

ANTIBODIES TO CANINE AND FELINE ONCOSTATIN M RECEPTOR BETA AND USES THEREOF

FIELD OF THE INVENTION

The present invention is in the field of immunotherapy. More specifically, it concerns a receptor
5 for Oncostatin M (OSM), and its modulators such as monoclonal antibodies that bind to the
canine and/or feline Oncostatin M receptor Beta subunit (OSMR- β). The invention also concerns
the diagnosis and/or treatment of diseases in canines and felines associated with OSM and IL-
31 using anti-OSMR- β antibodies.

BACKGROUND OF THE INVENTION

Cytokines comprise a large family of small proteins that play a critical role in the development
and control of the immune response. Certain cytokines are associated with the initiation and the
persistence of pathological pain behavior including nerve and skin injuries. A more recently
discovered cytokine, interleukin-31 (IL-31), has been linked to the induction of chronic skin
15 inflammation (Dillon et al. (2004) *Nat. Immunol.* 5, 752–760). Human and murine data have
shown high expression of IL-31 associated with severe inflammatory skin disorders including
pruritis, alopecia, skin lesion, and atopic dermatitis (AD) and with other regulated allergic
diseases such as asthma (Dillon et al. (2004) *supra*; Neis et al. (2006) *J. Allergy Clin. Immunol.*
118, 930–937; Rabenhorst et al. (2014) *Curr. Allergy Asthma Rep.* 14, 423; Cornelissen et al.
20 (2012) *Eur. J. Cell Biol.* 91, 552–566; Takaoka et al. (2006) *Exp. Dermatol.* 15, 161–167;
Sonkoly et al. (2006) *J. Allergy Clin. Immunol.* 117, 411–417; Lewis et al. (2017) *J. Eur. Acad.*
Dermatology Venereol. 31, 142–150).

An experimental animal model for human AD reported a strong correlation between itch-
25 associated scratching behavior in NC/Nga mice and expression of IL-31 mRNA (Takaoka et al.
supra). Elevated IL-31 serum levels were found in adult patients with AD compare to healthy
control subjects (Raap et al. (2008) *J. Allergy Clin. Immunol.* 122, 421–423) and in pediatric
patients during AD flare and quiescence (Ezzat et al. (2011) *J. Eur. Acad. Dermatology*
Venereol. 25, 334–339).

30 Together, these data suggest that IL-31 represents an important target for the development of
treatments against such skin inflammatory diseases in humans. Antagonist anti-IL-31
monoclonal antibodies (mAbs) are currently in development for human health (Pantazi et al.

(2017) *Nat. Rev. Drug Discov.* 17, 237–238; Nemoto et al. (2016) *Br. J. Dermatol.* 174, 296–304). Also, anti-IL-31 mAbs have already been developed in animal health (US Patent No. 8,790,651B2 to Bammert et al.; Michels et al. (2016) *Vet. Dermatol.* 27, 478–e129). For example, an anti-hIL-31RA mAb, CIM331, binds to IL-31RA, inhibits IL-31 signaling, and
5 reduces severe pruritus (Nemoto et al. (2016) *supra*). In veterinary medicine a “caninized” anti-IL-31 mAb, Lokivetmab, showed efficacy in clinical trials for canine pruritis and is currently approved as an AD therapy for dogs (Michels et al. (2016) *supra*).

IL-31 is a member of the IL-6 cytokine superfamily produced preferentially by T helper type 2
10 cells (Dillon et al. (2004) *supra*). Mature human IL-31 (hIL-31) is composed of 141 amino acids (Dillon et al. (2004) *supra*) with a predicted topology of four antiparallel helices (Le Saux et al. (2010) *J. Biol. Chem.* 285, 3470–3477). The IL-31 signaling pathway is thought to be mediated through a gp130-like type 1 cytokine receptor (IL-31RA, also known as GPL) and Oncostatin M receptor (OSMR) (Dillon et al. *supra*; Le Saux et al. (2010) *supra*; Diveu et al. (2004) *Eur.*
15 *Cytokine Netw.* 15, 291–302; Zhang et al. (2008) *Cytokine Growth Factor Rev.* 19, 347–356). Both receptors belong to the type I cytokine receptor family which share a common cytokine binding domain (CBD) formed by two fibronectin type III-like domains (Diveu et al. (2003) *J. Biol. Chem.* 278, 49850–49859).

20 Previous studies supplied immunoprecipitation evidence that human IL-31RA (hIL-31RA) binds directly to hIL-31. In these same studies, immunoprecipitation results failed to detect direct human OSMR (hOSMR) binding to hIL-31 (Le Saux et al. (2010) Molecular dissection of human interleukin-31-mediated signal transduction through site-directed mutagenesis. *J. Biol. Chem.* 285, 3470–3477; Diveu et al. (2004) *supra*). However, an increase in binding was distinguished
25 when hIL-31RA and hOSMR were combined, suggesting that hIL-31 binds first to hIL-31RA, at which time hOSMR is recruited to form the ternary complex (Le Saux et al. (2010) *supra*; Diveu et al. (2004) *supra*). In this model, the ternary complex activates numerous downstream signaling pathways (Dillon et al. (2004) *supra*; Le Saux et al. (2010) *supra*, Diveu et al. (2004) *supra*; Dambacher et al. (2007) *Gut* 56, 1257–1265; Dreuw et al. (2004) *J. Biol. Chem.* 279,
30 36112–36120).

Based on the structure of IL-6/IL-6 α -Receptor/gp130 complex (Boulanger et al. (2003) *Science*. 300, 2101–2104), the IL-6 cytokine superfamily is thought to interact with their receptors through three different contact binding sites (sites I, II, and III). Le Saux et al. used computational

analysis and sparse alanine scanning to delineate sites II and III only as critical binding sites for the interaction between hIL-31 and its receptors (Le Saux et al. (2010) *supra*). In particular, Glu44, Glu106, and His110 were identified as critical residues for binding site II while Lys134 was identified within the binding site III.

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Applicant initiated a study to gain insights into the interactions between feline IL-31 (fIL-31) and its feline receptors fOSMR and fIL-31RA and to map the conformational epitope for an anti-fIL-31 mAb termed mAb #1 or 15H05 (Medina-Cucurella AV et al; Feline Interleukin-31 Shares Overlapping Epitopes with the Oncostatin M Receptor and IL-31RA. *Biochemistry*. 2020 Jun 16;59(23):2171-2181) ; WO2019/177697A2 (Zoetis Services LLC). In contrast to previous studies conducted with human homologs, which showed that OSMR cannot interact with IL-31 in the absence of IL-31RA, Applicant discovered through multiple biophysical methods that fOSMR directly binds fIL-31 and partially interferes with fIL-31RA binding. Applicant identified the potential fIL-31 binding sites for fOSMR, fIL31-RA, and an anti-fIL-31 mAb using a predicted structural model combined with fine epitope mapping (Medina-Cucurella (2019) *Methods Mol. Biol.* 1764, 101–121), using yeast surface display (Chao et al. (2006) *Nat. Protoc.* 1, 755–768), nicking mutagenesis (Wrenbeck et al. (2016) *Nat. Methods* 13, 928–930), and deep sequencing (Araya and Fowler (2011) *Trends Biotechnol.* 29, 435–442). The constructed binding sites agreed with the sites previously found by Le Saux et al. *supra* and showed an additional overlapping site between both receptors which Applicant termed the “shared site”. Specifically, the binding site for IL31-RA contains fIL-31 positions E20 and K82, while the binding site for OSMR comprises the “PADNFERK” motif (P103-K110) and positions G39 and K100, which agreed with previous studies on the human homolog. However, Applicant’s results also revealed a new overlapping site, composed of positions R69, R72, P73, D76, D81, and E97, between both feline receptors which was not previously reported for human. The conformational epitope of an anti-feline IL-31 mAb that inhibits both OSMR and IL-31RA also mapped to this shared site. Combined, Applicant’s results show that fIL-31 binds IL-31RA and OSMR independently through a partially shared epitope. These results suggest that the mechanisms for IL-31 signaling in companion animals such as cats may be different from the mechanisms for IL-31 signaling in humans. This in turn implies that efficient therapeutic strategies to antagonize IL-31-mediated signaling in companion animals may be different from therapeutic strategies to antagonize IL-31-mediated signaling in higher animals.

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It would be desirable to provide therapeutic monoclonal antibodies that bind to the canine and/or feline Oncostatin M receptor Beta subunit (OSMR- β) and antagonize OSM-mediated and/or IL-31-mediated signaling in canines and/or felines. Such antibodies would preferably be useful for the diagnosis and/or treatment of diseases in canines and felines associated with OSM and IL-31, including severe inflammatory skin disorders, such as allergic dermatitis and atopic dermatitis and fibrotic conditions, such as dermal or renal fibrosis, in dogs and cats.

Cellular models describe the role of OSM in modulating human keratinocyte function by influencing the expression of genes encoding proteins involved in support structure/metabolism, inflammation/innate immunity, and tissue remodeling. (Boniface K et al; Oncostatin M secreted by skin infiltrating T lymphocytes is a potent keratinocyte activator involved in skin inflammation. J Immunol. 2007 Apr 1;178(7):4615-22). These investigators provide compelling evidence for the role of OSM-mediated OSMR activation in cutaneous inflammation resulting from keratinocyte activation in a cell models using primary keratinocytes in a reconstituted epidermis. OSM-mediated OSMR activation in these keratinocyte cultures upregulates gene expression patterns consistent with psoriatic and atopic disease in skin. These data were supported by increased expression of both OSM and OSMR in patients with inflammatory skin disorders (psoriatic or atopic dermatitis (AD). By targeting OSMR- β with a blocking monoclonal antibody, the function of both IL-31 and OSM cytokines will be blocked. By blocking the function of IL-31, it is anticipated that anti-pruritic and anti-inflammatory properties, similar to Lokivetmab will be achieved, but additional anti-inflammatory and anti-fibrotic activity beyond Lokivetmab may be achieved due to inhibition of OSM.

Summary of the Invention

The present invention provides an isolated antibody, or antigen-binding portion thereof that specifically binds to canine or feline Oncostatin M receptor Beta (OSMR- β) or both, wherein the antibody antagonizes IL-31-mediated signaling or OSM-mediated signaling or both in a canine and/or feline cell. In one embodiment, the antibody, or antigen-binding portion thereof antagonizes both IL-31-mediated signaling and OSM-mediated signaling in a canine and/or feline cell. In one embodiment, the IL-31-mediated signaling is pSTAT signaling, such as, but not limited to, pSTAT3 signaling. In another embodiment, the OSM-mediated signaling is pSTAT signaling, such as, but not limited to, pSTAT3 signaling.

In one embodiment, the antibody, or antigen-binding portion thereof of the present invention includes a combination of complementary determining region (CDR) sequences selected from the following:

- 5 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY), variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSI>NNLH), VL-CDR2 of SEQ NO: 5 (YASQSI), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);
- 10 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 15 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 20 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY), VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
- 25 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV), VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

In some embodiments of the CDR variants of the parent antibodies, amino acid residues located in parent antibodies 02D09, 09E09, 10F07, and 19F07 at the mutation positions denoted in Table A of the example section are conserved in the CDR variants of the respective parent antibody.

In other embodiments, CDR variants of the 19F07 parent antibody are provided in Table B of the example section. Specifically, Table B provides a generic description for each CDR of the 19F07 antibody and shows allowable amino acid substitutions for each CDR of 19F07.

In another embodiment, an antibody according to the present invention includes at least one of the following variable heavy chains and variable light chains:

(a) a variable heavy chain comprising

- SEQ ID NO: 31 (MU_02D09_VH)
EVQLVESGGGLVKPGGSLTSCAASGFTFSDYGMHWLRQAPEKGLEWWAYISSGSRAVFFAD
TVKGRFTISRDNANTLYLQMTSLRSDDTAMYYCARDRYDGRGFAYWGQGTTLTVSA,
- 5 SEQ ID NO: 35 (MU_09E09_VH)
DVQLVESGEGDLVKPGGSLKLSAASGFTFSSYAMSWVRQTPEKRLEWWAYISSGGDYIYYADT
VKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCTRDPITGTFAYWGQGTTLTVSA,
- SEQ ID NO: 39 (MU_10F07_VH)
DVQLVESGEGDLVKPGGSLKLSAASGFTFSSYAMSWVRQTPEKRLEWWTYISSGGDYFYAD
10 TVKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCTRDPITGTFAYWGQGTTLTVSA,
- SEQ ID NO: 43 (MU_14C04_VH)
EVQLQESGAELVKPGASVKISCKASGYAFSNYWMNWMKQRPKGLEWIGQIYPGHVNTNYN
GNFKDKATLTADK
SSSTAYMQLSSLTSEDSAVYFCARSADNSGFVLFAYWGQGTTLTVS,
- 15 SEQ ID NO: 47 (MU_19F07_VH)
EVKLVESEGGLVQPGSSMKLSCTASGFTFSDYYMAWVRQVPEKGLEWWANINYDGSSTYYLD
SLKSRFIISRDNANKILYLQMSSLKSEDTATYYCARGLTWDFDVWGTGTTVTVSS,
- SEQ ID NO: 51 (FEL_02D09_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSDYGMHWVRQAPGKGLQWWAYISSGSRAVFFA
20 DTVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDRYDGRGFAYWGQGTTLTVSS,
- SEQ ID NO: 53 (FEL_02D09_VH2)
DVQLVESGGDLVKPGGSLRLTCVASGFTFSDYGMHWVRQAPGKGLQWWAYISSGSRAVFFA
DTVKGRFTISRDNANTLYLQMNGLRTEDTATYYCARDRYDGRGFAYWGQGTTLTVSS,
- SEQ ID NO: 59 (CAN_09E09_VH1)
25 EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYIYYAD
TVKGRFTISRDNANTLYLQMNSLRAEDTAMYYCVRDPITGTFAYWGQGTTLTVSS,
- SEQ ID NO: 61 (CAN_09E09_VH2)
EVQLVESGGDLVKPAGSLTSLCLASGFTFSSYAMSWVRQTPEKGLQWWAYISSGGDYIYYADT
VKGRFTISRDNANTLYLQMNSLRDEDTAVYYCARDPITGTFAYWGQGTTLTVSS,
- 30 SEQ ID NO: 67 (FEL_09E09_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSSYAMSWVRQAPGKGLQWWAYISSGGDYIYYAD
TVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDPITGTFAYWGQGTTLTVSS,
- SEQ ID NO: 69 (FEL_09E09_VH2)
DVQLVESGGNLVKPGGSLRLTCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYIYYAD
35 TVKGRFTISKDNANTLYLQMNSLKTEDTATYYCARDPITGTFAYWGQGTTLTVSS,
- SEQ ID NO: 75 (CAN_10F07_VH1)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYFYA
DTVKGRFTISRDNANTLYLQMNSLRAEDTAMYYCVRDPITGTFAYWGQGTTLTVSS,
- SEQ ID NO: 79 (CAN_10F07_VH2)
40 EVQLVESGGDLVKPAGSLTSLCLASGFTFSSYAMSWVRQTPEKGLQWWAYISSGGDYFYAD
TVKGRFTISRDNANTLYLQMNSLRDEDTAVYYCARDPITGTFAYWGQGTTLTVSS,

SEQ ID NO: 83 (FEL_10F07_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSSYAMSWVRQAPGKGLQWWAYISSGGDYFYFA
DTVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDPITGTFAYWGQGLTVTVSS,

5 SEQ ID NO: 87 (FEL_10F07_VH2)
DVQLVESGGDLVKPGGSLRLTCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYFYFA
DTVKGRFTISRDDAKNTLYLQMSSLKTEDTATYYCTGDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 91 (CAN_19F07_VH1)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSDYYMAWVRQAPGKGLQWWANINYDGSSTYYLD
SLKSRFTISRDNANTLYLQMNSLRAEDTAMYYCVRGLTWDFDVWGQGLTVTVSS,

10 SEQ ID NO: 95 (CAN_19F07_VH2)
EVQLVESGGDLVKPAGSLTSLCLASGFTFSDYYMAWVRQTPEKGLQWWANINYDGSSTYYLD
SLKSRFTISRDNANTLYLQMNSLRDEDTAVYYCARGLTWDFDVWGQGLTVTVSS,

15 SEQ ID NO: 99 (FEL_19F07_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSDYYMAWVRQAPGKGLQWWANINYDGSSTYYL
DSLKSRFTISRDNANTLYLQMNSLKTEDTATYYCVRGLTWDFDVWGQGLTVTVSS,

SEQ ID NO: 103 (FEL_19F07_VH2)
DVQLVESGGDLVKPGGSLRLTCVASGFTFSDYYMAWVRQAPGKGLQWWANINYDGSSTYYLD
SLKSRFTISRDNANTLYLQMNSLKTEDTATYYCARGLTWDFDVWGQGLTVTVSS,

20 SEQ ID NO: 127. (CAN_14C04_VH1)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYWMNWRQAPGKGLQWWAQIYPGHVNTNYN
GNFKDRFTISRDNARNTVYLQMNSLRAEDTAVYYCARSADNSGFVLFAYWGQGLTVTVSS, or

25 SEQ ID NO: 129. (CAN_14C04_VH2)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYWMNWRQSPGKGLQWWAQIYPGHVNTNYN
GNFKDRFTISRDNANTLYLQMNSLRAEDTAVYFCARSADNSGFVLFAYWGQGLTVTVSS,
and

(b) a variable light chain comprising

SEQ ID NO: 33 (MU_02D09_VL)
DIVLTQSPATLSVTPGDSVSLSCRASQSSNNLHWYQQTSHEPRLITYASQSSIGIPSRFSGS
GSGTDFTLINSVETEDFGMYFCQQSNSWPLTFGAGTKLELK,

30 SEQ ID NO: 37 (MU_09E09_VL)
DLQMTQTSSLSASLGDRVITISCRASQDINNYLNWYQQKPDGTVKLLIYYTSTLHSGVPSRFSG
SGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIK,

35 SEQ ID NO: 41 (MU_10F07_VL)
DIQMTQTSSLSASLGDRVITISCRASQDITNYLNWYQQKPDGTVKLLIYYTSTLHSGVPSRFSG
SGSGTDFSLTISNLEQEDIATYFCQQGHMLPWTFGGGKLEIK,

SEQ ID NO: 45 (MU_14C04_VL)
DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYLHWYQQKPGQPPLKLLIFLASNLESGVPA
RFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPLTFGAGTKLELK,

40 SEQ ID NO: 49 (MU_19F07_VL)
DIVMTQSHKFMSPSVGDRVSITCKASQDQDVAWYQQKPGQSPKLLIYLASTRHTGVPDRFT
GSGSGTDFTLISNVQSEDLADYFCQQYSRFPLTFGAGTKLELK,

SEQ ID NO: 55 (FEL_02D09_VL1)
EIQMTQSPSSLSASPGDRVITICRASQSSISNNLHWYQQKPGKVPKLLIYYASQSISGVPSRFSG
SGSGTDFTLTISSELEPEDAATYYCQQSNSWPLTFGQGT,

5 SEQ ID NO: 57 (FEL_02D09_VL2)
DIVMTQTPLSLSVTPGESASISCRASQSSISNNLHWYQKSGQSPRRLIYYASQSISGVDPDRFSG
SGSGTDFTLRISRVEADDVGYYCQQSNSWPLTFGQGT,

SEQ ID NO: 63 (CAN_09E09_VL1)
EIVMTQSPASLSLSQEEKVTITCRASQDINNYLNWYQQKPGQAPKLLIYYTSTLHSGVPSRFSG
SGSGTDFSTISSLEPEDVAVYYCQQGNTLPWTFGQGT,

10 SEQ ID NO: 65 (CAN_09E09_VL2)
DIVLTQPTSVSGSLGQRVITICRASQDINNYLNWYQQLPKGAPKLLVYYTSTLHSGVPDRFSGS
NSGSSATLTITGLQAEDEADYYCQQGNTLPWTFGQGT,

15 SEQ ID NO: 71 (FEL_09E09_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDINNYLNWYQQKPGKVPKLLIYYTSTLHSGVPSRFSG
SGSGTDFTLTISSELEPEDAATYYCQQGNTLPWTFGQGT,

SEQ ID NO: 73 (FEL_09E09_VL2)
DITMTQSPGSLAGSPGQQVTMNCRASQDINNYLNWYQQKPGQHPKLLIYYTSTLHSGVPDRF
SGSGSGTDFTLTISNLQAEDVASYYCQQGNTLPWTFGQGT,

20 SEQ ID NO: 77 (CAN_10F07_VL1)
EIVMTQSPASLSLSQEEKVTITCRASQDITNYLNWYQQKPGQAPKLLIYYTSTLHSGVPSRFSG
SGSGTDFSTISSLEPEDVAVYYCQQGHMLPWTFGQGT,

SEQ ID NO: 81 (CAN_10F07_VL2)
DIVLTQPTSVSGSLGQRVITICRASQDITNYLNWYQQLPKGAPKLLVYYTSTLHSGVPDRFSGS
NSGSSATLTITGLQAEDEADYYCQQGHMLPWTFGQGT,

25 SEQ ID NO: 85 (FEL_10F07_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDITNYLNWYQQKPGKVPKLLIYYTSTLHSGVPSRFSG
SGSGTDFTLTISSELEPEDAATYYCQQGHMLPWTFGQGT,

30 SEQ ID NO: 89 (FEL_10F07_VL2)
DITMTQSPGSLAGSPGQQVTMNCRASQDITNYLNWYQQKPGQHPKLLIYYTSTLHSGVPDRFS
SGSGTDFTLTISNLQAEDVASYYCQQGHMLPWTFGQGT,

SEQ ID NO: 93 (CAN_19F07_VL1)
EIVMTQSPASLSLSQEEKVTITCKASQDVDTAVAWYQQKPGQAPKLLIYLASTRHTGVPSRFSG
SGSGTDFSTISSLEPEDVAVYYCQQYSRFPLTFGQGT,

35 SEQ ID NO: 97 (CAN_19F07_VL2)
DIVMTQTPLSLSVSPGETASISCKASQDVDTAVAWFRQKPGQSPQRLLIYLASTRHTGVDPDRFS
SGSGTDFTLRISRVEADDTGVYYCQQYSRFPLTFGQGT,

SEQ ID NO: 101 (FEL_19F07_VL1)
EIQMTQSPSSLSASPGDRVITITCKASQDVDTAVAWYQQKPGKVPKLLIYLASTRHTGVPSRFS
SGSGTDFTLTISSELEPEDAATYYCQQYSRFPLTFGQGT,

40 SEQ ID NO: 105 (FEL_19F07_VL2)
DITMTQSPGSLAGSPGQQVTMNCASQDVDTAVAWYQQKPGQHPKLLIYLASTRHTGVDPDRF
SGSGSGTDFTLTISNLQAEDVASYYCQQYSRFPLTFGQGT,

SEQ ID NO: 131. (CAN_14C04_VL1)

EIVMTQSPASLSLSQEEKVTITCRASKSVSTSGYSYLHWYQQKPGQAPKLLIYLASNLESGVPS
RFGSGSGTDFSTISSLEPEDVAVYYCQHSRELPLTFGQGT, or

SEQ ID NO: 133. (CAN_14C04_VL2)

- 5 DIVMTQTPLSLSVSPGETASISCRASKSVSTSGYSYLHWYLQKPGQSPQLLIYLASNLESGVSK
RFGSGSGTDFTLRISRVEADDTGIYYCQHSRELPLTFGQGT.

In one embodiment, the antibody is chimeric. In another embodiment, the antibody is caninized or felinized.

- 10 In one embodiment, the antibody inhibits or neutralizes an IL-31-mediated or OSM-mediated pruritic or allergic condition in a dog or cat. In one aspect, the IL-31-mediated or OSM-mediated pruritic condition is selected from atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis. In another aspect, the IL-31-mediated or OSM-mediated allergic condition is selected from
15 allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstruction pulmonary disease, and inflammatory processes resulting from autoimmunity.

- In one embodiment, the antibody inhibits an IL-31-mediated or OSM-mediated fibrotic or
20 inflammatory disorder. In one aspect, the IL-31-mediated or OSM-mediated fibrotic disorder is selected from renal fibrosis, pulmonary fibrosis, and dermal fibrosis. In another aspect, the IL-31-mediated or OSM-mediated inflammatory disorder is selected from inflammatory processes resulting from autoimmunity, inflammation in the skin or joint of animals affected by osteoarthritis, immune-mediated polyarthritis, chronic bronchitis, allergic asthma, atopic
25 dermatitis, allergic dermatitis, pyotraumatic dermatitis, atherosclerosis, and cardiovascular disease.

- In a further embodiment, the antibody reduces IL-31-mediated or OSM-mediated inflammatory pain. In one aspect, the IL-31-mediated or OSM-mediated inflammatory pain is osteoarthritis
30 pain.

The present invention also provides a veterinary composition comprising a therapeutically effective amount of an antibody as described above.

- 35 This invention also provides a method of treating an IL-31-mediated or OSM-mediated disorder in a subject, comprising administering an antibody according to the invention to the subject. In

one embodiment of this method, the IL-31-mediated or OSM-mediated disorder is selected from a pruritic condition, an allergic condition, a fibrotic disorder, an inflammatory disorder, or inflammatory pain.

- 5 In another embodiment of the method of treating, the IL-31-mediated or OSM-mediated pruritic condition is selected from the group consisting of atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis. In yet another embodiment of the method, the IL-31-mediated or OSM-mediated allergic condition is selected from allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-
- 10 responsiveness, chronic obstruction pulmonary disease, or inflammatory processes resulting from autoimmunity.

- In further embodiments of the method of treating, the IL-31-mediated or OSM-mediated fibrotic disorder is selected from renal fibrosis, pulmonary fibrosis, or dermal fibrosis. In another
- 15 embodiment of the method, the IL-31-mediated or OSM-mediated inflammatory disorder is selected from inflammatory processes resulting from autoimmunity, inflammation in the skin or joint of animals affected by osteoarthritis, immune-mediated polyarthritis, chronic bronchitis, allergic asthma, atopic dermatitis, allergic dermatitis, pyotraumatic dermatitis, atherosclerosis, or cardiovascular disease.

- 20 In a still further embodiment of the method of treating, the IL-31-mediated or OSM-mediated inflammatory pain is osteoarthritis pain.

- In one embodiment of the method of treating, the subject is a dog or a cat.

- 25 The present invention further provides a method of inhibiting IL-31 and/or OSM activity in a dog or cat comprising administering an antibody as described above to the dog or cat.

- Also provided is a method of detecting OSMR Beta in a sample. This method includes
- 30 incubating a sample comprising OSMR Beta in the presence of an antibody as described above; and detecting the antibody which is bound to OSMR Beta in the sample. In one embodiment of this method, the antibody comprises a label. In one embodiment, the detection method further includes quantitating the OSMR Beta in the sample.

The present invention also provides host cells and nucleic acids that can be used to produce the antibodies or antigen-binding portions thereof of this invention. In one embodiment, the invention provides a host cell that produces an isolated antibody or antigen-binding portion thereof which includes at least one of the following combinations of complementary determining

5 region (CDR) sequences:

- 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY), variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSISSNNLH), VL-CDR2 of SEQ NO: 5 (YASQSISS), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);
- 10 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 15 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 20 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY), VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
- 25 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV), VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFLPT); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

The present invention also provides an isolated nucleic acid including a nucleic acid sequence encoding at least one of the following combinations of variable heavy complementary

30 determining region (CDR) sequences:

- 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), and VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY);
- 35 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), and VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY);
- 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), and VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY);
- 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), and VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY);

- 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYDMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), and VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV); or
 - 6) CDR variants of 1, 2, 3, 4, or 5.
- 5 In one embodiment, the nucleic acid described above further includes a nucleic acid sequence encoding at least one of the following combinations of variable light CDR sequences:
- 1) 02D09: variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQISNNLH), VL-CDR2 of SEQ NO: 5 (YASQIS), and VL-CDR3 of SEQ ID NO: 6 (QQNSWPLT);
 - 10 2) 09E09: VL-CDR1 of VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
 - 3) 10F07: VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
 - 15 4) 14C04: VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
 - 5) 19F07: VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
 - 6) CDR variants of 1, 2, 3, 4, or 5.
- 20 In another embodiment, a nucleic acid sequence according to the invention encodes at least one of the following combinations of variable light CDR sequences:
- 1) 02D09: variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQISNNLH), VL-CDR2 of SEQ NO: 5 (YASQIS), and VL-CDR3 of SEQ ID NO: 6 (QQNSWPLT);
 - 25 2) 09E09: VL-CDR1 of VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
 - 3) 10F07: VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
 - 30 4) 14C04: VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
 - 5) 19F07: VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
 - 35 6) CDR variants of 1, 2, 3, 4, or 5.
- 40 The present invention further provides a vector that includes a nucleic acid as described above.

Furthermore, the present invention provides a method of producing an antibody. This method includes culturing the host cell described above under conditions that result in production of the antibody and isolating the antibody from the host cell or culture medium of the host cell.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the pSTAT3 dose-response produced by treatment of canine and feline OSM in a) canine DH82 cells and b) feline Fcwf4 cells.

Figure 2 is a flow chart showing the steps used to select the anti-OSMR antibodies described in this application.

- 10 Figure 3 is an alignment of the variable heavy and variable light chain sequences of the anti-OSMR antibodies described in this application.

Figure 4 are matrices showing the RMSD values of the CDRs following pairwise comparison of the structures of homology models generated for the 5 anti-OSMR antibodies described in this application.

- 15 Figure 5 A) is a homology model showing a dimer of canine OSM bound to a dimer of the canine OSMR protein. A black circle highlights the binding interface of OSM and OSMR. B) shows an enlarged region of the binding interface highlighting amino acids known to be important in the human OSM: OSMR interaction.

- 20 Figure 6 shows the epitope binding sites of the antibodies described herein based on the logical location of where OSM binds to OSMR and the sequence diversity between human, canine, and feline OSMR protein.

Figure 7 shows the quantitation of OSMR mRNA in feline skin using and RNAscope ISH probe. Control tissues are from normal feline skin biopsies and diseased tissues are from skin biopsies from felines with allergic skin disease.

- 25 Figure 8 shows a single representative image from IHC staining of the OSMR protein from control (non-allergic) versus diseased (allergic) skin tissue.

- Figure 9 A) shows the proliferation of canine synoviocytes in response to an increasing dose of the canine OSM protein over a 24-hour time period. B) shows the inhibition of OSM-induced canine synoviocyte proliferation with the anti-OSMR mouse antibody 10F07 (described herein) compared to a control antibody (also 24-hour incubation).
- 30

Figure 10 A) shows the induction of the canine MCP-1 protein in canine synoviocytes in response to an increasing dose of the canine OSM protein over a 24-hour time period. B) shows the inhibition of OSM-induced canine synoviocyte MCP-1 production with the anti-OSMR mouse antibody 10F07 (described herein) compared to a control antibody (also 24-hour incubation).

Figure 10 A) shows the induction of the canine MCP-1 protein in canine synoviocytes in response to an increasing dose of the canine OSM protein over a 24-hour time period. B) shows the inhibition of OSM-induced canine synoviocyte MCP-1 production with the anti-OSMR mouse antibody 10F07 (described herein) compared to a control antibody (also 24-hour incubation).

Figure 11 shows the induction of the canine MCP-1 protein in primary canine renal fibroblasts in response to an increasing dose of the canine OSM protein over a 72-hour time period.

Figure 12 A) shows the pre and post dosing pruritic scores in a canine model of IL-31 induced pruritus comparing the anti-OSMR mouse:canine chimera 19F07 (T01) and 10F07 (T02), both dosed subcutaneously to six animals per group on day zero with a dose of 12.0 mg/kg. B) shows the summary statistics for post-challenge scores by treatment group and paired t-test with mean and 90% confidence limits.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 is a variable heavy chain CDR1 referred to herein as 02D09-VH-CDR1;
SEQ ID NO: 2 is a variable heavy chain CDR2 referred to herein as 02D09-VH-CDR2;
SEQ ID NO: 3 is a variable heavy chain CDR3 referred to herein as 02D09-VH-CDR3;
SEQ ID NO: 4 is a variable light chain CDR1 referred to herein as 02D09-VL-CDR1;
SEQ ID NO: 5 is a variable light chain CDR2 referred to herein as 02D09-VL-CDR2;
SEQ ID NO: 6 is a variable light chain CDR3 referred to herein as 02D09-VL-CDR3;
SEQ ID NO: 7 is a variable heavy chain CDR1 referred to herein as 09E09-VH-CDR1;
SEQ ID NO: 8 is a variable heavy chain CDR2 referred to herein as 09E09-VH-CDR2;
SEQ ID NO: 9 is a variable heavy chain CDR3 referred to herein as 09E09-VH-CDR3;
SEQ ID NO: 10 is a variable light chain CDR1 referred to herein as 09E09-VL-CDR1;

- SEQ ID NO: 11 is a variable light chain CDR2 referred to herein as 09E09-VL-CDR2;
- SEQ ID NO: 12 is a variable light chain CDR3 referred to herein as 09E09-VL-CDR3;
- SEQ ID NO: 13 is a variable heavy chain CDR1 referred to herein as 10F07-VH-CDR1;
- SEQ ID NO: 14 is a variable heavy chain CDR2 referred to herein as 10F07-VH-CDR2;
- 5 SEQ ID NO: 15 is a variable heavy chain CDR3 referred to herein as 10F07-VH-CDR3;
- SEQ ID NO: 16 is a variable light chain CDR1 referred to herein as 10F07-VL-CDR1;
- SEQ ID NO: 17 is a variable light chain CDR2 referred to herein as 10F07-VL-CDR2;
- SEQ ID NO: 18 is a variable light chain CDR3 referred to herein as 10F07-VL-CDR3;
- SEQ ID NO: 19 is a variable heavy chain CDR1 referred to herein as 14C04-VH-CDR1;
- 10 SEQ ID NO: 20 is a variable heavy chain CDR2 referred to herein as 14C04-VH-CDR2;
- SEQ ID NO: 21 is a variable heavy chain CDR3 referred to herein as 14C04-VH-CDR3;
- SEQ ID NO: 22 is a variable light chain CDR1 referred to herein as 14C04-VL-CDR1;
- SEQ ID NO: 23 is a variable light chain CDR2 referred to herein as 14C04-VL-CDR2;
- SEQ ID NO: 24 is a variable light chain CDR3 referred to herein as 14C04-VL-CDR3;
- 15 SEQ ID NO: 25 is a variable heavy chain CDR1 referred to herein as 19F07-VH-CDR1;
- SEQ ID NO: 26 is a variable heavy chain CDR2 referred to herein as 19F07-VH-CDR2;
- SEQ ID NO: 27 is a variable heavy chain CDR3 referred to herein as 19F07-VH-CDR3;
- SEQ ID NO: 28 is a variable light chain CDR1 referred to herein as 19F07-VL-CDR1;
- SEQ ID NO: 29 is a variable light chain CDR2 referred to herein as 19F07-VL-CDR2;
- 20 SEQ ID NO: 30 is a variable light chain CDR3 referred to herein as 19F07-VL-CDR3;
- SEQ ID NO: 31 is a variable heavy chain sequence referred to herein as MU_02D09_VH;
- SEQ ID NO: 32 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU_02D09_VH;
- SEQ ID NO: 33 is a variable light chain sequence referred to herein as MU_02D09_VL;

SEQ ID NO: 34 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU_02D09_VL;

SEQ ID NO: 35 is a variable heavy chain sequence referred to herein as MU_09E09_VH;

5 SEQ ID NO: 36 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU_09E09_VH;

SEQ ID NO: 37 is a variable light chain sequence referred to herein as MU_09E09_VL;

SEQ ID NO: 38 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU_09E09_VL;

SEQ ID NO: 39 is a variable heavy chain sequence referred to herein as MU_10F07_VH;

10 SEQ ID NO: 40 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU_10F07_VH;

SEQ ID NO: 41 is a variable light chain sequence referred to herein as MU_10F07_VL;

SEQ ID NO: 42 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU_10F07_VL;

15 SEQ ID NO: 43 is a variable heavy chain sequence referred to herein as MU_14C04_VH;

SEQ ID NO: 44 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU_14C04_VH;

SEQ ID NO: 45 is a variable light chain sequence referred to herein as MU_14C04_VL;

20 SEQ ID NO: 46 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU_14C04_VL;

SEQ ID NO: 47 is a variable heavy chain sequence referred to herein as MU_19F07_VH;

SEQ ID NO: 48 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as MU_19F07_VH;

SEQ ID NO: 49 is a variable light chain sequence referred to herein as MU_19F07_VL;

25 SEQ ID NO: 50 is the nucleotide sequence encoding the variable light chain sequence referred to herein as MU_19F07_VL;

SEQ ID NO: 51 is a variable heavy chain sequence referred to herein as FEL_02D09_VH1;

SEQ ID NO: 52 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_02D09_VH1;

SEQ ID NO: 53 is a variable heavy chain sequence referred to herein as FEL_02D09_VH2;

5 SEQ ID NO: 54 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_02D09_VH2;

SEQ ID NO: 55 is a variable light chain sequence referred to herein as FEL_02D09_VL1;

SEQ ID NO: 56 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_02D09_VL1;

SEQ ID NO: 57 is a variable light chain sequence referred to herein as FEL_02D09_VL2;

10 SEQ ID NO: 58 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_02D09_VL2;

SEQ ID NO: 59 is a variable heavy chain sequence referred to herein as CAN_09E09_VH1;

SEQ ID NO: 60 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_09E09_VH1;

15 SEQ ID NO: 61 is a variable heavy chain sequence referred to herein as CAN_09E09_VH2;

SEQ ID NO: 62 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_09E09_VH2;

SEQ ID NO: 63 is a variable light chain sequence referred to herein as CAN_09E09_VL1;

20 SEQ ID NO: 64 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN_09E09_VL1;

SEQ ID NO: 65 is a variable light chain sequence referred to herein as CAN_09E09_VL2;

SEQ ID NO: 66 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN_09E09_VL2;

SEQ ID NO: 67 is a variable heavy chain sequence referred to herein as FEL_09E09_VH1;

25 SEQ ID NO: 68 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_09E09_VH1;

SEQ ID NO: 69 is a variable heavy chain sequence referred to herein as FEL_09E09_VH2;

SEQ ID NO: 70 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_09E09_VH2;

SEQ ID NO: 71 is a variable light chain sequence referred to herein as FEL_09E09_VL1;

5 SEQ ID NO: 72 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_09E09_VL1;

SEQ ID NO: 73 is a variable light chain sequence referred to herein as FEL_09E09_VL2;

SEQ ID NO: 74 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_09E09_VL2;

SEQ ID NO: 75 is a variable heavy chain sequence referred to herein as CAN_10F07_VH1;

10 SEQ ID NO: 76 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_10F07_VH1;

SEQ ID NO: 77 is a variable light chain sequence referred to herein as CAN_10F07_VL1;

SEQ ID NO: 78 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN_10F07_VL1;

15 SEQ ID NO: 79 is a variable heavy chain sequence referred to herein as CAN_10F07_VH2;

SEQ ID NO: 80 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_10F07_VH2;

SEQ ID NO: 81 is a variable light chain sequence referred to herein as CAN_10F07_VL2;

20 SEQ ID NO: 82 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN_10F07_VL2;

SEQ ID NO: 83 is a variable heavy chain sequence referred to herein as FEL_10F07_VH1;

SEQ ID NO: 84 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_10F07_VH1;

SEQ ID NO: 85 is a variable light chain sequence referred to herein as FEL_10F07_VL1;

25 SEQ ID NO: 86 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_10F07_VL1;

SEQ ID NO: 87 is a variable heavy chain sequence referred to herein as FEL_10F07_VH2;

SEQ ID NO: 88 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_10F07_VH2;

SEQ ID NO: 89 is a variable light chain sequence referred to herein as FEL_10F07_VL2;

5 SEQ ID NO: 90 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_10F07_VL2;

SEQ ID NO: 91 is a variable heavy chain sequence referred to herein as CAN_19F07_VH1;

SEQ ID NO: 92 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_19F07_VH1;

SEQ ID NO: 93 is a variable light chain sequence referred to herein as CAN_19F07_VL1;

10 SEQ ID NO: 94 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN_19F07_VL1;

SEQ ID NO: 95 is a variable heavy chain sequence referred to herein as CAN_19F07_VH2;

SEQ ID NO: 96 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_19F07_VH2;

15 SEQ ID NO: 97 is a variable light chain sequence referred to herein as CAN_19F07_VL2;

SEQ ID NO: 98 is the nucleotide sequence encoding the variable light chain sequence referred to herein as CAN_19F07_VL2;

SEQ ID NO: 99 is a variable heavy chain sequence referred to herein as FEL_19F07_VH1;

20 SEQ ID NO: 100 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_19F07_VH1;

SEQ ID NO: 101 is a variable light chain sequence referred to herein as FEL_19F07_VL1;

SEQ ID NO: 102 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_19F07_VL1;

SEQ ID NO: 103 is a variable heavy chain sequence referred to herein as FEL_19F07_VH2;

25 SEQ ID NO: 104 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as FEL_19F07_VH2;

SEQ ID NO: 105 is a variable light chain sequence referred to herein as FEL_19F07_VL2;

SEQ ID NO: 106 is the nucleotide sequence encoding the variable light chain sequence referred to herein as FEL_19F07_VL2;

SEQ ID NO: 107 is the amino acid sequence of a canine OSMR-Fc fusion protein designated herein as Canine_OSMR_hlgG1_Fc;

- 5 SEQ ID NO: 108 is the nucleotide sequence encoding the canine OSMR-Fc fusion protein designated herein as Canine_OSMR_hlgG1_Fc;

SEQ ID NO: 109 is the amino acid sequence corresponding to canine OSM, which is referred to herein as Canine_OSM;

- 10 SEQ ID NO: 110 is the amino acid sequence corresponding to feline OSM-Fc fusion protein, which is referred to herein as Feline_OSM_hlgG1_Fc;

SEQ ID NO: 111 is the nucleotide sequence corresponding to feline OSM-Fc fusion protein, which is referred to herein as Feline_OSM_hlgG1_Fc;

SEQ ID NO: 112 is the amino acid sequence of a feline OSMR-Fc fusion protein designated herein as Feline_OSMR_hlgG1_Fc;

- 15 SEQ ID NO: 113 is the nucleotide sequence encoding the feline OSMR-Fc fusion protein designated herein as Feline_OSMR_hlgG1_Fc;

SEQ ID NO: 114 is the amino acid sequence for the canine heavy chain constant region referred to herein as Canine_HC_65_1;

- 20 SEQ ID NO: 115 is the nucleotide sequence encoding the canine heavy chain constant region referred to herein as Canine_HC_65_1;

SEQ ID NO: 116 is the amino acid sequence for the canine light chain constant region referred to herein as Canine_LC_Kappa;

SEQ ID NO: 117 is the nucleotide sequence encoding the canine light chain constant region referred to herein as Canine_LC_Kappa;

- 25 SEQ ID NO: 118 is the amino acid sequence for the feline heavy chain constant region referred to herein as Feline_HC_AlleleA_1;

SEQ ID NO: 119 is the nucleotide sequence encoding the feline heavy chain constant region referred to herein as Feline_HC_AlleleA_1;

SEQ ID NO: 120 is the amino acid sequence for the feline light chain constant region referred to herein as Feline_LC_Kappa_G_minus;

SEQ ID NO: 121 is the nucleotide sequence encoding the amino acid sequence for the feline light chain constant region referred to herein as Feline_LC_Kappa_G_minus;

- 5 SEQ ID NO: 122 is the amino acid sequence for the human OSMR protein referred to herein as Human_OSMR;

SEQ ID NO: 123 is the amino acid sequence for the canine IL31 protein referred to herein as Canine_IL31;

- 10 SEQ ID NO: 124 is the nucleotide sequence encoding canine IL31 protein referred to herein as Canine_IL31;

SEQ ID NO: 125 is the amino acid sequence for the feline IL31 protein referred to herein as Feline_IL31;

SEQ ID NO: 126 is the nucleotide sequence encoding feline IL31 protein referred to herein as Feline_IL31;

- 15 SEQ ID NO: 127 is a variable heavy chain sequence referred to herein as CAN_14C04_VH1;

SEQ ID NO: 128 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_14C04_VH1;

SEQ ID NO: 129 is a variable heavy chain sequence referred to herein as CAN_14C04_VH2;

- 20 SEQ ID NO: 130 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_14C04_VH2;

SEQ ID NO: 131 is a variable heavy chain sequence referred to herein as CAN_14C04_VL1;

SEQ ID NO: 132 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_14C04_VL2;

SEQ ID NO: 133 is a variable heavy chain sequence referred to herein as CAN_14C04_VL1;

- 25 SEQ ID NO: 134 is the nucleotide sequence encoding the variable heavy chain sequence referred to herein as CAN_14C04_VL2;

SEQ ID NO: 135; is the amino acid sequence of the human LIFR protein designated herein as Human_LIFR;

SEQ ID NO: 136; is the amino acid sequence of the human LIF protein designated herein as Human_LIF;

DEFINITIONS

Before describing the present invention in detail, several terms used in the context of the
5 present invention will be defined. In addition to these terms, others are defined elsewhere in the specification, as necessary. Unless otherwise expressly defined herein, terms of art used in this specification will have their art-recognized meanings.

As used in the specification and claims, the singular form “a”, “an” and “the” include plural
10 references unless the context clearly dictates otherwise. For example, reference to “an antibody” includes a plurality of such antibodies.

As used herein, the term “comprising” is intended to mean that the compositions and methods
include the recited elements, but not excluding others.

15 Epitope, as used herein, refers to the antigenic determinant recognized by the CDRs of the antibody. In other words, epitope refers to that portion of any molecule capable of being recognized by, and bound by, an antibody. Unless indicated otherwise, the term “epitope” as used herein, refers to the region of OSMR Beta to which an anti-OSMR Beta agent is reactive
20 to.

An “antigen” is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of being recognized by, and bound by, an antibody (the corresponding antibody binding region may be referred to as a paratope). In general, epitopes
25 consist of chemically active surface groupings of molecules, for example, amino acids or sugar side chains, and have specific three-dimensional structural characteristics as well as specific charge characteristics.

The term “specifically” in the context of antibody binding, refers to high avidity and/or high
30 affinity binding of an antibody to a specific antigen, i.e., a polypeptide, or epitope. In many embodiments, the specific antigen is an antigen (or a fragment or subfraction of an antigen) used to immunize the animal host from which the antibody-producing cells were isolated. Antibody specifically binding an antigen is stronger than binding of the same antibody to other

antigens. Antibodies which bind specifically to a polypeptide may be capable of binding other polypeptides at a weak, yet detectable level (e.g., 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to a subject polypeptide, e.g. by use of appropriate controls. In
5 general, specific antibodies bind to an antigen with a binding affinity with a K_D of 10^{-7} M or less, e.g., 10^{-8} M or less (e.g., 10^{-9} M or less, 10^{-10} or less, 10^{-11} or less, 10^{-12} or less, or 10^{-13} or less, etc.).

As used herein, the term "antibody" refers to an intact immunoglobulin having two light and two
10 heavy chains. Thus, a single isolated antibody or fragment may be a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a recombinant antibody, a chimeric antibody, a heterochimeric antibody, a caninized antibody, or a felinized antibody. The term "antibody" preferably refers to monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof that can bind to the OSMR Beta protein and fragments thereof. The term
15 antibody is used both to refer to a homogeneous molecular, or a mixture such as a serum product made up of a plurality of different molecular entities.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H)
20 chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its
25 other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

30 The term "antibody fragment" refers to less than an intact antibody structure, including, without limitation, an isolated single antibody chain, an Fv construct, a Fab construct, an Fc construct, a light chain variable or complementarity determining region (CDR) sequence, etc.

The term "variable" region comprises framework and CDRs (otherwise known as hypervariables) and refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise multiple FRs, largely adopting a β -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the α -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (Kabat, et al. (1991), above) and/or those residues from a "hypervariable loop" (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the

antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) can recognize and bind antigen, although at a lower affinity than the entire binding site.

- 5 The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody
10 fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

- The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid
15 sequences of their constant domains.

- Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. Presently there are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into
20 subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2 (as defined by mouse and human designation). The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called *alpha*, *delta*, *epsilon*, *gamma*, and *mu*, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in multiple species. The prevalence of individual isotypes and functional activities
25 associated with these constant domains are species-specific and must be experimentally defined.

- "Monoclonal antibody" as defined herein is an antibody produced by a single clone of cells (specifically, a single clone of hybridoma cells) and therefore a single pure homogeneous type
30 of antibody. All monoclonal antibodies produced from the same clone are identical and have the same antigen specificity. The term "monoclonal" pertains to a single clone of cells, a single cell, and the progeny of that cell.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins)

in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species, as well as fragments of such antibodies, so long as they exhibit the
5 desired biological activity. Typically, chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to canine constant segments. In this embodiment, the antigen binding site is derived from mouse while the F_c portion is canine.

10

"Caninized" forms of non-canine (e.g., murine) antibodies are genetically engineered antibodies that contain minimal sequence derived from non-canine immunoglobulin. Caninized antibodies are canine immunoglobulin sequences (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-canine
15 species (donor antibody) such as mouse having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the canine immunoglobulin sequences are replaced by corresponding non-canine residues. Furthermore, caninized antibodies may include residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the caninized
20 antibody will include substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-canine immunoglobulin sequence and all or substantially all of the FRs are those of a canine immunoglobulin sequence. The caninized antibody optionally also will comprise a complete, or at least a portion of an immunoglobulin constant region (F_c), typically that of a canine
25 immunoglobulin sequence. In one embodiment, mouse CDRs are grafted onto canine frameworks.

30

"Felinized" forms of non-feline (e.g., murine) antibodies are genetically engineered antibodies that contain minimal sequence derived from non-feline immunoglobulin. Felinized antibodies are
feline immunoglobulin sequences (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-feline species (donor antibody) such as mouse having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the feline immunoglobulin sequences are replaced by corresponding non-feline residues. Furthermore, felinized antibodies may include residues

that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the felinized antibody will include substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-feline immunoglobulin sequence and all or substantially all of the FRs are those of a feline immunoglobulin sequence. The felinized antibody optionally also will comprise a complete, or at least a portion of an immunoglobulin constant region (Fc), typically that of a feline immunoglobulin sequence.

The term "heterochimeric" as defined herein, refers to an antibody in which one of the antibody chains (heavy or light) is caninized while the other is chimeric. In one embodiment, a caninized variable heavy chain (where all of the CDRs are mouse and all FRs are canine) is paired with a chimeric variable light chain (where all of the CDRs are mouse and all FRs are mouse. In this embodiment, both the variable heavy and variable light chains are fused to a canine constant region.

A "variant" anti-OSMR Beta antibody, refers herein to a molecule which differs in amino acid sequence from a "parent" anti-OSMR Beta antibody amino acid sequence by virtue of addition, deletion, and/or substitution of one or more amino acid residue(s) in the parent antibody sequence and retains at least one desired activity of the parent anti-OSMR Beta-antibody. Desired activities can include the ability to bind the antigen specifically, the ability to reduce, inhibit or neutralize OSM activity and/or IL-31 activity in an animal, and the ability to inhibit OSM-mediated and/or IL-31-mediated pSTAT signaling (STAT phosphorylation) in a cell-based assay. In one embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable (CDR) and/or framework region(s) of the parent antibody. For example, the variant may comprise at least one, e.g. from about one to about fifteen, and preferably from about two to about five, or from two to about ten substitutions in one or more hypervariable (CDR) and/or framework regions of the parent antibody. In one embodiment, the variant can include at least about the following number of amino acid substitutions in one or more of the CDR regions of the parent antibody: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In another embodiment, the variant can include up to about the following number of amino acid substitutions in one or more of the CDR regions of the parent antibody: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. Regarding a variant antibody, it is to be understood that the number of amino acid substitutions in any given CDR of the parent antibody can be different from the number of substitutions in other CDRs of the parent antibody. In one embodiment, the variant

will have variable heavy CDR1, variable heavy CDR2, and variable heavy CDR3 amino acid sequences which can each independently have at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% amino acid sequence identity with the variable heavy CDR1, variable heavy CDR2, and variable heavy CDR3 amino acid sequences of the parent antibody. In another embodiment, the variant will have variable light CDR1, variable light CDR2, and variable light CDR3 amino acid sequences which can each independently have at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% amino acid sequence identity with the variable light CDR1, variable light CDR2, and variable light CDR3 amino acid sequences of the parent antibody. Ordinarily, the variant will have an amino acid sequence having at least 50% amino acid sequence identity with the parent antibody heavy or light chain variable domain sequences, more preferably at least 65%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95% sequence identity. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind an OSMR Beta and preferably has desired activities which are superior to those of the parent antibody. For example, the variant may have a stronger binding affinity, enhanced ability to reduce, inhibit or neutralize OSM and/or IL-31 activity in an animal, and/or enhanced ability to inhibit OSM and/or IL-31-mediated pSTAT signaling in a cell-based assay.

A "variant" nucleic acid refers herein to a molecule which differs in sequence from a "parent" nucleic acid. Polynucleotide sequence divergence may result from mutational changes such as deletions, substitutions, or additions of one or more nucleotides. Each of these changes may occur alone or in combination, one or more times in a given sequence.

The "parent" antibody herein is one that is encoded by an amino acid sequence used for the preparation of the variant. Preferably, the parent antibody has a canine framework region and, if

present, has canine antibody constant region(s). For example, the parent antibody may be a caninized or canine antibody. As another example, the parent antibody may be a felinized or feline antibody. As yet another example, the parent antibody is a murine monoclonal antibody.

5 The term "isolated" means that the material (e.g., antibody or nucleic acid) is separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the material, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. With respect to nucleic acid, an isolated nucleic acid may include one that is separated
10 from the 5' to 3' sequences with which it is normally associated in the chromosome. In preferred embodiments, the material will be purified to greater than 95% by weight of the material, and most preferably more than 99% by weight. Isolated material includes the material *in situ* within recombinant cells since at least one component of the material's natural environment will not be present. Ordinarily, however, isolated material will be prepared by at least one purification step.

15

The word "label" when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody or nucleic acid. The label may itself be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that
20 is detectable.

The terms "nucleic acid", "polynucleotide", "nucleic acid molecule" and the like may be used interchangeably herein and refer to a series of nucleotide bases (also called "nucleotides") in DNA and RNA. The nucleic acid may contain deoxyribonucleotides, ribonucleotides, and/or their
25 analogs. The term "nucleic acid" includes, for example, single-stranded and double-stranded molecules. A nucleic acid can be, for example, a gene or gene fragment, exons, introns, a DNA molecule (e.g., cDNA), an RNA molecule (e.g., mRNA), recombinant nucleic acids, plasmids, and other vectors, primers and probes. Both 5' to 3' (sense) and 3' to 5' (antisense) polynucleotides are included.

30

A "subject" or "patient" refers to an animal in need of treatment that can be affected by molecules of the invention. Animals that can be treated in accordance with the invention include vertebrates, with mammals such as canine, feline, and equine animals being particularly preferred examples.

A "therapeutically effective amount" (or "effective amount") refers to an amount of an active ingredient, e.g., an agent according to the invention, sufficient to effect beneficial or desired results when administered to a subject or patient. An effective amount can be administered in one or more administrations, applications or dosages. A therapeutically effective amount of a composition according to the invention may be readily determined by one of ordinary skill in the art. In the context of this invention, a "therapeutically effective amount" is one that produces an objectively measured change in one or more parameters associated with treatment of a pruritic condition or an allergic condition including clinical improvement in symptoms. Of course, the therapeutically effective amount will vary depending upon the particular subject and condition being treated, the weight and age of the subject, the severity of the disease condition, the particular compound chosen, the dosing regimen to be followed, timing of administration, the manner of administration and the like, all of which can readily be determined by one of ordinary skill in the art.

As used herein, the term "therapeutic" encompasses the full spectrum of treatments for a disease or disorder. A "therapeutic" agent of the invention may act in a manner that is prophylactic or preventive, including those that incorporate procedures designed to target animals that can be identified as being at risk (pharmacogenetics); or in a manner that is ameliorative or curative in nature; or may act to slow the rate or extent of the progression of at least one symptom of a disease or disorder being treated.

"Treatment", "treating", and the like refers to both therapeutic treatment and prophylactic or preventative measures. Animals in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. The term "treatment" or "treating" of a disease or disorder includes preventing or protecting against the disease or disorder (that is, causing the clinical symptoms not to develop); inhibiting the disease or disorder (i.e., arresting or suppressing the development of clinical symptoms; and/or relieving the disease or disorder (i.e., causing the regression of clinical symptoms). As will be appreciated, it is not always possible to distinguish between "preventing" and "suppressing" a disease or disorder since the ultimate inductive event or events may be unknown or latent. Accordingly, the term "prophylaxis" will be understood to constitute a type of "treatment" that encompasses both "preventing" and "suppressing." The term "treatment" thus includes "prophylaxis".

The term “allergic condition” is defined herein as a disorder or disease caused by an interaction between the immune system and a substance foreign to the body. This foreign substance is termed “an allergen”. Common allergens include aeroallergens, such as pollens, dust, molds, dust mite proteins, injected saliva from insect bites, etc. Examples of allergic conditions include, but are not limited to, the following: allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstructive pulmonary disease, and inflammatory processes resulting from autoimmunity, such as Irritable bowel syndrome (IBS).

10 The term “pruritic condition” is defined herein as a disease or disorder characterized by an intense itching sensation that produces the urge to rub or scratch the skin to obtain relief. Examples of pruritic conditions include, but are not limited to, the following: atopic dermatitis, eczema, psoriasis, scleroderma, and pruritus.

15 The term “fibrotic disorder” is defined herein as a disease or disorder characterized by fibrosis, which is the accumulation of extracellular matrix components in organs or tissues, changing their structure and leading to a disruption of normal function. Fibrosis can occur in almost any organ or tissue and is associated with a wide variety of diseases. Examples of types of fibrotic disorders include, but are not limited to, renal fibrosis, pulmonary fibrosis, and dermal fibrosis.

20

The term “inflammatory disorder” includes a vast array of disorders and conditions that are characterized by inflammation. Examples include, but are not limited to, inflammatory processes resulting from autoimmunity, inflammation in the skin or joint of animals affected by osteoarthritis, immune-mediated polyarthritis, chronic bronchitis, allergic asthma, atopic dermatitis, allergic dermatitis, pyotraumatic dermatitis, atherosclerosis, and cardiovascular disease.

30 The term “inflammatory pain” as used herein is the spontaneous hypersensitivity to pain that occurs in response to tissue damage and inflammation (e.g., postoperative pain, trauma, arthritis). In one non-limiting example, the inflammatory pain is pain associated with osteoarthritis.

As used herein, the terms “cell”, “cell line”, and “cell culture” may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is

understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell (e.g., bacterial cells, yeast cells, mammalian cells, and insect cells) whether located *in vitro* or *in vivo*. For example, host cells may be located in a transgenic animal. Host cell can be used as a recipient for vectors and may include any transformable organism that is capable of replicating a vector and/or expressing a heterologous nucleic acid encoded by a vector.

A "composition" is intended to mean a combination of active agent and another compound or composition which can be inert (e.g., a label), or active, such as an adjuvant.

As defined herein, pharmaceutically acceptable carriers suitable for use in the invention are well known to those of skill in the art. Such carriers include, without limitation, water, saline, buffered saline, phosphate buffer, alcoholic/aqueous solutions, emulsions or suspensions. Other conventionally employed diluents, adjuvants and excipients, may be added in accordance with conventional techniques. Such carriers can include ethanol, polyols, and suitable mixtures thereof, vegetable oils, and injectable organic esters. Buffers and pH adjusting agents may also be employed. Buffers include, without limitation, salts prepared from an organic acid or base. Representative buffers include, without limitation, organic acid salts, such as salts of citric acid, e.g., citrates, ascorbic acid, gluconic acid, histidine-HCl, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid, Tris, trimethanmine hydrochloride, or phosphate buffers. Parenteral carriers can include sodium chloride solution, Ringer's dextrose, dextrose, trehalose, sucrose, and sodium chloride, lactated Ringer's or fixed oils. Intravenous carriers can include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose and the like. Preservatives and other additives such as, for example, antimicrobials, antioxidants, chelating agents (e.g., EDTA), inert gases and the like may also be provided in the pharmaceutical carriers. The present invention is not limited by the selection of the carrier. The preparation of these pharmaceutically acceptable compositions, from the above-described components, having appropriate pH isotonicity, stability and other conventional characteristics is within the skill of the art. See, e.g., texts such as Remington: The Science and Practice of Pharmacy, 20th ed, Lippincott Williams & Wilkins, publ., 2000; and The Handbook of Pharmaceutical Excipients, 4.sup.th edit., eds. R. C. Rowe et al, APhA Publications, 2003.

The term "conservative amino acid substitution" indicates any amino acid substitution for a given amino acid residue, where the substitute residue is so chemically similar to that of the given residue that no substantial decrease in polypeptide function (e.g., enzymatic activity) results. Conservative amino acid substitutions are commonly known in the art and examples thereof are described, e.g., in U.S. Pat. Nos. 6,790,639, 6,774,107, 6,194,167, or 5,350,576. In a preferred embodiment, a conservative amino acid substitution will be any one that occurs within one of the following six groups

- 1. Small aliphatic, substantially non-polar residues: Ala, Gly, Pro, Ser, and Thr;
- 2. Large aliphatic, non-polar residues: Ile, Leu, and Val; Met;
- 3. Polar, negatively charged residues and their amides: Asp and Glu;
- 4. Amides of polar, negatively charged residues: Asn and Gln; His;
- 5. Polar, positively charged residues: Arg and Lys; His; and
- 6. Large aromatic residues: Trp and Tyr; Phe.

In a preferred embodiment, a conservative amino acid substitution will be any one of the following, which are listed as Native Residue (Conservative Substitutions) pairs: Ala (Ser); Arg (Lys); Asn (Gln; His); Asp (Glu); Gln (Asn); Glu (Asp); Gly (Pro); His (Asn; Gln); Ile (Leu; Val); Leu (Ile; Val); Lys (Arg; Gln; Glu); Met (Leu; Ile); Phe (Met; Leu; Tyr); Ser (Thr); Thr (Ser); Trp (Tyr); Tyr (Trp; Phe); and Val (Ile; Leu).

Just as a polypeptide may contain conservative amino acid substitution(s), a polynucleotide hereof may contain conservative codon substitution(s). A codon substitution is considered conservative if, when expressed, it produces a conservative amino acid substitution, as described above. Degenerate codon substitution, which results in no amino acid substitution, is also useful in polynucleotides according to the present invention. Thus, e.g., a polynucleotide encoding a selected polypeptide useful in an embodiment of the present invention may be mutated by degenerate codon substitution in order to approximate the codon usage frequency exhibited by an expression host cell to be transformed therewith, or to otherwise improve the expression thereof.

DETAILED DESCRIPTION OF THE INVENTION

It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for

the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

Unless otherwise defined, scientific and technical terms used in connection with the antibodies described herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art.

Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transfection (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification, See e.g., Sambrook et al. MOLECULAR CLONING: LAB. MANUAL (3rd ed., Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y., 2001) and Ausubel et al. Current Protocols in Molecular Biology (New York: Greene Publishing Association/Wiley Interscience), 1993. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about."

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All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application.

The present invention provides for recombinant monoclonal antibodies and peptides and their uses in clinical and scientific procedures, including diagnostic procedures. With the advent of methods of molecular biology and recombinant technology, it is possible to produce antibody and antibody-like molecules by recombinant means and thereby generate gene sequences that
5 code for specific amino acid sequences found in the polypeptide structure of the antibodies. Such antibodies can be produced by either cloning the gene sequences encoding the polypeptide chains of said antibodies or by direct synthesis of said polypeptide chains, with assembly of the synthesized chains to form active tetrameric (H_2L_2) structures with affinity for specific epitopes and antigenic determinants. This has permitted the ready production of
10 antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

Regardless of the source of the antibodies, or how they are recombinantly constructed, or how they are synthesized, *in vitro* or *in vivo*, using transgenic animals, large cell cultures of
15 laboratory or commercial size, using transgenic plants, or by direct chemical synthesis employing no living organisms at any stage of the process, all antibodies have a similar overall 3 dimensional structure. This structure is often given as H_2L_2 and refers to the fact that antibodies commonly comprise two light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary
20 antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity. The variable regions of either H or L chains contain the amino acid sequences capable of specifically binding to antigenic targets.

25 As used herein, the term "antigen binding region" refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antibody binding region includes the "framework" amino acid residues necessary to maintain the proper conformation of the antigen-binding residues.

30 Within the variable regions of the H or L chains that provide for the antigen binding regions are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the

basic specificity of the antibody for a particular antigenic determinant structure.

The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have three CDR regions, each non-contiguous with the others.

In all mammalian species, antibody peptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs.

Regarding the antigenic determinate recognized by the CDR regions of the antibody, this is also referred to as the "epitope." In other words, epitope refers to that portion or portions of any molecule capable of being recognized by, and bound by, an antibody (the corresponding antibody binding region may be referred to as a paratope).

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce an antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" is meant to include both intact immunoglobulin molecules as well as portions, fragments, peptides and derivatives thereof such as, for example, Fab, Fab', F(ab')₂, Fv, Fse, CDR regions, paratopes, or any portion or peptide sequence of the antibody that is capable of binding an antigen or epitope. An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody.

Antibody also includes chimeric antibodies, heterochimeric antibodies, caninized antibodies, or felinized antibodies, as well as fragments, portions, regions, peptides or derivatives thereof,

provided by any known technique, such as, but not limited to, enzymatic cleavage, peptide synthesis, or recombinant techniques. Such antibodies of the present invention are capable of specifically binding at least one of canine OSMR Beta or feline OSMR Beta. Antibody fragments or portions may lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. Examples of antibody fragments may be produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). See, e.g., Wahl et al., 24 J. Nucl. Med. 316-25 (1983). Portions of antibodies may be made by any of the above methods, or may be made by expressing a portion of the recombinant molecule. For example, the CDR region(s) of a recombinant antibody may be isolated and subcloned into the appropriate expression vector. See, e.g., U.S. Pat. No. 6,680,053.

Clones 02D09, 09E09, 10F07, 14C04, and 19F07 Nucleotide and Amino Acid Sequences

In some embodiments, the present invention provides for novel monoclonal antibodies that specifically bind to at least one of canine OSMR Beta or feline OSMR Beta. In one embodiment, a monoclonal antibody of the invention binds to canine OSMR beta or feline OSMR Beta and prevents or inhibits activation of the IL-31 co-receptor complex comprising IL-31 receptor A (IL-31Ra) and Oncostatin-M-specific receptor (OsmR or OSMR Beta). The monoclonal antibodies of the present invention are identified herein as "02D09", "09E09", "10F07", "14C04", and "19F07" which refers to the number assigned to its hybridoma clone. Herein, "02D09", "09E09", "10F07", "14C04", and "19F07" also refers to the portion of the monoclonal antibody, the paratope or CDRs, that bind specifically with an OSMR Beta epitope identified as 02D09, 09E09, 10F07, 14C04, and 19F07 because of its ability to bind the 02D09, 09E09, 10F07, 14C04, and 19F07 antibodies, respectively. The several recombinant, chimeric, heterochimeric, caninized and/or felinized forms of 02D09, 09E09, 10F07, 14C04, and 19F07 described herein may be referred to by the same name.

In one embodiment, the antibody, or antigen-binding portion thereof of the present invention includes a combination of complementary determining region (CDR) sequences selected from the following:

- 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY),

variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQISNNLH), VL-CDR2 of SEQ NO: 5 (YASQIS), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);

- 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY), VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
- 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV), VL-CDR1 of SEQ ID NO: 28 (KASQDVTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPWT); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

In some embodiments, amino acid residues located in parent antibodies 02D09, 09E09, 10F07, and 19F07 at the mutation positions denoted in Table A of the example section are conserved in the CDR variants of the respective parent antibody. For example, based on the information in Table A and the sequence listing, in some embodiments the underlined amino acid residues shown below are conserved in CDR variants of parent antibodies 02D09, 09E09, 10F07, and 19F07.

02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY), variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSISNNLH), VL-CDR2 of SEQ NO: 5 (YASQIS), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);

09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);

10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);

19F07: VH-CDR1 of SEQ ID NO: 25 (DYMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV), VL-CDR1 of

SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASIRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSREPLT).

5 Based on the results described in Example 19, Alanine replacement mutations at the underlined positions negatively impacted binding of the antibody to the OSMR target. By inference, the residues which are not underlined can be substituted in the CDR variants since Alanine mutations at those positions did not negatively impact binding of the antibody to the OSMR target.

10 Furthermore, the information in Table B of the example section supports that in some embodiments CDR variants of the 19F07 parent antibody are those which can have the allowed substitutions specified in the sequence definitions in that table, which is reproduced below.

SEQ ID NO:	SEQUENCE DEFINITION WITH ALLOWED SUBSTITUTIONS	DESCRIPTION WITH (BINDING TARGET PROTEIN)
25	D ₁ Y ₂ Y ₃ M ₄ A ₅ wherein Y ₃ is Y	CDR-H1 (canine OSMR)
26	N ₁ I ₂ N ₃ Y ₄ D ₅ G ₆ S ₇ S ₈ T ₉ Y ₁₀ Y ₁₁ L ₁₂ D ₁₃ S ₁₄ L ₁₅ K ₁₆ S ₁₇ wherein Y ₁₀ is Y, C, F, H, T, or W	CDR-H2 (canine OSMR)
27	G ₁ L ₂ T ₃ W ₄ D ₅ F ₆ D ₇ V ₈ W ₉ wherein W ₄ is W, G, or C	CDR-H3 (canine OSMR)
27	G ₁ L ₂ T ₃ W ₄ D ₅ F ₆ D ₇ V ₈ W ₉ wherein W ₄ is W, G, or F	CDR-H3 (feline OSMR)
28	K ₁ A ₂ S ₃ Q ₄ D ₅ V ₆ D ₇ T ₈ A ₉ V ₁₀ A ₁₁ wherein V ₆ is V, L, M, A, N, S, E, I, H, or C; D ₇ is D, G, Y, N, or E; A ₉ is A, S, or G; V ₁₀ is V, L, I, A, D, T, M, or C; A ₁₁ is A, S, T, G, or C	CDR-L1 (canine OSMR)
28	K ₁ A ₂ S ₃ Q ₄ D ₅ V ₆ D ₇ T ₈ A ₉ V ₁₀ A ₁₁ wherein V ₆ is V, L, M, A, G, N, T, S, P, I, F, or H; D ₇ is D, G, Y, N, E, S, R, A, L, M, H, T, Q, I, or W; A ₉ is A, S, V, T, or G; V ₁₀ is V, L, F, I, A, S, G, T, M, Q, N, or C; A ₁₁ is A, S, T, V, G, C, N, or D	CDR-L1 (feline OSMR)
29	L ₁ A ₂ S ₃ T ₄ R ₅ H ₆ T ₇ wherein L ₁ is L, S, F, M, A, G, or Q; A ₂ is A, S, V, G, D, P, T, L, E, or C	CDR-L2 (canine OSMR)
29	L ₁ A ₂ S ₃ T ₄ R ₅ H ₆ T ₇ wherein A ₂ is A, S, V, G, D, T, L, E, R, C, Y	CDR-L2 (feline OSMR)
30	Q ₁ Q ₂ Y ₃ S ₄ R ₅ F ₆ P ₇ L ₈ T ₉ F ₁₀ wherein Q ₁ is Q, S, H, K, E, G, M, A, N, F, or Y; Q ₂ is Q or H; Y ₃ is Y or F; S ₄ is S, I, N, A, or T; F ₆ is F, L, I, V, Y, M, E, W, or D; P ₇ is P, S, C, A, G, or D; L ₈ is L, R, P, M, S, V, I, W, F, or E; F ₁₀ is F, L, M, I, Y, or W	CDR-L3 (canine OSMR)
30	Q ₁ Q ₂ Y ₃ S ₄ R ₅ F ₆ P ₇ L ₈ T ₉ F ₁₀ wherein Q ₁ is Q, L, S, H, K, V, E, G, M, A, T, N, F, Y, or C; Q ₂ is Q, M, H, S, Y, T, N, F, E, C, or A; Y ₃ is Y, L, R, M, F, W, H, I, or N; P ₇ is P, S, H, T, R, C, A, G, or D	CDR-L3 (feline OSMR)

In another embodiment, an antibody according to the present invention includes at least one of the following variable heavy chains and variable light chains:

5

(a) a variable heavy chain comprising

SEQ ID NO: 31 (MU_02D09_VH)
EVQLVESGGGLVKPGGSLTSCAASGFTFSDYGMHWLRQAPEKGLEWWAYISSGSRAVFFAD
TVKGRFTISRDNANTLYLQMTSLRSDDTAMYYCARDRYDGRGFAYWGQGLTVTSA,

10 SEQ ID NO: 35 (MU_09E09_VH)
DVKLVESGGLVKPGGSLKLSAASGFTFSSYAMSWVRQTPEKRLEWWAYISSGGDYIYYADT
VKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCTRPITGTFAYWGQGLTVTSA,

SEQ ID NO: 39 (MU_10F07_VH)
DVKLVESGGLVKPGGSLKLSAASGFTFSSYAMSWVRQTPEKRLEWWTYISSGGDYFYAD
15 TVKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCTRPITGTFAYWGQGLTVTSA,

SEQ ID NO: 43 (MU_14C04_VH)
EVQLQESGAELVKPGASVKISCKASGYAFSNYWMNWMKQRPKGLEWIGQIYPGHVNTNYN
GNFKDKATLTADK
SSSTAYMQLSSLTSEDSAVYFCARSADNSGFVLFAYWGQGLTVTVS,

20 SEQ ID NO: 47 (MU_19F07_VH)
EVKLVESEGGLVQPGSSMKLSCTASGFTFSDYYMAWVRQVPEKGLEWWANINYDGSSTYYLD
SLKSRFIISRDNANKILYLQMSSLKSEDTATYYCARGLTWDFDVWGTGTTVTVSS,

SEQ ID NO: 51 (FEL_02D09_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSDYGMHWVRQAPGKGLQWWAYISSGSRAVFFA
25 DTVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDRYDGRGFAYWGQGLTVTVSS,

SEQ ID NO: 53 (FEL_02D09_VH2)
DVQLVESGGDLVKPGGSLRLTCVASGFTFSDYGMHWVRQAPGKGLQWWAYISSGSRAVFFA
DTVKGRFTISRDNANTLYLQMNSLRTEDTATYYCARDRYDGRGFAYWGQGLTVTVSS,

SEQ ID NO: 59 (CAN_09E09_VH1)
30 EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYIYYAD
TVKGRFTISRDNANTLYLQMNSLRAEDTAMYYCVRDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 61 (CAN_09E09_VH2)
EVQLVESGGDLVKPAGSLTSLCLASGFTFSSYAMSWVRQTPEKGLQWWAYISSGGDYIYYADT
VKGRFTISRDNANTLYLQMNSLRDEDTAVYYCARDPITGTFAYWGQGLTVTVSS,

35 SEQ ID NO: 67 (FEL_09E09_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSSYAMSWVRQAPGKGLQWWAYISSGGDYIYYAD
TVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 69 (FEL_09E09_VH2)
40 DVQLVESGGNLVKPGGSLRLTCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYIYYAD
TVKGRFTISKDNANTLYLQMNSLKTEDTATYYCARDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 75 (CAN_10F07_VH1)
 EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYFYA
 DTVKGRFTISRDNANTLYLQMNSLRAEDTAMYYCVRDPITGTFAYWGQGLTVTVSS,

5 SEQ ID NO: 79 (CAN_10F07_VH2)
 EVQLVESGGDLVKPAGSLTLSCLASGFTFSSYAMSWVRQTPEKGLQWWAYISSGGDYFYAD
 TVKGRFTISRDNANTLYLQMNSLRDEDTAVYYCARDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 83 (FEL_10F07_VH1)
 DVQLVESGGDLVKPGGSLRLTCVASGFTYSSYAMSWVRQAPGKGLQWWAYISSGGDYFYA
 DTVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDPITGTFAYWGQGLTVTVSS,

10 SEQ ID NO: 87 (FEL_10F07_VH2)
 DVQLVESGGDLVKPGGSLRLTCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYFYA
 DTVKGRFTISRDDAKNTLYLQMSSLKTEDTATYYCTGDPITGTFAYWGQGLTVTVSS,

15 SEQ ID NO: 91 (CAN_19F07_VH1)
 EVQLVESGGDLVKPGGSLRLSCVASGFTFSDYYMAWVRQAPGKGLQWWANINYDGSSTYYLD
 SLKSRFTISRDNANTLYLQMNSLRAEDTAMYYCVRGLTWDFDVWGQGLTVTVSS,

SEQ ID NO: 95 (CAN_19F07_VH2)
 EVQLVESGGDLVKPAGSLTLSCLASGFTFSDYYMAWVRQTPEKGLQWWANINYDGSSTYYLD
 SLKSRFTISRDNANTLYLQMNSLRDEDTAVYYCARGLTWDFDVWGQGLTVTVSS,

20 SEQ ID NO: 99 (FEL_19F07_VH1)
 DVQLVESGGDLVKPGGSLRLTCVASGFTYSDYYMAWVRQAPGKGLQWWANINYDGSSTYYL
 DSLKSRFTISRDNANTLYLQMNSLKTEDTATYYCVRGLTWDFDVWGQGLTVTVSS,

SEQ ID NO: 103 (FEL_19F07_VH2)
 DVQLVESGGDLVKPGGSLRLTCVASGFTFSDYYMAWVRQAPGKGLQWWANINYDGSSTYYLD
 SLKSRFTISRDNANTLYLQMNSLKTEDTATYYCARGLTWDFDVWGQGLTVTVSS,

25 SEQ ID NO: 127. (CAN_14C04_VH1)
 EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYWMNWRQAPGKGLQWWAQIYPGHVNTNYN
 GNFKDRFTISRDNARNTVYLQMNSLRAEDTAVYYCARSADNSGFVLFAYWGQGLTVTVSS, or

SEQ ID NO: 129. (CAN_14C04_VH2)
 EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYWMNWRQSPGKGLQWWAQIYPGHVNTNYN
 30 GNFKDRFTISRDNANTLYLQMNSLRAEDTAVYFCARSADNSGFVLFAYWGQGLTVTVSS;
 and

(b) a variable light chain comprising

35 SEQ ID NO: 33 (MU_02D09_VL)
 DIVLTQSPATLSVTPGDSVSLSCRASQSSNNLHWYQQTSHEPRLLITYASQSISGIPSRFSGS
 GSGTDFTLISNVETEDFGMYFCQQSNSWPLTFGAGTKLELK,

SEQ ID NO: 37 (MU_09E09_VL)
 DLQMTQTSSLSASLGDRVTISCRASQDINNYLNWYQQKPDGTVKLLIYYTSTLHSGVPSRFSG
 SSGTDFSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIK,

40 SEQ ID NO: 41 (MU_10F07_VL)
 DIQMTQTSSLSASLGDRVTISCRASQDITNYLNWYQQKPDGTVKLLIYYTSTLHSGVPSRFSG
 SSGTDFSLTISNLEQEDIATYFCQQGHMLPWTFGGGKLEIK,

SEQ ID NO: 45 (MU_14C04_VL)
DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYLHWYQQKPGQPPKLLIFLASNLESGVPA
RFGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPLTFGAGTKLELK,

- 5 SEQ ID NO: 49 (MU_19F07_VL)
DIVMTQSHKFMSPSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIYLA STRHTGVPDRFT
GSGSGTDFTLTISNVQSEDLADYFCQQYSRFLTFGAGTKLELK,

SEQ ID NO: 55 (FEL_02D09_VL1)
EIQMTQSPSSLSASPGDRVITICRASQSISNNLHWYQQKPGKVPKLLIYYASQSISGVPSRFSG
SGSGTDFTLTISSLEPEDAATYYCQQSNSWPLTFGQGT,

- 10 SEQ ID NO: 57 (FEL_02D09_VL2)
DIVMTQTPLSLSVTPGESASISCRASQSISNNLHWYLLQKSGQSPRRLIYYASQSISGV PDRFSG
SGSGTDFTLRISRVEADDVGYYCQQSNSWPLTFGQGT,

- 15 SEQ ID NO: 63 (CAN_09E09_VL1)
EIVMTQSPASLSLSQEEKVTITCRASQDINNYLNWYQQKPGQAPKLLIYYTSTLHSGVPSRFSG
SGSGTDFSTFISSLEPEDVAVYYCQQGNTLPWTFGQGT,

SEQ ID NO: 65 (CAN_09E09_VL2)
DIVLTQPTSVSGSLGQRVTISCRASQDINNYLNWYQQLPKGAPKLLVYYTSTLHSGVPDRFSGS
NSGSSATLTITGLQAEDEADYYCQQGNTLPWTFGQGT,

- 20 SEQ ID NO: 71 (FEL_09E09_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDINNYLNWYQQKPGKVPKLLIYYTSTLHSGVPSRFSG
SGSGTDFTLTISSLEPEDAATYYCQQGNTLPWTFGQGT,

SEQ ID NO: 73 (FEL_09E09_VL2)
DITMTQSPGSLAGSPGQQVTMNCRASQDINNYLNWYQQKPGQHPKLLIYYTSTLHSGVPDRF
SGSGSGTDFTLTISNLQAEDVASYYCQQGNTLPWTFGQGT,

- 25 SEQ ID NO: 77 (CAN_10F07_VL1)
EIVMTQSPASLSLSQEEKVTITCRASQDITNYLNWYQQKPGQAPKLLIYYTSTLHSGVPSRFSG
SGSGTDFSTFISSLEPEDVAVYYCQQGHMLPWTFGQGT,

- 30 SEQ ID NO: 81 (CAN_10F07_VL2)
DIVLTQPTSVSGSLGQRVTISCRASQDITNYLNWYQQLPKGAPKLLVYYTSTLHSGVPDRFSGS
NSGSSATLTITGLQAEDEADYYCQQGHMLPWTFGQGT,

SEQ ID NO: 85 (FEL_10F07_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDITNYLNWYQQKPGKVPKLLIYYTSTLHSGVPSRFSG
SGSGTDFTLTISSLEPEDAATYYCQQGHMLPWTFGQGT,

- 35 SEQ ID NO: 89 (FEL_10F07_VL2)
DITMTQSPGSLAGSPGQQVTMNCRASQDITNYLNWYQQKPGQHPKLLIYYTSTLHSGVPDRFS
GSGSGTDFTLTISNLQAEDVASYYCQQGHMLPWTFGQGT,

SEQ ID NO: 93 (CAN_19F07_VL1)
EIVMTQSPASLSLSQEEKVTITCKASQDVDTAVAWYQQKPGQAPKLLIYLA STRHTGVPSRFSG
SGSGTDFSTFISSLEPEDVAVYYCQQYSRFLTFGQGT,

- 40 SEQ ID NO: 97 (CAN_19F07_VL2)
DIVMTQTPLSLSVSPGETASICKASQDVDTAVAWFRQKPGQSPQRLLIYLA STRHTGV PDRFS
GSGSGTDFTLRISRVEADDTGVYYCQQYSRFLTFGQGT,

SEQ ID NO: 101 (FEL_19F07_VL1)
 EIQMTQSPSSLSASPGDRVITICKASQDVDTAVAWYQQKPGKVPKLLIYLASTRHTGVPSRFS
 GSGSGTDFTLTISSLEPEDAATYYCQQYSRFLTFGQGT,

5 SEQ ID NO: 105 (FEL_19F07_VL2)
 DITMTQSPGSLAGSPGQQVTMNCASQDVDTAVAWYQQKPGQHPKLLIYLASTRHTGVPDFR
 SSGSGTDFTLTISNLQAEDVASYYCQQYSRFLTFGQGT,

SEQ ID NO: 131. (CAN_14C04_VL1)
 EIVMTQSPASLSLSQEEKVTITCRASKSVSTSGYSYLHWYQQKPGQAPKLLIYLASNLESGVPS
 RFSGSGSGTDFSTISSLEPEDVAVYYCQHSRELPLTFGQGT, or

10 SEQ ID NO: 133. (CAN_14C04_VL2)
 DIVMTQTPLSLSVSPGETASISCRASKSVSTSGYSYLHWYLLQKPGQSPQLLIYLASNLESGVSK
 RFSGSGSGTDFTLRISRVEADDTGIYYCQHSRELPLTFGQGT.

In other embodiments, the invention provides a host cell that produces an antibody described
 15 above.

The present invention also includes, within its scope, nucleotide sequences encoding the
 variable regions of the light and heavy chains of the anti-OSMR Beta antibody of the present
 invention. Included also within the scope of the invention is any nucleotide sequence that
 20 encodes the amino acid sequence of the 02D09, 09E09, 10F07, 14C04, and 19F07 antibodies
 or antigen-binding portions thereof.

The present invention also provides an isolated nucleic acid including a nucleic acid sequence
 encoding at least one of the following combinations of variable heavy complementary
 25 determining region (CDR) sequences:

- 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID
 NO: 2 (YISSGSRAVFFADTVKG), and VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY);
- 30 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8
 (YISSGGDYIYYADTVKG), and VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY);
- 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14
 (YISSGGDYFYADTVKG), and VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY);
- 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20
 (QIYPGHVNTNYNGNFKD), and VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY);
- 35 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYMA), VH-CDR2 of SEQ ID NO: 26
 (NINYDGSSTYYLDSLKS), and VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

In one embodiment, the nucleic acid described above further includes a nucleic acid sequence encoding at least one of the following combinations of variable light CDR sequences:

- 5 1) 02D09: variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSI>NNLH), VL-CDR2 of SEQ NO: 5 (YASQSI), and VL-CDR3 of SEQ ID NO: 6 (QQNSWPLT);
- 2) 09E09: VL-CDR1 of VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 3) 10F07: VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 10 4) 14C04: VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
- 5) 19F07: VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
- 15 6) CDR variants of 1, 2, 3, 4, or 5.

In another embodiment, a nucleic acid sequence according to the invention encodes at least one of the following combinations of variable light CDR sequences:

- 1) 02D09: variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSI>NNLH), VL-CDR2 of SEQ NO: 5 (YASQSI), and VL-CDR3 of SEQ ID NO: 6 (QQNSWPLT);
- 20 2) 09E09: VL-CDR1 of VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 3) 10F07: VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 25 4) 14C04: VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
- 5) 19F07: VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

- 30 The present invention further provides a vector that includes a nucleic acid as described above. A single expression vector can carry the nucleic acid sequence encoding the variable light CDR sequences as well as the nucleic acid sequence encoding the variable heavy CDR sequences. Alternatively, the nucleic acid sequence encoding the variable light CDRs may be carried by one vector whereas the nucleic acid sequence encoding the variable heavy CDRs may be carried by
- 35 a separate vector.

Because the genetic code is degenerate, more than one codon can be used to encode a

particular amino acid. Using the genetic code, one or more different nucleotide sequences can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual XXX-encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic or prokaryotic cells expressing an anti-OSMR Beta antibody or portion thereof. Such "codon usage rules" are disclosed by Lathe, et al., 183 J. Molec. Biol. 1-12 (1985). Using the "codon usage rules" of Lathe, a single nucleotide sequence, or a set of nucleotide sequences, that contains a theoretical "most probable" nucleotide sequence capable of encoding anti-OSMR Beta sequences can be identified.

It is also intended that the antibody coding regions for use in the present invention could also be provided by altering existing antibody genes using standard molecular biological techniques that result in variants (agonists) of the antibodies and antigen-binding portions described herein. Such variants include, but are not limited to deletions, additions and substitutions in the amino acid sequence of the anti-OSMR Beta antibodies or antigen-binding portions thereof.

For example, one class of substitutions is conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in an anti-OSMR Beta antibody sequence by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg, replacements among the aromatic residues Phe, Tyr, and the like. Guidance concerning which amino acid changes are likely to be phenotypically silent is found in Bowie et al., 247 Science 1306-10 (1990).

Variant or agonist anti-OSMR Beta antibodies or antigen-binding portions thereof may be fully functional or may lack function in one or more activities. Fully functional variants typically contain only conservative variations or variations in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a

substitution, insertion, inversion, or deletion in a critical residue or critical region.

Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis. Cunningham et al., 244 Science
5 1081-85 (1989). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as epitope binding or in vitro ADCC activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallography, nuclear magnetic resonance, or photoaffinity labeling. Smith et al., 224 J. Mol. Biol. 899-904 (1992); de Vos et al., 255 Science
10 306-12 (1992).

Moreover, polypeptides often contain amino acids other than the twenty "naturally occurring" amino acids. Further, many amino acids, including the terminal amino acids, may be modified by natural processes, such as processing and other post-translational modifications, or by
15 chemical modification techniques well known in the art. Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation,
20 formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, *gamma* carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

25 Such modifications are well known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, *gamma*-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as Proteins--Structure and
30 Molecular Properties (2nd ed., T. E. Creighton, W.H. Freeman & Co., NY, 1993). Many detailed reviews are available on this subject, such as by Wold, Posttranslational Covalent Modification of proteins, 1-12 (Johnson, ed., Academic Press, NY, 1983); Seifter et al. 182 Meth. Enzymol. 626-46 (1990); and Rattan et al. 663 Ann. NY Acad. Sci. 48-62 (1992).

Accordingly, the antibodies and antigen-binding portions thereof of the present invention also encompass derivatives or analogs in which a substituted amino acid residue is not one encoded by the genetic code.

- 5 Similarly, the additions and substitutions in the amino acid sequence as well as variations, and modifications just described may be equally applicable to the amino acid sequence of the OSM Beta antigen and/or epitopes thereof, and are thus encompassed by the present invention. As mentioned above, the genes encoding a monoclonal antibody according to the present invention is specifically effective in the recognition of OSMR Beta.

10

Antibody Derivatives

- Included within the scope of this invention are antibody derivatives. A "derivative" of an antibody contains additional chemical moieties not normally a part of the protein. Covalent modifications of the protein are included within the scope of this invention. Such modifications may be
- 15 introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. For example, derivatization with bifunctional agents, well-known in the art, is useful for cross-linking the antibody or fragment to a water-insoluble support matrix or to other macromolecular carriers.

20

- Derivatives also include radioactively labeled monoclonal antibodies that are labeled. For example, with radioactive iodine (^{125}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), indium (^{111}In), tritium (^3H) or the like; conjugates of monoclonal antibodies with biotin or avidin, with enzymes, such as horseradish peroxidase, alkaline phosphatase, *beta*-D-galactosidase, glucose oxidase,
- 25 glucoamylase, carboxylic acid anhydrase, acetylcholine esterase, lysozyme, malate dehydrogenase or glucose 6-phosphate dehydrogenase; and also conjugates of monoclonal antibodies with bioluminescent agents (such as luciferase), chemoluminescent agents (such as acridine esters) or fluorescent agents (such as phycobiliproteins).

- 30 Another derivative bifunctional antibody of the present invention is a bispecific antibody, generated by combining parts of two separate antibodies that recognize two different antigenic groups. This may be achieved by crosslinking or recombinant techniques. Additionally, moieties may be added to the antibody or a portion thereof to increase half-life *in vivo* (e.g., by lengthening the time to clearance from the blood stream. Such techniques include, for example,

adding PEG moieties (also termed pegylation), and are well-known in the art. See U.S. Patent. Appl. Pub. No. 20030031671.

Recombinant Expression of Antibodies

5 In some embodiments, the nucleic acids encoding a subject monoclonal antibody are introduced directly into a host cell, and the cell is incubated under conditions sufficient to induce expression of the encoded antibody. After the subject nucleic acids have been introduced into a cell, the cell is typically incubated, normally at 37°C, sometimes under selection, for a period of about 1-24 hours in order to allow for the expression of the antibody. In one embodiment, the antibody is
10 secreted into the supernatant of the media in which the cell is growing.

Traditionally, monoclonal antibodies have been produced as native molecules in murine hybridoma lines. In addition to that technology, the present invention provides for recombinant DNA expression of monoclonal antibodies. This allows the production of caninized and felinized
15 antibodies, as well as a spectrum of antibody derivatives and fusion proteins in a host species of choice.

A nucleic acid sequence encoding at least one anti-OSMR Beta antibody, or antigen-binding portion thereof of the present invention may be recombined with vector DNA in accordance with
20 conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Maniatis et al., MOLECULAR CLONING, LAB. MANUAL, (Cold Spring Harbor Lab. Press, NY, 1982 and 1989),
25 and Ausubel et al. 1993 *supra*, may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule or antigen binding region thereof.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory
30 information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as anti-OSMR beta polypeptides or antibody portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to

organism, as is well known in the analogous art. See, e.g., Sambrook et al., 2001 *supra*; Ausubel et al., 1993 *supra*.

The present invention accordingly encompasses the expression of an anti-OSMR Beta antibody
5 or antigen-binding portion thereof, in either prokaryotic or eukaryotic cells. Suitable hosts
include bacterial or eukaryotic hosts including bacteria, yeast, insects, fungi, bird and
mammalian cells either *in vivo*, or *in situ*, or host cells of mammalian, insect, bird or yeast origin.
The mammalian cell or tissue may be of human, primate, hamster, rabbit, rodent, cow, pig,
sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used.

10

In one embodiment, the introduced nucleotide sequence will be incorporated into a plasmid or
viral vector capable of autonomous replication in the recipient host. Any of a wide variety of
vectors may be employed for this purpose. See, e.g., Ausubel et al., 1993 *supra*. Factors of
importance in selecting a particular plasmid or viral vector include: the ease with which recipient
15 cells that contain the vector may be recognized and selected from those recipient cells which do
not contain the vector; the number of copies of the vector which are desired in a particular host;
and whether it is desirable to be able to "shuttle" the vector between host cells of different
species.

20

Example prokaryotic vectors known in the art include plasmids such as those capable of
replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, .pi.VX). Such
plasmids are, for example, disclosed by Maniatis et al., 1989 *supra*; Ausubel et al., 1993 *supra*.
Bacillus plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan,
in THE MOLEC. BIO. OF THE BACILLI 307-329 (Academic Press, NY, 1982). Suitable
25 Streptomyces plasmids include pIJ101 (Kendall et al., 169 J. Bacteriol. 4177-83 (1987)), and
Streptomyces bacteriophages such as .phi.C31 (Chater et al., in SIXTH INT'L SYMPOSIUM ON
ACTINOMYCETALES BIO. 45-54 (Akademiai Kiado, Budapest, Hungary 1986). Pseudomonas
plasmids are reviewed in John et al., 8 Rev. Infect. Dis. 693-704 (1986); Izaki, 33 Jpn. J.
Bacteriol. 729-42 (1978); and Ausubel et al., 1993 *supra*.

30

Alternatively, gene expression elements useful for the expression of cDNA encoding anti-OSMR
Beta antibodies or antigen-binding portions thereof include, but are not limited to (a) viral
transcription promoters and their enhancer elements, such as the SV40 early promoter
(Okayama et al., 3 Mol. Cell. Biol. 280 (1983)), Rous sarcoma virus LTR (Gorman et al., 79

Proc. Natl. Acad. Sci., USA 6777 (1982)), and Moloney murine leukemia virus LTR (Grosschedl et al., 41 Cell 885 (1985)); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayama et al., 1983), and (c) polyadenylation sites such as in SV40 (Okayama et al., 1983).

5

Immunoglobulin cDNA genes can be expressed as described by Weidle et al., 51 Gene 21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements.

10

For immunoglobulin genes comprised of part cDNA, part genomic DNA (Whittle et al., 1 Protein Engin. 499 (1987)), the transcriptional promoter can be human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin, and mRNA splicing and polyadenylation regions can be the native chromosomal immunoglobulin sequences.

15

In one embodiment, for expression of cDNA genes in rodent cells, the transcriptional promoter is a viral LTR sequence, the transcriptional promoter enhancers are either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, the splice region contains an intron of greater than 31 bp, and the polyadenylation and transcription termination regions are derived from the native chromosomal sequence corresponding to the immunoglobulin chain being synthesized. In other embodiments, cDNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

20

25

Each fused gene can be assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the chimeric immunoglobulin chain gene product are then transfected singly with a gene encoding an anti-OSMR Beta immunoglobulin chain or chimeric H or chimeric L chain-encoding gene, or are co-transfected with a chimeric H and a chimeric L chain gene.

30

The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulin chains or intact antibodies or fragments are recovered from the culture.

In one embodiment, the fused genes encoding the anti-OSMR Beta immunoglobulin H and L

chains or chimeric H and L chains, or portions thereof are assembled in separate expression vectors that are then used to co-transfect a recipient cell. Alternatively, the fused genes encoding the chimeric H and L chains can be assembled on the same expression vector.

- 5 For transfection of the expression vectors and production of the chimeric antibody, the recipient cell line may be a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites
- 10 fluid. Other suitable recipient cells include lymphoid cells such as B lymphocytes of human or non-human origin, hybridoma cells of human or non-human origin, or interspecies heterohybridoma cells.

- The expression vector carrying a chimeric, caninized or felinized antibody construct or anti-
- 15 OSMR Beta immunoglobulin gene construct of the present invention can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment.
- 20 Johnston et al., 240 Science 1538 (1988).

- Yeast can provide substantial advantages over bacteria for the production of immunoglobulin H and L chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies now exist which utilize strong promoter sequences and
- 25 high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides). Hitzman et al., 11th Int'l Conference on Yeast, Genetics & Molec. Biol. (Montpelier, France, 1982).

- 30 Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of anti-OSMR Beta antibody sequences, antibody and assembled murine and chimeric, heterochimeric, caninized, or felinized antibodies, fragments and regions thereof. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced

in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast. See Vol. II DNA Cloning, 45-66, (Glover, ed.,) IRL Press, Oxford, UK 1985).

Bacterial strains can also be utilized as hosts for the production of antibody molecules or peptides described by this invention. Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of murine, chimeric, heterochimeric, caninized or felinized antibodies, fragments and regions or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria (see Glover, 1985 *supra*; Ausubel, 1993 *supra*; Sambrook, 2001 *supra*; Colligan et al., eds. Current Protocols in Immunology, John Wiley & Sons, NY, NY (1994-2001); Colligan et al., eds. Current Protocols in Protein Science, John Wiley & Sons, NY, NY (1997-2001).

Host mammalian cells may be grown *in vitro* or *in vivo*. Mammalian cells provide post-translational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of H and L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein.

Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO-K1 (ATCC CRL 61) cells.

Many vector systems are available for the expression of cloned anti-OSMR Beta H and L chain genes in mammalian cells (see Glover, 1985 *supra*). Different approaches can be followed to obtain complete H₂L₂ antibodies. It is possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H₂L₂ antibodies and/or anti-OSMR Beta fragments (e.g., antigen-binding portion thereof). The co-expression can occur by using either the same or different plasmids in the same host. Genes for

both H and L chains and/or anti-OSMR Beta fragments can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with an H chain plasmid containing a
5 second selectable marker. Cell lines producing anti-OSMR Beta amino acids sequences and/or H₂L₂ molecules *via* either route could be transfected with plasmids encoding additional copies of peptides, H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled H₂L₂ antibody molecules or enhanced stability of the transfected cell lines.

10

For long-term, high-yield production of recombinant antibodies, stable expression may be used. For example, cell lines, which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with immunoglobulin expression cassettes and a selectable marker. Following the
15 introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into a chromosome and grow to form foci which in turn can be cloned and expanded into cell lines. Such engineered cell lines may be particularly useful in screening and evaluation
20 of compounds/components that interact directly or indirectly with the antibody molecule.

Once an antibody of the invention has been produced, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly affinity for the specific antigen after Protein A, and sizing
25 column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In many embodiments, antibodies are secreted from the cell into culture medium and harvested from the culture medium.

Pharmaceutical Applications

30 The anti-OSMR Beta antibodies or antigen-binding portions thereof of the present invention can be used for example in the treatment of various conditions in companion animals, such as dogs and cats. These conditions include pruritic conditions, allergic conditions, inflammatory conditions, fibrotic conditions, and pain associated with inflammation. Specific but non-limiting examples of these types of conditions are disclosed herein. It is to be understood that in some

cases, a particular disorder may be considered to fall under more than only one category. For example, certain allergic conditions can be considered inflammatory conditions as well. More specifically, the invention further provides for a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody or peptide according to the invention. The antibody can be a chimeric, heterochimeric, caninized, or
5 felinized antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are also envisioned. The antibody and pharmaceutical compositions thereof of this invention are useful for parenteral administration, e.g., subcutaneously, intramuscularly or intravenously.

10

Anti-OSMR Beta antibodies and/or antigen-binding portions thereof of the present invention can be administered either as individual therapeutic agents or in combination with other therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical
15 practice.

Administration of the antibodies disclosed herein may be carried out by any suitable means, including parenteral injection (such as intraperitoneal, subcutaneous, or intramuscular injection), orally, or by topical administration of the antibodies (typically carried in a pharmaceutical
20 formulation) to an airway surface. Topical administration to an airway surface can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler). Topical administration of the antibodies to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid and liquid particles) containing the antibodies as an aerosol suspension, and then causing the subject to
25 inhale the respirable particles. Methods and apparatus for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed. Oral administration may be, for example, in the form of an ingestible liquid or solid formulation.

In some desired embodiments, the antibodies are administered by parenteral injection. For
30 parenteral administration, anti-OSMR Beta antibodies or antigen-binding portions thereof can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. For example the vehicle may be a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, such as an aqueous carrier such vehicles are water, saline, Ringer's solution, dextrose solution, trehalose or sucrose

- solution, or 5% serum albumin, 0.4% saline, 0.3% glycine and the like. Liposomes and nonaqueous vehicles such as fixed oils can also be used. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable
- 5 auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually
- 10 at or at least about 1% to as much as 15% or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.
- 15 Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, REMINGTON'S PHARMA. SCI. (15th ed., Mack Pub. Co., Easton, Pa., 1980).

- The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable
- 20 carrier prior to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss and that use levels may have to be adjusted to compensate.

- 25 The compositions containing the present antibodies, or a cocktail thereof can be administered for prevention of recurrence and/or therapeutic treatments for existing disease. Suitable pharmaceutical carriers are described in the most recent edition of REMINGTON'S PHARMACEUTICAL SCIENCES, a standard reference text in this field of art.

- 30 In therapeutic application, compositions are administered to a subject already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose" or a "therapeutically effective amount". Amounts effective for this use will depend upon the severity of the disease and the general state of the subject's own immune system, but

generally range from about 0.1 mg antibody per kg body weight to about 10 mg antibody per kg body weight, preferably about 0.3 mg antibody per kg of body weight to about 5 mg of antibody per kg of body weight. In view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present canine-like and feline-like antibodies of this invention, it may be possible to administer substantial excesses of these antibodies.

The dosage administered will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms kind of concurrent treatment, frequency of treatment, and the effect desired.

As a non-limiting example, treatment of IL-31-related or OSM-related pathologies in dogs or cats can be provided as a biweekly or monthly dosage of anti-OSMR Beta antibodies of the present invention in the dosage range described above.

Example antibodies for canine or feline therapeutic use are high affinity (these may also be high avidity) antibodies, and fragments, regions and derivatives thereof having potent *in vivo* anti-OSMR Beta activity, according to the present invention.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating veterinarian. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the subject.

25

Diagnostic Applications

The present invention also provides the above anti-OSMR Beta antibodies and antigen-binding portions thereof for use in diagnostic methods for detecting OSMR Beta in companion animals known to be or suspected of having an OSM and/or IL-31-mediated condition, such as a pruritic condition, an allergic condition, an inflammatory condition, a fibrotic condition, or pain associated with inflammation.

Anti-OSMR Beta antibodies and/or antigen-binding portions thereof of the present invention are useful for immunoassays which detect or quantitate OSMR Beta, or anti-OSMR Beta antibodies,

in a sample. An immunoassay for OSMR Beta typically comprises incubating a clinical or biological sample in the presence of a detectably labeled high affinity (or high avidity) anti-OSMR Beta antibody of the present invention capable of selectively binding to OSMR Beta, and detecting the labeled antibody which is bound in a sample. Various clinical assay procedures are well known in the art. See, e.g., IMMUNOASSAYS FOR THE 80'S (Voller et al., eds., Univ. Park, 1981). Such samples include tissue biopsy, blood, serum, and fecal samples, or liquids collected from animal subjects and subjected to ELISA analysis as described below.

In some embodiments, the binding of antigen to antibody is detected without the use of a solid support. For example, the binding of antigen to antibody can be detected in a liquid format.

In other embodiments, an anti-OSMR Beta antibody can, for example, be fixed to nitrocellulose, or another solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled OSMR Beta-specific antibody. The solid phase support can then be washed with the buffer a second time to remove unbound labeled antibody. The amount of bound label on the solid support can then be detected by known method steps.

"Solid phase support" or "carrier" refers to any support capable of binding peptide, antigen, or antibody. Well-known supports or carriers, include glass, polystyrene, polypropylene, polyethylene, polyvinylidene fluoride (PVDF), dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material can have virtually any possible structural configuration so long as the coupled molecule is capable of binding to OSMR Beta or an anti-OSMR Beta antibody. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface can be flat, such as a sheet, culture dish, test strip, etc. For example, supports may include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody, peptide or antigen, or can ascertain the same by routine experimentation.

Well known method steps can determine binding activity of a given lot of anti-OSMR Beta antibody. Those skilled in the art can determine operative and optimal assay conditions by routine experimentation.

Detectably labeling an OSMR Beta-specific antibody can be accomplished by linking to an enzyme for use in an enzyme immunoassay (EIA), or enzyme-linked immunosorbent assay (ELISA). The linked enzyme reacts with the exposed substrate to generate a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the OSMR Beta-specific antibodies of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

By radioactively labeling the OSMR Beta-specific antibodies, it is possible to detect OSMR beta through the use of a radioimmunoassay (RIA). See Work et al., LAB. TECHNIQUES & BIOCHEM. 1N MOLEC. Bio. (No. Holland Pub. Co., NY, 1978). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention include: ^3H , ^{125}I , ^{131}I , ^{35}S , ^{14}C , and ^{125}I .

It is also possible to label the OSMR Beta-specific antibodies with a fluorescent compound. When the fluorescent labeled antibody is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The OSMR Beta-specific antibodies can also be detectably labeled using fluorescence-emitting metals such as ^{125}Eu , or others of the lanthanide series. These metals can be attached to the OSMR Beta-specific antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediamine-tetraacetic acid (EDTA).

The OSMR Beta-specific antibodies also can be detectably labeled by coupling to a chemiluminescent compound. The presence of the chemiluminescently labeled antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of useful chemiluminescent labeling compounds are luminol,

isoluminol, therrromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound can be used to label the OSMR Beta-specific antibody, portion, fragment, polypeptide, or derivative of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Detection of the OSMR Beta-specific antibody, portion, fragment, polypeptide, or derivative can be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorimetric methods which employ a substrate for the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

For the purposes of the present invention, the OSMR Beta which is detected by the above assays can be present in a biological sample. Any sample containing OSMR Beta may be used. For example, the sample is a biological fluid such as, for example, blood, serum, lymph, urine, feces, inflammatory exudate, cerebrospinal fluid, amniotic fluid, a tissue extract or homogenate, and the like. The invention is not limited to assays using only these samples, however, it being possible for one of ordinary skill in the art, in light of the present specification, to determine suitable conditions which allow the use of other samples.

In situ detection can be accomplished by removing a histological specimen from an animal subject, and providing the combination of labeled antibodies of the present invention to such a specimen. The antibody (or portion thereof) may be provided by applying or by overlaying the labeled antibody (or portion) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of OSMR Beta but also the distribution of OSMR Beta in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

The antibody, fragment or derivative of the present invention can be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support that is insoluble in the fluid being tested and a quantity of detectably labeled soluble antibody is
5 added to permit detection and/or quantification of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

The antibodies may be used to quantitatively or qualitatively detect the OSMR Beta in a sample or to detect presence of cells that express the OSMR Beta. This can be accomplished by
10 immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with fluorescence microscopy, flow cytometric, or fluorometric detection. For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the antibody, such as antibodies specific for canine or feline immunoglobulin constant regions.
15 Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays, such as those discussed previously are available and are well known to those skilled in the art.

20 In one embodiment, the diagnostic method for detecting OSMR Beta is a lateral flow immunoassay test. This is also known as the immunochromatographic assay, Rapid ImmunoMigration (RIM™) or strip test. Lateral flow immunoassays are essentially immunoassays adapted to operate along a single axis to suit the test strip format. A number of variations of the technology have been developed into commercial products, but they all operate
25 according to the same basic principle. A typical test strip consists of the following components: (1) sample pad – an absorbent pad onto which the test sample is applied; (2) conjugate or reagent pad – this contains antibodies specific to the target analyte conjugated to colored particles (usually colloidal gold particles, or latex microspheres); (3) reaction membrane – typically a hydrophobic nitrocellulose or cellulose acetate membrane onto which anti-target
30 analyte antibodies are immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing antibodies specific for the conjugate antibodies); and (4) wick or waste reservoir – a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it. The components of the strip are usually fixed to an inert backing material and may be presented in a simple dipstick

format or within a plastic casing with a sample port and reaction window showing the capture and control zones.

There are two main types of lateral flow immunoassay used in microbiological testing: double
5 antibody sandwich assays and competitive assays. In the double antibody sandwich format, the
sample migrates from the sample pad through the conjugate pad where any target analyte
present will bind to the conjugate. The sample then continues to migrate across the membrane
until it reaches the capture zone where the target/conjugate complex will bind to the immobilized
antibodies producing a visible line on the membrane. The sample then migrates further along
10 the strip until it reaches the control zone, where excess conjugate will bind and produce a
second visible line on the membrane. This control line indicates that the sample has migrated
across the membrane as intended. Two clear lines on the membrane is a positive result. A
single line in the control zone is a negative result. Competitive assays differ from the double
antibody sandwich format in that the conjugate pad contains antibodies that are already bound
15 to the target analyte, or to an analogue of it. If the target analyte is present in the sample it will
therefore not bind with the conjugate and will remain unlabeled. As the sample migrates along
the membrane and reaches the capture zone an excess of unlabeled analyte will bind to the
immobilized antibodies and block the capture of the conjugate, so that no visible line is
produced. The unbound conjugate will then bind to the antibodies in the control zone producing
20 a visible control line. A single control line on the membrane is a positive result. Two visible lines
in the capture and control zones is a negative result. However, if an excess of unlabeled target
analyte is not present, a weak line may be produced in the capture zone, indicating an
inconclusive result. There are a number of variations on lateral flow technology. The capture
zone on the membrane may contain immobilized antigens or enzymes - depending on the target
25 analyte - rather than antibodies. It is also possible to apply multiple capture zones to create a
multiplex test. For example, commercial test strips able to detect both EHEC Shiga toxins ST1
and ST2 separately in the same sample have been developed.

Importantly, the antibodies of the present invention may be helpful in diagnosing a pruritic
30 condition, an allergic condition, an inflammatory disorder, a fibrotic disorder, pain associated
with inflammation, or combinations thereof in dogs or cats. More specifically, the antibody of the
present invention may identify the overexpression of OSMR Beta in companion animals. Thus,
the antibody of the present invention may provide an important immunohistochemistry tool.

The antibodies of the present invention may be used on antibody arrays, highly suitable for measuring gene expression profiles.

Kits

5 Also included within the scope of the present invention are kits for practicing the subject methods. The kits at least include one or more of the antibodies of the present invention, a nucleic acid encoding the same, or a cell containing the same. In one embodiment, an antibody of the present invention may be provided, usually in a lyophilized form, in a container. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are typically included
10 in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like. Generally, these materials will be present in less than 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may
15 be present in from about 1% to 99% wt. of the total composition. Where a second antibody capable of binding to the primary antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above. The kit will generally also include a set of instructions for use.

20

In one embodiment, a kit according to the present invention is a test strip kit (lateral flow immunoassay kit) useful for detecting canine or feline OSMR Beta protein in a sample. Such a test strip will typically include a sample pad onto which the test sample is applied; a conjugate or reagent pad containing an antibody specific to canine or feline OSMR Beta, wherein the
25 antibody is conjugated to colored particles (usually colloidal gold particles); a reaction membrane onto which anti-OSMR Beta antibodies are immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing antibodies specific for the conjugate antibodies); and a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it. The test strip kit will
30 generally also include directions for use.

Cell-based Assays for Assessing Functional Activity of Canine or Feline OSMR Beta Inhibitor Candidates

The present invention also provides a cell-based assay for assessing the functional activity of a

- canine or feline OSMR Beta inhibitor candidate. This method includes: providing cells that endogenously express receptors for and are responsive to canine or feline OSM; incubating canine or feline OSM in the presence of a composition comprising an OSMR Beta inhibitor candidate or a vehicle control; treating the cells with the incubated canine or feline OSM;
- 5 measuring the direct phosphorylation of a protein induced by the canine or feline OSM treatment; and determining whether the composition comprising the canine or feline OSMR Beta inhibitor candidate inhibited the canine or feline OSM induced direct protein phosphorylation relative to the vehicle control.
- 10 In one embodiment, the measuring step comprises measuring direct phosphorylation of a STAT protein. In one specific embodiment, the STAT protein is STAT3.

In another embodiment of the cell-based assay, the cells are monocytes and/or macrophages. For example, in one embodiment, the cells are canine DH82 cells. DH82 cells are a

15 macrophage-monocyte cell line from a dog with malignant histiocytosis.

In one embodiment, the direct protein phosphorylation induced by the canine or feline OSM treatment results in an increase in signal. In another embodiment, the inhibition of the direct protein phosphorylation induced by the canine or feline OSMR Beta inhibitor candidate results in

20 a decrease in signal.

In one embodiment, the canine or feline OSMR Beta inhibitor candidate is a recombinant canine or feline anti-OSMR Beta antibody. In one specific embodiment, the recombinant canine or feline anti-OSMR Beta antibody is contained in a hybridoma culture supernatant. In other

25 embodiments, the canine or feline OSMR Beta inhibitor candidate is a small molecule pharmaceutical compound.

In one embodiment, the canine or feline OSM is co-incubated with the canine or feline OSMR Beta inhibitor candidate prior to treating the cells with the canine or feline OSM. In another

30 embodiment, the cells are preincubated with a gamma interferon prior to the canine or feline OSM treatment for a period of time sufficient to increase OSM Beta receptor expression. In one embodiment, the cells are preincubated with canine gamma interferon. In some embodiments, following the preincubation with canine gamma interferon, the cells are subsequently serum starved prior to the canine or feline OSM treatment.

In some embodiments, a canine or feline OSMR Beta inhibitor candidate that inhibits greater than 50% of the direct protein phosphorylation relative to the vehicle control is selected for further purification and/or characterization. In one embodiment, the method can further include identifying the IC₅₀ values of the canine or feline OSMR Beta inhibitor candidate *via* the cell-based assay for any that candidates that inhibited greater than 50% of the direct protein phosphorylation relative to the vehicle control, for example.

In one embodiment, inhibition of the canine or feline OSM induced direct protein phosphorylation observed in the assay correlates with inhibition of an IL-31-mediated or OSM-mediated condition in dogs or cats. Such disorders include, but are not limited to, the IL-31-mediated or OSM-mediated pruritic, allergic, inflammatory, and fibrotic disorders described herein, as well as OSM-mediated inflammatory pain, such as osteoarthritis pain.

In one specific embodiment, the present invention provides a cell-based assay for assessing the functional activity of a canine or feline IL-31 inhibitor candidate, including: providing canine DH82 cells that endogenously express receptors for and are responsive to canine or feline OSM; incubating canine or feline OSM in the presence of a composition comprising an OSMR Beta inhibitor candidate or a vehicle control; treating the DH82 cells with the incubated canine or feline OSM; measuring a biological activity in the DH82 cells induced by the canine or feline OSM treatment; and determining whether the composition comprising the canine or feline OSMR Beta inhibitor candidate inhibited the canine or feline OSM induced biological activity relative to the vehicle control. This cell-based assay is referred to as the DH82 assay below.

In one embodiment of the DH82 assay, the biological activity is direct phosphorylation of a protein induced by the canine or feline OSM treatment. In one embodiment of the DH82 assay, the protein is a STAT protein, such as STAT3. In another embodiment of the DH82 assay, the direct protein phosphorylation induced by canine or feline OSM treatment results in an increase in signal. In another embodiment of the DH82 assay, the inhibition of the direct protein phosphorylation induced by the canine or feline OSMR Beta inhibitor candidate results in a decrease in signal.

In a further embodiment of the DH82 assay, the canine or feline OSMR Beta inhibitor candidate is a recombinant canine or feline anti-OSMR Beta antibody. Such a recombinant canine or feline anti-OSMR Beta antibody may be contained in a hybridoma culture supernatant, for example. In

other embodiments of the DH82 assay, the canine or feline OSMR Beta inhibitor candidate is a small molecule pharmaceutical compound.

5 In another embodiment of the DH82 assay, the canine or feline OSM is co-incubated with the canine or feline OSMR Beta inhibitor candidate prior to treating the DH82 cells with the canine or feline OSM. In a further embodiment of the DH82 assay, the DH82 cells are preincubated with a gamma interferon, such as, but not limited to, canine gamma interferon prior to the canine or feline OSM treatment for a period of time sufficient to increase OSMR Beta receptor expression. In one embodiment, the DH82 cells are subsequently serum starved prior to the
10 canine or feline OSM treatment.

In one specific embodiment of the DH82 assay, a canine or feline OSMR Beta inhibitor candidate that inhibits greater than 50% of the direct protein phosphorylation relative to the vehicle control is selected for further purification and/or characterization. In one embodiment, for
15 example, the method can include identifying the IC50 values of the canine or feline OSMR Beta inhibitor candidate *via* the DH82 cell-based assay.

In one embodiment of the DH82 assay, inhibition of the canine or feline OSM induced direct protein phosphorylation observed in the assay correlates with inhibition of an IL-31-mediated or
20 OSM-mediated condition in dogs or cats. Such disorders include, but are not limited to, the IL-31-mediated or OSM-mediated pruritic, allergic, inflammatory, and fibrotic disorders described herein, as well as OSM-mediated inflammatory pain, such as osteoarthritis pain.

The invention will now be described further by the non-limiting examples below.
25

EXAMPLES

Example 1. Generation of recombinant proteins used for this work

Recombinant proteins were generated for the purpose of generating antibodies and to assess the affinity and potency of the antibody candidates. By homology to the human homologs, the
30 cytokine binding, Ig-like, and fibronectin III domains were identified for canine and feline OSMR. Synthetic DNA constructs were designed for optimal expression of canine (SEQ ID NO: 107; Canine_OSMR_hIgG1_Fc) the corresponding nucleotide sequence for which is (SEQ ID NO: 108; Canine_OSMR_hIgG1_Fc) and feline (SEQ ID NO: 112; Feline_OSMR_hIgG1_Fc) the corresponding nucleotide sequence for which is (SEQ ID NO: 113; Feline_OSMR_hIgG1_Fc)

OSMR proteins as human IgG1 Fc fusions. A synthetic DNA construct was also designed for optimal expression of the feline OSM gene (SEQ ID NO: 110; Feline_OSM_hIgG1_Fc) the corresponding nucleotide sequence for which is (SEQ ID NO: 111; Feline_OSM_hIgG1_Fc). Canine OSM was purchased from Kingfisher Biotech, Inc. (Saint Paul, MN) and the protein sequence is (SEQ ID NO: 109; Canine_OSM). All synthetic cassettes were cloned into pcDNA3.1 using standard molecular biology methods and expressed in one of two mammalian suspension cell systems, Freestyle 293F (Human Embryonic Kidney) cells or EXPICHO-S (Chinese Hamster Ovary) cells.

Suspension cells were maintained in Freestyle 293 expression medium (Gibco) between 0.15 and approximately 2.5×10^6 cells/ml. On transfection day the cells were diluted to 1.0×10^6 cells/ml and transfected with a mixture of plasmid DNA and FectoPRO (Polyplus Transfection) reagent described in the FectoPro Protocol following condition C. Approximately 24 hours later, deviating from the FectoPro protocol, a feed consisting of 20% w/v Tryptone, diluted in Freestyle 293 medium, was added to each culture. Following 7 days of incubation, the cultures were harvested and clarified. For suspension EXPICHO-S, cells were maintained in EXPICHO expression medium (Gibco) between 0.14 and 8.0×10^6 cells/ml. Cells are diluted following the ExpiCHO protocol user manual on Day -1 and transfection day. Diluted cells were transfected as described in the protocol using reagents sourced from ExpiFectamine CHO Transfection Kit (Gibco) following Max Titer conditions. Following 12-14 days of incubation, the cultures were harvested and clarified.

For purification of hexahistidine tagged proteins, conditioned media was adjusted to 500 mM sodium chloride, 5 mM imidazole, and pH 7.4, and loaded onto IMAC resin (either Ni Sepharose Excel (GE Healthcare) or HisPur Cobalt (Thermo Scientific)) which had been pre-equilibrated with buffer A (5 mM imidazole, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.4). Following load, IMAC resin was washed extensively with Buffer A and then eluted via increasing concentrations of imidazole from 5 to 500 mM, in the same buffer. Fractions were evaluated by SDS-PAGE. Pools were made and dialyzed out of imidazole into the final buffer. Fc fusion proteins and antibodies were purified using Protein A chromatography. Conditioned media was loaded onto MabSelect Sure LX (GE Healthcare) or AmMag Protein A Magnetic beads (Genscript) which had been pre-equilibrated with PBS. Following sample load, the resin was washed with PBS and then with 20 mM sodium acetate, pH 5.5. In the cases where magnetic beads were used, 0.05% tween-20 was added to the equilibration and wash buffers. Using either method, the samples were eluted from the

column with 20 mM acetic acid, pH 3.5. Following elution, pools were made and neutralized with the addition of 1 M sodium acetate to 4%. Depending on available volume and intended use, samples were sometimes exchanged into a final buffer (e.g. PBS, other). Final protein concentration was measured by absorbance at 280 nm or by BCA protein assay.

- 5 Synthesis and characterization of the canine IL-31 protein (SEQ ID NO: 123; Canine_IL31) the corresponding nucleotide sequence for which is (SEQ ID NO: 124; Canine_IL31) was described previously (US Patent No. 8,790,651 to Bammert, et al.). Synthesis and characterization of the feline IL-31 protein (SEQ ID NO: 125; Feline_IL31) the corresponding nucleotide sequence for which is (SEQ ID NO: 126; Feline_IL31) was described previously (US Patent Application No. 10 20190284272 to Bammert, et al.).

Example 2. Identification of mouse monoclonal antibodies recognizing canine and/or feline OSMR

- A mixture of recombinant canine and feline OSMR proteins represented by (SEQ ID NO: 107; Canine_OSMR_hlgG1_Fc), the corresponding nucleotide sequence for which is (SEQ ID NO: 15 108; Canine_OSMR_hlgG1_Fc) and (SEQ ID NO: 112; Feline_OSMR_hlgG1_Fc), the corresponding nucleotide sequence for which is (SEQ ID NO: 113; Feline_OSMR_hlgG1_Fc) respectively, were used to immunize female AJ and CD1 mice for the purpose of generating monoclonal antibodies. Mice were immunized using a 28 Day Rapid Immunization Protocol (RIMMS) which includes a series of low-dosage immunizations administered over a two-week period. Serum antibody titers from immunized mice were determined using an enzyme linked immuno- 20 sorbent assay (ELISA). Canine or feline OSMR (50 ng/well) was immobilized to polystyrene microplates and used as a capture antigen. A separate ELISA was performed to determine the antibody response to an unrelated human IgG Fc fusion protein. Prior to assay, each plate was blocked using casein and serum from immunized mice was diluted in phosphate buffered saline 25 with 0.05% tween-20 (PBST). The presence of anti-OSMR (or anti human IgG Fc) antibodies was detected with an anti-mouse HRP labeled secondary antibody. Following addition of a chromogenic substrate (SureBlue Reserve TMB 1-Component Microwell Peroxidase Substrate, KPL, Inc., Gaithersburg, MD) and after a 10-minute incubation at room temperature (RT) the reaction was stopped with the addition of 100 μ L of 0.1 N HCl. The absorbance of each well was 30 determined at an optical density (OD) of 450 nm.

Test bleeds were taken on Day 20 and antisera samples were assessed to determine whether a fusion-ready titer, as defined by an OD>0.1 above background at 1:31K serum dilution, has

- been reached. In addition, a human IgG absorption assay was utilized to evaluate anti-OSMR specificity. Serum samples were spiked with pooled/purified human IgG prior to application on the ELISA plate. Antibodies that recognize the “irrelevant” human IgG Fc component of the fusion proteins were absorbed from the system; antibodies that are OSMR-specific bound OSMR on the ELISA plate. Post-absorption anti-OSMR signal was indicative of target-specific responses. A single responsive CD-1 mouse was selected having a high specific titer to both canine and feline OSMR following absorption of the anti-human IgGs. This mouse received a pre-fusion boost and donor splenocytes were used for fusion on day 28.
- Hybridoma supernatants were screened for antibodies that bind to canine and/or feline OSMR proteins but do not bind to the irrelevant human IgG Fc by ELISA. Candidate mouse anti OSMR hybridomas that selectively bound canine and/or feline OSMR were further subcloned to generate a hybridomas producing homogeneous antibody and for sequencing of the variable heavy and light chains. Cells producing antibodies with these desired properties were chosen for sequence analysis of RNA transcripts of the variable heavy (VH) and variable light (VL) IgG chains.

Example 3. Method to determine affinity of anti-OSMR antibodies for OSMR using surface plasmon resonance

- The affinity with which candidate mAbs bind canine, feline, and human (SEQ ID NO: 122; Human_OSMR) (R&D Systems, Minneapolis, MN) OSMR was determined using surface plasmon resonance (SPR) on a Biacore system (Biacore Life Sciences (GE Healthcare), Uppsala, Sweden). To avoid affinity differences associated with differential surface preparation that can occur when immobilizing antibodies to surfaces; OSMR from each species was directly conjugated to individual surfaces. Immobilization was obtained by amine coupling 5 µg/mL OSMR using N-hydroxysuccinimide (NHS)/1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) chemistry. Chips were quenched with ethanolamine and the affinity with which all candidate mAbs bound to the immobilized OSMR was evaluated. All curves were fit to a 1:1 model. Affinity constants (KD) less than 1×10^{-11} M (1E-11 M) are below the lower limit of quantitation of detection for the instrument. Results for affinity measurements are described herein.

Example 4. Method to determine potency of anti-OSMR antibodies assessed by inhibition of canine and feline IL-31 induced pSTAT3 signaling in canine and feline macrophage cells

To identify candidates with inhibitory activity, antibodies were assessed for their ability to affect IL-31-mediated STAT3 phosphorylation in either a canine or feline cell-based assay. STAT3 phosphorylation was determined in canine DH-82 (ATCC® CRL-10389™) or feline Fcwf-4 macrophage-like cells (ATCC CRL-2787). DH82 and Fcwf-4 cells were primed with canine
5 interferon gamma (R&D Systems, Minneapolis, MN) at 10 ng/mL for 24 hours or feline interferon gamma (R&D Systems, Minneapolis, MN) at 125 ng/mL for 96 hours, respectively, to increase receptor expression. Both cell types were serum starved for 2 hours prior to IL-31 and mAb treatment. Using two independent methods, all candidate mAbs were evaluated for their ability to inhibit either 1 µg/mL canine or 42 ng/mL feline IL-31 induced STAT3 phosphorylation.
10 Assays were also run to demonstrate cross-reactivity of canine and feline cytokines and cross-functionality of the antibodies ability to inhibit signaling in both species. To ensure complex formation, a one-hour co-incubation of mAb and IL-31 cytokine prior to cell stimulation was completed. IL-31 cell stimulation was carried out for five minutes. STAT3 phosphorylation was measured using AlphaLISA SureFire ULTRA™ technology (Perkin Elmer, Waltham, MA). In the
15 case where antibody concentration and purity are unknown, hybridoma supernatants were qualitatively measured for their ability to inhibit STAT3 phosphorylation following a one-hour co-incubation with 1 µg/ml canine or 42 ng/ml feline IL-31. The potency of individual monoclonal antibodies defined by their ability to inhibit IL-31 mediated STAT3 phosphorylation in these assays was considered the key selection criteria for further advancement of select antibodies.
20 The term potency refers to the IC₅₀ value calculated from these assays and is the concentration of the antibody where signaling induced by IL-31 is reduced to one half its maximal value. Increased potency described herein correlates to a lower IC₅₀ value.

Example 5. Method to determine potency of anti-OSMR antibodies assessed by inhibition of canine and feline OSM induced pSTAT3 signaling in canine and feline macrophage cells

25 To identify candidates with inhibitory activity, antibodies were assessed for their ability to affect OSM-mediated STAT3 phosphorylation in either a canine or feline cell-based assay. STAT3 phosphorylation was determined in canine DH-82 (ATCC CRL-10389™) or feline Fcwf-4 macrophage-like cells (ATCC CRL-2787). To assess the dynamic range of these assays, a
30 dose response curve using canine and feline OSM was assessed in both cell types. Cells were serum starved for 2 hours followed by treatment for 10 minutes with a nine point, half log curve from 1 µg/mL to 0.0001 µg/mL canine or feline OSM to induced STAT3 phosphorylation. Reactions were quenched with lysis buffer and STAT3 phosphorylation was

measured using AlphaLISA SureFire ULTRA™ technology (Perkin Elmer, Waltham, MA). EC₅₀ values were determined as the concentration of OSM protein which induces a 50 percent maximal signal. Figures 1A and 1B show the dose-response curves and EC₅₀ values for canine and feline OSM in canine DH-82 and feline Fcwf-4 cells respectively.

5

To determine the potency of candidate antibodies by assaying their ability to inhibit OSM-mediated STAT3 phosphorylation, both canine and feline assays were performed. Cells were serum starved for 2 hours prior to OSM and mAb treatment. Using two independent methods, all candidate mAbs were evaluated for their ability to inhibit 0.02 µg/mL canine OSM induced
10 STAT3 phosphorylation. Assays were conducted allowing 30-minute incubation for DH-82 or 20-minute incubation for Fcwf-4 cells of the anti-OSMR mAb with the serum starved cells. At this time the supernatant was removed and media containing 0.02 µg/mL canine OSM added for cell stimulation for ten minutes. The reaction was quenched with lysis buffer and STAT3 phosphorylation was measured. In the case where antibody concentration and purity are
15 unknown, hybridoma supernatants were qualitatively measured for their ability to inhibit STAT3 phosphorylation following a 30 minute or 20-minute incubation on cells as above, followed by a 0.02 µg/mL stimulation with canine OSM. The potency of individual monoclonal antibodies defined by their ability to inhibit OSM mediated STAT3 phosphorylation in these assays was considered the key selection criteria for further advancement of select antibodies. The term
20 potency refers to the IC₅₀ value calculated from these assays and is the concentration of the antibody where signaling induced by OSM is reduced to one half its maximal value. Increased potency described herein correlates to a lower IC₅₀ value.

25 Example 6. Selection of anti-OSMR monoclonal antibodies capable of neutralizing IL-31 and OSM mediated STAT phosphorylation in canine and feline cells

Mouse anti-OSMR antibodies were selected based on the criteria outline in figure 2. Mice were immunized with a combination of canine and feline OSMR proteins which contain the three cytokine binding domains and a single fibronectin III domain fused to a human IgG1 Fc to
30 facilitate expression and purification. Antibodies produced from the hybridomas were screened for binding by ELISA to canine and feline OSMR and counter screened against an unrelated human IgG. Hybridomas producing antibodies which bound to canine and/or feline OSMR and did not bind to human IgG were selected for further analysis. Exhausted hybridoma supernatants were generated from these initial hits and antibody was purified for further

analysis. Antibodies from these non-clonal hybridomas were initially assayed for binding to canine and feline OSMR using Biacore to confirm results from the ELISA screen. These hybridoma candidates were further tested for their ability to inhibit IL-31 and OSM mediated STAT phosphorylation in canine DH82 and feline Fcwf-4 cells. Hybridoma candidates that
5 bound to canine and/or feline OSMR and had some inhibitory activity in one of the IL-31 and OSM cell based assays were selected for subcloning. Subcloning of hybridoma candidates was performed by limiting dilution of cells and monoclonality was confirmed. Supernatants from these cells producing monoclonal antibodies were confirmed for binding to canine and/or feline OSMR protein by ELISA and cells producing these antibodies were used to isolate RNA for
10 sequence analysis of the variable heavy and variable light IgG chains. Purified monoclonal antibodies from these cultures were also tested for binding to human OSMR. None of the antibody candidates described herein bound to human OSMR.

This hybridoma campaign initially produced five monoclonal antibodies with unique variable
15 heavy and variable light combinations. These mouse anti-OSMR antibodies are 02D09 with the variable heavy chain sequence (SEQ ID NO: 31; MU_02D09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 32; MU_02D09_VH) and a variable light chain sequence (SEQ ID NO: 33; MU_02D09_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 34; MU_02D09_VL); 09E09 with the variable heavy chain sequence (SEQ ID NO: 35;
20 MU_09E09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 36; MU_09E09_VH) and a variable light chain sequence (SEQ ID NO: 37; MU_09E09_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 38; MU_09E09_VL); 10F07 with the variable heavy chain sequence (SEQ ID NO: 39; MU_10F07_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 40; MU_10F07_VH) and a variable light chain
25 sequence (SEQ ID NO: 41; MU_10F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 42; MU_10F07_VL); 14C04 with the variable heavy chain sequence (SEQ ID NO: 43; MU_14C04_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 44; MU_14C04_VH) and a variable light chain sequence (SEQ ID NO: 45; MU_14C04_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 46; MU_14C04_VL); and 19F07
30 with the variable heavy chain sequence (SEQ ID NO: 47; MU_19F07_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 48; MU_19F07_VH) and a variable light chain sequence (SEQ ID NO: 49; MU_19F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 50; MU_19F07_VL). Figure 3 shows the clustalW alignment of these variable heavy and light chains with the CDRs highlighted in black boxes.

TABLE 1

<u>Antibody ID</u>	<u>Type</u>	<u>KD (M)</u>		
		<u>Human OSMR</u> (SEQ ID NO: 122)	<u>Canine OSMR</u> (SEQ ID NO: 107)	<u>Feline OSMR</u> (SEQ ID NO: 112)
Mu_02D09	Mouse	no binding	no binding	8.96E-09
Mu_09E09	Mouse	no binding	7.87E-09	3.75E-09
Mu_10F07	Mouse	no binding	2.03E-09	1.59E-09
Mu_14C04	Mouse	no binding	1.42E-08	no binding
Mu_19F07	Mouse	no binding	9.51E-10	2.07E-11

5

Table 1. Affinity (KD) of mouse anti-OSMR antibodies to the human, canine, and feline OSMR proteins using Biacore.

10 TABLE 2

<u>Inhibition of IL-31 and OSM pSTAT3 Signaling</u>					
<u>Cell type used:</u>	<u>Canine DH82</u>	<u>Feline IL-31 Fcwf-4</u>	<u>Canine DH82</u>		
<u>Cytokine used:</u>	<u>Canine IL-31</u>	<u>Feline IL-31</u>	<u>Canine OSM</u>	<u>Feline OSM</u>	
	(SEQ ID NO: 123)	(SEQ ID NO: 125)	(SEQ ID NO: 109)	(SEQ ID NO: 110)	
<u>Antibody ID</u>	<u>Antibody type</u>	<u>% inh</u>	<u>% inh</u>	<u>IC₅₀ (μg/ml)</u>	<u>% inh</u>
Mu_02D09	Mouse	0	67	>50	nt
Mu_09E09	Mouse	80	63	>50, 14	nt
Mu_10F07	Mouse	93	91	0.04	nt
Mu_14C04	Mouse	72	0	0.73	nt
Mu_19F07	Mouse	87	90	0.27	nt

nt = not tested

Table 2. Inhibition of IL-31 and OSM mediated pSTAT3 signaling in canine DH82 and feline Fcwf-4 cells with anti-canine and/or anti-feline OSMR antibodies.

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Example 7. Generation of chimeric antibodies

Antibody variable domains are responsible for antigen binding. Grafting of the full variable domain onto respective constant region is expected to have little or no impact on the antibody's ability to bind the OSMR immunogen. To simultaneously confirm that the correct sequence of the heavy and light chain variable regions was identified and to produce homogenous material, expression vectors were designed to produce recombinant chimeric antibodies in mammalian expression systems. Chimeric antibodies described here consist of the variable sequence (both CDR and framework) from the host species antibody grafted onto the respective heavy and light constant regions of a canine or feline IgG molecule (for example; mouse variable: canine constant is referred to as mouse: canine chimera). Synthetic DNA sequences were constructed for the variable heavy (VH) and variable light (VL) sequences of selected antibodies.

For mouse: canine chimeras, each mouse variable region was cloned into a mammalian expression plasmid containing either the canine IgG heavy (SEQ ID NO: 114; Canine_HC_65_1) the corresponding nucleotide sequence for which is (SEQ ID NO: 115; Canine_HC_65_1) or light chain (SEQ ID NO: 116; Canine_LC_Kappa) the corresponding nucleotide sequence for which is (SEQ ID NO: 117; Canine_LC_Kappa) constant regions. For mouse: feline chimeras, each respective variable region was cloned into a mammalian expression plasmid containing either the feline IgG heavy (SEQ ID NO: 118; Feline_HC_AlleleA_1) the corresponding nucleotide sequence for which is (SEQ ID NO: 119; Feline_HC_AlleleA_1) or light chain (SEQ ID NO: 120; Feline_LC_Kappa_G_minus) the corresponding nucleotide sequence for which is (SEQ ID NO: 121; Feline_LC_Kappa_G_minus) constant regions. These antibodies are mouse: canine 02D09 chimera with the variable heavy chain sequence (SEQ ID NO: 31; MU_02D09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 32; MU_02D09_VH) and a variable light chain sequence (SEQ ID NO: 33; MU_02D09_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 34; MU_02D09_VL); mouse: canine 09E09 chimera with the variable heavy chain sequence (SEQ ID NO: 35; MU_09E09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 36; MU_09E09_VH) and a variable light chain sequence (SEQ ID NO: 37; MU_09E09_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 38; MU_09E09_VL); mouse: canine 10F07 chimera with the variable heavy chain sequence (SEQ ID NO: 39; MU_10F07_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 40; MU_10F07_VH) and a variable light chain sequence (SEQ ID NO: 41; MU_10F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 42; MU_10F07_VL); mouse: canine 14C04 chimera with the variable heavy chain sequence (SEQ

ID NO: 43; MU_14C04_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 44; MU_14C04_VH) and a variable light chain sequence (SEQ ID NO: 45; MU_10F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 46; MU_10F07_VL); mouse: canine 19F07 chimera with the variable heavy chain sequence (SEQ ID NO: 47; MU_19F07_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 48; MU_19F07_VH) and a variable light chain sequence (SEQ ID NO: 49; MU_19F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 50; MU_19F07_VL); mouse: feline 02D09 chimera with the variable heavy chain sequence (SEQ ID NO: 31; MU_02D09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 32; MU_02D09_VH) and a variable light chain sequence (SEQ ID NO: 33; MU_02D09_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 34; MU_02D09_VL); mouse: feline 09E09 chimera with the variable heavy chain sequence (SEQ ID NO: 35; MU_09E09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 36; MU_09E09_VH) and a variable light chain sequence (SEQ ID NO: 37; MU_09E09_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 38; MU_09E09_VL); mouse: feline 10F07 chimera with the variable heavy chain sequence (SEQ ID NO: 39; MU_10F07_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 40; MU_10F07_VH) and a variable light chain sequence (SEQ ID NO: 41; MU_10F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 42; MU_10F07_VL); mouse: feline 14C04 chimera with the variable heavy chain sequence (SEQ ID NO: 43; MU_14C04_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 44; MU_14C04_VH) and a variable light chain sequence (SEQ ID NO: 45; MU_14C04_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 46; MU_14C04_VL); and mouse: feline 19F07 chimera with the variable heavy chain sequence (SEQ ID NO: 47; MU_19F07_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 48; MU_19F07_VH) and a variable light chain sequence (SEQ ID NO: 49; MU_19F07_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 50; MU_19F07_VL) These sequences contain unique restriction endonuclease sites, a Kozak consensus sequence and, an N-terminal secretion leader to facilitate expression and secretion of the chimeric recombinant antibody from a mammalian cell line. The plasmids encoding each heavy and light chain, under the control of the CMV promoter, were co-transfected into host cells and purified as described herein.

TABLE 3

Antibody ID	Chimera type	KD (M)								
		Human OSMR (SEQ ID NO: 122)			Canine OSMR (SEQ ID NO: 107)			Feline OSMR (SEQ ID NO: 112)		
		ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	KD (M)	ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	KD (M)	ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	KD (M)
02D09 *	Mouse:Canine		no binding		2.56E+05	3.28E-03	1.28E-08	1.42E+05	9.42E-04	6.65E-09
* 09E09	Mouse:Canine		no binding		1.96E+05	1.04E-03	5.29E-09	2.94E+05	6.97E-04	2.37E-09
10F07	Mouse:Canine		no binding		1.61E+05	2.22E-04	1.38E-09	2.00E+04	4.40E-07	2.20E-11
14C04	Mouse:Canine		nt			nt			nt	
19F07	Mouse:Canine		no binding		1.90E+03	8.40E-04	4.42E-07	4.51E+04	2.00E-03	4.45E-08

nt = not tested

* chimeric 02D09 showed weak binding to
canine OSMR with fast off rate

Table 3. Affinity (KD) of mouse: canine anti-OSMR chimeric antibodies to the human, canine, and feline OSMR proteins using Biacore.

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TABLE 4

Inhibition of IL-31 and OSM pSTAT3 Signaling					
Cell type used:		Canine DH82	Feline IL-31 Fcwf-4	Canine DH82	Feline IL-31 Fcwf-4
Cytokine used:		Canine IL-31 (SEQ ID NO: 123)	Feline IL-31 (SEQ ID NO: 125)	Canine OSM (SEQ ID NO: 109)	Canine OSM (SEQ ID NO: 109)
Antibody ID	Chimera type	IC ₅₀ (μg/ml)	IC ₅₀ (μg/ml)	IC ₅₀ (μg/ml)	IC ₅₀ (μg/ml)
02D09	Mouse:Canine	97.70	IC	IC	IC
* 09E09	Mouse:Canine	47.52	IC	IC	IC
10F07	Mouse:Canine	0.03	0.05	0.20	0.28
14C04	Mouse:Canine	nt	nt	nt	nt
19F07	Mouse:Canine	nt	nt	nt	nt

IC = incomplete curve

nt = not tested

Table 4. Inhibition of IL-31 and OSM mediated pSTAT3 signaling in canine DH82 and feline Fcwf-4 cells with anti-canine and/or anti-feline mouse: canine anti-OSMR chimeric antibodies.

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Example 8. Design and expression of speciated anti OSMR antibodies

The generation of anti-drug antibodies (ADAs) can be associated with loss of efficacy for any biotherapeutic protein including monoclonal antibodies. Comprehensive evaluation of the literature has shown that speciation of monoclonal antibodies can reduce the propensity for mAbs to be immunogenic although examples of immunogenic fully human mAbs and non-immunogenic chimeric mAbs can be found. We describe herein two methods of speciation;

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caninization meaning grafting of mouse CDRs onto frameworks of a *Canis* species (example, *Canis lupus familiaris* or dog) and felinization meaning grafting of mouse CDRs onto frameworks of a *Felis* species (example, *Felis catus* or cat). To help mitigate risks associated with ADA formation for the anti-OSMR monoclonal antibodies provided herein, a caninization and felinization strategy was employed. The caninization and felinization strategy was based on identifying the most appropriate canine or feline germline antibody sequence for CDR grafting. Following extensive analysis of all available germline sequences for both the variable heavy and light chain, germline candidates were selected based on their homology to the mouse anti-OSMR mAbs, and the CDRs from these mouse progenitor mAbs were used to replace native canine or feline CDRs. The objective was to retain high affinity and cell-based activity using canine or feline antibody frameworks to minimize the potential of immunogenicity *in vivo*.

Caninized and felinized mAbs are expressed and characterized for their affinity to canine and feline OSMR and their potency in cell-based assays. In the event that a caninized or felinized antibody loses its ability to bind canine or feline OSMR, a systematic dissection is undertaken to identify; 1) the chain responsible for the loss of function, 2) the framework responsible for the loss of function and 3) the amino acid(s) responsible for the loss function. Speciated antibodies described here consist of the variable sequence (both CDR and framework) expressed with the respective heavy and light constant regions of a canine or feline IgG molecule. Synthetic DNA sequences are constructed for the variable heavy (VH) and variable light (VL) sequences of selected antibodies. These sequences contain unique restriction endonuclease sites, a Kozak consensus sequence and, an N-terminal secretion leader to facilitate expression and secretion of the recombinant antibody from a mammalian cell line. For caninized antibodies, each caninized variable region are cloned into a mammalian expression plasmid containing either the canine IgG heavy (SEQ ID NO: 114; Canine_HC_65_1) the corresponding nucleotide sequence for which is (SEQ ID NO: 115; Canine_HC_65_1) or light chain (SEQ ID NO: 116; Canine_LC_Kappa) the corresponding nucleotide sequence for which is (SEQ ID NO: 117; Canine_LC_Kappa) constant regions. For felinized antibodies, each respective variable region are cloned into a mammalian expression plasmid containing either the feline IgG heavy (SEQ ID NO: 118; Feline_HC_AlleleA_1) the corresponding nucleotide sequence for which is (SEQ ID NO: 119; Feline_HC_AlleleA_1) or light chain (SEQ ID NO: 120; Feline_LC_Kappa_G_minus) the corresponding nucleotide sequence for which is (SEQ ID NO: 121; Feline_LC_Kappa_G_minus) constant regions. The plasmids encoding each heavy and light chain, under the control of the CMV promoter, are co-transfected into host cells, expressed, and purified as described herein.

Feline 02D09 1.1 is the variable heavy chain sequence (SEQ ID NO: 51; FEL_02D09_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 52; FEL_02D09_VH1) and a variable light chain sequence (SEQ ID NO: 55; FEL_02D09_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 56; FEL_02D09_VL1); Feline 02D09 2.1 is the variable heavy chain sequence (SEQ ID NO: 53; FEL_02D09_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 54; FEL_02D09_VH2) and a variable light chain sequence (SEQ ID NO: 55; FEL_02D09_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 56; FEL_02D09_VL1); Feline 02D09 1.2 is the variable heavy chain sequence (SEQ ID NO: 51; FEL_02D09_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 52; FEL_02D09_VH1) and a variable light chain sequence (SEQ ID NO: 57; FEL_02D09_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 58; FEL_02D09_VL2); Feline 02D09 2.2 is the variable heavy chain sequence (SEQ ID NO: 53; FEL_02D09_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 54; FEL_02D09_VH2) and a variable light chain sequence (SEQ ID NO: 57; FEL_02D09_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 58; FEL_02D09_VL2).

Canine 09E09 1.1 is the variable heavy chain sequence (SEQ ID NO: 59; CAN_09E09_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 60; CAN_09E09_VH1) and a variable light chain sequence (SEQ ID NO: 63; CAN_09E09_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 64; CAN_09E09_VL1); Canine 09E09 2.1 is the variable heavy chain sequence (SEQ ID NO: 61; CAN_09E09_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 62; CAN_09E09_VH2) and a variable light chain sequence (SEQ ID NO: 63; CAN_09E09_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 64; CAN_09E09_VL1); Canine 09E09 1.2 is the variable heavy chain sequence (SEQ ID NO: 59; CAN_09E09_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 60; CAN_09E09_VH1) and a variable light chain sequence (SEQ ID NO: 65; CAN_09E09_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 66; CAN_09E09_VL2); Canine 09E09 2.2 is the variable heavy chain sequence (SEQ ID NO: 61; CAN_09E09_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 62; CAN_09E09_VH2) and a variable light chain sequence (SEQ ID NO: 65; CAN_09E09_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 66; CAN_09E09_VL2). Feline 09E09 1.1 is the variable heavy chain sequence (SEQ ID NO: 67; FEL_09E09_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 68; FEL_09E09_VH1) and a variable light chain sequence (SEQ ID NO: 71; FEL_09E09_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 72; FEL_09E09_VL1); Feline 09E09 2.1 is the variable heavy chain sequence (SEQ ID

NO: 69; FEL_09E09_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 70; FEL_09E09_VH2) and a variable light chain sequence (SEQ ID NO: 71; FEL_09E09_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 72; FEL_09E09_VL1); Feline 09E09 1.2 is the variable heavy chain sequence (SEQ ID NO: 67; FEL_09E09_VH1) the
5 corresponding nucleotide sequence for which is (SEQ ID NO: 68; FEL_09E09_VH1) and a variable light chain sequence (SEQ ID NO: 73; FEL_09E09_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 74; FEL_09E09_VL2); Feline 09E09 2.2 is the variable heavy chain sequence (SEQ ID NO: 69; FEL_09E09_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 70; FEL_09E09_VH2) and a variable light chain sequence
10 (SEQ ID NO: 73; FEL_09E09_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 74; FEL_09E09_VL2).

Canine 10F07 1.1 is the variable heavy chain sequence (SEQ ID NO: 75; CAN_10F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 76; CAN_10F07_VH1) and a variable light chain sequence (SEQ ID NO: 77; CAN_10F07_VL1) the corresponding nucleotide
15 sequence for which is (SEQ ID NO: 78; CAN_10F07_VL1); Canine 10F07 2.1 is the variable heavy chain sequence (SEQ ID NO: 79; CAN_10F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 80; CAN_10F07_VH2) and a variable light chain sequence (SEQ ID NO: 77; CAN_10F07_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 78; CAN_10F07_VL1); Canine 10F07 1.2 is the variable heavy chain sequence (SEQ ID
20 NO: 75; CAN_10F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 76; CAN_10F07_VH1) and a variable light chain sequence (SEQ ID NO: 81; CAN_10F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 82; CAN_10F07_VL2); Canine 10F07 2.2 is the variable heavy chain sequence (SEQ ID NO: 79; CAN_10F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 80; CAN_10F07_VH2) and a
25 variable light chain sequence (SEQ ID NO: 81; CAN_10F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 82; CAN_10F07_VL2). Feline 10F07 1.1 is the variable heavy chain sequence (SEQ ID NO: 83; FEL_10F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 84; FEL_10F07_VH1) and a variable light chain sequence (SEQ ID NO: 85; FEL_10F07_VL1) the corresponding nucleotide sequence for which is (SEQ
30 ID NO: 86; FEL_10F07_VL1); Feline 10F07 2.1 is the variable heavy chain sequence (SEQ ID NO: 87; FEL_10F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 88; FEL_10F07_VH2) and a variable light chain sequence (SEQ ID NO: 85; FEL_10F07_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 86; FEL_10F07_VL1); Feline 10F07 1.2 is the variable heavy chain sequence (SEQ ID NO: 83; FEL_10F07_VH1) the

corresponding nucleotide sequence for which is (SEQ ID NO: 84; FEL_10F07_VH1) and a variable light chain sequence (SEQ ID NO: 89; FEL_10F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 90; FEL_10F07_VL2); Feline 10F07 2.2 is the variable heavy chain sequence (SEQ ID NO: 87; FEL_10F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 88; FEL_10F07_VH2) and a variable light chain sequence (SEQ ID NO: 89; FEL_10F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 90; FEL_10F07_VL2).

Canine 19F07 1.1 is the variable heavy chain sequence (SEQ ID NO: 91; CAN_19F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 92; CAN_19F07_VH1) and a variable light chain sequence (SEQ ID NO: 93; CAN_19F07_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 94; CAN_19F07_VL1); Canine 19F07 2.1 is the variable heavy chain sequence (SEQ ID NO: 95; CAN_19F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 96; CAN_19F07_VH2) and a variable light chain sequence (SEQ ID NO: 93; CAN_19F07_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 94; CAN_19F07_VL1); Canine 19F07 1.2 is the variable heavy chain sequence (SEQ ID NO: 91; CAN_19F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 92; CAN_19F07_VH1) and a variable light chain sequence (SEQ ID NO: 97; CAN_19F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 98; CAN_19F07_VL2); Canine 19F07 2.2 is the variable heavy chain sequence (SEQ ID NO: 95; CAN_19F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 96; CAN_19F07_VH2) and a variable light chain sequence (SEQ ID NO: 97; CAN_19F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 98; CAN_19F07_VL2). Feline 19F07 1.1 is the variable heavy chain sequence (SEQ ID NO: 99; FEL_19F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 100; FEL_19F07_VH1) and a variable light chain sequence (SEQ ID NO: 101; FEL_19F07_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 102; FEL_19F07_VL1); Feline 19F07 2.1 is the variable heavy chain sequence (SEQ ID NO: 103; FEL_19F07_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 104; FEL_19F07_VH2) and a variable light chain sequence (SEQ ID NO: 101; FEL_19F07_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 102; FEL_19F07_VL1); Feline 19F07 1.2 is the variable heavy chain sequence (SEQ ID NO: 99; FEL_19F07_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 100; FEL_19F07_VH1) and a variable light chain sequence (SEQ ID NO: 105; FEL_19F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 106; FEL_19F07_VL2); Feline 19F07 2.2 is the variable heavy chain sequence (SEQ ID NO: 103; FEL_19F07_VH2) the

corresponding nucleotide sequence for which is (SEQ ID NO: 104; FEL_19F07_VH2) and a variable light chain sequence (SEQ ID NO: 105; FEL_19F07_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 106; FEL_19F07_VL2).

Canine 14C04 1.1 is the variable heavy chain sequence (SEQ ID NO: 127; CAN_14C04_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 128; CAN_14C04_VH1) and a variable light chain sequence (SEQ ID NO: 131; CAN_14C04_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 132; CAN_14C04_VL1); Canine 14C04 2.1 is the variable heavy chain sequence (SEQ ID NO: 129; CAN_14C04_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 130; CAN_14C04_VH2) and a variable light chain sequence (SEQ ID NO: 131; CAN_14C04_VL1) the corresponding nucleotide sequence for which is (SEQ ID NO: 132; CAN_14C04_VL1); Canine 14C04 1.2 is the variable heavy chain sequence (SEQ ID NO: 127; CAN_14C04_VH1) the corresponding nucleotide sequence for which is (SEQ ID NO: 128; CAN_14C04_VH1) and a variable light chain sequence (SEQ ID NO: 133; CAN_14C04_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 134; CAN_14C04_VL2); Canine 14C04 2.2 is the variable heavy chain sequence (SEQ ID NO: 129; CAN_14C04_VH2) the corresponding nucleotide sequence for which is (SEQ ID NO: 130; CAN_14C04_VH2) and a variable light chain sequence (SEQ ID NO: 133; CAN_14C04_VL2) the corresponding nucleotide sequence for which is (SEQ ID NO: 134; CAN_14C04_VL2).

20 Example 9. Homology modeling of candidate anti-OSMR antibodies and comparison of structural variances between CDRs

Generation of homology models based on known protein structures is useful in understanding the three-dimensional structure of antibodies. Overlaying these protein models through superposition allows direct comparison of the areas where antibodies are alike and different from one another in three-dimensional space. These methods overcome the limitations of comparing proteins as strings of linear amino acid sequences which does not account for the collective representation of each amino acid's physicochemical property with respect to its surroundings. Antibody models of the 5 mouse anti-OSMR antibodies described herein were generated using the Molecular Operating Environment (MOE™) software (Chemical Computing Group, Montreal, Canada) which is capable of creating such models similar to other software that is available. See, for example, Almagro et al.; Antibody Modeling Assessment; *Proteins: Struct. Func. Bioinf.* 79 (2011) 3050–3066, of record.

To compare the structure of the 5 mouse anti-OSMR antibody models, the root-mean-square deviation (RMSD) value was determined from pairwise comparison between each antibodies CDRs which are considered the most critical for antigen binding. The RMSD value is known metric in the art used to determine the likeness of structures in three-dimensional space by comparing the coordinates of their alpha carbon atoms which act as the backbone scaffold of the protein. When calculating RMSD, the MOE software uses a comparison of each alpha carbon to the respectively aligned alpha carbons on the other structures to generate a root mean square deviation which represents the overall difference in the structures for the selected area of interest. We describe herein the comparison of only the CDR regions between each antibody and not the entire variable domains. When calculating the RMSD value between the three antibody structures the sequence alignment is done considering structural overlap and areas where gaps are introduced to optimize the alignment. In some circumstances this results in an overestimation of RMSD similarity (lower RMSD value) due to variation in loop length not being considered. This is the case when considering CDRH3 and CDRL1 from the 5 mouse anti-OSMR antibody candidates where CDR lengths are different (Figure 3). An RMSD greater than or equal to 2 angstroms represents a significant separation in three-dimensional space.

Figure 4 shows six matrices with the RMSD resulting from pairwise comparison of each CDR from the 5 mouse anti-OSMR antibodies described herein. Comparison of CDRH1 from these structural models indicates that these antibodies have a general structural similarity in this loop to one another with none of the RMSD values exceeding 2 angstroms. Mouse antibody 14C04 with the variable heavy chain sequence (SEQ ID NO: 43; MU_14C04_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 44; MU_14C04_VH) and a variable light chain sequence (SEQ ID NO: 45; MU_14C04_VL) the corresponding nucleotide sequence for which is (SEQ ID NO: 46; MU_14C04_VL); has the most distinct CDRH1 structure (largest RMSD compared to other antibodies) and is of interest to note this antibody is the only one which is specific for canine OSMR only. The RMSD difference between murine anti-OSMR antibody 14C04 and the others is more pronounced when comparing CDRH2 with RMSD values exceeding 2 for each comparison.

The RMSD results from comparing CDRH3 structures from the 5 mouse anti-OSMR antibodies highlights another important consideration of the structure function relationship with the antibodies and their target, OSMR. Mouse anti-OSMR antibody 14C04 is highly structurally distinct from the others in this CDR with RMSD values exceeding 2 angstroms in 3 of the 4 comparisons and as previously mentioned, 14C04 is canine OSMR specific (see table 1 for

affinity data). The other antibody having a high structural distinction from the other 4 in mouse anti-OSMR 02D09 with the variable heavy chain sequence (SEQ ID NO: 31; MU_02D09_VH) the corresponding nucleotide sequence for which is (SEQ ID NO: 32; MU_02D09_VH) and a variable light chain sequence (SEQ ID NO: 33; MU_02D09_VL) the corresponding nucleotide
5 sequence for which is (SEQ ID NO: 34; MU_02D09_VL). Mouse anti-OSMR 02D09 is interesting in that this antibody shows specificity for binding to feline OSMR (see table 1 and table 3, table 3 supports higher affinity of the chimeric 02D09 antibody to feline OSMR (compared to canine OSMR) as well as a slower off rate supporting its preferential specificity to the feline OSMR protein). To reiterate a point previously made, antibodies 02D09 and 14C04
10 also have longer CDRH3s and these additional amino acids are not considered in the direct structural comparison however they do highly impact the distinct structures of these two CDRH3 loops when compared to the other 3 antibodies which bind to both [emphasis added] canine and feline OSMR.

Continuing with the comparison of the light chain CDR structures, the figure 4 CDRL1 matrix
15 shows the RMSD comparison of the 5 anti OSMR antibodies do not exceed the 2-angstrom difference. Again, the canine OSMR antibody 14C04 has higher RMSD values indicating a trend towards structural distinction without consideration of the 4 additional amino acids in this CDR. The additional 4 amino acids in this CDR loop represent a significant structural difference compared to the other 4 antibodies and further support a unique structural composition of the
20 binding paratope for this antibody. Antibodies 02D09 and 14C04 have the greatest structural variance in the CDRL2 loop with the most significant (RMSD greater than 2 angstroms) existing in the comparison between them.

It is of interest to point out that the amino acid sequences of the CDRL2s from antibodies 09E09 and 10F07 are identical however the RMSD comparison between them is not zero (value is
25 0.31). An RMSD value of 0.31 represents structures that are highly similar to one another. Differences arise from the fact that antibodies 09E09 and 10F07 do not have identical variable light chains and therefore are not identical to one another. Structural changes introduced in other areas of the protein chain can be responsible for differences occurring distally and are reflected in subtle differences in the RMSD value.

30 Comparison of light chain CDRL3 reveals again that there is a significant structural difference between antibodies 02D09 and 09E09 and a trend towards these two species specific anti-OSMR mAbs to show greater structural variation when compared to those have dual specificity for canine and feline OSMR. While not wishing to be bound to one particular hypothesis we

present evidence supporting a link between the structure of CDR loops of these anti-OSMR antibodies and their functionality of specifically binding to, and blocking, a canine and/or feline OSMR whereby this binding inhibits the ability of pSTAT3 signaling induced by IL-31 or OSM proteins.

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Example 10. Homology modeling of the canine and feline OSM: OSMR receptors

The OSMR receptor has been characterized in the context of the cytokines to which it binds resulting in a cellular signaling response. These cytokines (including OSM) are members of the gp130 family of cytokines which elicit pleiotropic responses in the context of different cellular and tissue distributions including cellular differentiation, proliferation, hematopoiesis, immunologic, and inflammatory responses (Richards CD.; The Enigmatic Cytokine Oncostatin M and Roles in Disease; *ISRN Inflammation*. 2013:512103 (2013)). The OSMR protein shares similar structural architecture to LIFR which, in humans, forms a heterodimeric receptor complex with gp130 to signal in response to both LIF and OSM. Overlap in such functional characteristics allows for comparison of these receptors structure within and between species. A protein structure for OSMR is not presently available however a crystal structure (PDB ID: 2Q7N, 4 angstroms) of Leukemia inhibitory factor (LIF) in complex with LIF receptor (LIFR) is available (Huyton T et al.; An unusual cytokine: Ig-domain interaction revealed in the crystal structure of leukemia inhibitory factor (LIF) in complex with the LIF receptor. *Proc Natl Acad Sci U S A*. 2007 Jul 31;104(31):12737-42). While not wishing to be bound to one hypothesis we present herein structural homology data using this LIF: LIFR co-crystal structure as a template to guide the understanding of the interactions between IL-31, OSM and OSMR. These structural data in combination with reports of functional mapping of amino acid residues involved in the interaction of human OSM with human OSMR allow inference to the homologous regions in canine and feline OSMR and support the understanding of the epitopes to which inhibitory anti-OSMR antibodies bind.

Figure 5A shows a representative homology model generated using amino acids 28-329 of (SEQ ID NO: 107; Canine_OSMR_hlgG1_Fc) the corresponding nucleotide sequence for which is (SEQ ID NO: 108; Canine_OSMR_hlgG1_Fc) (the extracellular domain of canine OSMR) on the template structure 2Q7N of human LIFR (SEQ ID NO: 135; Human_LIFR). A similar homology model was generated using amino acids 28-329 of (SEQ ID NO: 112; Feline_OSMR_hlgG1_Fc) the corresponding nucleotide sequence for which is (SEQ ID NO:

113; Feline_OSMR_hlgG1_Fc) (the extracellular domain of feline OSMR) on the template structure 2Q7N of human LIFR (SEQ ID NO: 135; Human_LIFR). Figure 5 shows the homology model generated using canine OSM (SEQ ID NO: 109; Canine_OSM) on the template structure 2Q7N of human LIF (SEQ ID NO: 136; Human_LIF). A similar homology model was generated
5 using amino acids 23-234 of feline OSM (SEQ ID NO: 110; Feline_OSM_hlgG1_Fc) the corresponding nucleotide sequence for which is (SEQ ID NO: 111; Feline_OSM_hlgG1_Fc) on the template structure 2Q7N human LIF (SEQ ID NO: 136; Human_LIF).

Figure 5A shows the binding interface of the canine OSM homology model near the canine
10 OSMR protein, based on the LIF: LIFR co-crystal template. Figure 5B shows an enlarged picture of an area contained within the highlighted circle. In this enlarged representation are amino acids labeled that were identified from a previous study defining relevant amino acids involved with the human OSM: OSMR interaction (Adrian-Segarra JM et al.; The AB loop of Oncostatin M (OSM) determines species-specific signaling in humans and mice. J Biol Chem.
15 2018 Dec 28;293(52):20181-20199). It is important to note that while this publication used the human proteins to assess the functionality of important amino acid residues involved with OSM binding to OSMR, the homologous amino acids labeled on this canine OSM:OSMR homology model are indeed positioned in this binding interface and therefore provide supporting evidence that inference from the human protein structures is supportive of the canine and feline receptor
20 systems.

Example 11. Determination of anti-OSMR binding region on canine and feline OSMR based on homology to known OSM binding domain and amino acid sequence variation between species

25 Figure 6 shows a drawing to logically define the binding region(s) of the 5 mouse anti-OSMR antibodies on canine and/or feline OSMR. That is to say, because of the narrow number of amino acid residues defining the OSM (and presumably the IL-31) binding sites on OSMR, coupled to the sequence divergence in the human OSMR protein and limited divergence between canine and feline OSMR, there are only a few places where these antibodies can
30 specifically bind and elicit the functional activity described herein. In figure 6, the extracellular domains of human OSMR are represented with filled in black ovals and a hatched square which represents the cytokine binding domain and open circles which represents the fibronectin III domains. Mapping studies like those described herein define a specific area (key individual amino acid contacts) that are responsible for binding of human OSM to OSMR. Depicted here

are also the homologous canine and feline OSMR protein regions that were used to immunize mice for the purpose of generating anti-OSMR antibodies. None of the antibodies selected for binding to canine and/or feline OSMR described herein bind to human OSMR indicating that the binding occurs in an area on canine and/or feline OSMR that is divergent from human. That is to say that the amino acids in these areas of canine and feline OSMR are different from human. Knowing that these antibodies bind to canine and/or feline OSMR and inhibit pSTAT3 mediated signaling induced by canine and/or feline OSM (and/or IL-31), means they bind in the discrete area where OSM and/or IL-31 bind to OSMR to activate the receptor and this area is a different amino acid sequence in canine and feline versus human OSMR.

There are 3 types of mouse anti-OSMR antibodies described herein, 1) an antibody that preferentially binds to feline OSMR 2) an antibody that specifically binds to canine OSMR and 3) 3 antibodies that bind to canine and feline OSMR. Based on these 3 binding phenotypes, an antibody that specifically binds to canine OSMR must bind in a region of OSMR near the binding site of OSM (and IL-31) where the amino acid sequence is unique to canine versus feline OSMR. An antibody that binds preferentially to feline OSMR over canine OSMR will bind to an area that is unique to feline OSMR and near the binding site of OSM (and IL-31). Antibodies that bind both canine and feline OSMR, inhibit OSM (and/or) IL-31 mediated signaling, will bind to an area on OSMR where the amino acid sequence is conserved between canine and feline OSMR. Based on the modelling described herein and functional data that has been determined for these antibodies, these data define the epitope of the anti-OSMR antibodies described herein.

Example 12. Docking of anti-OSMR antibodies on the OSMR receptor model

Described herein are homology models generated for the 5-candidate mouse anti-OSMR antibodies selected that bind to canine and/or feline OSMR. Also described are the variation in the structure: function relationship between these antibodies with respect to CDR structures and differential binding and potency against neutralizing canine and/or feline IL-31 and/or OSM pSTAT3 signaling in canine and/or feline cells. Additionally, homology models of the OSM: OSMR binding interface are described which defines the regions on the OSM cytokine and receptor which are important for receptor activation. Use of these models and knowledge of the specific amino acid residues involved in these interactions can be translated to the homologous regions in the canine and feline OSM and OSMR proteins.

Docking of a protein onto another protein (or protein complex) is performed using software like MOE or related software currently available. An antibody homology model (or solved structure of an antibody) is docked onto a receptor using input from the user to define areas on the protein (example, specific amino acid residues) to guide the software. These areas are defined from knowledge of experimental data defining residues that are important for contact between a cytokine and the receptor to which it binds. These data can be derived from homologous protein structures and/or structures of proteins with similar functional characteristics and similar architecture (and not necessarily similar amino acid sequence). Docking of an anti-OSMR antibody onto the OSMR structure is performed in the MOE software with knowledge of the human OSM: OSMR interactions coupled with knowledge of which mouse anti canine and/or feline CDRs are important for species specificity. Placement of the antibody model on the relevant area of OSM:OSMR interaction is also supported by the fact that a) the mouse anti-OSMR antibodies described herein do bind to canine and/or feline OSMR and b) these antibodies block the ability of IL-31 and/or OSM to induce pSTAT3 signaling in canine and/or feline cells. These facts define a discrete area of the OSMR protein to which these antibodies can logically bind and limit the area to define where docking of the CDRs should take place.

Example 13. Analysis of OSMR gene expression in skin tissue biopsies from felines with allergic dermatitis using in situ hybridization (ISH)

Twenty skin biopsies from felines with allergic skin disease were formalin-fixed and paraffin-embedded (FFPE) prior to sectioning for ISH analysis. Nineteen biopsies from normal feline skin without signs of allergic disease were subjected to FFPE and sectioned for comparative analysis. Automated single chromogenic ISH protocol from Advanced Cell Diagnostics, RNAscope 2.5 LS Reagent Kit-Red (ACD, 322750-USM) was performed using the Leica Bond-RX system. Prior to probe hybridization, tissue samples were subjected to heat and enzymatic epitope retrieval using the ACD proprietary protease reagent. Positive control staining was performed on each sample to determine the RNA integrity using a probe to the Cyclophilin B (PPIB) gene (Advanced Cell Diagnostics, Inc., Newark, CA). A custom probe was designed to detect the expression of the feline OSMR gene (Advanced Cell Diagnostics, Inc., Newark, CA). Each slide was visualized and quantified on Leica Aperio ImageScope 12.3.2.8013. Each image was annotated, and RNA expression was analyzed with the macro algorithm Leica RNA ISH v2 embedded in the ImageScope software. Data was extracted from the ImageScope

software and reported as ratio of RNA signal to analyzed tissue area. Student one-tailed test was used to determine signal significance.

Figure 7 shows the quantitative results following imaging of OSMR mRNA using ISH from normal and allergic feline skin biopsies. ISH of mRNA using RNAScope is highly specific for detection of the targeted transcript, in this case feline OSMR mRNA. There is a clear, significant overexpression of OSMR mRNA in the skin of felines with allergic disease. These results indicate a correlation between a clinically relevant diagnosis of allergic skin disease in felines and the overexpression of the OSMR gene representing a link between OSMR and the disease pathophysiology. These results support the hypothesis that OSMR is involved in feline skin disorders and targeting OSMR with an antibody capable of blocking cytokine-mediated signaling through the receptor may be useful in treating such disorders including, but not limited to, allergic and atopic dermatitis.

Example 14. Analysis of OSMR protein in skin tissue biopsies from feline with allergic dermatitis using immunohistochemistry (IHC)

Five skin biopsies from felines with allergic skin disease were formalin-fixed and paraffin-embedded (FFPE) prior to sectioning for IHC analysis. Five biopsies from normal feline skin without signs of allergic disease were subjected to FFPE and sectioned for comparative analysis. Automated single chromogenic IHC refine red protocol was performed using the Leica Bond-RX system (Leica Biosystems, Buffalo Grove, IL). Prior to antibody staining, sample epitopes were exposed by heat induced epitope retrieval in EDTA buffer. Expression of the feline OSMR protein was carried out using a polyclonal rabbit anti-OSMR antibody (LSBio LS-B11477, Seattle, WA) and detected with Leica Bond refine red AP linked polymer and fast red chromogen (Leica Biosystems, Buffalo Grove, IL). Qualitative analysis was performed by inspection of each image for red staining indicating expression of the OSMR protein.

Figure 8 shows a representative image comparing a normal feline skin section to a skin section from a feline with allergic disease. Colored slide images of skin contain light brown epidermal regions, punctate dark purple spots which are Dapi staining of the nuclei, and red which is the colorimetric staining of the OSMR protein following development of the alkaline phosphatase (AP) labeled secondary antibody. Figure 8 shows staining of the feline OSMR protein in a control skin tissue image and an allergic skin tissue image following conversion of the colored

images to a grayscale format. In the control (non-allergic) skin sample there is minor expression of the OSMR protein in the sub-epidermal space and an ordinary distribution of cells near the epidermal layer. The image from the allergic skin sample clearly shows the elevated expression of the OSMR protein throughout the sub-epidermal space with a large infiltration of cells with minimal distinction between the epidermal and sub-epidermal spaces. The OSMR protein is visual in these images as a gray to black shading of space outside of the black defined staining of the nuclei. It is of interest to note that although the cellular boundaries in these images are not annotated there is an appearance of staining in interstitial areas which are presumably away from the cell indicating that there may be secreted soluble forms of the OSMR receptor present. While not wishing to be bound to one hypothesis, it is conceivable that such secreted receptors may have a regulatory role by acting as an antagonist or carrier protein for the proteins which OSMR interacts with. If these soluble forms have the same amino acid sequence and structure as the cell-associated form of the receptor then an anti-OSMR antibody like the ones described herein would also bind to the soluble form. Visual examination of the five control feline skin slides and five allergic skin slides indicate a similar pattern for OSMR staining as what is described here for the two representative images. The results support the quantitative results described herein showing overexpression of OSMR in the skin of felines with allergic disease when compared to feline skin samples without allergic disease. These results provide evidence of a role for the OSMR protein in the skin of felines with skin disorders including, but not limited to, allergic and atopic dermatitis.

Example 15. An IL-31 induced itch model in dogs and cats to determine in vivo efficacy of anti OSMR antibodies.

While not wishing to be bound to one particular hypothesis we present evidence that binding of selected antibodies to canine and/or feline OSMR, that are capable of blocking (or neutralizing) the biological activity of pSTAT3 induced signaling by IL-31 and/or OSM and that such IL-31 and/or OSM inhibition at the level of the OSMR receptor, may be beneficial as a therapeutic against atopic, allergic, inflammatory and other disorders described herein for canines and felines. The ability of an antibody to effectively neutralize its target can be assessed in vivo using an appropriate model for efficacy in a host species. Such a model to determine *in vivo* efficacy is described.

To determine in vivo efficacy in the dog, subcutaneous (SC) administration of an anti-canine

OSMR antibody is given to laboratory dogs. Baseline responses are performed with all dogs and dogs are randomized into groups and housed based on their pruritic score index (PSI). The dogs are then administered an anti-canine OSMR antibody on day 7 and IL-31 challenges are performed at day 8, 14 and 22. Reduction in mean PSI for day 8 and 14, relative to day 1, in antibody treated animals is considered for an efficacy endpoint when comparing the PSI scores for untreated animals. Assessing the PSI day to day variation associated with dogs' pruritic behaviors is controlled for variation with a 30-minute baseline PSI determined for each dog, on each day prior to IL-31 challenge. Such *in vivo* model data may provide evidence that; 1) anti-canine OSMR monoclonal antibodies can neutralize the ability of IL-31 to induce pruritus in dogs, 2) inhibition of IL-31 mediated signaling by blocking OSMR in a cell based assay correlates with *in vivo* efficacy and 3) the parameters necessary to utilize an IL-31 model for antibody evaluation are established for the evaluation of other candidate antibodies.

The efficacy of anti-feline OSMR is assessed in an IL-31 induced *in vivo* cat model. Pre-challenge pruritic behavior for the vehicle placebo and antibody groups is assessed from day -7 through day 28 with day zero being the day of antibody administration. On day zero cats are dosed with an anti-OSMR antibody subcutaneously which is seven days prior to the first feline IL-31 challenge. Pruritic behavior is assessed on days 7, 21, and 28 for 1 hour following an intravenous challenge of the IL-31 protein. Reduction in pruritus observed on days 7, 21 or 28 following IL-31 challenge, when compared to vehicle placebo control, is considered for efficacy. These data support the ability of an anti-OSMR antibody to neutralize pruritus induced by feline IL-31 *in vivo* and suggest the antibody may serve as a therapeutic in the treatment of IL-31 mediated disease in cats including, but not limited to, atopic dermatitis.

Example 16. Additional cell-based data with mouse and mouse:canine chimeric anti-OSMR antibodies

Additional cell-based experiments were performed to assess the potency of anti-OSMR antibodies in cell challenge assays for IL-31 and OSM-mediated pSTAT3 signaling. These data were generated in support of previous work described in Examples 6 and 7. The sequence of mouse and mouse:dog chimeric antibodies are described in Examples 6 and 7 respectively. These data demonstrate that both antibodies 10F07 and 19F07 possess superior potency towards inhibiting canine and feline IL-31 and OSM mediated pSTAT3 signaling in both canine and feline cells. Also of interest is the selective potency of antibody 14C04 towards inhibition of canine mediated signaling and 02D09 for feline indicating the potential for structural variance on

the OSMR receptor which leads to differential specificity on the epitope recognized by these two antibodies.

TABLE 5

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Inhibition of IL-31 and OSM pSTAT3 Signaling					
Cell type used:	Canine DH82	Feline IL-31 Fcwf-4	Canine DH82	Feline IL-31 Fcwf-4	
Cytokine used:	Canine IL-31	Feline IL-31	Canine OSM	Canine OSM	
	(SEQ ID NO: 123)	(SEQ ID NO: 125)	(SEQ ID NO: 109)	(SEQ ID NO: 109)	
Antibody ID	Antibody type	IC ₅₀ (μg/ml)	IC ₅₀ (μg/ml)	IC ₅₀ (μg/ml)	IC ₅₀ (μg/ml)
Mu_14C04	mouse	5.65	0 % inh @ 75 μg/ml	8.64	nt
14C04	mouse:dog chimera	nt	nt	nt	nt
Mu_02D09	mouse	0 % inh @ 75 μg/ml	67% inh @ 75 μg/ml	> 50 μg/ml	nt
02D09	mouse:dog chimera	97.7	nt	nt	nt
Mu_19F07	mouse	0.07	0.42	0.27	0.68
19F07	mouse:dog chimera	0.28	nt	0.26	nt
Mu_09E09	mouse	80% inh @ 75 μg/ml	63% inh @ 75 μg/ml	54% @ 15 μg/ml	nt
09E09	mouse:dog chimera	31.31	nt	nt	nt
Mu_10F07	mouse	93% inh @ 75 μg/ml	91% inh @ 75 μg/ml	0.05	0.23
10F07	mouse:dog chimera	0.05	0.32	0.16	nt

nt = not tested

% inh @ = percent inhibition at indicated concentration

Table 5. Inhibition of IL-31 and OSM mediated pSTAT3 signaling in canine DH82 and feline Fcwf-4 cells with anti-canine and/or anti-feline mouse: canine anti-OSMR chimeric antibodies.

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Example 17. Binding data for caninized and felinized anti-OSMR antibodies

Caninized (canine) and felinized (feline) anti-OSMR antibodies were cloned, expressed, and purified. The sequence of each speciated antibody in table 6 is described in Example 8.

- 15 Affinity of these speciated antibodies to canine and feline OSMR was measured using Biacore. Table 5 describes results of these Biacore experiments for speciated antibodies that expressed and bound canine and/or feline OSMR. As indicated in the table, this first round of speciation produced antibodies from the 19F07, 09E09, and 10F07 lineage that expressed and bound to their protein target. For comparison to the mouse and mouse:canine chimeras having the same
- 20 CDRs as these speciated versions, see tables 1 and 3 respectively. The highest affinity speciation for each of these three series antibodies are Fel_09E09_1.1 binding to feline OSMR with a KD of 7.73E-9 M, Fel_10F07_2.2 binding to feline OSMR with a KD of 1.21E-9 M, Can_19F07_2.1 binding to canine OSMR with a KD of 8.30E-9 M, and Fel_19F07_2.2 binding to feline OSMR with a KD of 3.32E-12 M. The objective during the speciation process is

maintain affinity of the speciated antibody form to its respective target protein, in this case to canine and/or feline OSMR. Detailed here are the binding kinetics for antibodies that exemplify this goal.

5 TABLE 6

Antibody ID	Antibody type	Canine OSMR (SEQ ID NO: 107)			Feline OSMR (SEQ ID NO: 112)		
		k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)
CAN_19F07_1.1	caninized	3.66E+04	3.34E-04	9.13E-09	1.11E+05	3.55E-05	3.20E-10
CAN_19F07_2.1	caninized	2.64E+04	2.20E-04	8.30E-09	5.67E+04	1.12E-05	1.98E-10
CAN_19F07_2.2	caninized		No binding		4.70E+04	1.13E-03	2.40E-08
FEL_19F07_1.1	felinized	2.85E+04	2.94E-04	1.03E-08	8.13E+04	6.37E-05	7.83E-10
FEL_19F07_1.2	felinized	1.05E+04	3.27E-04	3.10E-08	4.47E+04	1.95E-05	4.35E-10
FEL_19F07_2.1	felinized		No binding		3.79E+03	4.07E-07	1.08E-10
FEL_19F07_2.2	felinized	4.88E+04	2.15E-04	4.40E-09	1.69E+05	5.62E-07	3.32E-12
CAN_09E09_1.2	caninized	2.86E+04	1.08E-03	3.77E-08	1.67E+05	1.84E-03	1.10E-08
FEL_09E09_1.1	felinized	5.75E+04	1.05E-03	1.82E-08	1.27E+05	9.79E-04	7.73E-09
FEL_10F07_1.2	felinized	6.65E+04	8.85E-04	1.33E-08	1.28E+05	6.10E-04	4.75E-09
FEL_10F07_2.1	felinized	8.50E+04	8.51E-04	1.00E-08	1.91E+05	4.20E-04	2.20E-09
FEL_10F07_2.2	felinized	1.81E+05	9.03E-04	4.99E-09	3.76E+05	4.57E-04	1.21E-09

Sample ID	Sample type	Canine OSMR (SEQ ID NO: 107)			Feline OSMR (SEQ ID NO: 112)			Human OSMR (SEQ ID NO: 122)		
		k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	KD (M)
anti-hOSMR	control antibody	No binding			No binding			2.15E+05	2.03E-05	9.42E-11
Not Transfected	control	No binding			No binding			No binding		
cOSM	SEQ ID NO: 109	6.87E+04	3.00E-03	4.36E-08	1.03E+05	4.23E-03	4.11E-08	1.02E+05	3.90E-03	3.81E-08
Ctrl-HBS-EP	control	No binding			No binding			No binding		

Table 6. Biacore binding data for caninized and felinized anti-OSMR antibodies to canine and feline OSMR. Experimental controls show binding of an anti-human OSMR antibody to human OSMR only. None of the mouse progenitor mAbs with these CDRs showed binding to human OSMR (see table 1). Non-transfected controls were grown and purified identically to these speciated recombinant mAbs without a plasmid to express a functional antibody. cOSM binding shows these three OSMR receptor forms are capable of binding to the canine form of the OSM protein. Ctrl-HBS-EP is a buffer control for the Biacore instrument.

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Example 18. Mutational analysis of anti-OSMR CDRs using alanine substitution

To establish a functional relationship between each amino acid residue on an antibodies CDR and the involvement of that residue in binding of the antibody to its target protein, an alanine replacement mutational analysis was performed. Individual mammalian expression plasmids containing the heavy and light chain mouse variable sequence were synthesized for four of the

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five anti-OSMR antibodies described in table 1 (alanine replacement for antibody Mu_14C04 was not done). The variable heavy and variable light chains were cloned with the heavy and light chain canine constant regions respectively as described herein for the generation of chimeric antibodies (Example 7). Expression and purification of these chimeric antibodies was carried out according to the procedures described herein (Example 1). For comparative analysis, each chimera was made without any changes from the initial mouse variable heavy and variable light chain sequence (wildtype). For each mutant, an individual plasmid was generated substituting alanine for each non-alanine residue of each heavy and light variable chain CDR position. Each plasmid containing a single alanine substitution at a single position of each CDR was paired with the wildtype plasmid of the corresponding heavy or light chain and expressed using a transient CHO cell system.

Example 19. ELISA and Biacore binding results for alanine mutants of anti-OSMR antibodies

The objective of alanine replacement mutational analysis is to further understand the involvement of individual CDR amino acid residues in binding to the OSMR target protein. A single substitution in a CDR amino acid residue to alanine may result in a functional change whereby the binding of the antibody to its target is negatively impacted (meaning there is less binding, or lower binding affinity, or lower ELISA signal, to the antibodies protein target compared to that of the wildtype antibody which contains no CDR substitutions). An assessment of binding for each alanine mutant was performed using an indirect ELISA where the feline OSMR protein (SEQ ID NO:112) was passively immobilized to a polystyrene ELISA plate. The ELISAs were performed according to standard procedures of blocking and washing with one hour incubation times between steps. To determine binding, each wildtype or mutant antibody was added to individual wells of the ELISA plate as a purified antibody (pure antibody) or cell culture supernatant (supernatant) where indicated. As these antibodies were constructed as mouse:canine chimeras, a goat anti-canine HRP labeled secondary antibody was used to detect the amount of antibody bound to the feline OSMR protein after development using an HRP substrate. To determine whether antibody was produced from the transient CHO cultures a separate ELISA was performed using a mouse anti-canine antibody as a capture reagent on the ELISA plate. Each supernatant or pure antibody prep was added to an individual well and allowed to bind. The presence of antibody was determined using an HRP labeled goat anti-dog detection mAb (antibody expression control). The colorimetric signal produced by each ELISA plate was normalized to a background control which lacked the presence of the analyte. The

data were expressed as a ratio of optical density (OD) from the ELISA with binding to feline OSMR (ELISA signal) to the OD from the antibody control plate (antibody expression control). The alanine mutations resulting in an OD ratio (ELISA signal/mAb control) less than or equal to 1.3 were considered to negatively impact binding. The OD ratio for each wildtype expression control was greater than 1.6. In general, most mutations that negatively impacted binding resulted in a OD ratio of less than 0.5 (table A).

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TABLE A.

DESCRIPTION	CDR	ELISA RATIO BINDING TO:	ANTIBODY BINDING AFFINITY USING BIACORE, AFFINITY MEASURED TO:								
			cOSMR (SEQ ID NO: 107)			fOSMR (SEQ ID NO: 112)			ANTIBODY EXPRESSION CONTROL		
			FELINE OSMR								
		(SEQ ID NO: 112)	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
Mu_02D09_VH (SEQ ID NO:31)_D31A	H1	0.159	9.08E+05	3.56E-03	3.92E-09	1.14E+06	8.72E-04	7.67E-10	1.21E+06	8.32E-05	6.85E-11
Mu_02D09_VH (SEQ ID NO:31)_Y32A	H1	0.991	7.07E+05	2.46E-02	3.48E-08	1.20E+05	7.65E-04	6.39E-09	1.21E+05	4.43E-05	3.66E-10
Mu_02D09_VH (SEQ ID NO:31)_G33A	H1	0.158		no binding		2.29E+05	1.40E-03	6.13E-09	3.27E+05	7.35E-06	2.25E-11
Mu_02D09_VH (SEQ ID NO:31)_H35A	H1	0.030		no binding		1.34E+05	1.08E-03	8.05E-09	1.39E+05	1.12E-04	8.03E-10
Mu_02D09_VH (SEQ ID NO:31)_Y50A	H2	0.009		no binding		1.00E+05	1.20E-07	1.19E-12	1.42E+05	3.68E-07	2.59E-12
Mu_02D09_VH (SEQ ID NO:31)_S52A	H2	0.001		no binding		6.56E+05	4.83E-04	7.36E-10	3.23E+05	7.55E-06	2.33E-11
Mu_02D09_VH (SEQ ID NO:31)_D62A	H2	0.010		no binding		1.09E+05	2.31E-07	2.11E-12	1.29E+05	2.45E-07	1.90E-12
Mu_02D09_VH (SEQ ID NO:31)_D99A	H3	0.000		no binding		4.08E+05	4.63E-04	1.13E-09	2.35E+05	3.84E-04	1.63E-09
Mu_02D09_VH (SEQ ID NO:31)_D100A	H3	0.009		no binding		1.40E+05	1.63E-03	1.16E-08	1.01E+05	1.71E-05	1.70E-10
Mu_02D09_VH (SEQ ID NO:31)_Y101A	H3	0.320		no binding		1.69E+05	1.94E-03	1.15E-08	1.47E+05	4.11E-05	2.80E-10
Mu_02D09_VH (SEQ ID NO:31)_D102A	H3	0.058		no binding		1.25E+05	1.74E-03	1.39E-08	1.35E+05	7.40E-05	5.47E-10
Mu_02D09_VH (SEQ ID NO:31)_R104A	H3	0.000		no binding		1.29E+05	5.79E-05	4.49E-10	1.05E+05	2.41E-05	2.29E-10
Mu_02D09_VL (SEQ ID NO:33)_Q27A	L1	0.025	1.26E+05	4.41E-03	3.50E-08	1.44E+05	1.09E-03	7.57E-09	1.58E+05	1.90E-04	1.20E-09
Mu_02D09_VL (SEQ ID NO:33)_I29A	L1	0.377		no binding		1.55E+05	1.13E-03	7.26E-09	1.33E+05	9.47E-05	7.14E-10
Mu_02D09_VL (SEQ ID NO:33)_S56A	L2	0.000	4.12E+04	6.53E-03	1.59E-07	1.16E+05	1.07E-03	9.30E-09	1.32E+05	1.11E-04	8.44E-10
Mu_02D09_VL (SEQ ID NO:33)_W94A	L3	0.000		no binding		1.04E+05	7.94E-08	7.65E-13	3.23E+05	3.16E-05	9.77E-11
Mu_02D09_VL (SEQ ID NO:33)_L96A	L3	1.267		no binding		4.40E+05	2.85E-07	6.48E-13	3.70E+05	2.43E-05	6.59E-11
Mu_02D09_wildtype		1.615	2.09E+04	1.18E-02	2.74E-06	1.31E+05	1.19E-03	9.08E-09	1.53E+05	9.15E-05	6.00E-10
Mu_09E09_VH (SEQ ID NO:35)_S35A	H1	1.049		no binding		7.56E+05	1.04E-03	1.37E-09	2.97E+05	5.34E-05	1.80E-10
Mu_09E09_VH (SEQ ID NO:35)_Y50A	H2	0.310		no binding		6.22E+05	1.77E-03	2.85E-09	3.01E+05	5.40E-05	1.79E-10
Mu_09E09_VH (SEQ ID NO:35)_Y57A	H2	0.052		no binding		9.43E+05	2.40E-03	2.54E-09	3.05E+05	6.34E-05	2.08E-10
Mu_09E09_VH (SEQ ID NO:35)_Y59A	H2	0.011		no binding			no binding		3.07E+05	4.52E-05	1.47E-10
Mu_09E09_VH (SEQ ID NO:35)_D99A	H3	0.908		no binding		6.12E+05	1.08E-03	1.76E-09	3.14E+05	6.88E-05	2.19E-10
Mu_09E09_VH (SEQ ID NO:35)_P100A	H3	0.946		no binding		7.29E+05	9.26E-04	1.27E-09	3.00E+05	2.35E-05	7.86E-11
Mu_09E09_VH (SEQ ID NO:35)_I101A	H3	0.000		no binding			no binding		3.14E+05	5.40E-05	1.72E-10
Mu_09E09_VH (SEQ ID NO:35)_T102A	H3	0.388		no binding		9.64E+05	1.87E-03	1.94E-09	3.11E+05	5.33E-05	1.72E-10
Mu_09E09_VH (SEQ ID NO:35)_F105A	H3	0.256		no binding		7.72E+05	1.86E-03	2.40E-09	3.38E+05	4.66E-05	1.38E-10
Mu_09E09_VL (SEQ ID NO:37)_Y32A	L1	0.092		no binding		2.51E+06	1.58E-03	6.32E-10	2.98E+05	8.76E-05	2.94E-10
Mu_09E09_VL (SEQ ID NO:37)_N34A	L1	0.023		no binding		3.21E+05	1.61E-03	5.02E-09	2.81E+05	8.91E-05	3.17E-10
Mu_09E09_VL (SEQ ID NO:37)_Y50A	L2	0.000		no binding			no binding		3.10E+05	7.96E-05	2.57E-10
Mu_09E09_VL (SEQ ID NO:37)_Q90A	L3	0.183		no binding			no binding		2.86E+05	7.12E-05	2.49E-10
Mu_09E09_VL (SEQ ID NO:37)_G91A	L3	0.136		no binding		9.18E+05	2.03E-03	2.21E-09	2.92E+05	8.96E-05	3.06E-10
Mu_09E09_VL (SEQ ID NO:37)_N92A	L3	0.015		no binding			no binding		2.96E+05	7.59E-05	2.57E-10
Mu_09E09_VL (SEQ ID NO:37)_L94A	L3	0.055		no binding		2.68E+06	1.67E-03	6.21E-10	3.05E+05	7.63E-05	2.50E-10
Mu_09E09_VL (SEQ ID NO:37)_W96A	L3	0.000		no binding			no binding		2.83E+05	9.76E-05	3.45E-10
Mu_09E09_wildtype		1.613	5.72E+05	2.10E-03	3.83E-09	7.59E+05	9.45E-04	1.24E-09	3.74E+05	8.68E-07	2.30E-12
Mu_10F07_VH (SEQ ID NO:39)_D99A	H3	0.545	5.16E+04	5.76E-04	1.12E-08	2.21E+05	7.04E-04	3.18E-09	1.17E+05	1.18E-04	1.01E-09
Mu_10F07_VH (SEQ ID NO:39)_I101A	H3	0.000		no binding		1.02E+05	7.01E-04	6.88E-09	1.01E+05	4.49E-05	4.45E-10
Mu_10F07_VH (SEQ ID NO:39)_Y107A	H3	0.375	4.01E+04	6.76E-05	1.69E-09	1.73E+05	2.47E-04	1.43E-09	9.95E+04	4.44E-05	4.46E-10
Mu_10F07_VL (SEQ ID NO:41)_L33A	L1	0.150	4.05E+04	2.22E-04	5.48E-09	1.79E+05	2.74E-04	1.53E-09	1.04E+05	8.43E-05	8.12E-10
Mu_10F07_VL (SEQ ID NO:41)_Y50A	L2	0.000		no binding		2.42E+05	1.73E-06	7.14E-12	1.74E+05	1.05E-04	6.00E-10
Mu_10F07_VL (SEQ ID NO:41)_T53A	L2	0.217	6.50E+04	2.56E-04	3.94E-09	2.55E+05	4.68E-04	1.83E-09	1.32E+05	2.75E-04	2.07E-09
Mu_10F07_VL (SEQ ID NO:41)_W96A	L3	0.134	2.16E+08	8.63E+00	4.00E-08	2.25E+05	1.58E-03	7.03E-09	1.07E+05	9.49E-05	8.84E-10
Mu_10F07_VL (SEQ ID NO:41)_T97A	L3	0.298	4.12E+04	2.19E-04	5.32E-09	1.72E+05	2.82E-04	1.63E-09	1.00E+05	8.07E-05	8.05E-10
Mu_10F07_wildtype		1.763	4.94E+04	3.02E-04	6.13E-09	2.20E+05	3.46E-04	1.57E-09	1.34E+05	8.27E-05	6.17E-10
Mu_19F07_VH (SEQ ID NO:47)_Y33A	H1	1.058		no binding		2.66E+05	1.08E-04	4.07E-10	3.01E+05	6.08E-05	2.02E-10
Mu_19F07_VH (SEQ ID NO:47)_S56A	H2	0.277		no binding			no binding		1.59E+05	6.46E-04	4.08E-09
Mu_19F07_VH (SEQ ID NO:47)_Y59A	H2	0.740		no binding		3.51E+05	1.41E-04	4.00E-10	3.24E+05	1.16E-04	3.59E-10
Mu_19F07_VH (SEQ ID NO:47)_Y60A	H2	0.479		no binding			no binding		1.50E+05	6.10E-04	4.06E-09
Mu_19F07_VH (SEQ ID NO:47)_W102A	H3	0.057		no binding			no binding		3.12E+05	4.35E-05	1.39E-10
Mu_19F07_VH (SEQ ID NO:47)_F104A	H3	0.982		no binding		1.44E+05	5.10E-10	3.55E-15	3.26E+05	5.09E-05	1.56E-10
Mu_19F07_VL (SEQ ID NO:49)_L50A	L2	1.106		no binding		4.11E+04	5.86E-07	1.43E-11	2.93E+05	4.80E-05	1.64E-10
Mu_19F07_VL (SEQ ID NO:49)_T53A	L2	0.821		no binding		1.69E+05	9.05E-07	5.36E-12	3.05E+05	3.75E-05	1.23E-10
Mu_19F07_VL (SEQ ID NO:49)_Q89A	L3	0.985		no binding		4.65E+04	2.38E-06	5.11E-11	3.19E+05	4.30E-05	1.35E-10
Mu_19F07_VL (SEQ ID NO:49)_Q90A	L3	1.084		no binding		2.73E+05	1.31E-07	4.80E-13	3.29E+05	5.29E-05	1.61E-10
Mu_19F07_VL (SEQ ID NO:49)_F94A	L3	1.090		no binding		9.04E+04	4.70E-07	5.20E-12	3.35E+05	5.35E-05	1.60E-10
Mu_19F07_wildtype		1.724	8.84E+04	7.75E-04	7.49E-09	2.42E+05	8.06E-05	3.12E-10	2.81E+05	2.29E-05	8.20E-11

Table A. Summarizes the results from the ELISA and Biacore with the alanine replacement mutations on each CDR position for the four anti-OSMR chimeric antibodies described. The alanine replacement mutations indicated in the description column are numbered based on the position in the entire variable region which falls within the recited CDR (CDR column).

The ELISA data are the ratio of signal produced by binding of alanine replacement mutants to feline OSMR. This ratio is the signal produced from binding to feline OSMR divided by the signal from the the antibody expression control (which indicates that recombinant antibody is present). Also shown here are Biacore binding kinetics to canine and feline OSMR with the
5 corresponding signal from the expression control. The data shown in this table are from experiments using the same preparation of antibody (supernatant or pure antibody) and are only from those alanine replacement mutants where binding was negatively impacted in the ELISA assay.

10 The Biacore data in table A shows kinetic binding data for each alanine replacement mutation where binding was negatively impacted in the feline OSMR ELISA experiment. Data from an ELISA represents an equilibrium state where the antibody is allowed to associate and dissociate over a one-hour period resulting in steady-state binding. Using Biacore, the target protein is immobilized to a surface and the antibody is passed over this surface in a mobile liquid phase.
15 Protein-protein interaction is determined from changes in the surface resonance which allows for calculation of association (k_a), disassociation (k_d), and affinity constant (KD). While not wishing to be bound to one hypothesis we present data from ELISA and Biacore experiments, using the same preparation of antibody, that may result in agreement between the two methods with respect to binding but also may show contradictory results due to the nature of the binding
20 kinetics and different assay dynamics. The data presented in table A (in combination with experimental results from yeast display mutational analysis in Example 21) allow for the definition of a structure: function relationship to be defined between the anti-OSMR antibodies described here and their ability to bind and block IL-31 and OSM-mediated signaling through the OSMR receptor.

25

Example 20. Method for using yeast surface display (YSD) coupled to mutational scanning to determine structure-function relationship of anti-OSMR CDR residues

30 To further define the amino acid residues in the anti-OSMR antibody 19F07 that are important for interaction with the OSMR protein target, an independent experimental method was employed which uses display of an antibodies variable domain on the surface of the yeast *Saccharomyces cerevisiae*. A mutational tolerance epitope mapping was carried out for antibody Mu_19F07 using the approach defined by Klesmith et al. (2019) *Biochemistry* 58, 4869-4881. Heavy and light chain variable domains were purchased as gBlocks (IDT) and

linked via a (G₄S)₄ linker to construct the 19F07 single chain variable binding fragment (scFv). Genes were cloned using HiFi assembly into a minimal bacterial plasmid containing the BbvCI nicking restriction site (New England Biolabs). Using nicking mutagenesis, two single-site saturation libraries were created using this vector with two different oligo pools (IDT) encoding

5 NNK codons for each codon position within the VH and VL domains separately (Wrenbeck et al. (2016) *Nature Methods* 13, 928-930). For all cloning steps, sufficient numbers of transformants were present to oversample the designed library (theoretical library size of 3744 and 3424 variants for the VH and VL library respectively). The parental unmutated scFv gBlock (wildtype), and VH and VL mutant libraries were PCR amplified and electroporated into EBY100 yeast with

10 a linearized yeast surface display vector using homologous recombination. The linearized yeast display vector is a c-terminal display vector featuring a (PAS)₄₀-HA tag-(G₄S)₃ linker 5' of the gene insert and a myc tag 3' of the gene insert. The yeast cultures were grown in 50 mL synthetic defined media with glucose and casamino acids (SDCAA) at 30°C for a day and passaged once in SDCAA for a second day of growth. Cell pellets were then transferred to 50

15 mL SGCAA (galactose) cultures at 18°C for 2-3 days.

Sortable conditions were found via titrating recombinant canine OSMR-hFc1 on yeast displaying the parental sequence and reading the bound fraction using a flow cell analyzer (BD Accuri). Yeast cells displaying the parental scFv construct were titrated using a variable volume –

20 constant protein to display ligand approach for a sufficient number of labelling days until the expected labelling fraction was 0.9. For all concentrations the protein to display ligand ratio was at least 10:1 and kept constant between conditions. The cells were stained with a chicken anti-myc FITC antibody (ICLlab) and protein A – AlexaFlour 647 to visualize full-length display and recombinant protein binding respectively. The median 647 fluorescence of full-length displaying

25 cells calculated via FlowJo was utilized to calculate a binding profile of the ligands versus concentration. A labelling concentration of 100 nM ligand was used for both recombinant canine and feline OSMR for the two library sorts. Sorting of libraries was performed on a SH800 (Sony Biotechnology) in purity mode with a 100 µm chip. In short, cells were gated on scatter, single cells, full-length display, and then binding versus display. Cells below the binding versus display

30 diagonal were collected (i.e. variants with reduced binding relative to the bulk population normalized for display). The number of cells collected for all sorts was greater than 67-fold coverage of their respective theoretical library size.

Sorted and reference libraries were prepared for Illumina sequencing and sequenced to a read

depth of at least 75-fold oversampling of non-synonymous variants (Kowalsky et al. (2015) *PLOS ONE* 10, e0118193). Mutational fitness z-scores of the sorted versus reference libraries were calculated using PACT using a reference library read count threshold of 12 and excluding non-designed variants from the total read count (Klesmith et al. (2019) *Bioinformatics* 35, 2707-2712). Z-scores are defined as the $\log_2$ enrichment ratio of an individual mutation relative to the wild-type $\log_2$ enrichment, normalized by the variance of wild-type synonymous codon enrichment ($[\epsilon_i - \epsilon_{wt}]/\sigma_{wt, \text{synon}}$). A z-score threshold of 2.0 was utilized to separate enriched mutations collected via sorting that reduced binding from mutations that were tolerated. Tolerated mutations are therefore defined as mutations with a z-score less than 2.0.

Example 21. Results from YSD to determine amino acid residues on anti OSMR antibodies important for binding to canine and feline OSMR

The results from these yeast display experiments describe an additional approach to determine relevant amino acid residues in the CDR binding domains of antibody 19F07. As described in Example 19, each independent method of analyzing alteration in affinity of an antibody is subject to the experimental dynamics which take place during binding and disassociation events. Yeast display mutational analysis of an antibodies paratope takes place in a fluid phase environment with the binding portion of the antibody (the ScFv) displayed on the surface of the yeast cell allowing access to the target antigen protein while linking the genotypic information encoding the mutation within the cell. This allows for a link between the physical properties of the binding fragment displayed and the DNA which encodes the variable heavy and light chain fragments. This method not only allows for the coupling of an individual mutation at an individual position of the CDR with binding to the target protein but also allows for analysis of all amino acid mutations at individual positions of each CDR. These data allow for determining those mutations which are permissible (allow binding), non-permissible (negatively impact binding), or in some circumstances are advantageous to binding (gain of function mutations).

Table B describes mutations in the CDRs of antibody Mu_19F07 which are allowed according to the results of the yeast surface display experiment. Data for substitution of all amino acids at each position were derived from this experiment however this table only shows those mutations at the described positions where a smaller subset of amino acids are allowed in place of the wildtype amino acid. These amino acid positions where few (or no) amino acid substitutions are allowed indicate that (by this method of binding to the target OSMR protein) these positions are

more relevant to link the structure of the antibodies CDR to the functional outcome (binding to OSMR). Taken with the results from the alanine substitution ELISA and Biacore experiments, a more complete picture of the necessary structural elements on these anti-OSMR antibodies is observed.

TABLE B

SEQ ID NO:	SEQUENCE DEFINITION WITH ALLOWED SUBSTITUTIONS	DESCRIPTION WITH (BINDING TARGET PROTEIN)
25	D ₁ Y ₂ Y ₃ M ₄ A ₅ wherein Y ₃ is Y	CDR-H1 (canine OSMR)
26	N ₁ I ₂ N ₃ Y ₄ D ₅ G ₆ S ₇ S ₈ T ₉ Y ₁₀ Y ₁₁ L ₁₂ D ₁₃ S ₁₄ L ₁₅ K ₁₆ S ₁₇ wherein Y ₁₀ is Y, C, F, H, T, or W	CDR-H2 (canine OSMR)
27	G ₁ L ₂ T ₃ W ₄ D ₅ F ₆ D ₇ V ₈ W ₉ wherein W ₄ is W, G, or C	CDR-H3 (canine OSMR)
27	G ₁ L ₂ T ₃ W ₄ D ₅ F ₆ D ₇ V ₈ W ₉ wherein W ₄ is W, G, or F	CDR-H3 (feline OSMR)
28	K ₁ A ₂ S ₃ Q ₄ D ₅ V ₆ D ₇ T ₈ A ₉ V ₁₀ A ₁₁ wherein V ₆ is V, L, M, A, N, S, E, I, H, or C; D ₇ is D, G, Y, N, or E; A ₉ is A, S, or G; V ₁₀ is V, L, I, A, D, T, M, or C; A ₁₁ is A, S, T, G, or C	CDR-L1 (canine OSMR)
28	K ₁ A ₂ S ₃ Q ₄ D ₅ V ₆ D ₇ T ₈ A ₉ V ₁₀ A ₁₁ wherein V ₆ is V, L, M, A, G, N, T, S, P, I, F, or H; D ₇ is D, G, Y, N, E, S, R, A, L, M, H, T, Q, I, or W; A ₉ is A, S, V, T, or G; V ₁₀ is V, L, F, I, A, S, G, T, M, Q, N, or C; A ₁₁ is A, S, T, V, G, C, N, or D	CDR-L1 (feline OSMR)
29	L ₁ A ₂ S ₃ T ₄ R ₅ H ₆ T ₇ wherein L ₁ is L, S, F, M, A, G, or Q; A ₂ is A, S, V, G, D, P, T, L, E, or C	CDR-L2 (canine OSMR)
29	L ₁ A ₂ S ₃ T ₄ R ₅ H ₆ T ₇ wherein A ₂ is A, S, V, G, D, T, L, E, R, C, Y	CDR-L2 (feline OSMR)
30	Q ₁ Q ₂ Y ₃ S ₄ R ₅ F ₆ P ₇ L ₈ T ₉ F ₁₀ wherein Q ₁ is Q, S, H, K, E, G, M, A, N, F, or Y; Q ₂ is Q or H; Y ₃ is Y or F; S ₄ is S, I, N, A, or T; F ₆ is F, L, I, V, Y, M, E, W, or D; P ₇ is P, S, C, A, G, or D; L ₈ is L, R, P, M, S, V, I, W, F, or E; F ₁₀ is F, L, M, I, Y, or W	CDR-L3 (canine OSMR)
30	Q ₁ Q ₂ Y ₃ S ₄ R ₅ F ₆ P ₇ L ₈ T ₉ F ₁₀ wherein Q ₁ is Q, L, S, H, K, V, E, G, M, A, T, N, F, Y, or C; Q ₂ is Q, M, H, S, Y, T, N, F, E, C, or A; Y ₃ is Y, L, R, M, F, W, H, I, or N; P ₇ is P, S, H, T, R, C, A, G, or D	CDR-L3 (feline OSMR)

Table B. Results from yeast display mutational analysis of each CDR on antibody 19F07 showing permissible (allow binding) substitutions at each indicated position. The results

described here are from those mutations at amino acids positions where a limited number of allowed substitutions occurred which impact binding and not from those positions where many allowable substitutions occur.

5 Example 22. Results from YSD to determine amino acid residues on OSMR involved with anti-OSMR antibody binding

Example 20 describes the method used to determine amino acid residues on the binding paratope of antibody 19F07 that are important for interaction with canine and feline OSMR. A similar method was used to determine the amino acid residues on feline OSMR that interact
10 with feline IL-31. Feline OSMR (SEQ ID NO: 112 (Feline_OSMR_hIgG1_Fc)) was displayed on the surface of yeast in its wildtype form and conditions of binding to feline IL-31 (SEQ ID NO:125) were optimized. A mutational library of feline OSMR was generated as described and flow cytometry sorting coupled with deep sequencing was carried out to determine mutations on feline OSMR defining relevant amino acid contacts that are made with feline IL-31. These data
15 reveal the epitope for binding of feline IL-31 to feline OSMR is a region on feline OSMR located between Leucine 157 (L157) to Phenylalanine 229 (F229) of SEQ ID NO: 112 (data not shown). It is important to note that these data support the homology model put forth in Example 10 of this application. This homology model describes a region where canine and feline OSM interact with OSMR. Functional data from cell-based assays described herein indicate these select anti-
20 OSMR antibodies have inhibitory properties against both IL-31 and OSM-mediated pSTAT3 signaling indicating a close proximity in the binding site of IL-31 and OSM on the OSMR protein. These mapping data do indeed support a similar binding site of feline IL-31 on feline OSMR as that proposed by the homology model of canine and feline OSM on their respective OSMR proteins. Taken together these results support knowledge of the relevant epitope space on
25 canine and feline OSMR required for binding of an antibody to inhibit the signaling function of the OSMR *in vivo*.

Example 23. A pro-inflammatory role of canine OSM in canine synoviocytes

30 Antagonism of OSMR signaling has proven effective at inhibiting downstream activation of Monocyte Chemoattractant Protein-1 (MCP-1) in canine chondrocytes and canine joint-derived synovial cells and significantly decreases Oncostatin M (OSM) induced cell proliferation in joint-derived synovial cells *in vitro* indicating a potential novel therapeutic for reduction in pain and inflammation *in vivo*. Anti-OSMR monoclonal antibodies represent a new class of novel

therapeutics that block OSM signaling for alleviating pain and inflammation associated with canine and feline osteoarthritis (OA). For determining *in vitro* potency, anti-OSMR antibodies were evaluated for functional activity in joint-derived synovial cells in proliferation and inhibition cell-based assays. A CellTiter-GLO luminescent cell viability assay kit was employed to

5 evaluate the effects of anti-OSMR mAbs on canine OSM induced cell proliferation. The primary canine joint-derived synovial cells were isolated and determined to respond to OSM stimulation by activating STAT-3 pathway and to a lesser extent STAT-5, and STAT-1. Canine joint-derived synoviocytes respond to OSM stimulation by rapid proliferation in 24 and 72 hours when plated on collagen type-1 coated plates (figure 9a). The anti-OSMR antibody Mu_10F07 potently

10 inhibited OSM-induced cell proliferation in canine joint-derived synovial cells (figure 9b). Canine synoviocytes also respond to OSM stimulation by rapidly synthesizing MCP-1 which recruits monocytes, macrophages, dendritic cells, and T cells to sites of active inflammation (figure 10a). The anti-OSMR antibody 10F07 dose-dependently reduced canine OSM-induced MCP-1 synthesis. Overall, results from these *in vitro* studies confirm functional activity relevant to an

15 anti-inflammatory role of blocking OSMR signaling using novel anti-OSMR antibodies.

Example 24. The pro-fibrotic role of OSM

Activation of MCP-1 in canine primary canine renal fibroblasts was determined to investigate the

20 role of OSM in renal fibrosis relevant to chronic kidney disease in dogs and cats. Canine OSM (SEQ ID NO:109) showed a dose dependent increase in MCP-1 levels over a 72 hour time period (figure 11). Blockade of this chemoattractant protein in renal fibroblasts with an anti-OSMR antibody may be beneficial in altering the progression of fibrotic disease.

25 Example 25. Evaluation of Anti-Canine OSMR mAbs in a Canine Model of IL-31-Induced Pruritus

IL-31 challenge procedures were conducted on Days -7 to establish baseline pruritic scores and Day 7. The intravenous solution was prepared from stock concentrations of canine recombinant

30 IL-31. Recording of pruritic activity for all dogs began after the challenge had been administered to the last dog on Study Days -7 and 7. Individuals administering the IL-31 challenge that were masked to treatment allocation could also record pruritic activity. A pruritus score was determined for (Post Challenge Period) for each dog using the following categorical scoring system. Specifically, at consecutive 1-minute intervals, "yes"/"no" decisions

were made regarding whether a pruritic behavior was displayed by each dog. Displays of the following pruritic behavior were enough to elicit a “yes” response over each discrete 1-minute time interval within an observation period. These behaviors include, licking or chewing (e.g., of paws, flank, tail, anal region), scratching (e.g., of flank or neck), headshaking or body-shaking, and rubbing any part of the body (e.g., on the cage flooring/walls). A “yes” response was indicated by marking a “1” and a “no” response was indicated by marking a “0” in the space provided for the specific 1-minute interval. The cumulative number of yes responses determined the Pruritus Score. A live feed camera located directly above each pen in the Surveillance Room allowed the scorers to observe the dogs from a separate room. Each scorer observed 4 dogs simultaneously with a real time image of each dog displayed on a single monitor. The maximum possible Post Challenge Pruritus score for an animal over the 120-minute period was 120. After completion of the observation period, the dogs were returned to their normal housing locations.

Pruritus Score for each animal was calculated as the total number of one-minute time segments pruritus behavior was observed for each time point (Days -7 and 7). A paired t-test was used to test for differences in means between Day -7 and Day 7 pruritic scores. Mouse:canine chimeric anti-OSMR antibodies 19F07 (T01) and 10F07 (T02) were administered as a single SC injection at 12.0 mg/kg and pruritic responses were observed 7 days post dosing. Figure 12 shows the post challenge scores for the total 2-hour observation periods on Study Day -7 and Study Day 7. These results demonstrate that a single subcutaneous 12 mg/kg dose of chimeric 19F07 and 10F07 showed a significantly lower total pruritus score at Study Day 7 in a canine model of IL-31 induced pruritus compared to pruritus scores recorded on Study Day -7.

What is claimed is:

1. An isolated antibody, or antigen-binding portion thereof that specifically binds to canine or feline Oncostatin M receptor Beta (OSMR- β) or both, wherein the antibody antagonizes IL-31-mediated signaling or OSM-mediated signaling or both in a canine and/or feline cell.
2. The antibody, or antigen-binding portion thereof of claim 1, wherein the antibody antagonizes both IL-31-mediated signaling and OSM-mediated signaling in a canine and/or feline cell.
3. The antibody, or antigen-binding portion thereof of claim 1 or claim 2, comprising a combination of complementary determining region (CDR) sequences selected from the group consisting of:
 - 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY), variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSISSNNLH), VL-CDR2 of SEQ ID NO: 5 (YASQSISS), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);
 - 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
 - 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
 - 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY), VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
 - 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYDMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV), VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
 - 6) CDR variants of 1, 2, 3, 4, or 5.
4. The antibody of any one of claims 1 to 3, comprising at least one of the group consisting of:

(c) a variable heavy chain comprising

SEQ ID NO: 31 (MU_02D09_VH)

EVQLVESGGGLVKPGGSLTSCAASGFTFSDYGMHWLRQAPEKGLEWWAYISSGSRAVFFAD
TVKGRFTISRDNANTLYLQMTSLRSDDTAMYYCARDRYDGRGFAYWGQGLTVTVSA,

SEQ ID NO: 35 (MU_09E09_VH)

DVKLVESGGLVKPGGSLKLSAASGFTFSSYAMSWVRQTPEKRLEWWAYISSGGDIYYADT
VKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCTRDPITGTFAYWGQGLTVTVSA,

SEQ ID NO: 39 (MU_10F07_VH)

DVKLVESGGLVKPGGSLKLSAASGFTFSSYAMSWVRQTPEKRLEWWTYISSGGDYFYAD
TVKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCTRDPITGTFAYWGQGLTVTVSA,

SEQ ID NO: 43 (MU_14C04_VH)

EVQLQESGAELVKPGASVKISCKASGYAFSNYWMNWMKQRPKGLEWIGQIYPGHVNTNYN
GNFKDKATLTADK
SSSTAYMQLSSLTSEDSAVYFCARSADNSGFVLFAYWGQGLTVTVS,

SEQ ID NO: 47 (MU_19F07_VH)

EVKLVESEGGLVQPGSSMKLSCTASGFTFSDYYMAWVRQVPEKGLEWWANINYDGSSTYYLD
SLKSRFIISRDNANKILYLQMSSLKSEDTATYYCARGLTWDFDVWGTGTTVTVSS,

SEQ ID NO: 51 (FEL_02D09_VH1)

DVQLVESGGDLVKPGGSLRLTCVASGFTYSDYGMHWVRQAPGKGLQWWAYISSGSRAVFFA
DTVKGRFTISRDNANKNTLYLQMNSLKTEDTATYYCVRDRYDGRGFAYWGQGLTVTVSS,

SEQ ID NO: 53 (FEL_02D09_VH2)

DVQLVESGGDLVKPGGSLRLTCVASGFTFSDYGMHWVRQAPGKGLQWWAYISSGSRAVFFA
DTVKGRFTISRDNANKNTLYLQMNGLRTEDTATYYCARDRYDGRGFAYWGQGLTVTVSS,

SEQ ID NO: 59 (CAN_09E09_VH1)

EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDIYYAD
TVKGRFTISRDNANKNTLYLQMNSLRAEDTAMYYCVRDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 61 (CAN_09E09_VH2)

EVQLVESGGDLVKPAGSLTSLCLASGFTFSSYAMSWVRQTPEKGLQWWAYISSGGDIYYADT
VKGRFTISRDNANKNTLYLQMNSLRDEDTAVYYCARDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 67 (FEL_09E09_VH1)

DVQLVESGGDLVKPGGSLRLTCVASGFTYSSYAMSWVRQAPGKGLQWWAYISSGGDIYYAD
TVKGRFTISRDNANKNTLYLQMNSLKTEDTATYYCVRDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 69 (FEL_09E09_VH2)

DVQLVESGGNLVKPGGSLRLTCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDIYYAD
TVKGRFTISKDNANKNTLYLQMNSLKTEDTATYYCARDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 75 (CAN_10F07_VH1)

EVQLVESGGDLVKPGGSLRLSCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYFYA
DTVKGRFTISRDNANKNTLYLQMNSLRAEDTAMYYCVRDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 79 (CAN_10F07_VH2)

EVQLVESGGDLVKPAGSLTSLCLASGFTFSSYAMSWVRQTPEKGLQWWAYISSGGDYFYAD
TVKGRFTISRDNANKNTLYLQMNSLRDEDTAVYYCARDPITGTFAYWGQGLTVTVSS,

SEQ ID NO: 83 (FEL_10F07_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSSYAMSWVRQAPGKGLQWWAYISSGGDYFYFA
DTVKGRFTISRDNANTLYLQMNSLKTEDTATYYCVRDPITGTFAYWGQGTLLTVSS,

SEQ ID NO: 87 (FEL_10F07_VH2)
DVQLVESGGDLVKPGGSLRLTCVASGFTFSSYAMSWVRQAPGKGLQWWAYISSGGDYFYFA
DTVKGRFTISRDDAKNTLYLQMSSLKTEDTATYYCTGDPITGTFAYWGQGTLLTVSS,

SEQ ID NO: 91 (CAN_19F07_VH1)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSDYYMAWVRQAPGKGLQWWANINYDGSSTYYLD
SLKSRFTISRDNANTLYLQMNSLRAEDTAMYYCVRGLTWDFDVWGQGTLLTVSS,

SEQ ID NO: 95 (CAN_19F07_VH2)
EVQLVESGGDLVKPAGSLTLSCLASGFTFSDYYMAWVRQTPEKGLQWWANINYDGSSTYYLD
SLKSRFTISRDNANTLYLQMNSLRDEDTAVYYCARGLTWDFDVWGQGTLLTVSS,

SEQ ID NO: 99 (FEL_19F07_VH1)
DVQLVESGGDLVKPGGSLRLTCVASGFTYSDYYMAWVRQAPGKGLQWWANINYDGSSTYYL
DSLKSRFTISRDNANTLYLQMNSLKTEDTATYYCVRGLTWDFDVWGQGTLLTVSS,

SEQ ID NO: 103 (FEL_19F07_VH2)
DVQLVESGGDLVKPGGSLRLTCVASGFTFSDYYMAWVRQAPGKGLQWWANINYDGSSTYYLD
SLKSRFTISRDNANTLYLQMNSLKTEDTATYYCARGLTWDFDVWGQGTLLTVSS,

SEQ ID NO: 127. (CAN_14C04_VH1)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYWMNWRQAPGKGLQWWAQIYPGHVNTNYN
GNFKDRFTISRDNARNTVYLQMNSLRAEDTAVYYCARSADNSGFVLFAYWGQGTLLTVSS, or

SEQ ID NO: 129. (CAN_14C04_VH2)
EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYWMNWRQSPGKGLQWWAQIYPGHVNTNYN
GNFKDRFTISRDNANTLYLQMNSLRAEDTAVYYFCARSADNSGFVLFAYWGQGTLLTVSS;
and

(d) a variable light chain comprising

SEQ ID NO: 33 (MU_02D09_VL)
DIVLTQSPATLSVTPGDSVSLSCRASQSSISNNLHWYQQTSHEPRLLITYASQSSIGIPSRFSGS
GSGTDFTLSINSVETEDFGMYFCQQSNSWPLTFGAGTKLELK,

SEQ ID NO: 37 (MU_09E09_VL)
DLQMTQTSSLSASLGDRVTISCRASQDINNYLNWYQQKPDGTVKLLIYYTSTLHSGVPSRFSG
SGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIK,

SEQ ID NO: 41 (MU_10F07_VL)
DIQMTQTSSLSASLGDRVTISCRASQDITNYLNWYQQKPDGTVKLLIYYTSTLHSGVPSRFSG
SGSGTDFSLTISNLEQEDIATYFCQQGHMLPWTFGGGKLEIK,

SEQ ID NO: 45 (MU_14C04_VL)
DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYLHWYQQKPGQPPLKLLIFLASNLESGVPA
RFSGSGSGTDFTLNHPVEEEDAATYYCQHSRELPLTFGAGTKLELK,

SEQ ID NO: 49 (MU_19F07_VL)
DIVMTQSHKFMSPSVGDRVSITCKASQDQDVTAVAWYQQKPGQSPKLLIYLASTRHTGVPDRFT
GSGSGTDFTLTISNVQSEDLADYFCQQYSRFLTFGAGTKLELK,

SEQ ID NO: 55 (FEL_02D09_VL1)
EIQMTQSPSSLSASPGDRVITICRASQSSNNLHWYQQKPGKVPKLLIYYASQSISGVPSRFSG
SGSGTDFTLTISLEPEDAATYYCQQSNSWPLTFGQGT,

SEQ ID NO: 57 (FEL_02D09_VL2)
DIVMTQTPLSLSVTPGESASISCRASQSSNNLHWYQLKSGQSPRRLIYYASQSISGVDPDRFSG
SGSGTDFTLRISRVEADDVGYYCQQSNSWPLTFGQGT,

SEQ ID NO: 63 (CAN_09E09_VL1)
EIVMTQSPASLSLSQEEKVTITCRASQDINNYLNWYQQKPGQAPKLLIYYTSTLHSGVPSRFSG
SGSGTDFSTISLEPEDVAVYYCQQGNTLPWTFGQGT,

SEQ ID NO: 65 (CAN_09E09_VL2)
DIVLTQPTSVSGSLGQRVITICRASQDINNYLNWYQQLPKGAPKLLVYYTSTLHSGVPDRFSGS
NSGSSATLTITGLQAEDEADYYCQQGNTLPWTFGQGT,

SEQ ID NO: 71 (FEL_09E09_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDINNYLNWYQQKPGKVPKLLIYYTSTLHSGVPSRFSG
SGSGTDFTLTISLEPEDAATYYCQQGNTLPWTFGQGT,

SEQ ID NO: 73 (FEL_09E09_VL2)
DITMTQSPGSLAGSPGQQVTMNCRASQDINNYLNWYQQKPGQHPKLLIYYTSTLHSGVPDRF
SGSGTDFTLTISNLQAEDVASYYCQQGNTLPWTFGQGT,

SEQ ID NO: 77 (CAN_10F07_VL1)
EIVMTQSPASLSLSQEEKVTITCRASQDITNYLNWYQQKPGQAPKLLIYYTSTLHSGVPSRFSG
SGSGTDFSTISLEPEDVAVYYCQQGHMLPWTFGQGT,

SEQ ID NO: 81 (CAN_10F07_VL2)
DIVLTQPTSVSGSLGQRVITICRASQDITNYLNWYQQLPKGAPKLLVYYTSTLHSGVPDRFSGS
NSGSSATLTITGLQAEDEADYYCQQGHMLPWTFGQGT,

SEQ ID NO: 85 (FEL_10F07_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDITNYLNWYQQKPGKVPKLLIYYTSTLHSGVPSRFSG
SGSGTDFTLTISLEPEDAATYYCQQGHMLPWTFGQGT,

SEQ ID NO: 89 (FEL_10F07_VL2)
DITMTQSPGSLAGSPGQQVTMNCRASQDITNYLNWYQQKPGQHPKLLIYYTSTLHSGVPDRFS
SGSGTDFTLTISNLQAEDVASYYCQQGHMLPWTFGQGT,

SEQ ID NO: 93 (CAN_19F07_VL1)
EIVMTQSPASLSLSQEEKVTITCKASQDVDTAVAWYQQKPGQAPKLLIYLASTRHTGVPSRFSG
SGSGTDFSTISLEPEDVAVYYCQQYSRFPLTFGQGT,

SEQ ID NO: 97 (CAN_19F07_VL2)
DIVMTQTPLSLSVSPGETASISCKASQDVDTAVAWFRQKPGQSPQRLLIYLASTRHTGVDPDRFS
SGSGTDFTLRISRVEADDTGVYYCQQYSRFPLTFGQGT,

SEQ ID NO: 101 (FEL_19F07_VL1)
EIQMTQSPSSLSASPGDRVITICRASQDVDTAVAWYQQKPGKVPKLLIYLASTRHTGVPSRFS
SGSGTDFTLTISLEPEDAATYYCQQYSRFPLTFGQGT,

SEQ ID NO: 105 (FEL_19F07_VL2)
DITMTQSPGSLAGSPGQQVTMNCASQDVDTAVAWYQQKPGQHPKLLIYLASTRHTGVDPDRF
SGSGTDFTLTISNLQAEDVASYYCQQYSRFPLTFGQGT,

SEQ ID NO: 131. (CAN_14C04_VL1)

EIVMTQSPASLSLSQEEKVTITCRASKSVSTSGYSYLHWYQQKPGQAPKLLIYLASNLESGVPS
RFGSGSGTDFSTISSLEPEDVAVYYCQHSRELPLTFGQGT, or

SEQ ID NO: 133. (CAN_14C04_VL2)

DIVMTQTPLSLSVSPGETASISCRASKSVSTSGYSYLHWYLQKPGQSPQLLIYLASNLESGVSK
RFGSGSGTDFTLRISRVEADDTGIYYCQHSRELPLTFGQGT.

5. The antibody of any one of claims 1 to 4, wherein the antibody is chimeric.
6. The antibody of any one of claims 1 to 4, wherein the antibody is caninized or felinized.
7. The antibody of any one of claims 1 to 4, wherein the antibody inhibits or neutralizes an IL-31-mediated or OSM-mediated pruritic or allergic condition in a dog or cat.
8. The antibody of claim 7, wherein the IL-31-mediated or OSM-mediated pruritic condition is selected from the group consisting of atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis.
9. The antibody of claim 8, wherein the IL-31-mediated or OSM-mediated allergic condition is selected from the group consisting of allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstruction pulmonary disease, and inflammatory processes resulting from autoimmunity.
10. The antibody of any one of claims 1 to 4, wherein the antibody inhibits an IL-31-mediated or OSM-mediated fibrotic or inflammatory disorder.
11. The antibody of claim 10, wherein the IL-31-mediated or OSM-mediated fibrotic disorder is selected from the group consisting of renal fibrosis, pulmonary fibrosis, and dermal fibrosis.
12. The antibody of claim 10, wherein the IL-31-mediated or OSM-mediated inflammatory disorder is selected from the group consisting of inflammatory processes resulting from autoimmunity, inflammation in the skin or joint of animals affected by osteoarthritis, immune-mediated polyarthritis, chronic bronchitis, allergic asthma, atopic dermatitis,

allergic dermatitis, pyotraumatic dermatitis, atherosclerosis, and cardiovascular disease.

13. The antibody of any one of claims 1 to 4, wherein the antibody reduces IL-31-mediated or OSM-mediated inflammatory pain.
14. The antibody of claim 13, wherein the IL-31-mediated or OSM-mediated inflammatory pain is osteoarthritis pain.
15. A veterinary composition comprising a therapeutically effective amount of the antibody of any one of claims 1 to 4.
16. A method of treating an IL-31-mediated or OSM-mediated disorder in a subject, comprising administering an antibody of any one of claims 1 to 4 to the subject.
17. The method of claim 16, wherein the IL-31-mediated or OSM-mediated disorder is selected from the group consisting of a pruritic condition, an allergic condition, a fibrotic disorder, an inflammatory disorder, and inflammatory pain.
18. The method of claim 17, wherein the IL-31-mediated or OSM-mediated pruritic condition is selected from the group consisting of atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis.
19. The method of claim 17, wherein the IL-31-mediated or OSM-mediated allergic condition is selected from the group consisting of allergic dermatitis, summer eczema, urticaria, heaves, inflammatory airway disease, recurrent airway obstruction, airway hyper-responsiveness, chronic obstruction pulmonary disease, and inflammatory processes resulting from autoimmunity.
20. The method of claim 17, wherein the IL-31-mediated or OSM-mediated fibrotic disorder is selected from the group consisting of renal fibrosis, pulmonary fibrosis, and dermal fibrosis.
21. The method of claim 17, wherein the IL-31-mediated or OSM-mediated inflammatory disorder is selected from the group consisting of inflammatory processes resulting from autoimmunity, inflammation in the skin or joint of animals affected by osteoarthritis, immune-mediated polyarthritis, chronic bronchitis, allergic asthma, atopic dermatitis,

allergic dermatitis, pyotraumatic dermatitis, atherosclerosis, and cardiovascular disease.

22. The method of claim 17, wherein the IL-31-mediated or OSM-mediated inflammatory pain is osteoarthritis pain.

23. The method of any one of claims 16 to 22, wherein the subject is a dog or a cat.

24. A method of inhibiting IL-31 and/or OSM activity in a dog or cat comprising administering an antibody according to any one of claims 1 to 4 to the dog or cat.

25. A method of detecting OSMR Beta in a sample comprising:

- a) incubating a sample comprising OSMR Beta in the presence of an antibody according to any one of claims 1 to 4; and
- b) detecting the antibody which is bound to OSMR Beta in the sample.

26. The method of claim 25, wherein the antibody comprises a label.

27. The method of claim 25 or claim 26, further comprising quantitating the OSMR Beta in the sample.

28. A host cell that produces an isolated antibody or antigen-binding portion thereof comprising at least one of the following combinations of complementary determining region (CDR) sequences:

- 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRAVFFADTVKG), VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY), variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQISNNLH), VL-CDR2 of SEQ NO: 5 (YASQIS), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);
- 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY), VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY), VL-

CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);

- 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYYMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV), VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

29. An isolated nucleic acid comprising a nucleic acid sequence encoding at least one of the following combinations of complementary determining region (CDR) sequences:

- 1) 02D09: variable heavy (VH)-CDR1 of SEQ ID NO: 1 (DYGMH), VH-CDR2 of SEQ ID NO: 2 (YISSGSRVFFADTVKG), and VH-CDR3 of SEQ ID NO: 3 (DRYDGRGFAY);
- 2) 09E09: VH-CDR1 of SEQ ID NO: 7 (SYAMS), VH-CDR2 of SEQ ID NO: 8 (YISSGGDYIYYADTVKG), and VH-CDR3 of SEQ ID NO: 9 (DPITGTFAY);
- 3) 10F07: VH-CDR1 of SEQ ID NO: 13 (SYAMS), VH-CDR2 of SEQ ID NO: 14 (YISSGGDYFYADTVKG), and VH-CDR3 of SEQ ID NO: 15 (DPITGTFAY);
- 4) 14C04: VH-CDR1 of SEQ ID NO: 19 (NYWMN), VH-CDR2 of SEQ ID NO: 20 (QIYPGHVNTNYNGNFKD), and VH-CDR3 of SEQ ID NO: 21 (SADNSGFVLFAY);
- 5) 19F07: VH-CDR1 of SEQ ID NO: 25 (DYYMA), VH-CDR2 of SEQ ID NO: 26 (NINYDGSSTYYLDSLKS), and VH-CDR3 of SEQ ID NO: 27 (GLTWDFDV); or
- 6) CDR variants of 1, 2, 3, 4, or 5.

30. The nucleic acid of claim 29, further comprising a nucleic acid sequence encoding at least one of the following combinations of CDR sequences:

- 1) 02D09: variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSSISNNLH), VL-CDR2 of SEQ ID NO: 5 (YASQSSIS), and VL-CDR3 of SEQ ID NO: 6 (QQSNSWPLT);
- 2) 09E09: VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
- 3) 10F07: VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
- 4) 14C04: VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
- 5) 19F07: VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or

- 6) CDR variants of 1, 2, 3, 4, or 5.
31. A nucleic acid sequence encoding at least one of the following combinations of CDR sequences:
- 1) 02D09: variable light (VL)-CDR1 of SEQ ID NO: 4 (RASQSIENNHL), VL-CDR2 of SEQ NO: 5 (YASQSI), and VL-CDR3 of SEQ ID NO: 6 (QQNSWPLT);
 - 2) 09E09: VL-CDR1 of VL-CDR1 of SEQ ID NO: 10 (RASQDINNYLN), VL-CDR2 of SEQ ID NO: 11 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 12 (QQGNTLPWT);
 - 3) 10F07: VL-CDR1 of SEQ ID NO: 16 (RASQDITNYLN), VL-CDR2 of SEQ ID NO: 17 (YTSTLHS), and VL-CDR3 of SEQ ID NO: 18 (QQGHMLPWT);
 - 4) 14C04: VL-CDR1 of SEQ ID NO: 22 (RASKSVSTSGYSYLH), VL-CDR2 of SEQ ID NO: 23 (LASNLES), and VL-CDR3 of SEQ ID NO: 24 (QHSRELPLT);
 - 5) 19F07: VL-CDR1 of SEQ ID NO: 28 (KASQDVDTAVA), VL-CDR2 of SEQ ID NO: 29 (LASTRHT), and VL-CDR3 of SEQ ID NO: 30 (QQYSRFPLT); or
- 6) CDR variants of 1, 2, 3, 4, or 5.
32. A vector comprising the nucleic acid of any one of claims 29, 30, or 31.
33. A method of producing an antibody comprising culturing the host cell of claim 28 under conditions that result in production of the antibody and isolating the antibody from the host cell or culture medium of the host cell.