample may indicate the presence of a bone remodeling disease or disorder.

In accordance with the present invention, the polynucleotide sequence described herein may be used for somatic cell gene therapy or for stem cell gene therapy.

The invention also relates to a pharmaceutical composition comprising a polynucleotide described herein or a polypeptide encoded by the selected polynucleotide or portion thereof and a suitable pharmaceutical carrier.

Additionally, the invention relates to products, compositions, processes and methods that comprise a polynucleotide described herein, a polypeptide encoded by the polynucleotides, a portion thereof, their variants or derivatives, for research, biological, clinical and therapeutic purposes.

The NSEQs and PSEQs may be used in diagnosis, prognosis, treatment, prevention, and selection and evaluation of therapies for diseases and disorders involving bone remodeling including, but not limited to, osteoporosis, osteopenia, osteomalacia, hyperparathyroidism, hyperthyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Tumer syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome, Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets (including vitamin D dependent, type I and II, and x-linked hypophosphatemic rickets), fibrogenesis imperfecta ossium, osteosclerotic disorders such as pycnodysostosis and damage caused by macrophage-mediated inflammatory processes.

Use of NSEQ as a Screening Tool

The polynucleotides obtained by the present invention may be used to detect and isolate expression products, for example, mRNA, complementary DNAs (cDNAs) and proteins derived from or homologous to the NSEQs. In one embodiment, the expression of mRNAs homologous to the NSEQs of the present invention may be detected, for example, by hybridization analysis, reverse transcription and in vitro nucleic acid amplification methods. Such procedures permit detection of mRNAs in a variety of tissue types or at different stages of development. The subject nucleic acids which are expressed in a tissue-specific or a developmental-stage-specific manner are useful as tissue-specific markers or for defining the developmental stage of a sample of cells or tissues that may define a particular disease state. One of skill in the art may readily adapt the NSEQs for these purposes.

Those skilled in the art will also recognize that the NSEQs and its expression products such as cDNA nucleic acids and genomic DNA may be used to prepare short oligonucleotides sequences. For example, oligonucleotides having ten to twelve nucleotides or more may be prepared which hybridize specifically to the present NSEQs and cDNAs and allow detection, identification and isolation of unique nucleic sequences by hybridization. Sequences of for example, at least 15-20 nucleotides may be used and selected from regions that lack homology to other known sequences. Sequences of 20 or more nucleotides that lack such homology show an increased specificity toward the target sequence. Useful hybridization conditions for probes and primers are readily determinable by those of skill in the art. Stringent hybridization conditions encompassed herewith are those that may allow hybridization of nucleic acids that are greater than 90% homologous but which may prevent hybridization of nucleic acids that are less than 70% homologous. The specificity of a probe may be determined by whether it is made from a unique region, a regulatory region, or from a conserved motif. Both probe specificity and the stringency of diagnostic hybridization or amplification (maximal, high, intermediate, or low) reactions may be determined whether the probe identifies exactly complementary sequences, allelic variants, or related sequences. Probes designed to detect related sequences may have at least 50% sequence identity to any of the selected polynucleotides.

It is to be understood herein that the NSEQs (including substantially identical sequences and fragments thereof) may hybridize to a substantially complementary sequence found in a test sample. Additionally, a sequence substantially complementary to NSEQ may bind a NSEQ found in a test sample.

Furthermore, a probe may be labelled by any procedure known in the art, for example by incorporation of nucleotides linked to a "reporter molecule". A "reporter molecule", as used herein, may be a molecule that provides an analytically identifiable signal allowing detection of a hybridized probe. Detection may be either qualitative or quantitative. Commonly used reporter molecules include fluorophores, enzymes, biotin, chemiluminescent molecules, bioluminescent molecules, digoxigenin, avidin, streptavidin or radioisotopes. Commonly used enzymes include horseradish peroxidase, alkaline phosphatase, glucose oxidase and β-galactosidase, among others. Enzymes may be conjugated to avidin or streptavidin for use with a biotinylated probe. Similarly, probes may be conjugated to avidin or streptavidin for use with a biotinylated enzyme. Incorporation of a reporter molecule into a DNA probe may be by any method known to the skilled artisan, for example by nick translation, primer extension, random oligo priming, by 3' or 5' end labeling or by other means. In addition, hybridization probes include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro. The labelled polynucleotide sequences may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; and in micro arrays utilizing samples from subjects to detect altered expression. Oligonucleotides useful as probes for screening of samples by hybridization assays or as primers for amplification may be packaged into kits. Such kits may contain the probes or primers in a pre-measured or predetermined amount, as well as other suitably packaged reagents and materials needed for the particular hybridization or amplification protocol. In another embodiment, the invention entails a substantially purified polypeptide encoded by the polynucleotides of NSEQs, polypeptide analogs or polypeptide fragments thereof. The polypeptides whether in a premature, mature or fused form, may be isolated from lysed cells, or from the culture medium, and purified to the extent

needed for the intended use. One of skill in the art may readily purify these proteins, polypeptides and peptides by any available procedure. For example, purification may be accomplished by salt fractionation, size exclusion chromatography, ion exchange chromatography, reverse phase chromatography, affinity chromatography and the like.

Use of NSEQ for Development of an Expression System

In order to express a biologically active polypeptide, NSEQ, or derivatives thereof, may be inserted into an expression vector, i.e., a vector that contains the elements for transcriptional and translational control of the inserted coding sequence in a particular host. These elements may include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' un-translated regions. Methods that are well known to those skilled in the art may be used to construct such expression vectors. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination.

A variety of expression vector/host cell systems known to those of skill in the art may be utilized to express NSEQ. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with baculovirus vectors; plant cell systems transformed with viral or bacterial expression vectors; or animal cell systems. For long-term production of recombinant proteins in mammalian systems, stable expression in cell lines may be effected. For example, NSEQ may be transformed into cell lines using expression vectors that may contain viral origins of replication and/or endogenous expression elements and a selectable or visible marker gene on the same or on a separate vector. The invention is not to be limited by the vector or host cell employed.

In general, host cells that contain NSEQ and that express a polypeptide encoded by the NSEQ, or a portion thereof, may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques that include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or amino acid sequences. Immunological methods for detecting and measuring the expression of polypeptides using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). Those of skill in the art may readily adapt these methodologies to the present invention.

The present invention additionally relates to a bioassay for evaluating compounds as potential antagonists of the polypeptide described herein, the bioassay may comprise:

a) culturing test cells in culture medium containing increasing concentrations of at least one compound whose ability to inhibit the action of a polypeptide described herein is sought to be determined, wherein the test cells may contain a polynucleotide sequence described herein (for example, in a form having improved trans-activation transcription activity, relative to wild-type polynucleotide, and comprising a response element operatively linked to a reporter gene); and thereafter

b) monitoring in the cells the level of expression of the product of the reporter gene as a function of the concentration of the potential antagonist compound in the culture medium, thereby indicating the ability of the potential antagonist compound to inhibit activation of the polypeptide encoded by, the polynucleotide sequence described herein.

The present invention further relates to a bioassay for evaluating compounds as potential agonists for a polypeptide encoded by the polynucleotide sequence described herein, the bioassay may comprise:

a) culturing test cells in culture medium containing increasing concentrations of at least one compound whose ability to promote the action of the polypeptide encoded by the polynucleotide sequence described herein is sought to be determined, wherein the test cells may contain a polynucleotide sequence described herein (for example, in a form having improved trans-activation transcription activity, relative to wild-type polynucleotide, and comprising a response element operatively linked to a reporter gene); and thereafter

b) monitoring in the cells the level of expression of the product of the reporter gene as a function of the concentration of the potential agonist compound in the culture medium, thereby indicating the ability of the potential agonist compound to promote activation of a polypeptide encoded by the polynucleotide sequence described herein.

Host cells transformed with NSEQ may be cultured under conditions for the expression and recovery of the polypeptide from cell culture. The polypeptide produced by a transgenic cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing NSEQ may be designed to contain signal sequences that direct secretion of the polypeptide through a prokaryotic or eukaryotic cell membrane. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express the polypeptide encoded by NSEQ. The nucleotide sequences of the present invention may be engineered using methods generally known in the art in order to alter the nucleotide sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing, which cleaves a "prepro" form of the polypeptide, may also be used to specify protein targeting, folding, and/or activity. Different host cells that have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and W138) are available commercially and from the American Type Culture Collection (ATCC) and may be chosen to ensure the correct modification and processing of the expressed polypeptide.

Those of skill in the art will readily appreciate that natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence resulting in translation of a fusion polypeptide containing heterologous polypeptide moieties in any of the aforementioned host systems. Such

heterologous polypeptide moieties may facilitate purification of fusion polypeptides using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein, thioredoxin, calmodulin binding peptide, 6-His (His), FLAG, c-myc, hemaglutinin (HA), and monoclonal antibody epitopes.

In yet a further aspect, the present invention relates to an isolated polynucleotide which may comprise a nucleotide sequence encoding a fusion protein, the fusion protein may comprise a fusion partner fused to a peptide fragment of a protein encoded by, or a naturally occurring allelic variant polypeptide encoded by, the polynucleotide sequence described herein.

Those of skill in the art will also readily recognize that the nucleic acid and polypeptide sequences may be synthesized, in whole or in part, using chemical or enzymatic methods well known in the art. For example, peptide synthesis may be performed using various solid-phase techniques and machines such as the ABI 431A Peptide synthesizer (PE Biosystems) may be used to automate synthesis. If desired, the amino acid sequence may be altered during synthesis and/or combined with sequences from other proteins to produce a variant protein.

Use of NSEQ as a Diagnostic Screening Tool

The skilled artisan will readily recognize that NSEQ may be used for diagnostic purposes to determine the absence, presence, or altered expression (i.e. increased or decreased compared to normal) of the gene. The polynucleotides may be at least 10 nucleotides long or at least 12 nucleotides long or at least 15 nucleotides long up to any desired length and may comprise complementary RNA and DNA molecules, branched nucleic acids, and/or peptide nucleic acids (PNAs). In one alternative, the polynucleotides may be used to detect and quantify gene expression in samples in which expression of NSEQ is correlated with disease. In another alternative, NSEQ may be used to detect genetic polymorphisms associated with a disease. These polymorphisms may be detected in the transcript cDNA.

The invention provides for the use of at least one polynucleotide comprising NSEQ (e.g., an open reading frame of NSEQ, a substantially complementary sequence, a substantially identical sequence, and fragments thereof) on an array and for the use of that array in a method for diagnosing a bone remodeling disease or disorder by hybridizing the array with a patient sample under conditions to allow complex formation, detecting complex formation, and comparing the amount of complex formation in the patient sample to that of standards for normal and diseased tissues wherein the complex formation in the patient sample indicates the presence of a bone remodeling disease or disorder.

In another embodiment, the present invention provides one or more compartmentalized kits for detection of bone resorption disease states. A first kit may have a receptacle containing at least one isolated probe. Such a probe may be a nucleic acid fragment which is present/absent in the genomic DNA of normal cells but which is absent/present in the genomic DNA of affected cells. Such a probe may be specific for a DNA site that is normally active/inactive but which may be inactive/active in certain cell types. Similarly, such a probe may be specific for a DNA site that may be abnormally expressed in certain cell types. Finally, such a probe may identify a specific DNA mutation. By specific for a DNA site is meant that the probe may be capable of hybridizing to the DNA sequence which is mutated, or may be capable of hybridizing to DNA sequences adjacent to the mutated DNA sequences. The probes provided in the present kits may have a covalently attached reporter molecule. Probes and reporter molecules may be readily prepared as described above by those of skill in the art.

Use of NSEQ as a Therapeutic

One of skill in the art will readily appreciate that the expression systems and assays discussed above may also be used to evaluate the efficacy of a particular therapeutic treatment regimen, in animal studies, in clinical trials, or to monitor the treatment of an individual subject. Once the presence of disease is established and a treatment protocol is initiated, hybridization or amplification assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate the level observed in a healthy subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to many years.

In yet another aspect of the invention, an NSEQ, a portion thereof, or its complement, may be used therapeutically for the purpose of expressing mRNA and polypeptide, or conversely to block transcription or translation of the mRNA. Expression vectors may be constructed using elements from retroviruses, adenoviruses, herpes or vaccinia viruses, or bacterial plasmids, and the like. These vectors may be used for delivery of nucleotide sequences to a particular target organ, tissue, or cell population. Methods well known to those skilled in the art may be used to construct vectors to express nucleic acid sequences or their complements.

Alternatively, NSEQ, a portion thereof, or its complement, may be used for somatic cell or stem cell gene therapy. Vectors may be introduced in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors are introduced into stem cells taken from the subject, and the resulting transgenic cells are clonally propagated for autologous transplant back into that same subject. Delivery of NSEQ by transfection, liposome injections, or polycationic amino polymers may be achieved using methods that are well known in the art. Additionally, endogenous NSEQ expression may be inactivated using homologous recombination methods that insert an inactive gene sequence into the coding region or other targeted region of NSEQ.

Depending on the specific goal to be achieved, vectors containing NSEQ may be introduced into a cell or tissue to express a missing polypeptide or to replace a non-functional polypeptide. Of course, when one wishes to express PSEQ in a cell or tissue, one may use a NSEQ able to encode such PSEQ for that purpose or may directly administer PSEQ to that cell or tissue.

On the other hand, when one wishes to attenuate or inhibit the expression of PSEQ, one may use a NSEQ (e.g., an inhibitory NSEQ) which is substantially complementary to at least a portion of a NSEQ able to encode such PSEQ.

The expression of an inhibitory NSEQ may be done by cloning the inhibitory NSEQ into a vector and introducing the vector into a cell to down-regulate the expression of a polypeptide encoded by the target NSEQ.

Vectors containing NSEQ (e.g., including inhibitory NSEQ) may be transformed into a cell or tissue to express a missing polypeptide or to replace a non-functional polypeptide. Similarly a vector constructed to express the complement of NSEQ may be transformed into a cell to down-regulate the over-expression of a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof. Complementary or anti-sense sequences may consist of an oligonucleotide derived from the transcription initiation site; nucleotides between about positions −10 and +10 from the ATG are preferred. Similarly, inhibition may be achieved using triple helix base-pairing methodology. Triple helix pairing is useful

because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee et al. 1994)

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the cleavage of mRNA and decrease the levels of particular mRNAs, such as those comprising the polynucleotide sequences of the invention. Ribozymes may cleave mRNA at specific cleavage sites. Alternatively, ribozymes may cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The construction and production of ribozymes is well known in the art.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages within the backbone of the molecule. Alternatively, nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases, may be included.

In addition to the active ingredients, a pharmaceutical composition may contain pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations that may be used pharmaceutically.

For any compound, the therapeutically effective dose may be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the concentration range and route of administration. Such information may then be used to determine useful doses and routes for administration in humans. These techniques are well known to one skilled in the art and a therapeutically effective dose refers to that amount of active ingredient that ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating and contrasting the ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population) statistics. Any of the therapeutic compositions described above may be applied to any subject in need of such therapy, including, but not limited to, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

The term "treatment" for purposes of this disclosure refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

Use of NSEQ in General Research

The invention finally provides products, compositions, processes and methods that utilize an NSEQ, their open reading frame, or a polypeptide encoded by the polynucleotides of NSEQ or their open reading frame, or a portion thereof, their variants, analogs, derivatives and fragments for research, biological, clinical and therapeutic purposes. For example, to identify splice variants, mutations, and polymorphisms

NSEQ may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences such as promoters and other regulatory elements. Additionally, one may use an XL-PCR kit (PE Biosystems, Foster City Calif.), nested primers, and commercially available cDNA libraries (Life Technologies, Rockville Md.) or genomic libraries (Clontech, Palo Alto Calif.) to extend the sequence.

The polynucleotides may also be used as targets in a micro-array. The micro-array may be used to monitor the expression patterns of large numbers of genes simultaneously and to identify splice variants, mutations, and polymorphisms. Information derived from analyses of the expression patterns may be used to determine gene function, to understand the genetic basis of a disease, to diagnose a disease, and to develop and monitor the activities of therapeutic agents used to treat a disease. Microarrays may also be used to detect genetic diversity, single nucleotide polymorphisms which may characterize a particular population, at the genomic level.

In yet another embodiment, polynucleotides may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data.

The present invention more particularly relates in one aspect thereof to a method of representatively identifying an endogeneously differentially expressed sequence involved in osteoclast differentiation. The sequence may be, for example, differentially expressed in a differentiated osteoclast cell compared to an undifferentiated osteoclast precursor cell.

The method of the present invention may comprise;

a) separately providing total messenger RNA from (mature or intermediately) differentiated human osteoclast cell and undifferentiated human osteoclast precursor cell, the total messenger RNA may comprise, for example, at least one endogeneously differentially expressed sequence,

b) generating single-stranded cDNA from each messenger RNA of differentiated human osteoclast cell and (e.g., randomly) tagging the 3'-end of the single-stranded cDNA with a RNA polymerase promoter sequence and a first sequence tag;

c) generating single-stranded cDNA from each messenger RNA of undifferentiated human osteoclast precursor cell and (e.g., randomly) tagging the 3'-end of the single-stranded cDNA with a RNA polymerase promoter sequence and a second sequence tag;

d) separately generating partially or completely double-stranded 5'-tagged-DNA from each of b) and c), the double-stranded 5'-tagged-DNA may thus comprise in a 5' to 3' direction, a double-stranded RNA polymerase promoter, a first or second sequence tag and an endogenously expressed sequence,

e) separately linearly amplifying a first and second tagged sense RNA from each of d) with a RNA polymerase enzyme (which may be selected based on the promoter used for tagging),

f) generating single-stranded complementary first or second tagged DNA from one of e),

g) hybridizing the single-stranded complementary first or second tagged DNA of

f) with the other linearly amplified sense RNA of e),

h) recovering unhybridized RNA with the help of the first or second sequence tag (for example by PCR or hybridization), and;

i) identifying (determining) the nucleotide sequence of unhybridized RNA.

Steps b) and/or c), may comprise generating a single copy of a single-stranded cDNA.

The method may further comprise the step of comparatively determining the presence of the identified endogeneously and differentially expressed sequence in a differentiated osteoclast cell relative to an undifferentiated osteoclast precursor cell.

A sequence which is substantially absent (e.g., totally absent or present in very low quantity) from one of differentiated osteoclast cell or an undifferentiated osteoclast precursor cell and present in the other of differentiated osteoclast cell or an undifferentiated osteoclast precursor cell may therefore be selected.

The sequence thus selected may be a positive regulator of osteoclast differentiation and therefore may represent an attractive target which may advantageously be used to promote bone resorption or alternatively such target may be inhibited to lower or prevent bone resorption.

Alternatively, the sequence selected using the above method may be a negative regulator of osteoclast differentiation and may therefore represent an attractive target which may advantageously be induced (e.g., at the level of transcription, translation, activity etc.) or provided to a cell to lower or prevent bone resorption. Also such negative regulator may, upon its inhibition, serve as a target to promote bone resorption.

In accordance with the present invention, the sequence may be further selected based on a reduced or substantially absent expression in other normal tissue, therefore representing a candidate sequence specifically involved in osteoclast differentiation and bone remodeling.

The method may also further comprise a step of determining the complete sequence of the nucleotide sequence and may also comprise determining the coding sequence of the nucleotide sequence.

The present invention also relates in a further aspect, to the isolated endogeneously and differentially expressed sequence (polynucleotide and polypeptide) identified by the method of the present invention.

More particularly, the present invention encompasses a polynucleotide which may comprise the identified polynucleotide sequence, a polynucleotide which may comprise the open reading frame of the identified polynucleotide sequence, a polynucleotide which may comprise a nucleotide sequence substantially identical to the polynucleotide identified by the method of the present invention, a polynucleotide which may comprise a nucleotide sequence substantially complementary to the polynucleotide identified by the method of the present invention, fragments and splice variant thereof.

In accordance with the present invention, the isolated endogeneously and differentially expressed sequence of the present invention may be a complete or partial RNA molecule.

Isolated DNA molecule able to be transcribed into the RNA molecule of the present invention are also encompassed herewith as well as vectors (including expression vectors) comprising the such DNA or RNA molecule.

The present invention also relates to libraries comprising at least one isolated endogeneously and differentially expressed sequence identified herein (e.g., partial or complete RNA or DNA, substantially identical sequences or substantially complementary sequences (e.g., probes) and fragments thereof (e.g., oligonucleotides)).

In accordance with the present invention, the isolated endogeneously and differentially expressed sequence may be selected, for example, from the group consisting of a polynucleotide which may consist in or comprise;

a) any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107,

b) the open reading frame of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107,

c) a polynucleotide which may comprise a nucleotide sequence substantially identical to a) or b), and;

d) a polynucleotide which may comprise a nucleotide sequence substantially complementary to any one of a) to c),

e) fragments of any one of a) to d).

In a further aspect the present invention relates to a polypeptide which may be encoded by the isolated endogeneously and differentially expressed sequence of the present invention.

In yet a further aspect the present invention relates to a polynucleotide able to encode a polypeptide of the present invention. Due to the degeneracy of the genetic code, it is to be understood herein that a multiplicity of polynucleotide sequence may encode the same polypeptide sequence and thus are encompassed by the present invention.

Exemplary polypeptides may comprise a sequence selected from the group consisting of any one of SEQ ID NO.:2 and a SEQ ID NO.:2 variant (e.g., SEQ ID NO.:4, SEQ ID NO.:108).

The present invention also relates to an isolated non-human ortholog polynucleotide sequence (involved in bone remodeling), the open reading frame of the non-human ortholog, substantially identical sequences, substantially complementary sequences, fragments and splice variants thereof.

The present invention as well relates to an isolated polypeptide encoded by the non-human ortholog polynucleotide as well as biologically active analogs and biologically active fragments thereof.

Exemplary embodiments of non-human (e.g., mouse) ortholog polynucleotides encompassed herewith include, for example, SEQ ID NO.:3 or SEQ ID NO.:107.

Exemplary embodiments of isolated polypeptide encoded by some non-human orthologs identified herein include for example, a polypeptide such as SEQ ID NO.:4 or SEQ ID NO.:108.

Exemplary embodiments of SEQ ID NO.:2 variant having 80% identity with SEQ ID NO.:2 include for example and without limitation, SEQ ID NO.:4, SEQ ID NO.:108 as well as other analogues that are published in databases under gene bank accession numbers or NCBI reference sequence: AAY40743.1, XP_512109.2, XP_001089000.1, XP_601064.4, NP_001094508.1, XP_855238.1, XP_574176.2 and EAX01462.1.

The present invention also more particularly relates, in an additional aspect thereof, to an isolated polynucleotide which may be differentially expressed in differentiated osteoclast cell compared to undifferentiated human osteoclast precursor cell.

The isolated polynucleotide may comprise a member selected from the group consisting of;

a) a polynucleotide which may comprise any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107;

b) a polynucleotide which may comprise the open reading frame of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107;

c) a polynucleotide which may comprise a transcribed or transcribable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, which may be, for example, free of untranslated or untranslatable portion(s);

d) a polynucleotide which may comprise a translated or translatable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 (e.g., coding portion),

e) a polynucleotide which may comprise a sequence substantially identical (e.g., from about 50 to 100%, or about 60 to 100% or about 70 to 100% or about 80 to 100% or about 85, 90, 95 to 100% identical over the entire sequence or portion of sequences) to a), b) c) or d),

f) a polynucleotide which may comprise a sequence substantially complementary (e.g., from about 50 to 100%, or about 60 to 100% or about 70 to 100% or about 80 to 100% or about 85, 90, 95 to 100% complementarity over the entire sequence or portion of sequences) to a), b), c) or d) and;

g) a fragment of any one of a) to f)

h) including polynucleotides which consist in the above.

Exemplary polynucleotides fragments of those listed above comprise polynucleotides of at least 10 nucleic acids which may be substantially complementary to the nucleic acid sequence of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107.

The present invention also relates to an isolated polynucleotide involved in osteoclast differentiation, the isolated polynucleotide may be selected, for example, from the group consisting of;

a) a polynucleotide comprising any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107,

b) a polynucleotide comprising the open reading frame of any one of SEQ ID NO.:1, SEQ ID NO.: 3 or SEQ ID NO.:107,

c) a polynucleotide which may comprise a transcribed or transcribable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, which may be, for example, free of untranslated or untranslatable portion(s);

d) a polynucleotide which may comprise a translated or translatable portion of any one of SEQ. ID. NO.: SEQ ID NO.:3 or SEQ ID NO.:107 (e.g., coding portion),

e) a polynucleotide substantially identical to a), b), c) or d); and;

f) a sequence of at least 10 nucleic acids which may be substantially complementary to the nucleic acid sequence of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 or more particularly of a), b), c) or d).

In accordance with the present invention the isolated polynucleotide may be able to promote osteoclast differentiation (e.g., in a mammal or mammalian cell thereof), i.e, a positive regulator of osteoclast differenciation.

Further in accordance with the present invention, the isolated polynucleotide may be able to inhibit, prevent or lower osteoclast differentiation (e.g., in a mammal or mammalian cell thereof), i.e, a negative regulator of osteoclast differenciation.

In yet a further aspect, the present invention relates to an isolated polynucleotide which may be able to inhibit osteoclast differentiation (e.g., in a mammal or mammalian cell thereof). The polynucleotide may be selected, for example, from the group consisting of polynucleotides which may comprise a sequence of at least 10 nucleic acids which is complementary to the nucleic acid sequence of any one of NSEQ described herein.

Suitable polynucleotides may be those which may be able to inhibit osteoclast differentiation which has been induced by an inducer of osteoclast differentiation such as those listed herein.

In accordance with the present invention, the polynucleotide may be, for example, a RNA molecule, a DNA molecule, including those which are partial or complete, single-stranded or double-stranded, hybrids, etc.

The present invention also relates to a vector (e.g., an expression vector) comprising the polynucleotide of the present invention.

The present invention additionally relates in an aspect thereof to a library of polynucleotide sequences which may be differentially expressed in a differentiated osteoclast cell compared to an undifferentiated osteoclast precursor cell. The library may comprise, for example, at least one member selected from the group consisting of

a) a polynucleotide which may comprise any one of SEQ ID NO.:1, SEQ ID NO.: 3 or SEQ ID NO.:107,

b) a polynucleotide which may comprise the open reading frame of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107,

c) a polynucleotide which may comprise a transcribed or transcribable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, which may be, for example, free of untranslated or untranslatable portion(s);

d) a polynucleotide which may comprise a translated or translatable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 (e.g., coding portion),

e) a polynucleotide which may comprise a sequence substantially identical (e.g., from about 50 to 100%, or about 60 to 100% or about 70 to 100% or about 80 to 100% or about 85, 90, 95 to 100% identical over the entire sequence or portion of sequences) to a), b), c) or d);

f) a polynucleotide which may comprise a sequence substantially complementary (e.g., from about 50 to 100%, or about 60 to 100% or about 70 to 100% or about 80 to 100% or about 85, 90, 95 to 100% complementarity over the entire sequence or portion of sequences) to a), b), c) or d) and;

g) a fragment of any one of a) to d).

The present invention also relates to an expression library which may comprise a library of polynucleotides described herein. In accordance with the present invention, each of the polynucleotide may be contained within an expression vector.

Arrays and kits comprising a library of polynucleotide sequences (comprising at least one polynucleotide such as complementary sequences) of the present invention are also encompassed herewith.

The present invention also provides in an additional aspect, a pharmaceutical composition for inhibiting osteoclast differentiation (bone resorption and bone resorption related diseases or disorders), the pharmaceutical composition may comprise, for example;

a) an isolated polynucleotide as defined herein (e.g., able to inhibit osteoclast differentiation) and;

b) a pharmaceutically acceptable carrier.

The present invention also provides in yet an additional aspect, a method for inhibiting osteoclast differentiation (e.g., for inhibiting bone resorption or for ameliorating bone resorption) in a mammal (individual) in need thereof (or in a mammalian cell), the method may comprise administering an isolated polynucleotide (e.g., able to inhibit osteoclast differentiation) or a suitable pharmaceutical composition comprising such suitable polynucleotide.

In accordance with the present invention, the mammal in need may suffer, for example and without limitation, from a condition selected from the group consisting of osteoporosis, osteopenia, osteomalacia, hyperparathyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Turner syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome, Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets (including vitamin D dependent, type I and II, and x-linked hypophosphatemic rickets), fibrogenesis imperfecta ossium, osteosclerotic disorders such as pycnodysostosis and damage caused by macrophage-mediated inflammatory processes, etc.

In a further aspect, the present invention relates to the use of an isolated polynucleotide (e.g., able to inhibit osteoclast differentiation) for the preparation of a medicament for the treatment of a bone resorption disease.

The present invention in another aspect thereof, provides a pharmaceutical composition for promoting osteoclast differentiation in a mammal in need thereof. The pharmaceutical composition may comprise, for example;

a. an isolated polynucleotide (e.g., able to promote osteoclast differentiation) and;

b. a pharmaceutically acceptable carrier.

The present invention also further provides a method for promoting osteoclast differentiation in a mammal in need thereof (or in a mammalian cell), the method may comprise, for example, administering an isolated polynucleotide (e.g., able to promote osteoclast differentiation) or a suitable pharmaceutical composition as described above.

The present invention additionally relates to the use of an isolated polynucleotide (e.g., able to promote osteoclast differentiation) for the preparation of a medicament for the treatment of a disease associated with insufficient bone resorption (e.g., hyperostosis) or excessive bone growth.

The present invention also relates to the use of at least one polynucleotide which may be selected from the group consisting of;

a) a polynucleotide comprising any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107,

b) a polynucleotide comprising the open reading frame of any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107,

c) a polynucleotide which may comprise a transcribed or transcribable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, which may be, for example, free of untranslated or untranslatable portion(s);

d) a polynucleotide which may comprise a translated or translatable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 (e.g., coding portion),

e) a polynucleotide comprising a sequence substantially identical (e.g., from about 50 to 100%, or about 60 to 100% or about 70 to 100% or about 80 to 100% or about 85, 90, 95 to 100% identical over the entire sequence or portion of sequences) to a), b), c) or d);

f) a polynucleotide comprising a sequence substantially complementary (e.g., from about 50 to 100%, or about 60 to 100% or about 70 to 100% or about 80 to 100% or about 85, 90, 95 to 100% complementarity over the entire sequence or portion of sequences) to a), b), c) or d);

g) a fragment of any one of a) to f) and;

h) a library comprising any one of a) to g)

in the diagnosis of a condition related to bone remodeling (a bone disease).

Also encompassed by the present invention are kits for the diagnosis of a condition related to bone remodeling. The kit may comprise a polynucleotide as described herein.

The present invention also provides in an additional aspect, an isolated polypeptide (polypeptide sequence) involved in osteoclast differentiation (in a mammal or a mammalian cell thereof). The polypeptide may comprise (or consist in) a sequence selected from the group consisting of;

a) any one of SEQ ID NO.:2 or a SEQ ID NO.:2 variant (e.g., SEQ ID NO.:4, SEQ ID NO.:108),

b) a polypeptide able to be encoded and/or encoded by any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 (their coding portion)

c) a biologically active fragment of any one of a) or b),

d) a biologically active analog of any one of a) or b).

In accordance with the present invention, the biologically active analog may comprise, for example, at least one amino acid substitution (conservative or non conservative) compared to the original sequence. In accordance with the present invention, the analog may comprise, for example, at least one amino acid substitution, deletion or insertion in its amino acid sequence.

The substitution may be conservative or non-conservative. The polypeptide analog may be a biologically active analog or an immunogenic analog which may comprise, for example, at least one amino acid substitution (conservative or non conservative), for example, 1 to 5, 1 to 10, 1 to 15, 1 to 20, 1 to 50 etc. (including any number there between) compared to the original sequence. An immunogenic analog may comprise, for example, at least one amino acid substitution compared to the original sequence and may still be bound by an antibody specific for the original sequence.

In accordance with the present invention, a polypeptide fragment may comprise, for example, at least 6 consecutive amino acids, at least 8 consecutive amino acids or more of an amino acid sequence described herein.

In yet a further aspect, the present invention provides a pharmaceutical composition which may comprise, for example a polypeptide as described herein and a pharmaceutically acceptable carrier.

Methods for modulating osteoclast differentiation in a mammal in need thereof (or in a mammalian cell) are also provided by the present invention, which methods may comprise administering an isolated polypeptide (e.g., able to promote osteoclast differentiation) or suitable pharmaceutical composition described herein.

In additional aspects, the present invention relates to the use of an isolated polypeptide (e.g., able to promote osteoclast differentiation) for the preparation of a medicament for the treatment of a disease associated with insufficient bone resorption.

Methods for ameliorating bone resorption in an individual in need thereof are also encompassed herewith, which method may comprise, for example, administering an isolated polypeptide (e.g., able to inhibit osteoclast differentiation) or suitable pharmaceutical compositions which may comprise such polypeptide.

In accordance with the present invention, the mammal may suffer, for example, from a condition selected from the group consisting of osteoporosis, osteopenia, osteomalacia, hyperparathyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Turner syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome,

Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets (including vitamin D dependent, type I and II, and x-linked hypophosphatemic rickets), fibrogenesis imperfecta ossium, osteosclerotic disorders such as pycnodysostosis and damage caused by macrophage-mediated inflammatory processes, etc.

In yet a further aspect, the present invention relates to the use of a polypeptide able to inhibit osteoclast differentiation in the preparation of a medicament for the treatment of a bone resorption disease in an individual in need thereof.

The present invention also relates to a compound and the use of a compound able to inhibit (e.g., in an osteoclast precursor cell) the activity or expression of a polypeptide which may be selected, for example, from the group consisting of antibodies and antigen binding fragments thereof, in the preparation of a medicament for the treatment of a bone disease in an individual in need thereof.

In yet an additional aspect, the present invention relates to a method of diagnosing a condition related to a bone resorption disorder or disease in an individual in need thereof. The method may comprise, for example, quantifying a polynucleotide described herein, such as, for example, polynucleotide selected from the group consisting of those comprising or consisting of (a) SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, (b) a polynucleotide which may comprise the open reading frame of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107, (c) a polynucleotide which may comprise a transcribed or transcribable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107; (d) a polynucleotide which may comprise a translated or translatable portion of any one of SEQ. ID. NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107; (e) substantially identical sequences of any one of (a) to (d); (f) substantially complementary sequences of any one of (a) to (e), or a polypeptide sequence which may be selected, for example, from the group consisting of SEQ ID NO.:2 and a SEQ ID NO.:2 variant thereof in a sample from the individual compared to a standard or normal value.

The present invention also relates to an assay and method for identifying a gene and/or protein involved in bone remodeling. The assay and method may comprise silencing an endogenous gene of an osteoclast cell and providing the cell with a candidate gene (or protein). A candidate gene (or protein) positively involved in bone remodeling may be identified by its ability to complement the silenced endogenous gene. For example, a candidate gene involved in osteoclast differentiation provided to a cell for which an endogenous gene has been silenced, may enable the cell to differentiate in the presence of an inducer such as, for example, RANKL.

The present invention further relates to a cell expressing an exogenous form of any one of the polypeptide (including variants, analogs etc.) or polynucleotide of the present invention (including substantially identical sequences, substantially complementary sequences, fragments, variants, orthologs, etc).

In accordance with the present invention, the cell may be for example, a bone cell. Also in accordance with the present invention, the cell may be an osteoclast (at any level of differentiation).

As used herein the term "exogenous form" is to be understood herein as a form which is not naturally expressed by the cell in question.

Antibodies and Antigen Binding Fragments

The term "antibody" refers to intact antibody, monoclonal or polyclonal antibodies. The term "antibody" also encompasses multispecific antibodies such as bispecific antibodies. Human antibodies are usually made of two light chains and two heavy chains each comprising variable regions and constant regions. The light chain variable region comprises 3 CDRs, identified herein as CDRL1, CDRL2 and CDRL3 flanked by framework regions. The heavy chain variable region comprises 3 CDRs, identified herein as CDRH1, CDRH2 and CDRH3 flanked by framework regions.

The term "antigen-binding fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to bind to an antigen (e.g., SEQ ID NO.:2 or variants thereof). It has been shown that the antigen-binding function of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR), e.g., V_H CDR3. Furthermore, although the two domains of the Fv fragment, V_L and are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single polypeptide chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. Furthermore, the antigen-binding fragments include binding-domain immunoglobulin fusion proteins comprising (i) a binding domain polypeptide (such as a heavy chain variable region, a light chain variable region, or a heavy chain variable region fused to a light chain variable region via a linker peptide) that is fused to an immunoglobulin hinge region polypeptide, (ii) an immunoglobulin heavy chain CH2 constant region fused to the hinge region, and (iii) an immunoglobulin heavy chain CH3 constant region fused to the CH2 constant region. The hinge region may be modified by replacing one or more cysteine residues with serine residues so as to prevent dimerization. Such binding-domain immunoglobulin fusion proteins are further disclosed in US 2003/0118592 and US 2003/0133939. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

A typical antigen binding site is comprised of the variable regions formed by the pairing of a light chain immunoglobulin and a heavy chain immunoglobulin. The structure of the antibody variable regions is very consistent and exhibits very similar structures. These variable regions are typically comprised of relatively homologous framework regions (FR) interspaced with three hypervariable regions termed Complementarity Determining Regions (CDRs). The overall binding activity of the antigen binding fragment is often dictated by the sequence of the CDRs. The FRs often play a role in the proper positioning and alignment in three dimensions of the CDRs for optimal antigen binding. Antibodies and/or antigen binding fragments of the present invention may originate, for example, from a mouse, a rat or any other mammal or from other sources such as through recombinant DNA technologies.

In a further aspect, the present invention relates to an antibody (e.g., isolated antibody), or antigen-binding fragment

thereof, that may specifically bind to a protein or polypeptide described herein. The antibody may be, for example, a monoclonal antibody; a polyclonal antibody an antibody generated using recombinant DNA technologies. The antibody may originate for example, from a mouse, rat, rabbit or any other mammal.

The antibody may also be a human antibody which may be obtained, for example, from a transgenic non-human mammal capable of expressing human Ig genes. The antibody may also be a humanised antibody which may comprise, for example, one or more complementarity determining regions of non-human origin. It may also comprise a surface residue of a human antibody and/or framework regions of a human antibody. The antibody may also be a chimeric antibody which may comprise, for example, variable domains of a non-human antibody and constant domains of a human antibody.

Suitable antibodies may also include, for example, an antigen-binding fragment, an Fab fragment; an $F(ab')_2$ fragment, and Fv fragment; or a single-chain antibody comprising an antigen-binding fragment (e.g., a single chain Fv).

The antibody of the present invention may be mutated and selected based on an increased affinity and/or specificity for one of a polypeptide described herein and/or based on a reduced immunogenicity in a desired host.

The antibody may further comprise a detectable label attached thereto.

The present invention further relates to a method of producing antibodies able to bind to one of a polypeptide, polypeptide fragments, or polypeptide analogs described herein, the method may comprise:

a) immunizing a mammal (e.g., mouse, a transgenic mammal capable of producing human Ig, etc.) with a suitable amount of a PSEQ described herein including, for example, a polypeptide fragment comprising at least 6 consecutive amino acids of a PSEQ;
b) collecting the serum from the mammal; and
c) isolating the polypeptide-specific antibodies from the serum of the mammal.

The method may further comprise the step of administering a second dose to the animal.

The present invention also relates to a method of producing a hybridoma which secretes an antibody that binds to a polypeptide described herein, the method may comprise:

a) immunizing a mammal (e.g., mouse, a transgenic mammal capable of producing human Ig, etc.) with a suitable amount of a PSEQ thereof;
b) obtaining lymphoid cells from the immunized animal obtained from (a);
c) fusing the lymphoid cells with an immortalizing cell to produce hybrid cells; and
d) selecting hybrid cells which produce antibody that specifically binds to a PSEQ thereof.

The present invention further relates to a method of producing an antibody that binds to one of the polypeptide described herein, the method may comprise:

a) synthesizing a library of antibodies (antigen binding fragment) on phage or ribosomes;
b) panning the library against a sample by bringing the phage or ribosomes into contact with a composition comprising a polypeptide or polypeptide fragment described herein;
c) isolating phage which binds to the polypeptide or polypeptide fragment, and;
d) obtaining an antibody from the phage or ribosomes.

The antibody of the present invention may thus be obtained, for example, from a library (e.g., bacteriophage library) which may be prepared, for example, by

a) extracting cells which are responsible for production of antibodies from a host mammal;
b) isolating RNA from the cells of (a);
c) reverse transcribing mRNA to produce cDNA;
d) amplifying the cDNA using a (antibody-specific) primer; and
e) inserting the cDNA of (d) into a phage display vector or ribosome display cassette such that antibodies are expressed on the phage or ribosomes.

The host animal may be immunized with polypeptide and/or a polypeptide fragment and/or analog described herein to induce an immune response prior to extracting the cells which are responsible for production of antibodies.

The present invention also relates to a kit for specifically assaying a polypeptide described herein, the kit may comprise, for example, an antibody or antibody fragment capable of binding specifically to the polypeptide described herein.

The present invention further contemplates antibodies that may bind to PSEQ. Suitable antibodies may bind to unique antigenic regions or epitopes in the polypeptides, or a portion thereof. Epitopes and antigenic regions useful for generating antibodies may be found within the proteins, polypeptides or peptides by procedures available to one of skill in the art. For example, short, unique peptide sequences may be identified in the proteins and polypeptides that have little or no homology to known amino acid sequences. Preferably the region of a protein selected to act as a peptide epitope or antigen is not entirely hydrophobic; hydrophilic regions are preferred because those regions likely constitute surface epitopes rather than internal regions of the proteins and polypeptides. These surface epitopes are more readily detected in samples tested for the presence of the proteins and polypeptides. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. The production of antibodies is well known to one of skill in the art.

Peptides may be made by any procedure known to one of skill in the art, for example, by using in vitro translation or chemical synthesis procedures. Short peptides which provide an antigenic epitope but which by themselves are too small to induce an immune response may be conjugated to a suitable carrier. Suitable carriers and methods of linkage are well known in the art. Suitable carriers are typically large macromolecules such as proteins, polysaccharides and polymeric amino acids. Examples include serum albumins, keyhole limpet hemocyanin, ovalbumin, polylysine and the like. One of skill in the art may use available procedures and coupling reagents to link the desired peptide epitope to such a carrier. For example, coupling reagents may be used to form disulfide linkages or thioether linkages from the carrier to the peptide of interest. If the peptide lacks a disulfide group, one may be provided by the addition of a cysteine residue. Alternatively, coupling may be accomplished by activation of carboxyl groups.

The minimum size of peptides useful for obtaining antigen specific antibodies may vary widely. The minimum size must be sufficient to provide an antigenic epitope that is specific to the protein or polypeptide. The maximum size is not critical unless it is desired to obtain antibodies to one particular epitope. For example, a large polypeptide may comprise multiple epitopes, one epitope being particularly useful and a second epitope being immunodominant. Typically, antigenic peptides selected from the present proteins and polypeptides

will range from 5 to about 100 amino acids in length. More typically, however, such an antigenic peptide will be a maximum of about 50 amino acids in length, and preferably a maximum of about 30 amino acids. It is usually desirable to select a sequence of about 6, 8, 10, 12 or 15 amino acids, up to about 20 or 25 amino acids.

Amino acid sequences comprising useful epitopes may be identified in a number of ways. For example, preparing a series of short peptides that taken together span the entire protein sequence may be used to screen the entire protein sequence. One of skill in the art may routinely test a few large polypeptides for the presence of an epitope showing a desired reactivity and also test progressively smaller and overlapping fragments to identify a preferred epitope with the desired specificity and reactivity.

Antigenic polypeptides and peptides are useful for the production of monoclonal and polyclonal antibodies. Antibodies to a polypeptide encoded by the polynucleotides of NSEQ, polypeptide analogs or portions thereof, may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, such as those that inhibit dimer formation, are especially preferred for therapeutic use. Monoclonal antibodies may be prepared using any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma, the human B-cell hybridoma, and the EBV-hybridoma techniques. In addition, techniques developed for the production of chimeric antibodies may be used. Alternatively, techniques described for the production of single chain antibodies may be employed. Fabs that may contain specific binding sites for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof, may also be generated. Various immunoassays may be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art.

Since hybridoma cells are hybrid mouse cells, they are strictly used to produce murine antibodies. It is clear that the glycosyl side chains of such murine antibodies might significantly differ from the glycosylation pattern observed in human cells. Differences in phosphorylation pattern between human cells and hybridomas might also have an impact on the activity of the antibody. Furthermore, administration of murine antibodies to human usually induces an anti-antibody immune response that could potentially neutralize any of the biological activity that the murine antibody might have.

In order to minimize recognition of murine antibodies by the human immune system or for improving the biological activity of the antibodies in human, murine antibodies are advantageously converted into partially (e.g., chimeric) or fully humanized antibodies. Recombinant form of the light chain and heavy chain of the (partially or fully) humanized antibody may thus be introduced into a mammalian expression system other than hybridoma cells (such as 293 cells, CHO or else). Mammalian expression system may procure the advantage of having a resulting glycosylation pattern that is closer to that of naturally occurring human form of the antibodies.

For example, in the case of lytic IgG1 antibodies, the proper glycosylation of the immunoglobulin chains is necessary for effector functions. These biological functions of IgG1 monoclonal antibodies include antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), both of which will be greatly influenced by the type of glycosyl side chains that are grafted to the amino acids during expression in mammalian cells.

In addition, optimized mammalian cell expression systems will often secrete significantly a greater amounts of antibodies compared to hybridomas. Therefore, there is a practical and probably economical reason for adopting human cells for production.

To obtain polyclonal antibodies, a selected animal may be immunized with a protein or polypeptide. Serum from the animal may be collected and treated according to known procedures. Polyclonal antibodies to the protein or polypeptide of interest may then be purified by affinity chromatography. Techniques for producing polyclonal antisera are well known in the art.

Monoclonal antibodies (MAbs) may be made by one of several procedures available to one of skill in the art, for example, by fusing antibody producing cells with immortalized cells and thereby making a hybridoma. The general methodology for fusion of antibody producing B cells to an immortal cell line is well within the province of one skilled in the art. Another example is the generation of MAbs from mRNA extracted from bone marrow and spleen cells of immunized animals using combinatorial antibody library technology.

One drawback of MAbs derived from animals or from derived cell lines is that although they may be administered to a patient for diagnostic or therapeutic purposes, they are often recognized as foreign antigens by the immune system and are unsuitable for continued use. Antibodies that are not recognized as foreign antigens by the human immune system have greater potential for both diagnosis and treatment. Methods for generating human and humanized antibodies are now well known in the art.

Chimeric antibodies may be constructed in which regions of a non-human MAb are replaced by their human counterparts, e.g., constant region. A preferred chimeric antibody is one that has amino acid sequences that comprise one or more complementarity determining regions (CDRs) of a non-human Mab that binds to a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof, grafted to human framework (FW) regions. Methods for producing such antibodies are well known in the art. Amino acid residues corresponding to CDRs and FWs are known to one of average skill in the art.

A variety of methods have been developed to preserve or to enhance affinity for antigen of antibodies comprising grafted CDRs. One way is to include in the chimeric antibody the foreign framework residues that influence the conformation of the CDR regions. A second way is to graft the foreign CDRs onto human variable domains with the closest homology to the foreign variable region. Thus, grafting of one or more non-human CDRs onto a human antibody may also involve the substitution of amino acid residues which are adjacent to a particular CDR sequence or which are not contiguous with the CDR sequence but which are packed against the CDR in the overall antibody variable domain structure and which affect the conformation of the CDR. Humanized antibodies of the invention therefore include human antibodies which comprise one or more non-human CDRs as well as such antibodies in which additional substitutions or replacements have been made to preserve or enhance binding characteristics.

Chimeric antibodies of the invention also include antibodies that have been humanized by replacing surface-exposed residues to make the MAb appear human. Because the internal packing of amino acid residues in the vicinity of the

antigen-binding site remains unchanged, affinity is preserved. Substitution of surface-exposed residues of a polypeptide encoded by the polynucleotides of NSEQ (or a portion thereof)-antibody according to the invention for the purpose of humanization does not mean substitution of CDR residues or adjacent residues that influence affinity for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof.

Chimeric antibodies may also include antibodies where some or all non-human constant domains have been replaced with human counterparts. This approach has the advantage that the antigen-binding site remains unaffected. However, significant amounts of non-human sequences may be present where variable domains are derived entirely from non-human antibodies.

Antibodies of the invention include human antibodies (e.g., humanized) that are antibodies consisting essentially of human sequences. Human antibodies may be obtained from phage display libraries wherein combinations of human heavy and light chain variable domains are displayed on the surface of filamentous phage. Combinations of variable domains are typically displayed on filamentous phage in the form of Fab's or scFvs. The library may be screened for phage bearing combinations of variable domains having desired antigen-binding characteristics. Preferred variable domain combinations are characterized by high affinity for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof. Preferred variable domain combinations may also be characterized by high specificity for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof, and little cross-reactivity to other related antigens. By screening from very large repertoires of antibody fragments, ($2\text{-}10\times 10^{10}$) a good diversity of high affinity Mabs may be isolated, with many expected to have sub-nanomolar affinities for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof.

Alternatively, human antibodies may be obtained from transgenic animals into which un-rearranged human Ig gene segments have been introduced and in which the endogenous mouse Ig genes have been inactivated. Preferred transgenic animals contain very large contiguous Ig gene fragments that are over 1 Mb in size but human polypeptide-specific Mabs of moderate affinity may be raised from transgenic animals containing smaller gene loci. Transgenic animals capable of expressing only human Ig genes may also be used to raise polyclonal antiserum comprising antibodies solely of human origin.

Antibodies of the invention may include those for which binding characteristics have been improved by direct mutation or by methods of affinity maturation. Affinity and specificity may be modified or improved by mutating CDRs and screening for antigen binding sites having the desired characteristics. CDRs may be mutated in a variety of ways. One way is to randomize individual residues or combinations of residues so that in a population of otherwise identical antigen binding sites, all twenty amino acids may be found at particular positions. Alternatively, mutations may be induced over a range of CDR residues by error prone PCR methods. Phage display vectors containing heavy and light chain variable region gene may be propagated in mutator strains of *E. coli*. These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

Antibodies of the invention may include complete anti-polypeptide antibodies as well as antibody fragments and derivatives that comprise a binding site for a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof. Derivatives are macromolecules that comprise a binding site linked to a functional domain. Functional domains may include, but are not limited to signalling domains, toxins, enzymes and cytokines.

The antibodies obtained by the means described herein may be useful for detecting proteins, variant and derivative polypeptides in specific tissues or in body fluids. Moreover, detection of aberrantly expressed proteins or protein fragments is probative of a disease state. For example, expression of the present polypeptides encoded by the polynucleotides of NSEQ, or a portion thereof, may indicate that the protein is being expressed at an inappropriate rate or at an inappropriate developmental stage. Hence, the present antibodies may be useful for detecting diseases associated with protein expression from NSEQs disclosed herein.

A variety of protocols for measuring polypeptides, including ELISAs, RIAs, and FACS, are well known in the art and provide a basis for diagnosing altered or abnormal levels of expression. Standard values for polypeptide expression are established by combining samples taken from healthy subjects, preferably human, with antibody to the polypeptide under conditions for complex formation. The amount of complex formation may be quantified by various methods, such as photometric means. Quantities of polypeptide expressed in disease samples may be compared with standard values. Deviation between standard and subject values may establish the parameters for diagnosing or monitoring disease.

Design of immunoassays is subject to a great deal of variation and a variety of these are known in the art. Immunoassays may use a monoclonal or polyclonal antibody reagent that is directed against one epitope of the antigen being assayed. Alternatively, a combination of monoclonal or polyclonal antibodies may be used which are directed against more than one epitope. Protocols may be based, for example, upon competition where one may use competitive drug screening assays in which neutralizing antibodies capable of binding a polypeptide encoded by the polynucleotides of NSEQ, or a portion thereof, specifically compete with a test compound for binding the polypeptide. Alternatively one may use, direct antigen-antibody reactions or sandwich type assays and protocols may, for example, make use of solid supports or immunoprecipitation. Furthermore, antibodies may be labelled with a reporter molecule for easy detection. Assays that amplify the signal from a bound reagent are also known. Examples include immunoassays that utilize avidin and biotin, or which utilize enzyme-labelled antibody or antigen conjugates, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labelled reagents include antibodies directed against the polypeptide protein epitopes or antigenic regions, packaged appropriately with the remaining reagents and materials required for the conduct of the assay, as well as a suitable set of assay instructions.

The present invention therefore provides a kit for specifically assaying a polypeptide described herein, the kit may comprise, for example, an antibody or antibody fragment capable of binding specifically to the polypeptide described herein.

In accordance with the present invention, the kit may be a diagnostic kit, which may comprise:

a) one or more antibodies described herein; and

b) a detection reagent which may comprise a reporter group.

In accordance with the present invention, the antibodies may be immobilized on a solid support. The detection reagent may comprise, for example, an anti-immunoglobulin, protein G, protein A or lectin etc. The reporter group may be selected,

without limitation, from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

In an additional aspect, the present invention provides a method for identifying an inhibitory compound (inhibitor, antagonist) which may be able to impair the function (activity) or expression of a polypeptide described herein, such as, for example, those which may be selected from the group consisting of SEQ ID NO.:2 or SEQ ID NO.:2 variant. The method may comprise contacting the polypeptide or a cell expressing the polypeptide with a candidate compound and measuring the function (activity) or expression of the polypeptide. A reduction in the function or activity of the polypeptide (compared to the absence of the candidate compound) may positively identify a suitable inhibitory compound.

In accordance with the present invention, the impaired function or activity may be associated with a reduced ability of the polypeptide to promote osteoclast differentiation, such as osteoclast differentiation induced by an inducer described herein or known in the art.

In accordance with the present invention the cell may not naturally (endogenously) express (polypeptide may substantially be unexpressed in a cell) the polypeptide or analog or alternatively, the expression of a naturally expressed polypeptide analog may be repressed.

For example, suitable method of screening for an inhibitor of SEQ ID NO.:1 may comprise repressing the expression of the mouse ortholog in a mouse osteoclast cell and evaluating differentiation of the osteoclast cell comprising SEQ ID NO.:1 in the presence or absence of a candidate inhibitor and for example, an inducer of osteoclast differentiation (e.g., RANKL).

The present invention also provides a method for identifying an inhibitory compound (inhibitor, antagonist) able to impair the function (activity) or expression of a polypeptide such as, for example SEQ ID NO.:2 or a SEQ ID NO.:2 variant such as SEQ ID NO.:4 or SEQ ID NO.:108. The method may comprise, for example, contacting the (isolated) polypeptide or a cell expressing the polypeptide with a candidate compound and measuring the function (activity) or expression of the polypeptide. A reduction in the function or activity of the polypeptide (compared to the absence of the candidate compound) may thus positively identify a suitable inhibitory compound.

In accordance with the present invention, the impaired function or activity may be associated, for example, with a reduced ability of the polypeptide to inhibit or promote osteoclast differentiation.

The cell used to carry the screening test may not naturally (endogenously) express the polypeptide or analogs, or alternatively the expression of a naturally expressed polypeptide analog may be repressed.

The present invention also relates to a method of identifying a positive or a negative regulator of osteoclast differentiation. The method may comprise, for example, performing a knockdown effect as described herein. The method may more particularly comprise a) providing an osteoclast cell with a compound (e.g., siRNA) able to specifically inhibit a target sequence (e.g., a polynucleotide or polypeptide as described herein), b) inducing differentiation (e.g., with an inducer such as, for example, RANKL) and c) determining the level of differentiation of the osteoclast cell (e.g., measuring the number of differentiated cells, their rate of differentiation, specific marker of differentiation etc).

Upon inhibition of a positive regulator, the levels of osteoclast differentiation will appear lowered. Upon inhibition of a negative regulator, the level of osteoclast differentiation will appear increased.

Another method of identifying a positive or a negative regulator of osteoclast differentiation is to a) provide a cell with one of a target sequence described herein (polypeptide or polynucleotide able to express a polypeptide) b) to induce differentiation (e.g., with an inducer such as, for example, RANKL) and c) to determine the level of differentiation of the osteoclast cell (e.g., measuring the number of differentiated cells, their rate of differentiation, specific marker of differentiation etc).

A cell provided with a positive regulator of osteoclast differentiation may have an increased level of differentiation. A cell provided with a negative regulator of osteoclast differentiation may have a decreased level of differentiation.

The present invention also provides a method of identifying a compound capable of interfering with osteoclast differentiation, the method may comprise contacting a cell including therein a non-endogenous polynucleotide sequence comprising any one of SEQ ID NO.:1, SEQ ID NO.:3 or SEQ ID NO.:107 (a coding portion) and quantifying (e.g. the number of) differentiated osteoclasts. A reduction in osteoclast differentiation in the presence of the compound in comparison to the absence of the compound may be indicative of an antagonist of osteoclast differentiation, while an increase in osteoclast differentiation in the presence of the compound in comparison to the absence of the compound may be indicative of an agonist of osteoclast differentiation.

In accordance with the present invention, the cell may also comprise an endogenous form of a polynucleotide.

As used herein the term "endogenous" means a substance that naturally originates from within an organism, tissue or cell. The term "endogenous polynucleotide" refers to a chromosomal form of a polynucleotide or RNA version (hnRNA, mRNA) produced by the chromosal form of the polynucleotide. The term "endogenous polypeptide" refers to the form of the protein encoded by an "endogenous polynucleotide".

As used herein the term "non-endogenous" or "exogenous" is used in opposition to "endogenous" in that the substance is provided from an external source although it may be introduced within the cell. The term "non-endogenous polynucleotide" refers to a synthetic polynucleotide introduced within the cell and include for example and without limitation, a vector comprising a sequence of interest, a synthetic mRNA, an oligonucleotide comprising a NSEQ etc. The term "non-endogenous polypeptide" refers to the form of the protein encoded by a "non-endogenous polynucleotide".

The present invention also relates to a method of identifying a compound capable of interfering with osteoclast differentiation, the method may comprise contacting a cell including therein a non-endogenous polypeptide sequence comprising any one of SEQ ID NO.:2 or SEQ ID NO.:2 variant with the compound and quantifying (e.g. the number of) differentiated osteoclasts. A reduction in osteoclast differentiation in the presence of the compound in comparison to the absence of the compound may be indicative of an antagonist of osteoclast differentiation while an increase in osteoclast differentiation in the presence of the compound in comparison to the absence of the compound may be indicative of an agonist of osteoclast differentiation.

As used herein the term "sequence identity" relates to (consecutive) nucleotides of a nucleotide sequence which with reference to an original nucleotide sequence. The identity may be compared over a region or over the total sequence of a nucleic acid sequence.

Thus, "identity" may be compared, for example, over a region of 3, 4, 5, 10, 19, 20 nucleotides or more (and any number there between). It is to be understood herein that gaps of non-identical nucleotides may be found between identical nucleic acids. For example, a polynucleotide may have 100% identity with another polynucleotide over a portion thereof. However, when the entire sequence of both polynucleotides is compared, the two polynucleotides may have 50% of their overall (total) sequence identical to one another.

Polynucleotides of the present invention or portion thereof having from about 50 to about 100%, or about 60 to about 100% or about 70 to about 100% or about 80 to about 100% or about 85%, about 90%, about 95% to about 100% sequence identity with an original polynucleotide are encompassed herewith. It is known by those of skill in the art, that a polynucleotide having from about 50% to 100% identity may function (e.g., anneal to a substantially complementary sequence) in a manner similar to an original polynucleotide and therefore may be used in replacement of an original polynucleotide. For example a polynucleotide (a nucleic acid sequence) may comprise or have from about 50% to 100% identity with an original polynucleotide over a defined region and may still work as efficiently or sufficiently to achieve the present invention.

Percent identity may be determined, for example, with an algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights.

As used herein the terms "sequence complementarity" refers to (consecutive) nucleotides of a nucleotide sequence which are complementary to a reference (original) nucleotide sequence. The complementarity may be compared over a region or over the total sequence of a nucleic acid sequence.

Polynucleotides of the present invention or portion thereof having from about 50 to about 100%, or about 60 to about 100% or about 70 to about 100% or about 80 to about 100% or about 85%, about 90%, about 95% to about 100% sequence complementarity with an original polynucleotide are thus encompassed herewith. It is known by those of skill in the art, that an polynucleotide having from about 50% to 100% complementarity with an original sequence may anneal to that sequence in a manner sufficient to carry out the present invention (e.g., inhibit expression of the original polynucleotide).

An "analogue" is to be understood herein as a molecule having a biological activity and chemical structure similar to that of a polypeptide described herein. An "analogue" may have sequence similarity with that of an original sequence or a portion of an original sequence and may also have a modification of its structure as discussed herein. For example, an "analogue" may have at least 90% sequence similarity with an original sequence or a portion of an original sequence. An "analogue" may also have, for example; at least 70% or even 50% sequence similarity (or less, i.e., at least 40%) with an original sequence or a portion of an original sequence.

Also, an "analogue" with reference to a polypeptide may have, for example, at least 50% sequence similarity to an original sequence with a combination of one or more modification in a backbone or side-chain of an amino acid, or an addition of a group or another molecule, etc.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribo-nucleotide, which may be unmodified RNA or DNA, or modified RNA or DNA.

"Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" includes but is not limited to linear and end-closed molecules. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptides" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds (i.e., peptide isosteres). "Polypeptide" refers to both short chains, commonly referred as peptides, oligopeptides or oligomers, and to longer chains generally referred to as proteins. As described above, polypeptides may contain amino acids other than the 20 gene-encoded amino acids.

As used herein the term "polypeptide analog" relates to mutants, variants, chimeras, fusions, deletions, additions and any other type of modifications made relative to a given polypeptide.

As used herein the term "biologically active" refers to a variant or fragment which retains some or all of the biological activity of the natural polypeptide, i.e., to be able to promote or inhibit osteoclast differentiation. Polypeptides or fragments of the present invention may also include "immunologically active" polypeptides or fragments. "Immunologically active polypeptides or fragments may be useful for immunization purposes (e.g. in the generation of antibodies).

Thus, biologically active polypeptides in the form of the original polypeptides, fragments (modified or not), analogues (modified or not), derivatives (modified or not), homologues, (modified or not) of the polypeptides described herein are encompassed by the present invention.

Therefore, any polypeptide having a modification compared to an original polypeptide which does not destroy significantly a desired biological activity is encompassed herein. It is well known in the art, that a number of modifications may be made to the polypeptides of the present invention without deleteriously affecting their biological activity. These modifications may, on the other hand, keep or increase the biological activity of the original polypeptide or may optimize one or more of the particularity (e.g. stability, bioavailability, etc.) of the polypeptides of the present invention which, in some instance might be desirable. Polypeptides of the present invention may comprise for example, those containing amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side-chains and the amino- or carboxy-terminus. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. It is to be understood herein that more than one modification to the polypeptides described herein are encompassed by the present invention to the extent that the biological activity is similar to the original (parent) polypeptide.

As discussed above, polypeptide modification may comprise, for example, amino acid insertion (i.e., addition), deletion and substitution (i.e., replacement), either conservative or non-conservative (e.g., D-amino acids, desamino acids) in the polypeptide sequence where such changes do not substantially alter the overall biological activity of the polypeptide.

Example of substitutions may be those, which are conservative (i.e., wherein a residue is replaced by another of the same general type or group) or when wanted, non-conservative (i.e., wherein a residue is replaced by an amino acid of another type). In addition, a non-naturally occurring amino acid may substitute for a naturally occurring amino acid (i.e., non-naturally occurring conservative amino acid substitution or a non-naturally occurring non-conservative amino acid substitution).

As is understood, naturally occurring amino acids may be sub-classified as acidic, basic, neutral and polar, or neutral and non-polar. Furthermore, three of the encoded amino acids are aromatic. It may be of use that encoded polypeptides differing from the determined polypeptide of the present invention contain substituted codons for amino acids, which are from the same type or group as that of the amino acid to be replaced. Thus, in some cases, the basic amino acids Lys, Arg and H is may be interchangeable; the acidic amino acids Asp and Glu may be interchangeable; the neutral polar amino acids Ser, Thr, Cys, Gln, and Asn may be interchangeable; the non-polar aliphatic amino acids Gly, Ala, Val, Ile, and Leu are interchangeable but because of size Gly and Ala are more closely related and Val, Ile and Leu are more closely related to each other, and the aromatic amino acids Phe, Trp and Tyr may be interchangeable.

It should be further noted that if the polypeptides are made synthetically, substitutions by amino acids, which are not naturally encoded by DNA (non-naturally occurring or unnatural amino acid) may also be made.

A non-naturally occurring amino acid is to be understood herein as an amino acid which is not naturally produced or found in a mammal. A non-naturally occurring amino acid comprises a D-amino acid, an amino acid having an acetylaminomethyl group attached to a sulfur atom of a cysteine, a pegylated amino acid, etc. The inclusion of a non-naturally occurring amino acid in a defined polypeptide sequence will therefore generate a derivative of the original polypeptide. Non-naturally occurring amino acids (residues) include also the omega amino acids of the formula $NH_2(CH_2)_nCOOH$ wherein n is 2-6, neutral nonpolar amino acids, such as sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, norleucine, etc. Phenylglycine may substitute for Trp, Tyr or Phe; citrulline and methionine sulfoxide are neutral nonpolar, cysteic acid is acidic, and ornithine is basic. Proline may be substituted with hydroxyproline and retain the conformation conferring properties.

It is known in the art that analogues may be generated by substitutional mutagenesis and retain the biological activity of the polypeptides of the present invention. These analogues have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. For example, one site of interest for substitutional mutagenesis may include but are not restricted to sites identified as the active site(s), or immunological site(s). Other sites of interest may be those, for example, in which particular residues obtained from various species are identical. These positions may be important for biological activity. Examples of substitutions identified as "conservative substitutions" are shown in Table A. If such substitutions result in a change not desired, then other type of substitutions, denominated "exemplary substitutions" in Table A, or as further described herein in reference to amino acid classes, are introduced and the products screened.

In some cases it may be of interest to modify the biological activity of a polypeptide by amino acid substitution, insertion, or deletion. For example, modification of a polypeptide may result in an increase in the polypeptide's biological activity, may modulate its toxicity, may result in changes in bioavailability or in stability, or may modulate its immunological activity or immunological identity. Substantial modifications in function or immunological identity are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation. (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

(1) hydrophobic: norleucine, methionine (Met), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile)
(2) neutral hydrophilic: Cysteine (Cys), Serine (Ser), Threonine (Thr)
(3) acidic: Aspartic acid (Asp), Glutamic acid (Glu)
(4) basic: Asparagine (Asn), Glutamine (Gln), Histidine (His), Lysine (Lys), Arginine (Arg)
(5) residues that influence chain orientation: Glycine (Gly), Proline (Pro); and aromatic: Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe)

Non-conservative substitutions will entail exchanging a member of one of these classes for another.

TABLE A

Examplary amino acid substitution

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

It is to be understood herein, that if a "range" or "group" of substances (e.g. amino acids), substituents" or the like is mentioned or if other types of a particular characteristic (e.g. temperature, pressure, chemical structure, time, etc.) is mentioned, the present invention relates to and explicitly incorporates herein each and every specific member and combination of sub-ranges or sub-groups therein whatsoever. Thus, any specified range or group is to be understood as a shorthand way of referring to each and every member of a range or group individually as well as each and every possible sub-

ranges or sub-groups encompassed therein; and similarly with respect to any sub-ranges or sub-groups therein. Thus, for example, with respect to a percentage (%) of identity of from about 80 to 100%, it is to be understood as specifically incorporating herein each and every individual %, as well as sub-range, such as for example 80%, 81%, 84.78%, 93%, 99% etc.; and similarly with respect to other parameters such as, concentrations, elements, etc.

It is in particular to be understood herein that the methods of the present invention each include each and every individual steps described thereby as well as those defined as positively including particular steps or excluding particular steps or a combination thereof; for example an exclusionary definition for a method of the present invention, may read as follows: "provided that said polynucleotide does not comprise or consist in SEQ ID NO.:XX or the open reading frame of SEQ ID NO.:XX" or "provided that said polypeptide does not comprise or consist in SEQ ID NO.:XX" or "provided that said polynucleotide fragment or said polypeptide fragment is less than X unit (e.g., nucleotides or amino acids) long or more than X unit (e.g., nucleotides or amino acids) long".

Other objects, features, advantages, and aspects of the present invention will become apparent to those skilled in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

In the appended drawings:

FIG. **1** is a picture of the macroarray hybridization results and quantitation of the signal intensities showing the differential expression data for STAR selected osteoclast-specific human SEQ. ID. NO.:1. The hybridization results obtained confirms its upregulation in all of the human osteoclast samples with generally higher expression in the more mature osteoclasts (A-F 2-4) compared to the precursors (A-F1) and little or no expression in all or most normal tissues (A-H 5-6 and A-G 7-8). In FIG. **1**, macroarrays were prepared using RAMP amplified RNA from human precursor cells (A-F1), and differentiated intermediate (A-F 2-3) and mature osteoclasts for four human donors (A-F 4), and 30 different normal human tissues (adrenal (A5), liver (B5), lung (C5), ovary (D5), skeletal muscle (E5), heart (F5), cervix (G5), thyroid (H5), breast (A6), placenta (B6), adrenal cortex (C6), kidney (D6), vena cava (E6), fallopian tube (F6), pancreas (G6), testicle (H6), jejunum (A7), aorta (B7), esophagus (C7), prostate (D7), stomach (E7), spleen (F7), ileum (G7), trachea (A8), brain (B8), colon (C8), thymus (D8), small intestine (E8), bladder (F8) and duodenum (G8)). The STAR dsDNA clone representing the respective SEQ ID NOs. was labeled with ^{32}P and hybridized to the macroarray. The probe labeling reaction was also spiked with a dsDNA sequence for *Arabidopsis*, which hybridizes to the same sequence spotted on the macroarray (M) in order to serve as a control for the labeling reaction. Quantitation of the hybridization signal at each spot was performed using a STORM 820 phosphorimager and the ImageQuant TL software (Amersham Biosciences, Piscataway, N.J.). A $\log_2$ value representing the average of the signals for the precursors (A-F1) was used as the baseline and was subtracted from the $\log_2$ value obtained for each of the remaining samples in order to determine their relative abundancies compared to the precursors and plotted as a bar graph (right panel).

FIG. **2** is a picture showing the knockdown effects on osteoclastogenesis by attenuating the endogenous expression of SEQ. ID. NO.:1 (AB0326). A significant decrease in the number of multinucleated osteoclasts was observed from precursor cells infected with the AB0326 shRNA (FIG. **2**A; bottom panel) compared to those with the lacZ shRNA (FIGS. **2**A and B; top panels). These results clearly indicated that expression of the gene encoding SEQ. ID. NO.:1 (AB0326) is required for osteoclast differentiation;

FIG. **3** is a picture showing the knockdown effects on osteoclastogenesis of the mouse orthologue for AB0326 in the RAW 264.7 model using shRNA-0326.2 (SEQ. ID. NO.: 5). The RAW-0326.2 cell line produced significantly less osteoclasts (FIG. **3**; bottom panel) compared to the cell line containing the scrambled shRNA (FIG. **3**; top panel). This result, coupled with that obtained in the human osteoclast precursor cells using the lentiviral shRNA delivery system demonstrate that in both human and mouse, AB0326 gene product is clearly required for osteoclastogenesis;

FIG. **4** is a picture showing the results of a functional complementation assay for SEQ. ID. NO.:1 (AB0326) in RAW-0326.2 cells to screen for inhibitors of osteoclastogenesis. The RAW-0326.2 cells transfected with the empty pd2 vector are unable to form osteoclasts in the presence of RANK ligand (center panel) indicating that the mouse AB0326 shRNA is still capable of silencing the AB0326 gene expression in these cells. Conversely, the cells transfected with the cDNA for the human AB0326 (pd2-hAB0326) are rescued and thus, differentiate more efficiently into osteoclasts in response to RANK ligand (right panel). Wild-type RAW 264.7 cells containing the empty vector (pd2) did not adversely affect the formation of osteoclasts in the presence of RANK ligand (left panel) ruling out an effect due to pd2. Thus, this complementation assay can be used to screen for inhibitors of the human AB0326 polypeptide;

FIG. **5** presents a Coomassie-stained polyacrylamide gel containing a sample of the purified human recombinant Siglec-15 that was expressed as a Fc fusion protein in 293-6E cells. This preparation was used to generate the monoclonal antibodies described herein

FIG. **6** shows the results of an Fc-Siglec-15 ELISA of the individual monoclonal antibodies selected from the 96-well plate from Omniclonal library #25 containing anti-Siglec-15 Fabs. The wells indicated by bold numbers contained the exemplary monoclonals 25A1, 25B4, 25B8, 25C1, 25D8, 25E5, 25E6, and 25E9. Also shown is an ELISA on the same plate using the Fc moiety alone to identify those monoclonals that were specific for the Fc portion of the Fc-Siglec-15 fusion protein.

FIG. **7** presents a scheme that illustrates the steps involved to convert the mouse Fabs into IgG2 mouse-human chimeric mAbs.

FIG. **8** shows drawings that compare the binding of the mouse anti-Siglec-15 Fabs with the binding of the corresponding IgG2 chimeric monoclonal antibodies for exemplary antibodies 25B4, 25B8, 25C1, 25D8, 25E6, and 25E9. The results indicate that the relative binding of the Fab variable regions was maintained when transferred to a full human IgG2 scaffold.

FIG. **9** shows the inhibition of the differentiation of human osteoclasts upon treatment with increasing concentrations of anti-Siglec-15 IgG2 chimeric monoclonal antibodies 25B8, 25E6, and 25E9. After treatment, the osteoclasts were stained for TRAP expression.

FIG. **10** shows the inhibition of the differentiation of mouse osteoclasts upon treatment with increasing concentrations of anti-Siglec-15 IgG2 chimeric monoclonal antibodies 25B8, 25E6, and 25D8. After treatment, the osteoclasts were stained for TRAP expression.

FIG. **11** shows the comparative binding of the human and mouse Siglec-15 in the presence of the exemplary antibody 25C8. The result indicates that the binding of the antibodies generated against the human Siglec-15 also interact with the mouse Siglec-15.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The applicant employed a carefully planned strategy to identify and isolate genetic sequences involved in osteoclastogenesis and bone remodeling. The process involved the following steps: 1) preparation of highly representative cDNA libraries using mRNA isolated from precursors and differentiated intermediate and mature osteoclasts of human origin; 2) isolation of sequences upregulated during osteoclastogenesis; 3) identification and characterization of upregulated sequences; 4) selection of upregulated sequences for tissue specificity; and 5) determination of knock-down effects on osteoclastogenesis. The results discussed in this disclosure demonstrate the advantage of targeting osteoclast genes that are specific to this differentiated cell type and provide a more efficient screening method when studying the genetic basis of diseases and disorders. Genes that are known to have a role in other areas of biology have been shown to play a critical role in osteoclastogenesis and osteoclast function. Genes that are known but have not had a role assigned to them until the present disclosure have also been isolated and shown to have a critical role in osteoclastogenesis and osteoclast function. Finally, novel genes have been identified and play a role, however, applicant reserves their disclosure until further study has been completed.

The present invention is illustrated in further details below in a non-limiting fashion.

Material and Methods

Commercially available reagents referred to in the present disclosure were used according to supplier's instructions unless otherwise indicated. Throughout the present disclosure certain starting materials were prepared as follows:

Example 1

Preparation of Osteoclast Differentiated Cells

The RAW 264.7 (RAW) osteoclast precursor cell line and human precursor cells (peripheral blood mononuclear cells or CD34+ progenitors) are well known in the art as murine and human models of osteoclastogenesis. These murine and human osteoclasts are therefore excellent sources of materials for isolating and characterizing genes specialized for osteoclast function.

Human primary osteoclasts were differentiated from G-CSF-mobilized peripheral blood mononuclear cells (Cambrex, East Rutherford, N.J.) as described by the supplier in the presence of 35 ng/ml M-CSF and 100 ng/ml RANK ligand. Multinucleated TRAP-staining osteoclasts were visible by 11-14 days. Osteoclasts were also derived from human osteoclasts precursor cells (CD34+ progenitors) (Cambrex, East Rutherford, N.J.) and cultured as described by the supplier. In the latter case, osteoclasts were obtained after 7 days.

RAW cells were purchased from American Type Culture Collection and maintained in high glucose DMEM containing 10% fetal bovine serum and antibiotics. The cells were sub-cultured bi-weekly to a maximum of 10-12 passages. For osteoclast differentiation experiments, RAW cells were seeded in 96-well plates at a density of 4×10^3 cells/well and allowed to plate for 24 h. Differentiation was induced in high glucose DMEM, 10% charcoal-treated foetal bovine serum (Hyclone, Logan, Utah), 0.05% BSA, antibiotics, 10 ng/ml macrophage colony stimulating factor (M-CSF), and 100 ng/ml receptor activator of NF-kB (RANK) ligand. The plates were re-fed on day 3 and osteoclasts were clearly visible by day 4. Typically, the cells were stained for tartrate-resistant acid phosphatase (TRAP) on day 4 or 5 unless otherwise indicated. For TRAP staining, the cells were washed with PBS and fixed in 10% formaldehyde for 1 h. After two PBS washes, the cells were rendered lightly permeable in 0.2% Triton X-100 in PBS for 5 min before washing in PBS. Staining was conducted at 37° C. for 20-25 min in 0.01% Naphtol AS-MX phosphate, 0.06% Fast Red Violet, 50 mM sodium tartrate, 100 mM sodium acetate, pH 5.2. Cells were visualized microscopically.

Example 2

Method of Isolating Differentially Expressed mRNA

Key to the discovery of differentially expressed sequences unique to osteoclasts is the use of the applicant's patented STAR technology (Subtractive Transcription-based Amplification of mRNA; U.S. Pat. No. 5,712,127 Malek et al., issued on Jan. 27, 1998). In this procedure, mRNA isolated from intermediate and mature osteoclasts is used to prepare "tester RNA", which is hybridized to complementary single-stranded "driver DNA" prepared from osteoclast precursor mRNA and only the un-hybridized "tester RNA" is recovered, and used to create cloned cDNA libraries, termed "subtracted libraries". Thus, the "subtracted libraries" are enriched for differentially expressed sequences inclusive of rare and novel mRNAs often missed by micro-array hybridization analysis. These rare and novel mRNA are thought to be representative of important gene targets for the development of better diagnostic and therapeutic strategies.

The clones contained in the enriched "subtracted libraries" are identified by DNA sequence analysis and their potential function assessed by acquiring information available in public databases (NCBI and GeneCard). The non-redundant clones are then used to prepare DNA micro-arrays, which are used to quantify their relative differential expression patterns by hybridization to fluorescent cDNA probes. Two classes of cDNA probes may be used, those which are generated from either RNA transcripts prepared from the same subtracted libraries (subtracted probes) or from mRNA isolated from different osteoclast samples (standard probes). The use of subtracted probes provides increased sensitivity for detecting the low abundance mRNA sequences that are preserved and enriched by STAR. Furthermore, the specificity of the differentially expressed sequences to osteoclast is measured by hybridizing radio-labeled probes prepared from each selected sequence to macroarrays containing RNA from different osteoclast samples and different normal human tissues. Additionally, Northern blot analysis is performed so as to confirm the presence of one or more specific mRNA species in the osteoclast samples. Following this, the full-length cDNAs representative of the mRNA species and/or spliced variants are cloned in *E. coli* DH10B.

A major challenge in gene expression profiling is the limited quantities of RNA available for molecular analysis. The amount of RNA isolated from many osteoclast samples or

human specimens (needle aspiration, laser capture microdissection (LCM) samples and transfected cultured cells) is often insufficient for preparing: 1) conventional tester and driver materials for STAR; 2) standard cDNA probes for DNA micro-array analysis; 3) RNA macroarrays for testing the specificity of expression; 4) Northern blots and; 5) full-length cDNA clones for further biological validation and characterization etc. Thus, the applicant has developed a proprietary technology called RAMP (RNA Amplification Procedure) (U.S. patent application Ser. No. 11/000,958 published under No. US 2005/0153333A1 on Jul. 14, 2005 and entitled "Selective Terminal Tagging of Nucleic Acids"), which linearly amplifies the mRNA contained in total RNA samples yielding microgram quantities of amplified RNA sufficient for the various analytical applications. The RAMP RNA produced is largely full-length mRNA-like sequences as a result of the proprietary method for adding a terminal sequence tag to the 3'-ends of single-stranded cDNA molecules, for use in linear transcription amplification. Greater than 99.5% of the sequences amplified in RAMP reactions show <2-fold variability and thus, RAMP provides unbiased RNA samples in quantities sufficient to enable the discovery of the unique mRNA sequences involved in osteoclastogenesis.

Example 3

Preparation of Human Osteoclasts Subtracted Library

Two human primary precursor cells from two different donors (Cambrex, East Rutherford, N.J.), and the corresponding intermediate (day 3 and day 7) and mature (days 11-14) osteoclasts were prepared as described above. Isolation of cellular RNA followed by mRNA purification from each was performed using standard methods (Qiagen, Mississauga, ON). Following the teachings of Malek et al. (U.S. Pat. No. 5,712,127), 2 μg of poly A+ mRNA from each sample were used to prepare highly representative (>2×10^6 CFU) cDNA libraries in specialized plasmid vectors necessary for preparing tester and driver materials. In each case, first-strand cDNA was synthesized using an oligo dT_{11} primer with 3' locking nucleotides (e.g., A, G or C) and containing a Not I recognition site. Next, second-strand cDNA synthesis was performed according to the manufacturer's procedure for double-stranded cDNA synthesis (Invitrogen, Burlington, ON) and the resulting double-stranded cDNA ligated to linkers containing an Asc I recognition site (New England Biolabs, Pickering, ON). The double-stranded cDNAs were then digested with Asc I and Not I restriction enzymes (New England Biolabs, Pickering, ON), purified from the excess linkers using the cDNA fractionation column from Invitrogen (Burlington, ON) as specified by the manufacturer and each ligated into specialized plasmid vectors—p14 (SEQ. ID. NO.:6) and p17+ (SEQ. ID. NO.:7) used for preparing tester and driver materials respectively. Thereafter, the ligated cDNAs were transformed into *E. coli* DH10B resulting in the desired cDNA libraries (RAW 264.7-precursor-p14, RAW 264.7-precursor-p17+, RAW 264.7-osteoclasts-p14 and RAW 264.7-osteoclasts-p17+). The plasmid DNA pool for each cDNA library was purified and a 2-μg aliquot of each linearized with Not I restriction enzyme. In vitro transcription of the Not I digested p14 and p17+ plasmid libraries was then performed with T7 RNA polymerase and sp6 RNA polymerase respectively (Ambion, Austin, Tex.).

Next, in order to prepare 3'-represented tester and driver libraries, a 10-μg aliquot of each of the in vitro synthesized RNA was converted to double-stranded cDNA by performing first-strand cDNA synthesis as described above followed by primer-directed (primer OGS 77 for p14 (SEQ. ID. NO.:8) and primer OGS 302 for p17+ (SEQ. ID. NO.:9)) second-strand DNA synthesis using Advantage-2 Taq polymerase (BD Biosciences Clontech, Mississauga, ON). The sequences corresponding to OGS 77 and OGS 302 were introduced into the in vitro synthesized RNA by way of the specialized vectors used for preparing the cDNA libraries. Thereafter, 6×1-μg aliquots of each double-stranded cDNA was digested individually with one of the following 4-base recognition restriction enzymes Rsa I, Sau3A1, Mse I, Msp I, MinPI I and Bsh 12361 (MBI Fermentas, Burlington, ON), yielding up to six possible 3'-fragments for each RNA species contained in the cDNA library. Following digestion, the restriction enzymes were inactivated with phenol and the set of six reactions pooled. The restriction enzymes sites were then blunted with T4 DNA polymerase and ligated to linkers containing an Asc I recognition site. Each linker-adapted pooled DNA sample was digested with Asc I and Not I restriction enzymes, desalted and ligated to specialized plasmid vectors, p14 and p17 (p17 plasmid vector is similar to the p17+ plasmid vector except for the sequence corresponding to SEQ. ID. NO.:9), and transformed into *E. coli* DH10B. The plasmid DNA pool for each p14 and p17 3'-represented library was purified (Qiagen, Mississauga, ON) and a 2-μg aliquot of each digested with Not I restriction enzyme, and transcribed in vitro with either T7 RNA polymerase or sp6 RNA polymerase (Ambion, Austin, Tex.). The resulting p14 3'-represented RNA was used directly as "tester RNA" whereas, the p17 3'-represented RNA was used to synthesize first-strand cDNA as described above, which then served as "driver DNA". Each "driver DNA" reaction was treated with RNase A and RNase H to remove the RNA, phenol extracted and desalted before use.

The following 3'-represented libraries were prepared:

Tester 1 (donor 1-day 3)—human intermediate osteoclast-3' in p14

Tester 2 (donor 1-day 7—human intermediate osteoclast)-3' in p14

Tester 3 (donor 1-day 11—human mature osteoclast)-3' in p14

Tester 4 (donor 2-day 3—human intermediate osteoclast)-3' in p14

Tester 5 (donor 2-day 7—human intermediate osteoclast)-3' in p14

Tester 6 (donor 2-day 13—human mature osteoclast)-3' in p14

Driver 1 (donor 1-day 3)—human precursor-3' in p17

Driver 2 (donor 2-day 3)—human precursor-3' in p17

The tester RNA samples were subtracted following the teachings of U.S. Pat. No. 5,712,127 with the corresponding driver DNA in a ratio of 1:100 for either 1- or 2-rounds following the teachings of Malek et al. (U.S. Pat. No. 5,712,127). Additionally, control reactions containing tester RNA and no driver DNA, and tester RNA plus driver DNA but no RNase H was prepared. The tester RNA remaining in each reaction after subtraction was converted to double-stranded DNA and a volume of 5% removed and amplified in a standard PCR reaction for 30-cycles for analytical purposes. The remaining 95% of only the driver plus RNase H subtracted samples were amplified for 4-cycles in PCR, digested with Asc I and Not I restriction enzymes, and one half ligated into the pCATRMAN (SEQ. ID. NO.:10) plasmid vector and the other half, into the p20 (SEQ. ID. NO.:11) plasmid vector. The ligated materials were transformed into *E. coli* DH10B and individual clones contained in the pCATRMAN libraries

were picked for further analysis (DNA sequencing and hybridization) whereas, clones contained in each p20 library were pooled for use as subtracted probes. Each 4-cycles amplified cloned subtracted library contained between 25,000 and 40,000 colonies.

The following cloned subtracted libraries were prepared:
SL90-tester 1 (day 3 osteoclast) minus driver 1 (precursor) (1-round) in pCATRMAN;
SL91-tester 2 (day 7 osteoclast) minus driver 1 (precursor) (1-round) in pCATRMAN;
SL92-tester 3 (day 11 osteoclast) minus driver 1 (precursor) (1-round) in pCATRMAN;
SL108-tester 1 (day 3 osteoclast) minus driver 1 (precursor) (2-rounds) in pCATRMAN;
SL109-tester 2 (day 7 osteoclast) minus driver 1 (precursor) (2-rounds) in pCATRMAN;
SL110-tester 3 (day 11 osteoclast) minus driver 1 (precursor) (2-rounds) in pCATRMAN;
SL93-tester 4 (day 3 osteoclast) minus driver 2 (precursor) (1-round) in pCATRMAN;
SL94-tester 5 (day 7 osteoclast) minus driver 2 (precursor) (1-round) in pCATRMAN;
SL95-tester 6 (day 13 osteoclast) minus driver 2 (precursor) (1-round) in pCATRMAN;
SL87-tester 4 (day 3 osteoclast) minus driver 2 (precursor) (2-rounds) in pCATRMAN;
SL88-tester 5 (day 7 osteoclast) minus driver 2 (precursor) (2-rounds) in pCATRMAN;
SL89-tester 6 (day 11 osteoclast) minus driver 2 (precursor) (2-rounds) in pCATRMAN

A 5-4 aliquot of the 30-cycles PCR amplified subtracted materials described above were visualized on a 1.5% agarose gel containing ethidium bromide and then transferred to Hybond N+ (Amersham Biosciences, Piscataway, N.J.) nylon membrane for Southern blot analysis. Using radiolabeled probes specific to the CTSK (cathepsin K; NM_000396.2) gene, which is known to be upregulated in osteoclasts, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase; M32599.1), which is a non-differentially expressed house-keeping gene, it was evident that there was subtraction of GAPDH but not CTSK. Based on these results, it was anticipated that the subtracted libraries would be enriched for differentially expressed upregulated sequences.

Example 4

Sequence Identification and Annotation of Clones Contained in the Subtracted Libraries

A total of 6,912 individual colonies contained in the pCATRMAN subtracted libraries (SL87-95 and SL108-110) described above were randomly picked using a Qbot (Genetix Inc., Boston, Mass.) into 60 μL of autoclaved water. Then, 42 μL of each was used in a 100-μL standard PCR reaction containing oligonucleotide primers, OGS 1 and OGS 142 and amplified for 40-cycles (94° C. for 10 minutes, 40× (94° C. for 40 seconds, 55° C. for 30 seconds and 72° C. for 2 minutes) followed by 72° C. for 7 minutes) in 96-wells microtitre plates using HotStart™ Taq polymerase (Qiagen, Mississauga, ON). The completed PCR reactions were desalted using the 96-well filter plates (Corning) and the amplicons recovered in 100 μL 10 mM Tris (pH 8.0). A 5-4 aliquot of each PCR reaction was visualized on a 1.5% agarose gel containing ethidium bromide and only those reactions containing a single amplified product were selected for DNA sequence analysis using standard DNA sequencing performed on an ABI 3100 instrument (Applied Biosystems, Foster City, Calif.). Each DNA sequence obtained was given a Sequence Identification Number and entered into a database for subsequent tracking and annotation.

Each sequence was selected for BLAST analysis of public databases (e.g. NCBI). Absent from these sequences were the standard housekeeping genes (GAPDH, actin, most ribosomal proteins etc.), which was a good indication that the subtracted library was depleted of at least the relatively abundant non-differentially expressed sequences.

Once sequencing and annotation of the selected clones were completed, the next step involved identifying those sequences that were actually upregulated in osteoclasts compared to precursors.

Example 5

Hybridization Analysis for Identifying Upregulated Sequences

The PCR amplicons representing the annotated sequences from the pCATRMAN libraries described above were used to prepare DNA microarrays. The purified PCR amplicons contained in 70 μL of the PCR reactions prepared in the previous section was lyophilized and each reconstituted in 20 μL of spotting solution comprising 3×SSC and 0.1% sarkosyl. DNA micro-arrays of each amplicon in triplicate were then prepared using CMT-GAP2 slides (Corning, Corning, N.Y.) and the GMS 417 spotter (Affymetrix, Santa Clara, Calif.).

The DNA micro-arrays were then hybridized with either standard or subtracted cy3 and cy5 labelled cDNA probes as recommended by the supplier (Amersham Biosciences, Piscataway, N.J.). The standard cDNA probes were synthesized using RAMP amplified RNA prepared from the different human osteoclast samples and the corresponding precursors. It is well known to the skilled artisan that standard cDNA probes only provide limited sensitivity of detection and consequently, low abundance sequences contained in the cDNA probes are usually missed. Thus, the hybridization analysis was also performed using cy3 and cy5 labelled subtracted cDNA probes prepared from subtracted libraries representing the different tester and driver materials. These subtracted libraries may be enriched for low abundance sequences as a result of following the teachings of Malek et al., and therefore, may provide increased detection sensitivity.

All hybridization reactions were performed using the dye-swap procedure as recommended by the supplier (Amersham Biosciences, Piscataway, N.J.) and approximately 500 putatively differentially expressed upregulated (>2-fold) sequences were selected for further analysis.

Example 6

Determining Osteoclast Specificity of the Differentially Expressed Sequences Identified

The differentially expressed sequences identified in Section F for the different human osteoclast subtracted libraries were tested for osteoclast specificity by hybridization to nylon membrane-based macroarrays. The macroarrays were prepared using RAMP amplified RNA from human precursors and osteoclasts (intermediate and mature) of six independent experiments from 4 different donors (3 males and 1 female), and 30 normal human tissues (adrenal, liver, lung, ovary, skeletal muscle, heart, cervix, thyroid, breast, placenta, adrenal cortex, kidney, vena cava, fallopian tube, pancreas, testicle, jejunum, aorta, esophagus, prostate, stomach, spleen, ileum, trachea, brain, colon, thymus, small intestine, bladder

and duodenum) purchased commercially (Ambion, Austin, Tex.). Because of the limited quantities of mRNA available for many of these samples, it was necessary to first amplify the mRNA using the RAMP methodology. Each amplified RNA sample was reconstituted to a final concentration of 250 ng/μL in 3×SSC and 0.1% sarkosyl in a 96-well microtitre plate and 1 μL spotted onto Hybond N+ nylon membranes using the specialized MULTI-PRINT™ apparatus (VP Scientific, San Diego, Calif.), air dried and UV-cross linked. A total of 400 different sequences selected from SL87-95 and SL108-110 were individually radiolabeled with α-^{32}P-dCTP using the random priming procedure recommended by the supplier (Amersham, Piscataway, N.J.) and used as probes on the macroarrays. Hybridization and washing steps were performed following standard procedures well known to those skilled in the art.

Of the 500 sequences tested, approximately 85% were found to be upregulated in all of the osteoclast RNA samples that were used to prepare the macroarrays. However, many of these sequences were also readily detected in a majority of the different normal human tissues. Based on these results, those sequences that appeared to be associated with experimental variability and those that were detected in many of the other human tissues at significantly elevated levels were eliminated. Consequently, only 35 sequences, which appeared to be upregulated and highly osteoclast-specific, were selected for biological validation studies. Included in this set of 35 genes were 4 where there was a significant upregulation in mature osteoclasts compared to most normal tissues but because the expression of these genes were overall lower in the precursor cells, they appeared to be elevated in the normal tissues after quantitation. However, their expression in the normal tissues was still relatively lower than that of the mature osteoclasts. Thus, these genes may still be important regulators in osteoclastogenesis and bone resorption and were therefore selected for biological validation. This subset of 35 sequences does not include genes also identified such as, CTSK, TRAP, MMP9, CST3 and CKB amongst others since these were previously reported in the literature to be upregulated in osteoclasts. FIG. **1** shows the macroarray pattern and quantitation of the hybridization signals of the osteoclasts and normal human tissues relative to precursor cells for the sequence selected for biological validation. Amongst the 35 sequences studied were 24 genes with functional annotation 9 genes with no functional annotation and 2 novel sequences (genomic hits). The identification of gene products involved in regulating osteoclast differentiation and function has thus led to the discovery of novel targets for the development of new and specific therapies of disease states characterized by abnormal bone remodeling.

SEQ. ID. NO.:1:

SEQ. ID. NO.:1 corresponds to a previously identified gene that encodes a hypothetical protein, LOC284266 with an unknown function. We have demonstrated that this gene is markedly upregulated in intermediate and mature osteoclast compared to precursor cells and other normal human tissues (FIG. **1**), which have not been previously reported. Thus, it is believed that this gene may be required for osteoclastogenesis and/or bone remodeling.

Nucleotide Sequence No.	NCBI Unigene #/Gene Symbol/Gene ID	Accession Number	ORF Nucleotide Positions/ Polypeptide sequence No.	Function
SEQ ID NO.: 1	Hs.287692/ CD33L3/	NM_213602	150-1136 encoding SEQ	hypothetical protein

-continued

Nucleotide Sequence No.	NCBI Unigene #/Gene Symbol/Gene ID	Accession Number	ORF Nucleotide Positions/ Polypeptide sequence No.	Function
	284266/ SIGLEC-15		ID NO.: 2	LOC284266; membrane associated function unknown

Example 7

Cloning of Full-Length cDNAs of Selected Sequences from Osteoclast mRNA

It was necessary to obtain full-length cDNA sequences in order to perform functional studies of the expressed proteins. Spliced variants are increasingly being implicated in tissue specific functions and as such, it is important to work with cDNA clones from the system under study. Applicant also recognizes that spliced variants may not always be involved. Thus, the applicant's approach has been to isolate the relevant full-length cDNA sequences directly from osteoclasts in order to identify variants and their potential role with respect to specificity.

Coding cDNA clones were isolated using both a 5'-RACE strategy (Invitrogen, Burlington, ON) and a standard two-primer gene specific approach in PCR. The 5'-RACE strategy used cDNA prepared from cap-selected osteoclast RNA and/or RAMP amplified osteoclast RNA. For amplification using gene specific primers, either cDNA prepared from RAMP RNA or total RNA was used. All cDNAs were synthesized following standard reverse transcription procedures (Invitrogen, Burlington, ON). The cDNA sequences obtained were cloned in *E. coli* DH10B and the nucleotide sequences for multiple clones determined. Thereafter, the cDNA sequences for each set were aligned and the open reading frame(s) (ORF) identified using standard software (e.g. ORF Finder-NCBI). The cDNA clones for the coding region for SEQ. ID. NO.:1 obtained from a human osteoclast sample, were identical to that of the published sequences corresponding to Accession#NM_213602.

Example 8

RNA Interference Studies

RNA interference is a recently discovered gene regulation mechanism that involves the sequence-specific decrease in a gene's expression by targeting the mRNA for degradation and although originally described in plants, it has been discovered across many animal kingdoms from protozoans and invertebrates to higher eukaryotes (reviewed in Agrawal et al., 2003). In physiological settings, the mechanism of RNA interference is triggered by the presence of double-stranded RNA molecules that are cleaved by an RNAse III-like protein active in cells, called Dicer, which releases the 21-23 bp siRNAs. The siRNA, in a homology-driven manner, complexes into a RNA-protein amalgamation termed RISC (RNA-induced silencing complex) in the presence of mRNA to cause degradation resulting in attenuation of that mRNA's expression (Agrawal et al., 2003).

Current approaches to studying the function of genes, such as gene knockout mice and dominant negatives, are often

inefficient, and generally expensive, and time-consuming. RNA interference is proving to be a method of choice for the analysis of a large number of genes in a quick and relatively inexpensive manner. Although transfection of synthetic siRNAs is an efficient method, the effects are often transient at best (Hannon G. J., 2002). Delivery of plasmids expressing short hairpin RNAs by stable transfection has been successful in allowing for the analysis of RNA interference in longer-term studies (Brummelkamp et al., 2002; Elbashir et al., 2001). In addition, more recent advances have permitted the expression of siRNA molecules, in the form of short hairpin RNAs, in primary human cells using viral delivery methods such as lentivirus (Lee et al., 2004; Rubinson et al., 2003).

Example 9

Determination of Knockdown Effects on Osteoclastogenesis

In order to develop a screening method for the human candidate genes, RNA interference was adapted to deliver shRNAs into human osteoclast precursor cells so that the expression of the candidate genes could be attenuated. This approach would then allow osteoclast differentiation to be carried out in cells containing decreased expression of these genes to determine their requirement, if any, in this process.

To this end, a commercial lentiviral shRNA delivery system (Invitrogen, Burlington, ON) was utilized to introduce specific shRNAs into human osteoclast precursor cells. The techniques used were as described by the manufacturer unless otherwise stated. In this example, the results obtained for the candidate gene, SEQ. ID. NO.:1 (AB0326) are presented. The protein encoded by this gene has no known function. The shRNA sequence used to specifically target SEQ. ID. NO.:1 is 5'-CAGGCCCAGGAGTCCAATT-3' (SEQ. ID. NO.:12). Briefly, a template for the expression of the shRNA was cloned into the lentiviral expression vector and co-transfected in 293FT cells with expression vectors for the viral structural proteins. After two days, supernatants containing the lentivirus were collected and stored at −80° C. Human osteoclast precursors purchased from Cambrex (East Rutherford, N.J.) were seeded in 24-well plates and cultured in complete medium containing macrophage-colony stimulating factor and allowed to adhere for three days. After washing with PBS, the cells were infected with 20 MOIs (multiplicity of infection) of either lentiviral particles containing a shRNA specific for the bacterial lacZ gene as a control (lacZ shRNA) or SEQ. ID. NO.:1 (AB0326 shRNA). After 24 h, the infected cells were treated with same medium containing 100 ng/ml RANK ligand for 5-8 days to allow for differentiation of osteoclast from precursor cells. Mature osteoclasts were fixed with formaldehyde and stained for TRAP expression as follows: the cells were washed with PBS and fixed in 10% formaldehyde for 1 h. After two PBS washes, the cells were lightly permeabilized in 0.2% Triton X-100 in PBS for 5 min before washing in PBS. Staining was conducted at 37° C. for 20-25 min in 0.01% Naphtol AS-MX phosphate, 0.06% Fast Red Violet, 50 mM sodium tartrate, 100 mM sodium acetate, pH 5.2. The stained cells were visualized by light microscopy and photographed (magnification: 40×). A significant decrease in the number of multinucleated osteoclasts was observed from precursor cells infected with the AB0326 shRNA (FIG. **2**A; bottom panel) compared to those with the lacZ shRNA (FIG. **2**A top panel). Therefore, the lentiviral shRNA perturbed osteoclastogenesis. These results clearly indicated that expression of the gene encoding SEQ. ID. NO.:1 (AB0326) is required for osteoclast differentiation.

Example 10

Biological Validation of the Mouse Orthologue (SEQ ID NO.:4 or 108) for AB0326 (SEQ. ID. NO.: 2) in Osteoclastogenesis Using the RAW 264.7 Model

As a means of developing a drug screening assay for the discovery of therapeutic molecules capable of attenuating human osteoclasts differentiation and activity using the targets identified, another osteoclast differentiation model was used. The RAW 264.7 (RAW) osteoclast precursor cell line is well known in the art as a murine model of osteoclastogenesis. However, due to the difficulty in transiently transfecting RAW cells, stable transfection was used as an approach where shRNA are expressed in the RAW cells constitutively. This permitted long term studies such as osteoclast differentiation to be carried out in the presence of specific shRNAs specific to the mouse orthologues of the human targets identified.

RAW cells were purchased from American Type Culture Collection (Manassass, Va.) and maintained in high glucose DMEM containing 10% fetal bovine serum and antibiotics. The cells were sub-cultured bi-weekly to a maximum of 10-12 passages. For osteoclast differentiation experiments, RAW cells were seeded in 96-well plates at a density of 4×10^3 cells/well and allowed to plate for 24 h. Differentiation was induced in high glucose DMEM, 10% charcoal-treated foetal bovine serum (obtained from Hyclone, Logan, Utah), 0.05% BSA, antibiotics, 10 ng/ml macrophage colony stimulating factor (M-CSF), and 100 ng/ml RANK ligand. The plates were re-fed on day 3 and osteoclasts were clearly visible by day 4. Typically, the cells were stained for TRAP on day 4 or 5 unless otherwise indicated.

To incorporate the shRNA-expression cassettes into the RAW cell chromosomes, the pSilencer 2.0 plasmid (SEQ. ID. NO.:15) was purchased from Ambion (Austin, Tex.) and sequence-specific oligonucleotides were ligated as recommended by the manufacturer. Two shRNA expression plasmids were designed and the sequences used for attenuating the mouse ortholog of AB0326 (SEQ. ID. NO.:4 or 108) gene expression were 5'-GCGCCGCGGATCGTCAACA-3' (SEQ. ID. NO.:13) and 5'-ACACGTGCACGGCGGCCAA-3' (SEQ. ID. NO.:14). A plasmid supplied by Ambion containing a scrambled shRNA sequence with no known homology to any mammalian gene was also included as a negative control in these experiments. RAW cells were seeded in 6-well plates at a density of 5×10^5 cells/well and transfected with 1 μg of each plasmid using Fugene6 (Roche, Laval, QC) as described in the protocol. After selection of stable transfectants in medium containing 2 μg/ml puromycin, the cell lines were expanded and tested in the presence of RANK ligand for osteoclastogenesis.

The stably transfected cell lines were designated RAW-0326.1, RAW-0326.2 and RAW-ctl. In 96-well plates in triplicate, 4 000 cells/well were seeded and treated with 100 ng/ml RANK ligand. After 4 days, osteoclasts were stained for TRAP expression and visualized by light microscopy (magnification was 40× and 100× as depicted in the left and right panels, respectively).

The representative results for the RAW-0326.2 line are shown in FIG. **3**. The RAW-0326.2 cell line produced significantly less osteoclasts (FIG. **3**; bottom panel) compared to the cell line containing the scrambled shRNA (FIG. **3**; top panel). The RAW-0326.1 cell line also showed attenuation of the mouse ortholog of AB0326 but not as pronounced (data not

shown). Therefore, as observed for the human gene, siRNAs to the mouse orthologue appear to phenotypically perturb osteoclast differentiation in the mouse model as well. These results, coupled with that obtained in the human osteoclast precursor cells using the lentiviral shRNA delivery system (section J), demonstrate that in both human and mouse, AB0326 gene product is clearly required for osteoclastogenesis.

Example 11

A Functional Complementation Assay for SEQ. ID. NO.:1 (AB0326) in RAW 264.6 Cells to Screen for Inhibitors of Osteoclastogenesis

To establish a screening assay based on SEQ. ID. NO.:1 and SEQ ID NO.:2 (AB0326) to find small molecules capable of attenuating osteoclast differentiation, the cDNA encoding human AB0326 was introduced into the RAW-0326.2 cell line. Thus, if the human AB0326 plays an identical functional role as the mouse orthologue in RAW 264.7 cells, it should restore the osteoclastogenesis capabilities of the RAW-0326.2 cell line.

To accomplish this task, the RAW-0326.2 cell line was transfected with an eukaryotic expression vector encoding the full length cDNA for human AB0326, termed pd2-hAB0326. This expression vector pd2; (SEQ. ID. NO.:15) was modified from a commercial vector, pd2-EGFP-N1 (Clontech, Mountain View, Calif.) where the EGFP gene was replaced by the full length coding sequence of the human AB0326 cDNA. The AB0326 gene expression was driven by a strong CMV promoter. Stable transfectants were selected using the antibiotic, G418. This resulted in a RAW-0326.2 cell line that expressed the human AB0326 gene product in which, the mouse orthologue of AB0326 was silenced. As a control, RAW-0326.2 cells were transfected with the pd2 empty vector, which should not complement the AB0326 shRNA activity. Also, the pd2 empty vector was transfected into RAW 264.7 cells to serve as a further control. After selection of stable pools of cells, 4 000 cells/well were seeded in 96-well plates and treated for 4 days with 100 ng/ml RANK ligand. Following fixation with formaldehyde, the cells were stained for TRAP, an osteoclast-specific marker gene. As shown in FIG. **4**, the RAW-0326.2 cells transfected with the empty pd2 vector are still unable to form osteoclasts in the presence of RANK ligand (center panel) indicating that the mouse AB0326 shRNA is still capable of silencing the AB0326 gene expression in these cells. Conversely, the cells transfected with human AB0326 (pd2-hAB0326) are rescued and thus, differentiate into more osteoclasts in response to RANK ligand (right panel). RAW 264.7 cells containing the empty vector (pd2) did not adversely affect the formation of osteoclasts in the presence of RANK ligand (left panel). These results confirm that the mouse and human orthologues of AB0326 are functionally conserved in osteoclast differentiation.

This particular type of cell-based assay can now serve as the basis for screening compounds capable of binding to and inhibiting the function of human AB0326. A compound library could be applied to this 'rescued' cell line in order to identify molecules (small molecule drugs, peptides, or antibodies) capable of inhibiting AB0326. Any reduction in osteoclast differentiation measured by a reduction in the expression of TRAP would be indicative of a decrease in human AB0326 activity. This assay is applicable to any gene required for proper osteoclast differentiation in RAW cells. A complementation assay can be developed for any human gene and used as the basis for drug screening.

One of skill in the art will readily recognize that orthologues for all mammals may be identified and verified using well-established techniques in the art, and that this disclosure is in no way limited to one mammal. The term "mammal(s)" for purposes of this disclosure refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

The sequences in the experiments discussed above are representative of the NSEQ being claimed and in no way limit the scope of the invention. The disclosure of the roles of the NSEQs in osteoclastogenesis and osteoclast function satisfies a need in the art to better understand the bone remodeling process, providing new compositions that are useful for the diagnosis, prognosis, treatment, prevention and evaluation of therapies for bone remodeling and associated disorders.

The art of genetic manipulation, molecular biology and pharmaceutical target development have advanced considerably in the last two decades. It will be readily apparent to those skilled in the art that newly identified functions for genetic sequences and corresponding protein sequences allows those sequences, variants and derivatives to be used directly or indirectly in real world applications for the development of research tools, diagnostic tools, therapies and treatments for disorders or disease states in which the genetic sequences have been implicated.

Example 12

Antibodies and Antigen Binding Fragments Binding to Siglec-15 to a Siglec-15 Analogue

This example provides details pertaining to the family of monoclonal antibodies that bind to Siglec-15.

To generate monoclonal antibodies, recombinant human Siglec-15 was produced in 293E cells using the large-scale transient transfection technology (Durocher et al., 2002; Durocher, 2004). A cDNA encoding amino acids 20-259 of SEQ ID NO.:2 (see SEQ ID NO.:16) was amplified by PCR using a forward primer that incorporated a BamHI restriction site (SEQ ID NO.:17) and a reverse primer that incorporated a NotI restriction site (SEQ ID NO.:18). The resulting PCR product was digested with BamHI and NotI and the fragment was ligated into the expression vector pYD5 (SEQ ID NO.: 19) that was similarly digested with the same restriction enzymes to create a vector called pYD5-0326. The pYD5 expression plasmid contains the coding sequence for the human Fc domain that allows fusion proteins to be generated as well as the sequence encoding the IgG1 signal peptide to allow the secretion of the fusion protein into the culture medium. For each milliliter of cells, one microgram of the expression vector, called pYD5-$0326_{20\text{-}259}$, was transfected in 293E cells grown in suspension to a density of 1.5-2.0 million cells/ml. The transfection reagent used was polyethylenimine (PEI), (linear, MW 25,000, Cat#23966 Polysciences, Inc., Warrington, Pa.) which was included at a DNA:PEI ratio of 1:3. Growth of the cells was continued for 5 days after which the culture medium was harvested for purification of the recombinant Fc-$0326_{20\text{-}259}$ fusion protein. The protein was purified using Protein-A agarose as instructed by the manufacturer (Sigma-Aldrich Canada Ltd., Oakville, ON). A representative polyacrylamide gel showing a sample of the purified Fc-$0326_{20\text{-}259}$ (indicated as Fc-Siglec-$15_{20\text{-}259}$) is shown in FIG. **3**.

The antibodies that bind Siglec-15 were generated using the Biosite phage display technology. A detailed description of the technology and the methods for generating these antibodies can be found in the U.S. Pat. No. 6,057,098. Briefly, the technology utilizes stringent panning of phage libraries that display the antigen binding fragments (Fabs). After a several rounds of panning, a library, termed the Omniclonal, was obtained that was enriched for recombinant Fabs containing light and heavy chain variable regions that bound to Siglec-15 with very high affinity and specificity. From this library, more precisely designated Omniclonal AL0025Z1, 96 individual recombinant monoclonal Fabs were prepared from *E. coli* and tested for Siglec-15 binding.

To measure the relative binding of each individual monoclonal antibody, recombinant human Fc-Siglec-$15_{20\text{-}259}$ was produced in 293E cells using the large-scale transient transfection technology (Durocher et al., 2002; Durocher, 2004). The 96-well master plate of monoclonal preparations contained different concentrations of purified anti-Siglec-15 Fabs in each well. A second stock master plate was prepared by diluting the Fabs to a final concentration of 10 μg/ml from which all subsequent dilutions were performed for ELISA measurements. To carry out the binding of Fc-Siglec-15 to the monoclonal preparations, the Fc-Siglec-$15_{20\text{-}259}$ was biotinylated with NHS-biotin (Pierce, Rockford, Ill.) and 10 ng/well was coated in a streptavidin 96-well plate. One nanogram of each Fab monoclonal preparation was added to each well and incubated at room temperature for 30 minutes. Bound antibody was detected with HRP-conjugated mouse anti-kappa light chain antibody in the presence of TMB liquid substrate (Sigma-Aldrich Canada Ltd., Oakville, ON) and readings were conducted at 450 nm in microtiter plate reader. As shown in FIG. **4**A, a total of 53 (highlighted dark grey) monoclonal antibodies displayed significant binding in this assay (>0.2 arbitrary OD_{450} units). The antibodies were purposely diluted to 1 ng/well to accentuate the binding of those antibodies with the most affinity for Siglec-15. Since the antibodies were generated using a Fc fusion protein, the monoclonals were also tested in an ELISA using biotinylated Fc domain only. As shown on FIG. **4**B, 17 antibodies interacted with the Fc moiety of the Fc-Siglec-$15_{20\text{-}259}$ (highlighted light grey). The values presented in bold (see FIG. **4**) represent the exemplary antibodies 25A1, 25B4, 25B8, 25C1, 25D8, 25E5, 25E6, and 25E9. These data also revealed that the binding of the antibodies varied from well to well indicating that they exhibited different affinities for Siglec-15.

The applicant noted that the antibody or antigen binding fragment of the present invention may bind efficiently to the antigen, in fact it was found that 1 ng of antibody is capable of binding to less than 500 ng of SEQ ID NO.:2.

The nucleic acid and amino acid sequence of selected antibodies light chain or heavy chain is listed in Table 1. The nucleic acid and amino acid sequence of selected antibodies light chain variable region or heavy chain variable region is listed in Table 2

TABLE 1

Complete sequences of light and heavy chain immunoglobulins that bind to Siglec-15

Antibody designation	Chain type	Nucleotide sequence (SEQ ID NO.:)	Amino acid sequence (SEQ ID NO.:)
25A1	Light (L)	20	21
25A1	Heavy (H)	22	23
25B4	Light	24	25
25B4	Heavy	26	27

TABLE 1-continued

Complete sequences of light and heavy chain immunoglobulins that bind to Siglec-15

Antibody designation	Chain type	Nucleotide sequence (SEQ ID NO.:)	Amino acid sequence (SEQ ID NO.:)
25B8	Light	28	29
25B8	Heavy	30	31
25C1	Light	32	33
25C1	Heavy	34	35
25D8	Light	36	37
25D8	Heavy	38	39
25E5	Light	40	41
25E5	Heavy	42	43
25E6	Light	44	45
25E6	Heavy	46	47
25E9	Light	48	49
25E9	Heavy	50	51

TABLE 2

Sequences of light and heavy chain variable regions that bind to Siglec-15

Antibody designation	Chain type	Nucleotide sequence (SEQ ID NO.:)	Amino acid sequence (SEQ ID NO.:)
25A1	Light (L)	52	53
25A1	Heavy (H)	54	55
25B4	Light	56	57
25B4	Heavy	58	59
25B8	Light	60	61
25B8	Heavy	62	63
25C1	Light	64	65
25C1	Heavy	66	67
25D8	Light	68	69
25D8	Heavy	70	71
25E5	Light	72	73
25E5	Heavy	74	75
25E6	Light	76	77
25E6	Heavy	78	79
25E9	Light	80	81
25E9	Heavy	82	83

Example 13

Conversion of Fabs into Chimeric Antibodies

This example discloses the methods used to convert the Fabs into full IgG2 chimeric monoclonal antibodies. A scheme of the methodology is presented in FIG. **5**.

In order to conduct in vitro and in vivo studies to validate the biological function of the antigen the light and heavy chain variable regions contained in the Fabs was transferred to full antibody scaffolds, to generate mouse-human chimeric IgG2s. The expression vectors for both the light and heavy immunoglobulin chains were constructed such that i) the original bacterial signal peptide sequences upstream of the Fab expression vectors were replaced by mammalian signal peptides and ii) the light and heavy chain constant regions in the mouse antibodies were replaced with human constant regions. The methods to accomplish this transfer utilized standard molecular biology techniques that are familiar to those skilled in the art. A brief overview of the methodology is described here (see FIG. **5**).

Light chain expression vector—an existing mammalian expression plasmid, called pTTVH8G (Durocher et al., 2002), designed to be used in a 293E transient transfection system was modified to accommodate the mouse light chain variable region. The resulting mouse-human chimeric light chain contained a mouse variable region followed by the

human kappa constant domain. The cDNA sequence encoding the human kappa constant domain was amplified by PCR with primers OGS1773 and OGS1774 (SEQ ID NOS:84 and 85, respectively). The nucleotide sequence and the corresponding amino acid sequence for the human kappa constant region are shown in SEQ ID NOS:86 and 87, respectively. The resulting 321 base pair PCR product was ligated into pTTVH8G immediately downstream of the signal peptide sequence of human VEGF A (NM_003376). This cloning step also positioned unique restriction endonuclease sites that permitted the precise positioning of the cDNAs encoding the mouse light chain variable regions. The sequence of the final expression plasmid, called pTTVK1, is shown in SEQ ID NO.:88. Based on the sequences disclosed in Table 2, PCR primers specific for the light chain variable regions of antibodies 25A1, 25B4, 25B8, 25C1, 25D8, 25E5, 25E6, and 25E9 were designed that incorporated, at their 5'-end, a sequence identical to the last 20 base pairs of the VEGF A signal peptide. The sequences of these primers are shown in SEQ ID NO.:89 for 25A1; SEQ ID NO.:90 for 25B4, 25B8, 25C1, 25D8, and 25E9; SEQ ID NO.:91 for 25E5, and SEQ ID NO.:92 for 25E6, respectively. The same reverse primer was used to amplify all four light chain variable regions since the extreme 3'-ends were identical. This primer (SEQ ID NO.:93) incorporated, at its 3'-end, a sequence identical to the first 20 base pairs of the human kappa constant domain. Both the PCR fragments and the digested pTTVK1 were treated with the 3'-5' exonuclease activity of T4 DNA polymerase resulting in complimentary ends that were joined by annealing. The annealing reactions were transformed into competent *E. coli* and the expression plasmids were verified by sequencing to ensure that the mouse light chain variable regions were properly inserted into the pTTVK1 expression vector. Those skilled in the art will readily recognize that the method used for construction of the light chain expression plasmids applies to all anti-Siglec-15 antibodies contained in the original Fab library.

Heavy chain expression vector—the expression vector that produced the heavy chain immunoglobulins was designed in a similar manner to the pTTVK1 described above for production of the light chain immunoglobulins. In the case of the chimeric anti-Siglec-15 antibodies, IgG2 isotype was required which is the preferred type for stable, blocking antibodies. To this end, the constant regions (CH1, CH2, and CH3) of the human IgG2 immunoglobulin were amplified and ligated into a pre-existing IgG1 expression vector and the detailed methods are described herein. Plasmid pYD11 (Durocher et al., 2002), which contains the human IgGK signal peptide sequence as well as the CH2 and CH3 regions of the human Fc domain of IgG1, was modified by ligating the cDNA sequence encoding the human constant CH1 region. PCR primers OGS1769 and OGS1770 (SEQ ID NOS:94 and 95), designed to contain unique restriction endonuclease sites, were used to amplify the human IgG1 CH1 region containing the nucleotide sequence and corresponding amino acid sequence shown in SEQ ID NOS:96 and 97. Following ligation of the 309 base pair fragment of human CH1 immediately downstream of the IgGK signal peptide sequence, the resulting plasmid was digested with the restriction enzymes ApaI and NsiI. These enzymes that digest both the constant IgG1 and IgG2 cDNAs in exactly the same positions that permits the IgG1 constant sequence to be replaced by the human IgG2 sequence in the expression vector. The cDNA encoding the human IgG2 constant domains was obtained from a commercially available source (Open Biosystems, Huntsville, Ala.). The final plasmid used to express the IgG2 immunoglobulin heavy chain was designated pYD19 and the sequence is shown in SEQ ID NO.:98. When a selected heavy chain variable region is ligated into this vector, the resulting plasmid encodes a full IgG2 heavy chain immunoglobulin with human constant regions. Based on the sequences disclosed in Table 2, PCR primers specific for the heavy chain variable regions of antibodies 25A1, 25B4, 25B8, 25C1, 25D8, 25E5, 25E6, and were designed that incorporated, at their 5'-end, a sequence identical to the last 20 base pairs of the IgGK signal peptide. The sequences of these primers are shown in SEQ ID NO.:99 for 25A1; SEQ ID NO.:100 for 24B4 and 25D8; SEQ ID NO.:101 for 25B8, 25C1, and 25E9; SEQ ID NO.:102 for 25E5; and SEQ ID NO.:103 for 25E6, respectively. The same reverse primer was used to amplify all four heavy chain variable regions since the extreme 3'-ends were identical. This primer (SEQ ID NO.:104) incorporated, at its 3'-end, a sequence identical to the first 20 base pairs of the human CH1 constant domain. Both the PCR fragments and the digested pYD19 were treated with the 3'-5' exonuclease activity of T4 DNA polymerase resulting in complimentary ends that were joined by annealing. The annealing reactions were transformed into competent *E. coli* and the expression plasmids were verified by sequencing to ensure that the mouse heavy chain variable regions were properly inserted into the pYD19 expression vector. Those skilled in the art will readily recognize that the method used for construction of the heavy chain expression plasmids applies to all anti-Siglec-15 antibodies contained in the original Fab library.

Expression of human IgG2s in 293E cells—The expression vectors prepared above that encoded the light and heavy chain immunoglobulins were expressed in 293E cells using the transient transfection system (Durocher et al., 2002). By virtue of the signal peptides incorporated at the amino-termini of both immunoglobulin chains, the mature IgG2 was harvested from the serum-free culture medium of the cells. The methods used for co-transfecting the light and heavy chain expression vectors were described herein. For each milliliter of cells, one microgram of a combination of both the light and heavy chain expression plasmids was transfected in 293E cells grown in suspension to a density of 1.5-2.0 million cells/ml. The ratio of light to heavy chain plasmid was optimized in order to achieve the most yield of antibody in the tissue culture medium and it was found to be 9:1 (L:H). The transfection reagent used was polyethylenimine (PEI), (linear, MW 25,000, Cat#23966 Polysciences, Inc., Warrington, Pa.) which was included at a DNA:PEI ratio of 1:3. Growth of the cells was continued for 5 days after which the culture medium was harvested for purification of the IgG2 chimeric monoclonal antibodies. The protein was purified using Protein-A agarose as instructed by the manufacturer (Sigma-Aldrich Canada Ltd., Oakville, ON).

To determine the relative binding affinity of selected monoclonals more accurately, increasing concentration of the Fabs was incubated with biotinylated Fc-Siglec-15_{20-259}. Ten nanograms of biotinylated Fc-Siglec-15_{20-259} was coated in streptavidin microtiter plates and increasing amounts of either Fabs or the chimeric IgG2 monoclonals 25B4, 25B8, 25C1, 25D8, 25E6, and 25E9 were added as indicated in FIG. **6**. As depicted in FIG. **6**, the binding of the 25B4, 2588, 25C1, 25D8, 25E6, and 25E9 chimeric IgG2 monoclonal antibodies was very similar to the Fabs. This result shows that the transposition of the variable domains from the mouse Fabs into a human IgG2 backbone did not significantly affect the capacity of the light and heavy chain variable regions to confer Siglec-15 binding.

Example 14

Inhibition of Siglec-15 Activity

This example describes the use of anti-Siglec-15 antibodies for inhibiting the differentiation of osteoclasts.

Human PBMNCs (AllCells, Emoryville, Calif.) were placed in the appropriate culture medium for 24 h at 37 C in a 5% CO_2 atmosphere. The cells were seeded in 96-well plates at a cell density of 100,000 cells/ml and treated with increasing concentration (0.01 μg/ml-100 μg/ml) of anti-Siglec-15 IgG2 chimeric monoclonal antibodies in the presence of 35 ng/ml M-CSF and 30 ng/ml RANKL. Undifferentiated precursor cells were treated only with M-CSF, The control wells were treated with a non-Siglec-15 binding IgG2. The cells were fixed, stained for TRAP, and multinucleated cells counted and photographed (magnification 40×). As depicted in FIG. **7**, mAbs targeting Siglec-15 could efficiently inhibit the differentiation of human osteoclasts in a dose-dependent manner. Inhibition of osteoclast differentiation was observed to varying extents with every exemplary Siglec-15 antibody that was tested but the most active monoclonals were 25B8, 25E6, and 25E9. Cells treated with a control chimeric IgG2 were not inhibited (see lower right panels in FIG. **8**, Control IgG2). This result is in complete agreement with the experiments disclosed by Sooknanan (Sooknanan et al., 2007) that showed that knockdown of Siglec-15 expression by RNA interference caused inhibition of human osteoclast differentiation.

In a parallel experiment, mouse PBMNCs were treated in a similar manner. As depicted in FIG. **8**, anti-Siglec-15 chimeric antibodies could inhibit the differentiation of mouse osteoclasts as exemplified by the chimeric mAbs designated 25B8, 25E6, and 25D8. This result confirms that the monoclonal antibodies that were generated against the human orthologue of Siglec-15 are cross-reactive against the mouse Siglec-15 protein as well. This was experimentally verified using an ELISA. A fragment of the mouse Siglec-15 cDNA was amplified corresponding to amino acids 21-256 using oligonucleotides containing the sequences shown in SEQ ID NOS: 105 and 106. This PCR fragment was ligated into the pYD5 expression vector as was described for the human Siglec-15 fragment for expression in 293-6E cells. The recombinant Fc-mouseSiglec-15 was purified using Protein-A affinity chromatography.

An exemplary anti-Siglec-15 monoclonal Fab designated 25C8 was incubated with either Fc-human(h)Siglec-15_{20-259} or Fc-mouse(m)Siglec-15_{21-256}. The results (see FIG. **9**) indicate that the binding activity of the antibodies that were generated against the human Siglec-15 also cross-react with the mouse orthologue of Siglec-15.

The results described above clearly demonstrate the importance of Siglec-15 in osteoclastogenesis. Attenuation of Siglec-15 expression in osteoclast precursor cells results in cells that are highly impaired in their ability to form multinucleated mature osteoclasts. Thus, targeting Siglec-15 with an inhibitor, in particular a therapeutic monoclonal antibody, would prove to be a very selective way to target those cells that are directly responsible for bone degradation during acute metastatic bone cancer or chronic osteoporosis.

Example 5

Inhibition of Siglec-15 Activity

This example evaluates the ability of anti-Siglec-15 antibodies in inhibiting bone resorption activity.

The OsteoLyse™ Assay (Human Collagen) made by Lonza provides a 96-well OsteoLyse™ Cell Culture Plate coated with fluorophore-derivatized human bone matrix (europiumconjugated collagen) for use in assays of osteoclast differentiation and function. The assay is a direct measure of the release of matrix metalloproteinases into the resorption lacuna of the osteoclast1. Cells can be seeded onto the surface of the OsteoLyse™ Plate in a manner identical to that used in traditional cell culture protocols. The resorptive activity of the osteoclasts, as reflected by the release of Eu-labeled collagen fragments, can be measured by simply sampling the cell culture supernatant after an appropriate period of cell culture. The cell culture supernatants are added to Fluorophore-Releasing Reagent in a second 96-well assay plate and counted using time-resolved fluorescence2.

Human PBMNCs (AllCells, Emoryville, Calif.) are placed in the appropriate culture medium for 24 h at 37 C in a 5% CO_2 atmosphere. The cells are seeded in a osteolysis assay plate at a cell density of 100,000 cells/ml and treated with increasing concentration (0.01 μg/ml-100 μg/ml) of anti-Siglec-15 IgG2 chimeric monoclonal antibodies in the presence of 35 ng/ml M-CSF and 30 ng/ml RANKL and appropriate culture medium.

After 3 days left in culture, 10 μL of the culture supernatant is removed, and treated with 200 μL of the Fluorophore Releasing Reagent. The quantity of free fluorescent collagen fragments released in the culture supernatant is determined by measuring the fluorescence intensity using a fluorescent plate reader.

Although the present invention has been described hereinabove by way of preferred embodiments thereof, it may be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

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U.S. Pat. No. 6,498,024, Malek et al., Dec. 24, 2002

U.S. patent application Ser. No. 11/000,958 field on Dec. 2, 2003 published under No. US 2005/0153333A1 on Jul. 14, 2005 and entitled "Selective Terminal Tagging of Nucleic Acids"

U.S. Pat. No. 6,617,434 Duffy, Sep. 9, 2003

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SEQUENCE LISTING

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Glu Phe Ala Gly Asp Val His Asp Arg Tyr Glu Ser Arg His Gly Val
145                 150                 155                 160

Arg Leu His Val Thr Ala Ala Pro Arg Ile Val Asn Ile Ser Val Leu
                165                 170                 175

Pro Ser Pro Ala His Ala Phe Arg Ala Leu Cys Thr Ala Glu Gly Glu
            180                 185                 190

Pro Pro Pro Ala Leu Ala Trp Ser Gly Pro Ala Leu Gly Asn Ser Leu
        195                 200                 205

Ala Ala Val Arg Ser Pro Arg Glu Gly His Gly His Leu Val Thr Ala
    210                 215                 220

Glu Leu Pro Ala Leu Thr His Asp Gly Arg Tyr Thr Cys Thr Ala Ala
225                 230                 235                 240

Asn Ser Leu Gly Arg Ser Glu Ala Ser Val Tyr Leu Phe Arg Phe His
                245                 250                 255

Gly Ala Ser Gly Ala Ser Thr Val Ala Leu Leu Leu Gly Ala Leu Gly
            260                 265                 270

Phe Lys Ala Leu Leu Leu Leu Gly Val Leu Ala Ala Arg Ala Ala Arg
        275                 280                 285

Arg Arg Pro Glu His Leu Asp Thr Pro Asp Thr Pro Pro Arg Ser Gln
    290                 295                 300

Ala Gln Glu Ser Asn Tyr Glu Asn Leu Ser Gln Met Asn Pro Arg Ser
305                 310                 315                 320

Pro Pro Ala Thr Met Cys Ser Pro
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<210> SEQ ID NO 3
<211> LENGTH: 1029
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

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gcgcagcgct ggtccatgca ggtgcccgcg gaggtgaacg cggaggctgg cgacgcggcg    180

gtgctgccct gcaccttcac gcacccgcac cgccactacg acgggccgct gacggccatc    240

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tcgaccctag cgctcctgct gggcgcgctg ggcctcaagg ccttgctgct gcttggcatt    840

ctgggagcgc gtgccacccg acgccgacta gatcacctgg tcccccagga cacccctcca    900
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cggtctcagg ctcaggagtc caattatgaa aatttgagcc agatgagtcc tccaggccac 960

cagctgccac gtgtttgctg tgaggaactc ctcagccatc accatctagt cattcaccat 1020

gagaaataa 1029

<210> SEQ ID NO 4
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

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1 5 10 15

Met Gly Ser Leu Val Lys Thr Arg Arg Asp Ala Ser Gly Asp Leu Leu
20 25 30

Asn Thr Glu Ala His Ser Ala Pro Ala Gln Arg Trp Ser Met Gln Val
35 40 45

Pro Ala Glu Val Asn Ala Glu Ala Gly Asp Ala Ala Val Leu Pro Cys
50 55 60

Thr Phe Thr His Pro His Arg His Tyr Asp Gly Pro Leu Thr Ala Ile
65 70 75 80

Trp Arg Ser Gly Glu Pro Tyr Ala Gly Pro Gln Val Phe Arg Cys Thr
85 90 95

Ala Ala Pro Gly Ser Glu Leu Cys Gln Thr Ala Leu Ser Leu His Gly
100 105 110

Arg Phe Arg Leu Leu Gly Asn Pro Arg Arg Asn Asp Leu Ser Leu Arg
115 120 125

Val Glu Arg Leu Ala Leu Ala Asp Ser Gly Arg Tyr Phe Cys Arg Val
130 135 140

Glu Phe Thr Gly Asp Ala His Asp Arg Tyr Glu Ser Arg His Gly Val
145 150 155 160

Arg Leu Arg Val Thr Ala Ala Ala Pro Arg Ile Val Asn Ile Ser Val
165 170 175

Leu Pro Gly Pro Ala His Ala Phe Arg Ala Leu Cys Thr Ala Glu Gly
180 185 190

Glu Pro Pro Pro Ala Leu Ala Trp Ser Gly Pro Ala Pro Gly Asn Ser
195 200 205

Ser Ala Ala Leu Gln Gly Gln Gly His Gly Tyr Gln Val Thr Ala Glu
210 215 220

Leu Pro Ala Leu Thr Arg Asp Gly Arg Tyr Thr Cys Thr Ala Ala Asn
225 230 235 240

Ser Leu Gly Arg Ala Glu Ala Ser Val Tyr Leu Phe Arg Phe His Gly
245 250 255

Ala Pro Gly Thr Ser Thr Leu Ala Leu Leu Leu Gly Ala Leu Gly Leu
260 265 270

Lys Ala Leu Leu Leu Leu Gly Ile Leu Gly Ala Arg Ala Thr Arg Arg
275 280 285

Arg Leu Asp His Leu Val Pro Gln Asp Thr Pro Pro Arg Ser Gln Ala
290 295 300

Gln Glu Ser Asn Tyr Glu Asn Leu Ser Gln Met Ser Pro Pro Gly His
305 310 315 320

Gln Leu Pro Arg Val Cys Cys Glu Glu Leu Leu Ser His His His Leu
325 330 335

Val Ile His His Glu Lys
340

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<210> SEQ ID NO 5
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: shRNA sequence

<400> SEQUENCE: 5

acacgtgcac ggcggccaa 19

<210> SEQ ID NO 6
<211> LENGTH: 2996
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid vectors - p14

<400> SEQUENCE: 6

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ggagacgaga gcacctggat aggttcgcgt ggcgcgccgc atgcgtcgac ggatcctgag 120

aacttcaggc tcctgggcaa cgtgctggtt attgtgctgt ctcatcattt tggcaaagaa 180

ttcactcctc aggtgcaggc tgcctatcag aaggtggtgg ctggtgtggc caatgccctg 240

gctcacaaat accactgaga tcttttttccc tctgccaaaa attatgggga catcatgaag 300

ccccttgagc atctgacttc tggctaataa aggaaattta ttttcattgc aaaaaaaaaa 360

agcggccgct aactgttggt gcaggcgctc ggaccgctag cttggcgtaa tcatggtcat 420

agctgtttcc tgtgtgaaat tgttatccgc tcacaattcc acacaacata cgagccggaa 480

gcataaagtg taaagcctgg ggtgcctaat gagtgagcta actcacatta attgcgttgc 540

gctcactgcc cgctttccag tcgggaaacc tgtcgtgcca gctgcattaa tgaatcggcc 600

aacgcgcggg gagaggcggt ttgcgtattg ggcgctcttc cgcttcctcg ctcactgact 660

cgctgcgctc ggtcgttcgg ctgcggcgag cggtatcagc tcactcaaag gcggtaatac 720

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ccccgttca gcccgaccgc tgcgccttat ccggtaacta tcgtcttgag tccaacccgg 1140

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cagtatttgg tatctgcgct ctgctgaagc cagttacctt cggaaaaaga gttggtagct 1320

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ttacgcgcag aaaaaaagga tctcaagaag atcctttgat cttttctacg gggtctgacg 1440

ctcagtggaa cgaaaactca cgttaaggga ttttggtcat gagattatca aaaaggatct 1500

tcacctagat ccttttaaat taaaaatgaa gttttaaatc aatctaaagt atatatgagt 1560

aaacttggtc tgacagttac caatgcttaa tcagtgaggc acctatctca gcgatctgtc 1620

tatttcgttc atccatagtt gcctgactcc ccgtcgtgta gataactacg atacgggagg 1680

gcttaccatc tggccccagt gctgcaatga taccgcgaga cccacgctca ccggctccag 1740

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tatccgcctc catccagtct attaattgtt gccgggaagc tagagtaagt agttcgccag 1860

ttaatagttt gcgcaacgtt gttgccattg ctacaggcat cgtggtgtca cgctcgtcgt 1920

ttggtatggc ttcattcagc tccggttccc aacgatcaag gcgagttaca tgatccccca 1980

tgttgtgcaa aaaagcggtt agctccttcg gtcctccgat cgttgtcaga agtaagttgg 2040

ccgcagtgtt atcactcatg gttatggcag cactgcataa ttctcttact gtcatgccat 2100

ccgtaagatg cttttctgtg actggtgagt actcaaccaa gtcattctga gaatagtgta 2160

tgcggcgacc gagttgctct tgcccggcgt caatacggga taataccgcg ccacatagca 2220

gaactttaaa agtgctcatc attggaaaac gttcttcggg gcgaaaactc tcaaggatct 2280

taccgctgtt gagatccagt tcgatgtaac ccactcgtgc acccaactga tcttcagcat 2340

cttttacttt caccagcgtt tctgggtgag caaaaacagg aaggcaaaat gccgcaaaaa 2400

agggaataag ggcgacacgg aaatgttgaa tactcatact cttccttttt caatattatt 2460

gaagcattta tcagggttat tgtctcatga gcggatacat atttgaatgt atttagaaaa 2520

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gcgggtgtcg gggctggctt aactatgcgg catcagagca gattgtactg agagtgcacc 2820

atatgcggtg tgaaataccg cacagatgcg taaggagaaa ataccgcatc aggcgccatt 2880

cgccattcag gctgcgcaac tgttgggaag ggcgatcggt gcgggcctct tcgctattac 2940

gccagctggc gaaaggggga tgtgctgcaa ggcgattaag ttgggtaacg ccaggg 2996

<210> SEQ ID NO 7
<211> LENGTH: 2992
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: plasmid vector p17+

<400> SEQUENCE: 7

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tgagaacttc aggctcctgg gcaacgtgct ggttattgtg ctgtctcatc attttggcaa 180

agaattcact cctcaggtgc aggctgccta tcagaaggtg gtggctggtg tggccaatgc 240

cctggctcac aaataccact gagatctttt tccctctgcc aaaaattatg gggacatcat 300

gaagcccctt gagcatctga cttctggcta ataaaggaaa tttattttca ttgcaaaaaa 360

aaaaagcggc cgctagagtc ggccgcagcg gccgagcttg gcgtaatcat ggtcatagct 420

gtttcctgtg tgaaattgtt atccgctcac aattccacac aacatacgag ccggaagcat 480

aaagtgtaaa gcctggggtg cctaatgagt gagctaactc acattaattg cgttgcgctc 540

actgcccgct ttccagtcgg gaaacctgtc gtgccagctg cattaatgaa tcggccaacg 600

cgcggggaga ggcggtttgc gtattgggcg ctcttccgct tcctcgctca ctgactcgct 660

gcgctcggtc gttcggctgc ggcgagcggt atcagctcac tcaaaggcgg taatacggtt 720

atccacagaa tcaggggata acgcaggaaa gaacatgtga gcaaaaggcc agcaaaaggc 780

caggaaccgt aaaaaggccg cgttgctggc gtttttccat aggctccgcc cccctgacga 840

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gcatcacaaa aatcgacgct caagtcagag gtggcgaaac ccgacaggac tataaagata 900

ccaggcgttt ccccctggaa gctccctcgt gcgctctcct gttccgaccc tgccgcttac 960

cggatacctg tccgcctttc tcccttcggg aagcgtggcg ctttctcaaa gctcacgctg 1020

taggtatctc agttcggtgt aggtcgttcg ctccaagctg ggctgtgtgc acgaaccccc 1080

cgttcagccc gaccgctgcg ccttatccgg taactatcgt cttgagtcca acccggtaag 1140

acacgactta tcgccactgg cagcagccac tggtaacagg attagcagag cgaggtatgt 1200

aggcggtgct acagagttct tgaagtggtg gcctaactac ggctacacta gaagaacagt 1260

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atccggcaaa caaaccaccg ctggtagcgg tggttttttt gtttgcaagc agcagattac 1380

gcgcagaaaa aaaggatctc aagaagatcc tttgatcttt tctacggggt ctgacgctca 1440

gtggaacgaa aactcacgtt aagggatttt ggtcatgaga ttatcaaaaa ggatcttcac 1500

ctagatcctt ttaaattaaa aatgaagttt taaatcaatc taaagtatat atgagtaaac 1560

ttggtctgac agttaccaat gcttaatcag tgaggcacct atctcagcga tctgtctatt 1620

tcgttcatcc atagttgcct gactccccgt cgtgtagata actacgatac gggagggctt 1680

accatctggc cccagtgctg caatgatacc gcgagaccca cgctcaccgg ctccagattt 1740

atcagcaata aaccagccag ccggaagggc cgagcgcaga agtggtcctg caactttatc 1800

cgcctccatc cagtctatta attgttgccg ggaagctaga gtaagtagtt cgccagttaa 1860

tagtttgcgc aacgttgttg ccattgctac aggcatcgtg gtgtcacgct cgtcgtttgg 1920

tatggcttca ttcagctccg gttcccaacg atcaaggcga gttacatgat cccccatgtt 1980

gtgcaaaaaa gcggttagct ccttcggtcc tccgatcgtt gtcagaagta agttggccgc 2040

agtgttatca ctcatggtta tggcagcact gcataattct cttactgtca tgccatccgt 2100

aagatgcttt tctgtgactg gtgagtactc aaccaagtca ttctgagaat agtgtatgcg 2160

gcgaccgagt tgctcttgcc cggcgtcaat acgggataat accgcgccac atagcagaac 2220

tttaaaagtg ctcatcattg gaaaacgttc ttcggggcga aaactctcaa ggatcttacc 2280

gctgttgaga tccagttcga tgtaacccac tcgtgcaccc aactgatctt cagcatcttt 2340

tactttcacc agcgtttctg ggtgagcaaa aacaggaagg caaaatgccg caaaaaaggg 2400

aataagggcg acacggaaat gttgaatact catactcttc ctttttcaat attattgaag 2460

catttatcag ggttattgtc tcatgagcgg atacatattt gaatgtattt agaaaaataa 2520

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gctggcgaaa gggggatgtg ctgcaaggcg attaagttgg gtaacgccag gg 2992

<210> SEQ ID NO 8
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer OGS 77 for p14

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<400> SEQUENCE: 8

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<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer OGS 302 for p17+

<400> SEQUENCE: 9

gcctgcacca acagttaaca 20

<210> SEQ ID NO 10
<211> LENGTH: 2757
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pCATRMAN plasmid vector

<400> SEQUENCE: 10

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ggagatggag aaaaaaatca ctggacgcgt ggcgcgccat taattaatgc ggccgctagc 120

tcgagtgata ataagcggat gaatggctgc aggcatgcaa gcttggcgta atcatggtca 180

tagctgtttc ctgtgtgaaa ttgttatccg ctcacaattc cacacaacat acgagccgga 240

agcataaagt gtaaagcctg gggtgcctaa tgagtgagct aactcacatt aattgcgttg 300

cgctcactgc ccgctttcca gtcgggaaac ctgtcgtgcc agctgcatta atgaatcggc 360

caacgcgcgg ggagaggcgg tttgcgtatt gggcgctctt ccgcttcctc gctcactgac 420

tcgctgcgct cggtcgttcg gctgcggcga gcggtatcag ctcactcaaa ggcggtaata 480

cggttatcca cagaatcagg ggataacgca ggaaagaaca tgtgagcaaa aggccagcaa 540

aaggccagga accgtaaaaa ggccgcgttg ctggcgtttt tccataggct ccgccccct 600

gacgagcatc acaaaaatcg acgctcaagt cagaggtggc gaaacccgac aggactataa 660

agataccagg cgtttccccc tggaagctcc ctcgtgcgct ctcctgttcc gaccctgccg 720

cttaccggat acctgtccgc ctttctccct tcgggaagcg tggcgctttc tcaatgctca 780

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tcttgatccg gcaaacaaac caccgctggt agcggtggtt tttttgtttg caagcagcag 1140

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gctcagtgga acgaaaactc acgttaaggg attttggtca tgagattatc aaaaaggatc 1260

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taaacttggt ctgacagtta ccaatgctta atcagtgagg cacctatctc agcgatctgt 1380

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atgttgtgca aaaaagcggt tagctccttc ggtcctccga tcgttgtcag aagtaagttg 1800

gccgcagtgt tatcactcat ggttatggca gcactgcata attctcttac tgtcatgcca 1860

tccgtaagat gcttttctgt gactggtgag tactcaacca agtcattctg agaatagtgt 1920

atgcggcgac cgagttgctc ttgcccggcg tcaatacggg ataataccgc gccacatagc 1980

agaactttaa aagtgctcat cattggaaaa cgttcttcgg ggcgaaaact ctcaaggatc 2040

ttaccgctgt tgagatccag ttcgatgtaa cccactcgtg cacccaactg atcttcagca 2100

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aataaacaaa taggggttcc gcgcacattt ccccgaaaag tgccacctga cgtctaagaa 2340

accattatta tcatgacatt aacctataaa aataggcgta tcacgaggcc ctttcgtctc 2400

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gcttgtctgt aagcggatgc cgggagcaga caagcccgtc agggcgcgtc agcgggtgtt 2520

ggcgggtgtc ggggctggct taactatgcg gcatcagagc agattgtact gagagtgcac 2580

catatgcggt gtgaaatacc gcacagatgc gtaaggagaa aataccgcat caggcgccat 2640

tcgccattca ggctgcgcaa ctgttgggaa gggcgatcgg tgcgggcctc ttcgctatta 2700

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<210> SEQ ID NO 11
<211> LENGTH: 2995
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: p20 plasmid vector

<400> SEQUENCE: 11

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ggctcctggg caacgtgctg gttattgtgc tgtctcatca ttttggcaaa gaattcactc 180

ctcaggtgca ggctgcctat cagaaggtgg tggctggtgt ggccaatgcc ctggctcaca 240

aataccactg agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg 300

agcatctgac ttctggctaa taaaggaaat ttattttcat tgcaaaaaaa aaaagcggcc 360

gctcttctat agtgtcacct aaatggccca gcggccgagc ttggcgtaat catggtcata 420

gctgtttcct gtgtgaaatt gttatccgct cacaattcca cacaacatac gagccggaag 480

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gttatccaca gaatcagggg ataacgcagg aaagaacatg tgagcaaaag gccagcaaaa 780

ggccaggaac cgtaaaaagg ccgcgttgct ggcgtttttc cataggctcc gccccctga 840

cgagcatcac aaaaatcgac gctcaagtca gaggtggcga aacccgacag gactataaag 900

ataccaggcg tttccccctg gaagctccct cgtgcgctct cctgttccga ccctgccgct 960

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taccggatac ctgtccgcct ttctcccttc gggaagcgtg gcgctttctc aaagctcacg 1020

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<210> SEQ ID NO 12
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: shRNA sequence

<400> SEQUENCE: 12

caggcccagg agtccaatt 19

<210> SEQ ID NO 13
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: shRNA sequence

<400> SEQUENCE: 13

gcgccgcgga tcgtcaaca 19

<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: shRNA sequence

<400> SEQUENCE: 14

acacgtgcac ggcggccaa 19

<210> SEQ ID NO 15
<211> LENGTH: 4002
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: expression vector pd2

<400> SEQUENCE: 15

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gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180

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atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg 480

ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt 540

acggtgggag gtctatataa gcagagctgg tttagtgaac cgtcagatcc gctagcgcta 600

ccggactcag atctcgagct caagcttcga attctgcagt cgacggtacc gcgggcccgg 660

gatccaccgg ggccgcgact ctagatcata atcagccata ccacatttgt agaggtttta 720

cttgctttaa aaaacctccc acacctcccc ctgaacctga aacataaaat gaatgcaatt 780

gttgttgtta acttgtttat tgcagcttat aatggttaca aataaagcaa tagcatcaca 840

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aaagaacgtg gactccaacg tcaaagggcg aaaaaccgtc tatcagggcg atggcccact 1140

acgtgaacca tcaccctaat caagtttttt ggggtcgagg tgccgtaaag cactaaatcg 1200

gaaccctaaa gggagccccc gatttagagc ttgacgggga aagccggcga acgtggcgag 1260

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<210> SEQ ID NO 16
<211> LENGTH: 242
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Siglec-15 fragment

<400> SEQUENCE: 16

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His Ser Ser Pro Ala Gln Arg Trp Ser Met Gln Val Pro Pro Glu Val
20 25 30

Ser Ala Glu Ala Gly Asp Ala Ala Val Leu Pro Cys Thr Phe Thr His
35 40 45

Pro His Arg His Tyr Asp Gly Pro Leu Thr Ala Ile Trp Arg Ala Gly
50 55 60

Glu Pro Tyr Ala Gly Pro Gln Val Phe Arg Cys Ala Ala Ala Arg Gly
65 70 75 80

Ser Glu Leu Cys Gln Thr Ala Leu Ser Leu His Gly Arg Phe Arg Leu
85 90 95

Leu Gly Asn Pro Arg Arg Asn Asp Leu Ser Leu Arg Val Glu Arg Leu
100 105 110

Ala Leu Ala Asp Asp Arg Arg Tyr Phe Cys Arg Val Glu Phe Ala Gly
115 120 125

Asp Val His Asp Arg Tyr Glu Ser Arg His Gly Val Arg Leu His Val
130 135 140

Thr Ala Ala Pro Arg Ile Val Asn Ile Ser Val Leu Pro Ser Pro Ala
145 150 155 160

His Ala Phe Arg Ala Leu Cys Thr Ala Glu Gly Glu Pro Pro Pro Ala
165 170 175

Leu Ala Trp Ser Gly Pro Ala Leu Gly Asn Ser Leu Ala Ala Val Arg
180 185 190

Ser Pro Arg Glu Gly His Gly His Leu Val Thr Ala Glu Leu Pro Ala
195 200 205

Leu Thr His Asp Gly Arg Tyr Thr Cys Thr Ala Ala Asn Ser Leu Gly
210 215 220

Arg Ser Glu Ala Ser Val Tyr Leu Phe Arg Phe His Gly Ala Ser Gly
225 230 235 240

Ala Ser

<210> SEQ ID NO 17
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer incorporating a BamHI
restriction site

<400> SEQUENCE: 17

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<210> SEQ ID NO 18

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<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer incorporating a NotI restriction site

<400> SEQUENCE: 18

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<210> SEQ ID NO 19
<211> LENGTH: 5138
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: expression vector pYD5

<400> SEQUENCE: 19

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ttattaatag taatcaatta cggggtcatt agttcatagc ccatatatgg agttccgcgt 120

tacataactt acggtaaatg gcccgcctgg ctgaccgccc aacgaccccc gcccattgac 180

gtcaataatg acgtatgttc ccatagtaac gccaataggg actttccatt gacgtcaatg 240

ggtggagtat ttacggtaaa ctgcccactt ggcagtacat caagtgtatc atatgccaag 300

tccgcccccct attgacgtca atgacggtaa atggcccgcc tggcattatg cccagtacat 360

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<210> SEQ ID NO 20
<211> LENGTH: 642
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 light chain sequence

<400> SEQUENCE: 20

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tcctccccca aaccctggat ttatcgcaca tccaacctgg cttctggagt ccctgctcgc 180

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gatgctgcca cttattactg ccagcagtgg agtagtaacc cactcacgtt cggtgctggg 300

accaagctgg agctgaaacg ggctgtggct gcaccatctg tcttcatctt cccgccatct 360

gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc 420

agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa ctcccaggag 480

agtgtcacag agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg 540

agcaaagcag actacgagaa acacaaagtc tacgcctgcg aagtcaccca tcagggcctg 600

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<210> SEQ ID NO 21
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 light chain sequence

<400> SEQUENCE: 21

Glu Asn Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Ile Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

```
Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
        35                  40                  45

Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Val Ala Ala Pro Ser Val
            100                 105                 110

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
        115                 120                 125

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
    130                 135                 140

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val
145                 150                 155                 160

Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
                165                 170                 175

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
            180                 185                 190

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
        195                 200                 205

Gly Glu Cys
    210
```

<210> SEQ ID NO 22
<211> LENGTH: 1353
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 heavy chain sequence

<400> SEQUENCE: 22

```
gaggtccagc tgcaacaatc tgggactgag ctggtgaggc ctgggtcctc agtgaagatt     60

tcctgcaagg cttctggcta caccttcacc aggtactgga tggactgggt gaagcagagg    120

cctggacaag gccttgagtg gatcggagag attgatcctt ctgatagtta tactaactac    180

aatcaaaagt tcaagggcaa ggccacattg actgtagata aattctccag aacagcctat    240

atggaactca gcagcctgac atctgaggac tctgcggtct attactgtgc aagatcgggg    300

gcctactcta gtgactatag ttacgacggg tttgcttact ggggccaagg gactctggtc    360

actgtctctg cagcctcaac gaagggccca tcggtcttcc ccctggcgcc ctgctccagg    420

agcacctccg agagcacagc cgccctgggc tgcctggtca aggactactt ccccgaaccg    480

gtgacggtgt cgtggaactc aggcgctctg accagcggcg tgcacacctt cccagctgtc    540

ctacagtcct caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcaacttc    600

ggcacccaga cctacacctg caacgtagat cacaagccca gcaacaccaa ggtggacaag    660

acagttgagc gcaaatgttg tgtcgagtgc ccaccgtgcc cagcaccacc tgtggcagga    720

ccgtcagtct tccgcttccc cccaaaaccc aaggacaccc gcatgatctc ccggaccccct    780

gaggtcacgt gcgtggtggt ggatgtgagc cacgaagacc ccgaggtcca gttcaactgg    840

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cacgggagga gcagttcaac    900

agcacgttcc gtgtggtcag cgtcctcacc gttgtgcacc aggactggct gaacggcaag    960

gagtacaagt gcaaggtctc caacaaaggc ctcccagccc ccatcgagaa aaccatctcc   1020
```

-continued

```
aaaaccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag   1080

atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctaccc cagcgacatc   1140

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac acctcccatg   1200

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg   1260

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg   1320

cagaagagcc tctccctgtc tccgggtaaa tga                                1353
```

<210> SEQ ID NO 23
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 heavy chain sequence

<400> SEQUENCE: 23

```
Glu Val Gln Leu Gln Gln Ser Gly Thr Glu Leu Val Arg Pro Gly Ser
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Trp Met Asp Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Phe Ser Arg Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Gly Ala Tyr Ser Ser Asp Tyr Ser Tyr Asp Gly Phe Ala
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys
        115                 120                 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
    130                 135                 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145                 150                 155                 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                165                 170                 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
            180                 185                 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
        195                 200                 205

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
    210                 215                 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
    290                 295                 300
```

-continued

```
Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450
```

<210> SEQ ID NO 24
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 light chain sequence

<400> SEQUENCE: 24

```
gatattgtga tgacccaggc tgcattctcc aatccagtca ctcttggaac atcagcttcc     60

atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg    120

tatctgcaga agccaggcca gtctcctcag ctcctgattt atcagatgtc caaccttgcc    180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc    240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatccg    300

tacacgttcg gaggggggac caagctggaa ataaaacggg ctgtggctgc accatctgtc    360

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg    420

ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa    480

tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc    540

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa    600

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag    660
```

<210> SEQ ID NO 25
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 light chain sequence

<400> SEQUENCE: 25

```
Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
1               5                   10                  15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
```

-continued

```
Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
                85                  90                  95

Leu Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
        115                 120                 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
    130                 135                 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145                 150                 155                 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
                165                 170                 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
            180                 185                 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
        195                 200                 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210> SEQ ID NO 26
<211> LENGTH: 1335
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 heavy chain sequence

<400> SEQUENCE: 26

caggtccaag tgcagcagcc tggggctgaa attgtgaggc ctggggcttc agtgaagctg     60

tcctgcaagg cttctggcta caccttcacc agctactgga tgcactgggt gaagcagagg    120

cctggacaag gccttgagtg gattggactg attaatccta ccaacggtcg tactaactac    180

aatgagaagt tcaagagcaa ggccacactg actgtagaca aatcctccag cacagcctac    240

atgcaactca gcagcctgac atctgaggac tctgcggtct attactgtgc aagaggggag    300

gacggggact actttgacta ctggggccaa ggcaccactc tcacagtctc ctcagcctca    360

acgaagggcc catcggtctt ccccctggcg ccctgctcca ggagcacctc cgagagcaca    420

gccgccctgg gctgcctggt caaggactac ttccccgaac cggtgacggt gtcgtggaac    480

tcaggcgctc tgaccagcgg cgtgcacacc ttcccagctg tcctacagtc ctcaggactc    540

tactccctca gcagcgtggt gaccgtgccc tccagcaact tcggcaccca gacctacacc    600

tgcaacgtag atcacaagcc cagcaacacc aaggtggaca agacagttga gcgcaaatgt    660

tgtgtcgagt gcccaccgtg cccagcacca cctgtggcag gaccgtcagt cttccgcttc    720

cccccaaaac ccaaggacac ccgcatgatc tcccggaccc ctgaggtcac gtgcgtggtg    780

gtggatgtga gccacgaaga ccccgaggtc cagttcaact ggtacgtgga cggcgtggag    840

gtgcataatg ccaagacaaa gccacgggag gagcagttca acagcacgtt ccgtgtggtc    900

agcgtcctca ccgttgtgca ccaggactgg ctgaacggca aggagtacaa gtgcaaggtc    960

tccaacaaag gcctcccagc ccccatcgag aaaaccatct ccaaaaccaa agggcagccc   1020

cgagaaccac aggtgtacac cctgccccca tcccgggagg agatgaccaa gaaccaggtc   1080
```

-continued

agcctgacct gcctggtcaa aggcttctac cccagcgaca tcgccgtgga gtgggagagc 1140

aatgggcagc cggagaacaa ctacaagacc acacctccca tgctggactc cgacggctcc 1200

ttcttcctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc 1260

tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg 1320

tctccgggta aatga 1335

<210> SEQ ID NO 27
<211> LENGTH: 444
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 heavy chain sequence

<400> SEQUENCE: 27

Gln Val Gln Val Gln Gln Pro Gly Ala Glu Ile Val Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Asn Pro Thr Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Asp Gly Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
210 215 220

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
290 295 300

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

-continued

```
Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
                325                 330                 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
            340                 345                 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
        355                 360                 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
    370                 375                 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
385                 390                 395                 400

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
                405                 410                 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
            420                 425                 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440
```

<210> SEQ ID NO 28
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 light chain sequence

<400> SEQUENCE: 28

```
gatattgtga tgacccaggc tgcaccctct gtacctgtca ctcctggaga gtcagtatcc     60

atctcctgca ggtctactaa gagtctcctg catagtaatg gcaacactta cttgtattgg    120

ttcctgcaga ggccaggcca gtctcctcag ctcctgatat atcggatgtc caaccttgcc    180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc    240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatcct    300

ttcacgttcg gaggggggac caagctggaa ataaaacggg ctgtggctgc accatctgtc    360

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg    420

ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa    480

tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc    540

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa    600

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag    660
```

<210> SEQ ID NO 29
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 light chain sequence

<400> SEQUENCE: 29

```
Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Thr Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80
```

-continued

```
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
                85                  90                  95

Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
        115                 120                 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
    130                 135                 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145                 150                 155                 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
                165                 170                 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
            180                 185                 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
        195                 200                 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> SEQ ID NO 30
<211> LENGTH: 1350
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 heavy chain sequence

<400> SEQUENCE: 30

```
gagatccagc tgcagcagtc tggagttgag ctggtgaggc ctggggcttc agtgacgctg     60

tcctgcaagg cttcgggcta cacatttact gactatgaca tgcactgggt gaagcagaca    120

cctgttcatg gcctggaatg gattggaact attgatcctg aaactggtgg tactgcctac    180

aatcagaagt tcaagggcaa ggccacactg actgcggaca gatcctccac cacagcctac    240

atggagctca gcagcctgac atctgaggac tctgccgtct attactgtac aactttctac    300

tatagtcact ataattacga cgtggggttt gcttactggg gccaagggac tctggtcact    360

gtctctgcag cctcaacgaa gggcccatcg gtcttccccc tggcgccctg ctccaggagc    420

acctccgaga gcacagccgc cctgggctgc ctggtcaagg actacttccc cgaaccggtg    480

acggtgtcgt ggaactcagg cgctctgacc agcggcgtgc acaccttccc agctgtccta    540

cagtcctcag gactctactc cctcagcagc gtggtgaccg tgccctccag caacttcggc    600

acccagacct acacctgcaa cgtagatcac aagcccagca acaccaaggt ggacaagaca    660

gttgagcgca aatgttgtgt cgagtgccca ccgtgcccag caccacctgt ggcaggaccg    720

tcagtcttcc gcttcccccc aaaacccaag gacacccgca tgatctcccg gacccctgag    780

gtcacgtgcg tggtggtgga tgtgagccac gaagaccccg aggtccagtt caactggtac    840

gtggacggcg tggaggtgca taatgccaag acaaagccac gggaggagca gttcaacagc    900

acgttccgtg tggtcagcgt cctcaccgtt gtgcaccagg actggctgaa cggcaaggag    960

tacaagtgca aggtctccaa caaaggcctc ccagccccca tcgagaaaac catctccaaa   1020

accaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg   1080

accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctaccccag cgacatcgcc   1140

gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacacc tcccatgctg   1200

gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag   1260
```

-continued

```
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag   1320

aagagcctct ccctgtctcc gggtaaatga                                    1350


<210> SEQ ID NO 31
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 heavy chain sequence

<400> SEQUENCE: 31

Glu Ile Gln Leu Gln Gln Ser Gly Val Glu Leu Val Arg Pro Gly Ala
1               5                   10                  15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30

Asp Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Arg Ser Ser Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Thr Thr Phe Tyr Tyr Ser His Tyr Asn Tyr Asp Val Gly Phe Ala Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val
        195                 200                 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys
    210                 215                 220

Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350
```

-continued

```
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> SEQ ID NO 32
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 light chain sequence

<400> SEQUENCE: 32

```
gatattgtga tgacccaggc tgcaccctct gtacctgtca ctcctggaga gtcagtatcc     60

atctcctgca ggtctagtaa gagtctcctg catagtaatg gcaacactta cttgtattgg    120

ttcctgcaga ggccaggcca gtcccctcag ctcctgatat atcggatgtc caaccttgcc    180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc    240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatcct    300

ttcacgttcg gaggggggac caagctggaa ataaaacggg ctgtggctgc accatctgtc    360

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg    420

ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa    480

tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc    540

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa    600

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag    660
```

<210> SEQ ID NO 33
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 light chain sequence

<400> SEQUENCE: 33

```
Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
                85                  90                  95
```

-continued

```
Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
        115                 120                 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
    130                 135                 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145                 150                 155                 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
                165                 170                 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
            180                 185                 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
        195                 200                 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210> SEQ ID NO 34
<211> LENGTH: 1422
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 heavy chain sequence

<400> SEQUENCE: 34

gagatccagc tgcagcagtc tggagctgag ctggtgaggc ctggggcttc agtgacgctg     60

tcctgcaagg cttcgggcta cacatttact gactatgaaa tgcactgggt gaagcagaca    120

cctgttcatg gcctggaatg gattggagct attgatcctg aaactggtgg tactgcctac    180

aatcagaagt tcaagggcaa ggccacactg actgcagaca aatcctccag cacagcctac    240

atggagctca gcagcctgac atctgaggac tctgccgtct attactgtac aagtttctac    300

tatacttact ataattacga cgtggggttt gcttactggg gccaagggac tctggtcact    360

gtctctgcag cctcaactgg ggcgtcttat tactatgcta tggaccactg gggtcaagga    420

acctcagtca ccgtctcctc agcctcaacg aagggcccat cggtcttccc cctggcgccc    480

tgctccagga gcacctccga gagcacagcc gccctgggct gcctggtcaa ggactacttc    540

cccgaaccgg tgacggtgtc gtggaactca ggcgctctga ccagcggcgt gcacaccttc    600

ccagctgtcc tacagtcctc aggactctac tccctcagca gcgtggtgac cgtgccctcc    660

agcaacttcg gcacccagac ctacacctgc aacgtagatc acaagcccag caacaccaag    720

gtggacaaga cagttgagcg caaatgttgt gtcgagtgcc caccgtgccc agcaccacct    780

gtggcaggac cgtcagtctt ccgcttcccc ccaaaaccca aggacacccg catgatctcc    840

cggacccctg aggtcacgtg cgtggtggtg gatgtgagcc acgaagaccc cgaggtccag    900

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc acgggaggag    960

cagttcaaca gcacgttccg tgtggtcagc gtcctcaccg ttgtgcacca ggactggctg   1020

aacggcaagg agtacaagtg caaggtctcc aacaaaggcc tcccagcccc catcgagaaa   1080

accatctcca aaaccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc    1140

cgggaggaga tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctacccc   1200

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccaca   1260

cctcccatgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag   1320

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac   1380
```

-continued

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1422

<210> SEQ ID NO 35
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 heavy chain sequence

<400> SEQUENCE: 35

Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
35 40 45

Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Thr Ser Phe Tyr Tyr Thr Tyr Tyr Asn Tyr Asp Val Gly Phe Ala Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
130 135 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145 150 155 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
165 170 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180 185 190

Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val
195 200 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys
210 215 220

Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val
290 295 300

Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

-continued

```
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> SEQ ID NO 36
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 light chain sequence

<400> SEQUENCE: 36

```
gatattgtga tgacccaggc tgcattctcc aatccagtca ctcttggaac atcagcttcc     60

atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg    120

tatctgcaga agccaggcca gtctcctcag ctcctgattt atcagatgtc caaccttgcc    180

tcaggagtcc cagacaggtt cagtagcagt gggtcaggaa ctgatttcac actgagaatc    240

agcagagtgg aggctgagga tgtgggtgtt tattactgtg ctcaaaatct agaacttccg    300

tacacgttcg gaggggggac caagctggaa ataaaacggg ctgtggctgc accatctgtc    360

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg    420

ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa    480

tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc    540

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa    600

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag    660
```

<210> SEQ ID NO 37
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 light chain sequence

<400> SEQUENCE: 37

```
Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
1               5                   10                  15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
                85                  90                  95

Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

-continued

```
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
        115                 120                 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
    130                 135                 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145                 150                 155                 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
                165                 170                 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
            180                 185                 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
        195                 200                 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> SEQ ID NO 38
<211> LENGTH: 1335
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 heavy chain sequence

<400> SEQUENCE: 38

```
caggtccaag tgcagcagcc tggggctgag cttgtgaagc ctggggcttc ggtgaagctg     60

tcctgcaagg cttctggcta caccttcacc agctactgga tgcactgggt gaagcagagg    120

cctggacaag gccttgagtg gattggactg attaatccta gcaacgctcg tactaactac    180

aatgagaagt tcaataccaa ggccacactg actgtagaca aatcctccag cacagcctac    240

atgcaactca gcagcctgac atctgaggac tctgcggtct attactgtgc aagaggggag    300

gacggggact actttgacta ctggggccaa ggcaccactc tcacagtctc ctcagcctca    360

acgaagggcc catcggtctt ccccctggcg ccctgctcca ggagcacctc cgagagcaca    420

gccgccctgg gctgcctggt caaggactac ttccccgaac cggtgacggt gtcgtggaac    480

tcaggcgctc tgaccagcgg cgtgcacacc ttcccagctg tcctacagtc ctcaggactc    540

tactccctca gcagcgtggt gaccgtgccc tccagcaact tcggcaccca gacctacacc    600

tgcaacgtag atcacaagcc cagcaacacc aaggtggaca agacagttga gcgcaaatgt    660

tgtgtcgagt gcccaccgtg cccagcacca cctgtggcag gaccgtcagt cttccgcttc    720

cccccaaaac ccaaggacac ccgcatgatc tcccggaccc ctgaggtcac gtgcgtggtg    780

gtggatgtga gccacgaaga ccccgaggtc cagttcaact ggtacgtgga cggcgtggag    840

gtgcataatg ccaagacaaa gccacgggag gagcagttca acagcacgtt ccgtgtggtc    900

agcgtcctca ccgttgtgca ccaggactgg ctgaacggca aggagtacaa gtgcaaggtc    960

tccaacaaag gcctcccagc ccccatcgag aaaaccatct ccaaaaccaa agggcagccc   1020

cgagaaccac aggtgtacac cctgccccca tcccgggagg agatgaccaa gaaccaggtc   1080

agcctgacct gcctggtcaa aggcttctac cccagcgaca tcgccgtgga gtgggagagc   1140

aatgggcagc cggagaacaa ctacaagacc acacctccca tgctggactc cgacggctcc   1200

ttcttcctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc   1260

tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg   1320

tctccgggta aatga                                                    1335
```

<210> SEQ ID NO 39

-continued

<211> LENGTH: 444
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 heavy chain sequence

<400> SEQUENCE: 39

Gln Val Gln Val Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Asn Pro Ser Asn Ala Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Asn Thr Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Asp Gly Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
210 215 220

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
290 295 300

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

-continued

```
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
385                 390                 395                 400

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
                405                 410                 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
            420                 425                 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440
```

<210> SEQ ID NO 40
<211> LENGTH: 642
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 light chain sequence

<400> SEQUENCE: 40

```
caaattgttc tcacccagtc tccaacactc atgtctgcat ctccagggga gaaggtcacc     60

atgacctgca gtgccagctc aagtgtaagt tacatgtact ggtaccagca gaagccaaga    120

tcctccccca aaccctggat ttatcgcaca tccaacctgg tttctggagt ccctgtacgc    180

ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcagcagcat ggaggctgaa    240

gatgctgcca cttattactg ccagcagtgg agtagtaacc cacccacgtt cggtgctggg    300

accaagctgg agctgaaacg ggctgtggct gcaccatctg tcttcatctt cccgccatct    360

gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc    420

agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa ctcccaggag    480

agtgtcacag agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg    540

agcaaagcag actacgagaa acacaaagtc tacgcctgcg aagtcaccca tcagggcctg    600

agctcgcccg tcacaaagag cttcaacagg ggagagtgtt ag                       642
```

<210> SEQ ID NO 41
<211> LENGTH: 211
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 light chain sequence

<400> SEQUENCE: 41

```
Gln Ile Val Leu Thr Gln Ser Pro Thr Leu Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
        35                  40                  45

Arg Thr Ser Asn Leu Val Ser Gly Val Pro Val Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Val Ala Ala Pro Ser Val
            100                 105                 110

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
        115                 120                 125

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
    130                 135                 140
```

-continued

```
Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val
145                 150                 155                 160

Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
                165                 170                 175

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
            180                 185                 190

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
        195                 200                 205

Gly Glu Cys
    210
```

<210> SEQ ID NO 42
<211> LENGTH: 1341
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 heavy chain sequence

<400> SEQUENCE: 42

```
gaagtgaagc ttgaggagtc tggaggtggc ctggtgcagc ctggaggatc cctgaaactc     60

tcctgtgcag cctcaggatt cgattttagt aaagactgga tgagttgggt ccggcaggct    120

ccagggaaag ggctagaatg gattggagaa attaatccag atagcagtac gataaactat    180

gcaccatctc ttaaggataa attcatcatc tccagagaga acgccaaaaa tacgctgtac    240

ctgcaaatga gcaaagtgag atctgaggac acagcccttt attactgttc aagactagag    300

gactacgaag actggtactt cgatgtctgg ggcgcaggga ccacggtcac cgtctcctca    360

gcctcaacga agggcccatc ggtcttcccc ctggcgccct gctccaggag cacctccgag    420

agcacagccg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg    480

tggaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca    540

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcaacttcgg cacccagacc    600

tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagac agttgagcgc    660

aaatgttgtg tcgagtgccc accgtgccca gcaccacctg tggcaggacc gtcagtcttc    720

cgcttccccc caaaacccaa ggacacccgc atgatctccc ggacccctga ggtcacgtgc    780

gtggtggtgg atgtgagcca cgaagacccc gaggtccagt tcaactggta cgtggacggc    840

gtggaggtgc ataatgccaa gacaaagcca cgggaggagc agttcaacag cacgttccgt    900

gtggtcagcg tcctcaccgt tgtgcaccag gactggctga acggcaagga gtacaagtgc    960

aaggtctcca acaaaggcct cccagccccc atcgagaaaa ccatctccaa aaccaaaggg   1020

cagccccgag aaccacaggt gtacaccctg cccccatccc gggaggagat gaccaagaac   1080

caggtcagcc tgacctgcct ggtcaaaggc ttctacccca gcgacatcgc cgtggagtgg   1140

gagagcaatg ggcagccgga gaacaactac aagaccacac ctcccatgct ggactccgac   1200

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac   1260

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc   1320

tccctgtctc cgggtaaatg a                                             1341
```

<210> SEQ ID NO 43
<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 heavy chain sequence

-continued

<400> SEQUENCE: 43

```
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Lys Asp
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Ala Pro Ser Leu
    50                  55                  60

Lys Asp Lys Phe Ile Ile Ser Arg Glu Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95

Ser Arg Leu Glu Asp Tyr Glu Asp Trp Tyr Phe Asp Val Trp Gly Ala
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val
    210                 215                 220

Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
225                 230                 235                 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                245                 250                 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
            260                 265                 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
    290                 295                 300

Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305                 310                 315                 320

Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                325                 330                 335

Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            340                 345                 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        355                 360                 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
    370                 375                 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp
385                 390                 395                 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                405                 410                 415
```

-continued

```
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
            420                 425                 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> SEQ ID NO 44
<211> LENGTH: 645
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 light chain sequence

<400> SEQUENCE: 44

```
agtattgtga tgacccagac tcccaaattc ctgcttgtat cagcaggaga cagggttacc     60

ataacctgca aggccagtca gagtgtgagt aatgctgtag cttggtacca acagaagcca    120

gggcagtctc ctaaactgct gatatactat acatccaatc gctacactgg agtccctgat    180

cgcttcactg gcagtggata tgggacggat ttcactttca ccatcaccac tgtgcaggct    240

gaagacctgg cagtttattt ctgtcagcag gattatacct ctccgtggac gttcggtgga    300

ggcaccaagc tggaaatcaa acgggctgtg gctgcaccat ctgtcttcat cttcccgcca    360

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat    420

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag    480

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg    540

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc    600

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag                    645
```

<210> SEQ ID NO 45
<211> LENGTH: 212
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 light chain sequence

<400> SEQUENCE: 45

```
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Ser Asn Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Thr Thr Val Gln Ala
65                  70                  75                  80

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Thr Ser Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Val Ala Ala Pro Ser
            100                 105                 110

Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
        115                 120                 125

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
    130                 135                 140

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
145                 150                 155                 160

Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr
                165                 170                 175
```

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```
Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys
            180                 185                 190

Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
        195                 200                 205

Arg Gly Glu Cys
    210


<210> SEQ ID NO 46
<211> LENGTH: 1317
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 heavy chain sequence

<400> SEQUENCE: 46

caggtccaac tgcagcagcc tggggctgaa ctggcgaagc ctggggcttc agtgaagttg     60

tcctgcaagg cttctggcta caccttcaac acctataata tgtactggtt gaaacagagg    120

cctgggcaag gccttgagtg gattgggggg attgatccta gcaatggtga tactaaaatc    180

aatgagaagt tcaagaacaa ggccacactg actgttgaca aatcctccag tacagcctat    240

atgcaactca gcggcctgac atctgaggac tctgcggtct attactgtac aagccatacg    300

tactggggcc aagggactct ggtcactgtc tctgcagcct caacgaaggg cccatcggtc    360

ttccccctgg cgccctgctc caggagcacc tccgagagca cagccgccct gggctgcctg    420

gtcaaggact acttccccga accggtgacg gtgtcgtgga actcaggcgc tctgaccagc    480

ggcgtgcaca ccttcccagc tgtcctacag tcctcaggac tctactccct cagcagcgtg    540

gtgaccgtgc cctccagcaa cttcggcacc cagacctaca cctgcaacgt agatcacaag    600

cccagcaaca ccaaggtgga caagacagtt gagcgcaaat gttgtgtcga gtgcccaccg    660

tgcccagcac cacctgtggc aggaccgtca gtcttccgct tccccccaaa acccaaggac    720

acccgcatga tctcccggac ccctgaggtc acgtgcgtgg tggtggatgt gagccacgaa    780

gaccccgagg tccagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca    840

aagccacggg aggagcagtt caacagcacg ttccgtgtgg tcagcgtcct caccgttgtg    900

caccaggact ggctgaacgg caaggagtac aagtgcaagg tctccaacaa aggcctccca    960

gcccccatcg agaaaaccat ctccaaaacc aaagggcagc cccgagaacc acaggtgtac   1020

accctgcccc catcccggga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc   1080

aaaggcttct accccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac   1140

aactacaaga ccacacctcc catgctggac tccgacggct ccttcttcct ctacagcaag   1200

ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat   1260

gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctccggg taaatga      1317


<210> SEQ ID NO 47
<211> LENGTH: 438
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 heavy chain sequence

<400> SEQUENCE: 47

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Ala Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Thr Tyr
            20                  25                  30
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```
Asn Met Tyr Trp Leu Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Gly Ile Asp Pro Ser Asn Gly Asp Thr Lys Ile Asn Glu Lys Phe
    50                  55                  60

Lys Asn Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Thr Ser His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
            100                 105                 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
        115                 120                 125

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
    130                 135                 140

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
145                 150                 155                 160

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
                165                 170                 175

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
            180                 185                 190

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
        195                 200                 205

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
    210                 215                 220

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
225                 230                 235                 240

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
                245                 250                 255

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
            260                 265                 270

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
        275                 280                 285

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
    290                 295                 300

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
305                 310                 315                 320

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
                325                 330                 335

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
            340                 345                 350

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
        355                 360                 365

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
    370                 375                 380

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
385                 390                 395                 400

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                405                 410                 415

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
            420                 425                 430

Ser Leu Ser Pro Gly Lys
        435
```

<210> SEQ ID NO 48

-continued

<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 light chain sequence

<400> SEQUENCE: 48

gatattgtga tgacccaggc tgcaccctct gtacctgtca ctcctggaga gtcagtatcc 60

atctcctgca ggtctactaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

ttcctgcaga ggccaggcca gtctcctcag ctcctgatat atcggatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatcct 300

ttcacgttcg gaggggggac caagctggaa ataaaacggg ctgtggctgc accatctgtc 360

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg 420

ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa 480

tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc 540

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa 600

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag 660

<210> SEQ ID NO 49
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 light chain sequence

<400> SEQUENCE: 49

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Thr Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
115 120 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130 135 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
145 150 155 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
180 185 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
195 200 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys

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210 215

<210> SEQ ID NO 50
<211> LENGTH: 1422
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 heavy chain sequence

<400> SEQUENCE: 50

gagatccagc tgcagcagtc tggagttgag ctggtgaggc ctggggcttc agtgacgctg 60

tcctgcaagg cttcgggcta cacatttact gactatgaca tgcactgggt gaagcagaca 120

cctgttcatg gcctggaatg gattggaact attgatcctg aaactggtgg tactgcctac 180

aatcagaagt tcaagggcaa ggccacactg actgcggaca gatcctccac cacagcctac 240

atggagctca gcagcctgac atctgaggac tctgccgtct attactgtac aagtttctac 300

tatacttact ctaattacga cgtggggttt gcttactggg gccaagggac tctggtcact 360

gtctctgcag cctcaactgg ggcgtcttat tactatgcta tggaccactg gggtcaagga 420

acctcagtca ccgtctcctc agcctcaacg aagggcccat cggtcttccc cctggcgccc 480

tgctccagga gcacctccga gagcacagcc gccctgggct gcctggtcaa ggactacttc 540

cccgaaccgg tgacggtgtc gtggaactca ggcgctctga ccagcggcgt gcacaccttc 600

ccagctgtcc tacagtcctc aggactctac tccctcagca gcgtggtgac cgtgccctcc 660

agcaacttcg gcacccagac ctacacctgc aacgtagatc acaagcccag caacaccaag 720

gtggacaaga cagttgagcg caaatgttgt gtcgagtgcc caccgtgccc agcaccacct 780

gtggcaggac cgtcagtctt ccgcttcccc ccaaaaccca aggacacccg catgatctcc 840

cggacccctg aggtcacgtg cgtggtggtg gatgtgagcc acgaagaccc cgaggtccag 900

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc acgggaggag 960

cagttcaaca gcacgttccg tgtggtcagc gtcctcaccg ttgtgcacca ggactggctg 1020

aacggcaagg agtacaagtg caaggtctcc aacaaaggcc tcccagcccc catcgagaaa 1080

accatctcca aaaccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc 1140

cgggaggaga tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctacccc 1200

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccaca 1260

cctcccatgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1320

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1380

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1422

<210> SEQ ID NO 51
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 heavy chain sequence

<400> SEQUENCE: 51

Glu Ile Gln Leu Gln Gln Ser Gly Val Glu Leu Val Arg Pro Gly Ala
1 5 10 15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asp Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
35 40 45

Gly Thr Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe

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    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Arg Ser Ser Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Thr Ser Phe Tyr Tyr Thr Tyr Ser Asn Tyr Asp Val Gly Phe Ala Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val
        195                 200                 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys
    210                 215                 220

Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
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<210> SEQ ID NO 52
<211> LENGTH: 318
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 light chain variable region sequence

<400> SEQUENCE: 52

gaaaatgtgc tcacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc 60

atatcctgca gtgccagctc aagtgtaagt tacatgtact ggtaccagca gaagccagga 120

tcctccccca aaccctggat ttatcgcaca tccaacctgg cttctggagt ccctgctcgc 180

ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcagcagcat ggaggctgaa 240

gatgctgcca cttattactg ccagcagtgg agtagtaacc cactcacgtt cggtgctggg 300

accaagctgg agctgaaa 318

<210> SEQ ID NO 53
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 light chain variable region sequence

<400> SEQUENCE: 53

Glu Asn Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Ile Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> SEQ ID NO 54
<211> LENGTH: 372
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 heavy chain variable region sequence

<400> SEQUENCE: 54

gaggtccagc tgcaacaatc tgggactgag ctggtgaggc ctgggtcctc agtgaagatt 60

tcctgcaagg cttctggcta caccttcacc aggtactgga tggactgggt gaagcagagg 120

cctggacaag gccttgagtg gatcggagag attgatcctt ctgatagtta tactaactac 180

aatcaaaagt tcaagggcaa ggccacattg actgtagata aattctccag aacagcctat 240

atggaactca gcagcctgac atctgaggac tctgcggtct attactgtgc aagatcgggg 300

gcctactcta gtgactatag ttacgacggg tttgcttact ggggccaagg gactctggtc 360

actgtctctg ca 372

<210> SEQ ID NO 55
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25A1 heavy chain variable region sequence

<400> SEQUENCE: 55

Glu Val Gln Leu Gln Gln Ser Gly Thr Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Trp Met Asp Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Phe Ser Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Gly Ala Tyr Ser Ser Asp Tyr Ser Tyr Asp Gly Phe Ala
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> SEQ ID NO 56
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 light chain variable region sequence

<400> SEQUENCE: 56

gatattgtga tgacccaggc tgcattctcc aatccagtca ctcttggaac atcagcttcc 60

atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg 120

tatctgcaga agccaggcca gtctcctcag ctcctgattt atcagatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatccg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> SEQ ID NO 57
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 light chain variable region sequence

<400> SEQUENCE: 57

Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
1 5 10 15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 58
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 heavy chain variable region sequence

<400> SEQUENCE: 58

caggtccaag tgcagcagcc tggggctgaa attgtgaggc ctggggcttc agtgaagctg 60

tcctgcaagg cttctggcta caccttcacc agctactgga tgcactgggt gaagcagagg 120

cctggacaag gccttgagtg gattggactg attaatccta ccaacggtcg tactaactac 180

aatgagaagt tcaagagcaa ggccacactg actgtagaca aatcctccag cacagcctac 240

atgcaactca gcagcctgac atctgaggac tctgcggtct attactgtgc aagaggggg 300

gacggggact actttgacta ctggggccaa ggcaccactc tcacagtctc ctca 354

<210> SEQ ID NO 59
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B4 heavy chain variable region sequence

<400> SEQUENCE: 59

Gln Val Gln Val Gln Gln Pro Gly Ala Glu Ile Val Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Asn Pro Thr Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Asp Gly Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser
115

<210> SEQ ID NO 60
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 light chain variable region sequence

<400> SEQUENCE: 60

gatattgtga tgacccaggc tgcaccctct gtacctgtca ctcctggaga gtcagtatcc 60

atctcctgca ggtctactaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

ttcctgcaga ggccaggcca gtctcctcag ctcctgatat atcggatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatcct 300

ttcacgttcg gaggggggac caagctggaa ataaaa 336

<210> SEQ ID NO 61

-continued

<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 light chain variable region sequence

<400> SEQUENCE: 61

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Thr Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 62
<211> LENGTH: 369
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 heavy chain variable region sequence

<400> SEQUENCE: 62

gagatccagc tgcagcagtc tggagttgag ctggtgaggc ctggggcttc agtgacgctg 60

tcctgcaagg cttcgggcta cacatttact gactatgaca tgcactgggt gaagcagaca 120

cctgttcatg gcctggaatg gattggaact attgatcctg aaactggtgg tactgcctac 180

aatcagaagt tcaagggcaa ggccacactg actgcggaca gatcctccac cacagcctac 240

atggagctca gcagcctgac atctgaggac tctgccgtct attactgtac aactttctac 300

tatagtcact ataattacga cgtggggttt gcttactggg gccaagggac tctggtcact 360

gtctctgca 369

<210> SEQ ID NO 63
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25B8 heavy chain variable region sequence

<400> SEQUENCE: 63

Glu Ile Gln Leu Gln Gln Ser Gly Val Glu Leu Val Arg Pro Gly Ala
1 5 10 15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asp Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
35 40 45

Gly Thr Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Arg Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

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Thr Thr Phe Tyr Tyr Ser His Tyr Asn Tyr Asp Val Gly Phe Ala Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
        115                 120


<210> SEQ ID NO 64
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 light chain variable region sequence

<400> SEQUENCE: 64

gatattgtga tgacccaggc tgcaccctct gtacctgtca ctcctggaga gtcagtatcc     60

atctcctgca ggtctagtaa gagtctcctg catagtaatg gcaacactta cttgtattgg    120

ttcctgcaga ggccaggcca gtcccctcag ctcctgatat atcggatgtc caaccttgcc    180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc    240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatcct    300

ttcacgttcg gaggggggac caagctggaa ataaaa                              336


<210> SEQ ID NO 65
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 light chain variable region sequence

<400> SEQUENCE: 65

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
                85                  90                  95

Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 66
<211> LENGTH: 369
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 heavy chain variable region sequence

<400> SEQUENCE: 66

gagatccagc tgcagcagtc tggagctgag ctggtgaggc ctggggcttc agtgacgctg     60

tcctgcaagg cttcgggcta cacatttact gactatgaaa tgcactgggt gaagcagaca    120

cctgttcatg gcctggaatg gattggagct attgatcctg aaactggtgg tactgcctac    180

aatcagaagt tcaagggcaa ggccacactg actgcagaca aatcctccag cacagcctac    240

atggagctca gcagcctgac atctgaggac tctgccgtct attactgtac aagtttctac    300
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tatacttact ataattacga cgtggggttt gcttactggg gccaagggac tctggtcact 360

gtctctgca 369

<210> SEQ ID NO 67
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25C1 heavy chain variable region sequence

<400> SEQUENCE: 67

Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
35 40 45

Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Thr Ser Phe Tyr Tyr Thr Tyr Tyr Asn Tyr Asp Val Gly Phe Ala Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> SEQ ID NO 68
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 light chain variable region sequence

<400> SEQUENCE: 68

gatattgtga tgacccaggc tgcattctcc aatccagtca ctcttggaac atcagcttcc 60

atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg 120

tatctgcaga agccaggcca gtctcctcag ctcctgattt atcagatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtagcagt gggtcaggaa ctgatttcac actgagaatc 240

agcagagtgg aggctgagga tgtgggtgtt tattactgtg ctcaaaatct agaacttccg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> SEQ ID NO 69
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 light chain variable region sequence

<400> SEQUENCE: 69

Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
1 5 10 15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro

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    50                  55                  60

Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
                85                  90                  95

Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 70
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 heavy chain variable region sequence

<400> SEQUENCE: 70

caggtccaag tgcagcagcc tggggctgag cttgtgaagc ctggggcttc ggtgaagctg     60

tcctgcaagg cttctggcta caccttcacc agctactgga tgcactgggt gaagcagagg    120

cctggacaag gccttgagtg gattggactg attaatccta gcaacgctcg tactaactac    180

aatgagaagt tcaataccaa ggccacactg actgtagaca aatcctccag cacagcctac    240

atgcaactca gcagcctgac atctgaggac tctgcggtct attactgtgc aagaggggg     300

gacggggact actttgacta ctggggccaa ggcaccactc tcacagtctc ctca          354


<210> SEQ ID NO 71
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25D8 heavy chain variable region sequence

<400> SEQUENCE: 71

Gln Val Gln Val Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Leu Ile Asn Pro Ser Asn Ala Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Asn Thr Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Gly Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Leu Thr Val Ser Ser
        115


<210> SEQ ID NO 72
<211> LENGTH: 318
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 light chain variable region sequence

<400> SEQUENCE: 72

caaattgttc tcacccagtc tccaacactc atgtctgcat ctccagggga gaaggtcacc     60

atgacctgca gtgccagctc aagtgtaagt tacatgtact ggtaccagca gaagccaaga    120
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tcctccccca aaccctggat ttatcgcaca tccaacctgg tttctggagt ccctgtacgc 180

ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcagcagcat ggaggctgaa 240

gatgctgcca cttattactg ccagcagtgg agtagtaacc cacccacgtt cggtgctggg 300

accaagctgg agctgaaa 318

<210> SEQ ID NO 73
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 light chain variable region sequence

<400> SEQUENCE: 73

Gln Ile Val Leu Thr Gln Ser Pro Thr Leu Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Arg Thr Ser Asn Leu Val Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> SEQ ID NO 74
<211> LENGTH: 360
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 heavy chain variable region sequence

<400> SEQUENCE: 74

gaagtgaagc ttgaggagtc tggaggtggc ctggtgcagc ctggaggatc cctgaaactc 60

tcctgtgcag cctcaggatt cgattttagt aaagactgga tgagttgggt ccggcaggct 120

ccagggaaag ggctagaatg gattggagaa attaatccag atagcagtac gataaactat 180

gcaccatctc ttaaggataa attcatcatc tccagagaga acgccaaaaa tacgctgtac 240

ctgcaaatga gcaaagtgag atctgaggac acagcccttt attactgttc aagactagag 300

gactacgaag actggtactt cgatgtctgg ggcgcaggga ccacggtcac cgtctcctca 360

<210> SEQ ID NO 75
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E5 heavy chain variable region sequence

<400> SEQUENCE: 75

Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Lys Asp
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

```
Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Ala Pro Ser Leu
    50                  55                  60

Lys Asp Lys Phe Ile Ile Ser Arg Glu Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95

Ser Arg Leu Glu Asp Tyr Glu Asp Trp Tyr Phe Asp Val Trp Gly Ala
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 76
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 light chain variable region sequence

<400> SEQUENCE: 76

agtattgtga tgacccagac tcccaaattc ctgcttgtat cagcaggaga cagggttacc     60

ataacctgca aggccagtca gagtgtgagt aatgctgtag cttggtacca acagaagcca    120

gggcagtctc ctaaactgct gatatactat acatccaatc gctacactgg agtccctgat    180

cgcttcactg gcagtggata tgggacggat ttcactttca ccatcaccac tgtgcaggct    240

gaagacctgg cagtttattt ctgtcagcag gattatacct ctccgtggac gttcggtgga    300

ggcaccaagc tggaaatcaa a                                              321


<210> SEQ ID NO 77
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 light chain variable region sequence

<400> SEQUENCE: 77

Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Ser Asn Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Thr Thr Val Gln Ala
65                  70                  75                  80

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Thr Ser Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210> SEQ ID NO 78
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 heavy chain variable region sequence

<400> SEQUENCE: 78

caggtccaac tgcagcagcc tggggctgaa ctggcgaagc ctggggcttc agtgaagttg     60
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tcctgcaagg cttctggcta caccttcaac acctataata tgtactggtt gaaacagagg 120

cctgggcaag gccttgagtg gattgggggg attgatccta gcaatggtga tactaaaatc 180

aatgagaagt tcaagaacaa ggccacactg actgttgaca aatcctccag tacagcctat 240

atgcaactca gcggcctgac atctgaggac tctgcggtct attactgtac aagccatacg 300

tactggggcc aagggactct ggtcactgtc tctgca 336

<210> SEQ ID NO 79
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E6 heavy chain variable region sequence

<400> SEQUENCE: 79

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Ala Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Thr Tyr
20 25 30

Asn Met Tyr Trp Leu Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gly Ile Asp Pro Ser Asn Gly Asp Thr Lys Ile Asn Glu Lys Phe
50 55 60

Lys Asn Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Thr Ser His Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
100 105 110

<210> SEQ ID NO 80
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 light chain variable region sequence

<400> SEQUENCE: 80

gatattgtga tgacccaggc tgcaccctct gtacctgtca ctcctggaga gtcagtatcc 60

atctcctgca ggtctactaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

ttcctgcaga ggccaggcca gtctcctcag ctcctgatat atcggatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct agaatatcct 300

ttcacgttcg gaggggggac caagctggaa ataaaa 336

<210> SEQ ID NO 81
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 light chain variable region sequence

<400> SEQUENCE: 81

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Thr Lys Ser Leu Leu His Ser
20 25 30

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Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
                85                  90                  95

Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 82
<211> LENGTH: 369
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 heavy chain variable region sequence

<400> SEQUENCE: 82

gagatccagc tgcagcagtc tggagttgag ctggtgaggc ctggggcttc agtgacgctg     60

tcctgcaagg cttcgggcta cacatttact gactatgaca tgcactgggt gaagcagaca    120

cctgttcatg gcctggaatg gattggaact attgatcctg aaactggtgg tactgcctac    180

aatcagaagt tcaagggcaa ggccacactg actgcggaca gatcctccac cacagcctac    240

atggagctca gcagcctgac atctgaggac tctgccgtct attactgtac aagtttctac    300

tatacttact ctaattacga cgtggggttt gcttactggg gccaagggac tctggtcact    360

gtctctgca                                                            369


<210> SEQ ID NO 83
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 25E9 heavy chain variable region sequence

<400> SEQUENCE: 83

Glu Ile Gln Leu Gln Gln Ser Gly Val Glu Leu Val Arg Pro Gly Ala
1               5                   10                  15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30

Asp Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Arg Ser Ser Thr Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Thr Ser Phe Tyr Tyr Thr Tyr Ser Asn Tyr Asp Val Gly Phe Ala Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
        115                 120


<210> SEQ ID NO 84
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer OGS1773
```

<400> SEQUENCE: 84

gtaagcgcta gcgcctcaac gaagggccca tctgtctttc ccctggcccc 50

<210> SEQ ID NO 85
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer OGS1774

<400> SEQUENCE: 85

gtaagcgaat tcacaagatt tgggctcaac tttcttg 37

<210> SEQ ID NO 86
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 86

gctgtggctg caccatctgt cttcatcttc ccgccatctg atgagcagtt gaaatctgga 60

actgcctctg ttgtgtgcct gctgaataac ttctatccca gagaggccaa agtacagtgg 120

aaggtggata acgccctcca atcgggtaac tcccaggaga gtgtcacaga gcaggacagc 180

aaggacagca cctacagcct cagcagcacc ctgacgctga gcaaagcaga ctacgagaaa 240

cacaaagtct acgcctgcga agtcacccat cagggcctga gctcgcccgt cacaaagagc 300

ttcaacaggg gagagtgtta g 321

<210> SEQ ID NO 87
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 87

Ala Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> SEQ ID NO 88
<211> LENGTH: 6385
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: expression plasmid pTTVK1

<400> SEQUENCE: 88

cttgagccgg cggatggtcg aggtgaggtg tggcaggctt gagatccagc tgttggggtg 60

agtactccct ctcaaaagcg ggcattactt ctgcgctaag attgtcagtt tccaaaaacg 120

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aggaggattt gatattcacc tggcccgatc tggccataca cttgagtgac aatgacatcc 180

actttgcctt tctctccaca ggtgtccact cccaggtcca agtttaaacg gatctctagc 240

gaattcatga actttctgct gtcttgggtg cattggagcc ttgccttgct gctctacctc 300

caccatgcca agtggtccca ggcttgagac ggagcttaca gcgctgtggc tgcaccatct 360

gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420

ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480

caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 540

ctcagcagca ccctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600

gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gcttcaacag gggagagtgt 660

tagggtaccg cggccgcttc gaatgagatc ccccgacctc gacctctggc taataaagga 720

aatttatttt cattgcaata gtgtgttgga atttttttgtg tctctcactc ggaaggacat 780

atgggagggc aaatcatttg gtcgagatcc ctcggagatc tctagctaga gccccgccgc 840

cggacgaact aaacctgact acggcatctc tgccccttct tcgcggggca gtgcatgtaa 900

tcccttcagt tggttggtac aacttgccaa ctgggccctg ttccacatgt gacacggggg 960

gggaccaaac acaaaggggt tctctgactg tagttgacat ccttataaat ggatgtgcac 1020

atttgccaac actgagtggc tttcatcctg gagcagactt tgcagtctgt ggactgcaac 1080

acaacattgc ctttatgtgt aactcttggc tgaagctctt acaccaatgc tgggggacat 1140

gtacctccca ggggcccagg aagactacgg gaggctacac caacgtcaat cagaggggcc 1200

tgtgtagcta ccgataagcg gaccctcaag agggcattag caatagtgtt tataaggccc 1260

ccttgttaac cctaaacggg tagcatatgc ttcccgggta gtagtatata ctatccagac 1320

taaccctaat tcaatagcat atgttaccca acgggaagca tatgctatcg aattagggtt 1380

agtaaaaggg tcctaaggaa cagcgatatc tcccacccca tgagctgtca cggttttatt 1440

tacatggggt caggattcca cgagggtagt gaaccatttt agtcacaagg gcagtggctg 1500

aagatcaagg agcgggcagt gaactctcct gaatcttcgc ctgcttcttc attctccttc 1560

gtttagctaa tagaataact gctgagttgt gaacagtaag gtgtatgtga ggtgctcgaa 1620

aacaaggttt caggtgacgc ccccagaata aaatttggac ggggggttca gtggtggcat 1680

tgtgctatga caccaatata accctcacaa acccccttggg caataaatac tagtgtagga 1740

atgaaacatt ctgaatatct ttaacaatag aaatccatgg ggtggggaca agccgtaaag 1800

actggatgtc catctcacac gaatttatgg ctatgggcaa cacataatcc tagtgcaata 1860

tgatactggg gttattaaga tgtgtcccag gcagggacca agacaggtga accatgttgt 1920

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<210> SEQ ID NO 89
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce a VEGF A signal peptide in the 25A1 light chain

<400> SEQUENCE: 89

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<210> SEQ ID NO 90
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce a VEGF A signal peptide in the 25B4, 25B8, 25C1, 25D8, and 25E9 light chains

<400> SEQUENCE: 90

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<210> SEQ ID NO 91
<211> LENGTH: 43
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce a VEGF A signal peptide in the 25E5 light chain

<400> SEQUENCE: 91

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<210> SEQ ID NO 92
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce a VEGF A signal peptide in the 25E6 light chain

<400> SEQUENCE: 92

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<210> SEQ ID NO 93
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer to amplify light chain variable regions

<400> SEQUENCE: 93

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<210> SEQ ID NO 94
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer OGS1769

<400> SEQUENCE: 94

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<210> SEQ ID NO 95
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer OGS1770

<400> SEQUENCE: 95

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<210> SEQ ID NO 96
<211> LENGTH: 309
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 96

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ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 240

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aaatcttgt 309

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<210> SEQ ID NO 97
<211> LENGTH: 103
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 97

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20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
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Lys Val Glu Pro Lys Ser Cys
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<210> SEQ ID NO 98
<211> LENGTH: 5367
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: plasmid pYD19

<400> SEQUENCE: 98

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<210> SEQ ID NO 99
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce IgGK signal peptide in the heavy chain variable region of 25A1

<400> SEQUENCE: 99

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<210> SEQ ID NO 100
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce IgGK signal peptide in the heavy chain variable regions of 24B4 and 25D8

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<400> SEQUENCE: 100

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<210> SEQ ID NO 101
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce IgGK signal peptide in the heavy chain variable regions of 25B8, 25C1 and 25E9

<400> SEQUENCE: 101

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<210> SEQ ID NO 102
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce IgGK signal peptide in the heavy chain variable region of 25E5

<400> SEQUENCE: 102

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<210> SEQ ID NO 103
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to introduce IgGK signal peptide in the heavy chain variable region of 25E6

<400> SEQUENCE: 103

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<210> SEQ ID NO 104
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer to amplify heavy chain variable regions

<400> SEQUENCE: 104

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<210> SEQ ID NO 105
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to amplify a fragment of murine Siglec 15 sequence

<400> SEQUENCE: 105

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<210> SEQ ID NO 106
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer to amplify a fragment of murine Siglec 15 sequence

<400> SEQUENCE: 106

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<210> SEQ ID NO 107
<211> LENGTH: 981
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: murine Siglec-15 (SEQ ID NO.:2 variant)

<400> SEQUENCE: 107

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cgtctgcgcg tgactgctgc gccgcggatc gtcaacatct cggtgctgcc gggccccgcg 540

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ggtcccgccc caggcaacag ctccgctgcc ctgcagggcc agggtcacgg ctaccaggtg 660

accgccgagt tgcccgcgct gacccgcgac ggccgctaca cgtgcacggc ggccaatagc 720

ctgggccgcg ccgaggccag cgtctacctg ttccgcttcc acggcgcccc cggaacctcg 780

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<210> SEQ ID NO 108
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: murine Siglec-15 (SEQ ID NO.:2 variant)

<400> SEQUENCE: 108

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Asn Thr Glu Ala His Ser Ala Pro Ala Gln Arg Trp Ser Met Gln Val
35 40 45

Pro Ala Glu Val Asn Ala Glu Ala Gly Asp Ala Ala Val Leu Pro Cys
50 55 60

Thr Phe Thr His Pro His Arg His Tyr Asp Gly Pro Leu Thr Ala Ile
65 70 75 80

Trp Arg Ser Gly Glu Pro Tyr Ala Gly Pro Gln Val Phe Arg Cys Thr
85 90 95

Ala Ala Pro Gly Ser Glu Leu Cys Gln Thr Ala Leu Ser Leu His Gly
100 105 110

Arg Phe Arg Leu Leu Gly Asn Pro Arg Arg Asn Asp Leu Ser Leu Arg
115 120 125

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Glu Phe Thr Gly Asp Ala His Asp Arg Tyr Glu Ser Arg His Gly Val
145                 150                 155                 160

Arg Leu Arg Val Thr Ala Ala Pro Arg Ile Val Asn Ile Ser Val Leu
                165                 170                 175

Pro Gly Pro Ala His Ala Phe Arg Ala Leu Cys Thr Ala Glu Gly Glu
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Pro Pro Pro Ala Leu Ala Trp Ser Gly Pro Ala Pro Gly Asn Ser Ser
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Ala Ala Leu Gln Gly Gln Gly His Gly Tyr Gln Val Thr Ala Glu Leu
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Pro Ala Leu Thr Arg Asp Gly Arg Tyr Thr Cys Thr Ala Ala Asn Ser
225                 230                 235                 240

Leu Gly Arg Ala Glu Ala Ser Val Tyr Leu Phe Arg Phe His Gly Ala
                245                 250                 255

Pro Gly Thr Ser Thr Leu Ala Leu Leu Leu Gly Ala Leu Gly Leu Lys
            260                 265                 270

Ala Leu Leu Leu Leu Gly Ile Leu Gly Ala Arg Ala Thr Arg Arg Arg
        275                 280                 285

Leu Asp His Leu Val Pro Gln Asp Thr Pro Pro Arg Ala Asp Gln Asp
    290                 295                 300

Thr Ser Pro Ile Trp Gly Ser Ala Glu Glu Ile Glu Asp Leu Lys Asp
305                 310                 315                 320

Leu His Lys Leu Gln Arg
                325
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What is claimed is:

1. A method of impairing osteoclast differentiation in a mammal in need thereof, the method comprising administering an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108) to said mammal.

2. The method of claim **1**, wherein the antibody or antigen binding fragment impairs an osteoclast differentiation activity of human Siglec-15 or murine Siglec 15.

3. The method of claim **2**, wherein the osteoclast differentiation activity is characterized by differentiation of osteoclast precursor cells into differentiated osteoclasts.

4. The method of claim **2**, wherein the antibody is a polyclonal antibody.

5. The method of claim **2**, wherein the antibody or antigen binding fragment is a monoclonal antibody or an antigen binding fragment thereof.

6. The method of claim **5**, wherein the monoclonal antibody or antigen binding fragment is produced from an isolated mammalian cell.

7. The method of claim **6**, wherein the isolated mammalian cell is a human cell.

8. The method of claim **6**, wherein the antibody or antigen binding fragment comprises a constant region of a human antibody or a fragment thereof.

9. The method of claim **8**, wherein the antibody or antigen binding fragment comprises a framework region of a human antibody.

10. The method of claim **2**, wherein the antibody or antigen binding fragment is a FV, a Fab, a Fab' or a $(Fab')_2$.

11. The method of claim **3**, wherein the osteoclast precursor cells are human osteoclast precursor cells.

12. The method of claim **11**, wherein the human osteoclast precursor cells are primary human osteoclast precursor cells.

13. The method of claim **2**, wherein the antibody or antigen binding fragment binds to human Siglec-15 with a greater affinity than to murine Siglec-15.

14. The method of claim **2**, wherein the antibody or antigen binding fragment binds to human Siglec-15 and does not bind murine Siglec-15.

15. A method for inhibiting bone resorption comprising administering to a subject in need thereof, an antibody or antigen binding fragment which specifically binds to human Siglec-15 (SEQ ID NO.:2) or murine Siglec-15 (SEQ ID NO.:108).

16. The method of claim **15**, wherein the antibody or antigen binding fragment impairs an activity of human Siglec-15 or murine Siglec-15 in osteoclast precursor cells or in osteoclasts.

17. The method of claim **16**, wherein the activity is osteoclastogenesis.

18. The method of claim **15**, wherein the antibody or antigen binding fragment inhibits osteoclast differentiation.

19. The method of claim **15**, wherein the antibody or antigen binding fragment is administered in combination with a drug or an hormone.

20. The method of claim **19**, wherein the drug is an antiresorptive drug or a drug increasing bone mineral density.

21. The method of claim **15**, wherein the subject in need thereof, suffers from a bone remodelling disorder.

22. The method of claim **21**, wherein the bone remodelling disorder is associated with a decrease in bone mass.

23. The method of claim **21**, wherein the bone remodelling disorder is selected from the group consisting of osteoporosis,

osteopenia, osteomalacia, hyperparathyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, Paget's disease, Cushing's disease/syndrome, Turner syndrome, Gaucher disease, Ehlers-Danlos syndrome, Marfan's syndrome, Menkes' syndrome, Fanconi's syndrome, multiple myeloma, hypercalcemia, hypocalcemia, arthritides, periodontal disease, rickets, fibrogenesis imperfecta ossium, osteosclerotic disorders, pycnodysostosis, and damage caused by macrophage-mediated inflammatory processes.

24. The method of claim **15**, wherein the antibody or antigen binding fragment binds to human Siglec-15 with a greater affinity than to murine Siglec-15.

25. The method of claim **15**, wherein the antibody or antigen binding fragment binds to human Siglec-15 and does not bind murine Siglec-15.

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