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(54) Title: CANINIZED RAT ANTIBODIES TO CANINE INTERLEUKIN-31 RECEPTOR *ALPHA*(57) Abstract: The present invention provides caninized rat antibodies to canine IL-31 receptor *alpha* that have a high binding affinity for canine IL-31 receptor *alpha*, and that can block the binding of canine IL-31 to canine IL-31 receptor *alpha*. The present invention further provides the use of the antibodies for the treatment of atopic dermatitis in dogs.

CANINIZED RAT ANTIBODIES TO CANINE INTERLEUKIN-31 RECEPTOR *ALPHA*

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) of provisional applications:

- 5 U.S. Serial 63/092,294, filed October 15, 2020, U.S. Serial 63/092,296, filed October 15, 2020, U.S. Serial 63/127,184, filed December 18, 2020, U.S. Serial 63/235,258, filed August 20, 2021, U.S. Serial 63/235,257, filed August 20, 2021, the contents of which are hereby incorporated by reference in their entireties.

## 10 SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 1, 2021, is named 25070-WO-PCT\_SL.txt and is 104,159 bytes in size.

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## FIELD OF THE INVENTION

- The present invention relates to antibodies to canine IL-31 receptor *alpha* that have a high binding affinity for canine IL-31 receptor *alpha*, and that can block the binding of canine IL-31 to the canine IL-31 receptor *alpha*. The present invention also relates to use of the antibodies of the present invention in the treatment of atopic dermatitis in dogs.

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## BACKGROUND OF THE INVENTION

- The immune system comprises a network of resident and recirculating specialized cells that function collaboratively to protect the host against infectious diseases and cancer. The ability of the immune system to perform this function depends to a large extent on the biological activities of a group of proteins secreted by leukocytes and collectively referred to as interleukins. Among the well-studied interleukins are four important molecules identified as interleukin-31 (IL-31), interleukin-4 (IL-4), interleukin-13 (IL-13), and interleukin-22 (IL-22). Although IL-4, IL-13, IL-22, and IL-31, are critical cytokines for the development of immune responses that are required for protection against extracellular pathogens (*e.g.*, tissue or lumen dwelling parasites), these cytokines also have been implicated in the pathogenesis of allergic diseases in humans and animals, including atopic dermatitis.

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Atopic dermatitis (AD) is a relapsing pruritic and chronic inflammatory skin disease, that is characterized by immune system dysregulation and epidermal barrier abnormalities in humans. The pathological and immunological attributes of atopic dermatitis have been the subject of extensive investigations [reviewed in Rahman *et al.* *Inflammation & Allergy-drug target* 10:486-496 (2011) and Harskamp *et al.*, *Seminar in Cutaneous Medicine and Surgery* 32:132-139 (2013)]. Atopic dermatitis is also a common condition in companion animals, especially dogs, where its prevalence has been estimated to be approximately 10-15% of the canine population. The pathogenesis of atopic dermatitis in dogs and cats [reviewed in Nuttall *et al.*, *Veterinary Records* 172(8):201-207 (2013)] shows significant similarities to that of atopic dermatitis in man including skin infiltration by a variety of immune cells and CD4<sup>+</sup> Th2 polarized cytokine milieu including the preponderance of IL-31, IL-4, and IL-13. In addition, IL-22 has been implicated in the exaggerated epithelial proliferation leading to epidermal hyperplasia that is characteristic of atopic dermatitis.

For example, antibodies against canine IL-31 have been shown to have an effect on pruritus associated with atopic dermatitis in dogs [US 8,790,651 B2; US 10,093,731 B2]. In addition, an antibody against human IL-31 receptor *alpha* (IL-31RA) has been tested and found to have an effect on pruritus associated with atopic dermatitis in humans [Ruzicka, *et al.*, *New England Journal of Medicine*, 376(9),826–835 (2017)].

IL-4 and IL-13 are closely related proteins that can be secreted by many cell types including CD4<sup>+</sup> Th2 cells, natural killer T cells (NKT), macrophages, mast cells, and basophils. IL-4 and IL-13 display many overlapping functions and are critical to the development of T cell-dependent humoral immune responses. It is known that IL-4 binds with high affinity to two receptors *i.e.*, type-I and type-II IL-4 receptors. Monoclonal antibodies raised against human IL-4 receptor *alpha* (IL-4 R<sub>α</sub>) have been developed and some of these antibodies have been extensively tested for their therapeutic effects for treating atopic dermatitis in humans [*see, e.g.*, US2015/0017176 A1]. More recently, caninized antibodies to canine IL-4 R<sub>α</sub> that block the binding of canine IL-4 to canine IL-4 R<sub>α</sub> also have been disclosed [US2018/0346580A1, hereby incorporated by reference in its entirety]. Because the Type II IL-4 receptor consists of the IL-4 receptor α chain and the IL-13 receptor α1 chain, antibodies to canine IL-4 R<sub>α</sub> have been obtained that can block both canine IL-4 and canine IL-13 from binding the Type II canine IL-4 receptor, thereby serving to help block the inflammation associated with atopic dermatitis [US2018/0346580A1].

Interleukin-22 (IL-22), also known as IL-10-related T cell-derived inducible factor (IL-TIF), belongs to the IL-10 cytokine family. IL-22 is produced by normal T cells upon anti-CD3 stimulation in humans. Mouse IL-22 expression is also induced in various organs upon lipopolysaccharide injection, suggesting that IL-22 may be involved in inflammatory responses.

5 IL-22 binds specifically to, and signals through, a receptor complex consisting of a heterodimeric complex of IL-10R2 (also known as IL-10R *beta*) and the Interleukin-22 receptor (IL-22R) [see, Lee *et al.*, *Pharmacology Research & Perspectives*, Pages 1-13 (2018:e00434)]. The Interleukin-22 receptor is also known as Interleukin-22R, *alpha* 1; IL-22RA1; IL-22R1; zcytor11; and CRF2-9 [Xu *et al.*, *Proc. Nat. Acad. Sci.* 98 (17) 9511-9516 (2001); Gelebart and  
10 Lai, *Atlas of Genetics and Cytogenetics* 14(12):1106-1110 (2010)]. IL-22 induces epithelial cell proliferation during wound healing, and its deficiency can enable enhanced tumor development [Huber *et al.*, *Nature* 491:259-263 (2012)]. IL-22 has been shown to activate STAT-1 and STAT-3 in several hepatoma cell lines and upregulate the production of acute phase proteins. Antibodies to Interleukin-22 and IL-22R act as anti-proliferative agents by blocking the  
15 interaction of IL-22 with IL-22R and thereby the related signaling pathway that leads to the epithelial proliferation.

Pharmaceuticals that have either proven to aid in the treatment of atopic dermatitis and/or have shown promise to do so include: Janus kinase (JAK) inhibitors [see *e.g.*, U.S. 8,133,899; U.S. 8,987,283; WO 2018/108969], spleen tyrosine kinase (SYK) inhibitors [see *e.g.*, U.S.  
20 8,759,366], and antagonists to a chemoattractant receptor-homologous molecule expressed on TH2 cells [see *e.g.*, U.S. 7,696,222, U.S. 8,546,422, U.S. 8,637,541, and U.S. 8,546,422].

However, despite some recent success in treating atopic dermatitis, there remains a need to design alternative and/or better therapies that can address one or more of the symptoms of canine atopic dermatitis.

25 The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

## SUMMARY OF THE INVENTION

The present invention provides new mammalian antibodies, including caninized  
30 antibodies, to IL-31 receptor *alpha* (IL-31RA) from canines. In certain embodiments, the mammalian antibodies to canine IL-31 receptor *alpha* (cIL-31RA) are isolated antibodies. In preferred embodiments, the mammalian antibodies or antigen binding fragments thereof bind canine IL-31RA. In more particular embodiments, the mammalian antibodies or antigen binding

fragments also block the binding of canine IL-31RA to canine interleukin-31. In particular embodiments, the antibodies are rat antibodies to canine IL-31RA. In more particular embodiments, the mammalian antibodies are caninized rat antibodies to canine IL-31RA.

Accordingly, the present invention provides mammalian antibodies or antigen binding  
 5 fragments thereof that bind canine interleukin-31 receptor *alpha* and that comprise a heavy chain that comprises a set of three heavy chain complementary determining regions (CDRs), a CDR heavy 1 (HCDR1), a CDR heavy 2 (HCDR2), and a CDR heavy 3 (HCDR3) in which the HCDR1 comprises the amino acid sequence of SEQ ID NO: 31; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 41; and the HCDR3 comprises the  
 10 amino acid sequence of SEQ ID NO: 33, SEQ ID NO: 39, or SEQ ID NO: 43.

In particular embodiments of this type, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 33. In related embodiments, the  
 15 mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 39. In still other embodiments, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2  
 20 that comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 43. In yet other embodiments, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 41, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 39.

Any of the mammalian antibodies of the present invention can further comprises a set of  
 25 three light chain complementary determining regions (LCDRs): a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3), in which the LCDR1 comprises the amino acid sequence of SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, or SEQ ID NO: 44; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 37;  
 30 and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 36 or SEQ ID NO: 45. In particular embodiments, the mammalian antibody or antigen binding fragment further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 34, SEQ ID NO: 38, or SEQ

ID NO: 44; a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35 or 37; and a LCDR3 that comprises the amino acid sequence of SEQ ID NO: 36.

In certain embodiments, the mammalian antibody or antigen binding fragment comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that  
5 comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 33; and further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 34, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35, and the LCDR3 that comprises the amino acid sequence of SEQ ID NO: 36. In related  
10 embodiments, the mammalian antibody or antigen binding fragment comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 33; and further comprises a LCDR1 that comprises the amino acid sequence of  
15 SEQ ID NO: 34, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 37, and the LCDR3 that comprises the amino acid sequence of SEQ ID NO: 36. In other embodiments, the mammalian antibody or antigen binding fragment comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of  
20 SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 33; and further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 38, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35, and a LCDR3 that  
comprises the amino acid sequence of SEQ ID NO: 36.

In other embodiments, the mammalian antibody or antigen binding fragment comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that  
comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 39; and further comprises a LCDR1 that comprises the amino acid  
25 sequence of SEQ ID NO: 40, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35, and a LCDR3 that comprises the amino acid sequence of SEQ ID NO: 36. In still other embodiments, the mammalian antibody or antigen binding fragment comprises an HCDR1 that  
comprises the amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 41, and an HCDR3 that comprises the amino acid sequence of  
30 SEQ ID NO: 39; and further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 42, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35, and a LCDR3 that comprises the amino acid sequence of SEQ ID NO: 36. In yet other embodiments, the mammalian antibody or antigen binding fragment comprises an HCDR1 that comprises the

amino acid sequence of SEQ ID NO: 31, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 32, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 43; and further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 44, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35, and a LCDR3 that  
5 comprises the amino acid sequence of SEQ ID NO: 45. In specific embodiments, when bound to canine IL-31RA, the antibody binds to an epitope comprised by the amino acid of SEQ ID NO: 104, or SEQ ID NO: 105, or to both SEQ ID NO: 104 and SEQ ID NO: 105. In related embodiments, when bound to canine IL-31RA, the antibody binds at least one amino acid residue, preferably one to three amino acid residues, more preferably two to five amino acid  
10 residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 104 or SEQ ID NO: 105, or both SEQ ID NO: 104 and SEQ ID NO: 105.

In specific embodiments, the mammalian antibody to canine IL-31RA is a rat antibody. In particular embodiments, the mammalian antibody to canine IL-31RA is a caninized rat  
15 antibody. In certain embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D cFc, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising the amino acid sequence of SEQ ID NO: 79. In other embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D cFc, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising the amino acid sequence  
20 of SEQ ID NO: 80. In still other embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D cFc, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising the amino acid sequence of SEQ ID NO: 81. In yet other embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D cFc, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising the amino acid sequence  
25 of SEQ ID NO: 82.

In certain embodiments, the caninized antibody comprises a heavy chain comprising a modified canine IgG-B (IgG-Bm) that comprises the amino acid sequence of SEQ ID NO: 78. In alternative embodiments, the caninized antibody comprises a heavy chain comprising a non-modified canine IgG-B that comprises the amino acid sequence of SEQ ID NO: 77.

30 The present invention also provides nucleic acids, including isolated nucleic acids, that encode the CDRs, the heavy chains of the caninized antibodies or antigen binding fragments thereof, and/or the light chains of the caninized antibodies or antigen binding fragments thereof.

Accordingly, the present invention further provides a nucleic acid that encodes a set of the three heavy chain complementary determining regions (CDRs), a CDR heavy 1 (HCDR1), a CDR heavy 2 (HCDR2), and a CDR heavy 3 (HCDR3) of a mammalian antibody or an antigen binding fragment thereof of the present invention. In a more specific embodiment of this type, the nucleic acid encodes an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 31; an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 41; and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 33, SEQ ID NO: 39, or SEQ ID NO: 43.

In certain embodiments of the compositions, the caninized antibody against canine IL-31RA comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 or SEQ ID NO: 85 and a light chain comprising the amino acid sequence of SEQ ID NO: 83, SEQ ID NO: 86, or SEQ ID NO: 87. The present invention further provides antigen binding fragments of these caninized antibodies.

In particular embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 84. In other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 85. In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 86 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 84. In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 86 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 85. In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 87 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 84. In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 87 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 85. In specific embodiments, when bound to canine IL-31RA, the antibody binds to an epitope comprised by the amino acid of SEQ ID NO: 104, or SEQ ID NO: 105, or to both SEQ ID NO: 104 and SEQ ID NO: 105. In related embodiments, when bound to canine IL-31RA, the antibody binds at least one amino acid residue, preferably one to three amino acid residues, more preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID

NO: 104 or SEQ ID NO: 105, or both SEQ ID NO: 104 and SEQ ID NO: 105. The present invention further provides antigen binding fragments of these caninized antibodies.

The present invention further provides a nucleic acid that encodes a set of the three light chain complementary determining regions (CDRs), a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3) of a mammalian antibody or an antigen binding fragment thereof of the present invention. In a more specific embodiment of this type, the nucleic acid encodes an LCDR1 that comprises the amino acid sequence of SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, or SEQ ID NO: 44; an LCDR2 that comprises the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 37; and an LCDR3 that comprises the amino acid sequence of SEQ ID NO: 36 or SEQ ID NO: 45.

The present invention also provides a nucleic acid that encodes the heavy chain of a mammalian antibody or an antigen binding fragment thereof of the present invention. The present invention also provides a nucleic acid that encodes the light chain of a mammalian antibody or an antigen binding fragment thereof of the present invention. In addition, the present invention provides expression vectors that comprise one or more of the nucleic acids of the present invention, and host cells that comprise such expression vectors.

The present invention further provides pharmaceutical compositions that comprise the caninized antibodies and antigen binding fragments thereof of the present invention along with a pharmaceutically acceptable carrier and/or diluent. The present invention also provides methods of treating atopic dermatitis comprising administering one of the aforesaid compositions to a canine that has atopic dermatitis. In particular embodiments, the present invention provides methods of aiding in blocking of the pruritus associated with atopic dermatitis, comprising administering to a canine in need thereof a therapeutically effective amount of a pharmaceutical composition of the present invention.

These and other aspects of the present invention will be better appreciated by reference to the following Brief Description of the Drawings and the Detailed Description.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The extracellular domain (ECD) of canine IL-31RA was tested for its ability to bind to canine IL-31. The results indicate that canine IL-31RA ECD binds in a dose-dependent manner to biotinylated canine IL-31.

Figures 2A-2E. The selected rat mAbs to canine IL-31RA were tested for their reactivity to canine IL-31RA. The results indicate that the selected rat mAbs bind to canine IL-31RA in a

dose-dependent manner. All the 14 rat monoclonal antibodies tested have strong binding reactivity to canine IL-31RA. Figure 2A: 4G7 (●), 20B8 (■), 22B4 (▲), 27A10 (▼), and the Rat IgG2a/*Kappa* (◆) control. Figure 2B: 38B6 (●), 48B1 (■), 49D3 (▲), and the Rat IgG2a/*Kappa* (◆) control. Figure 2C: 10A12 (■), 44E2 (▼) and the Rat IgG2a/*Kappa* (◆) control.

5 Figure 2D: 47F3 (●), 51G4 (■), and the Rat IgG2a/*Kappa* (▼) control. Figure 2E: 7D7(●), 28F12 (■), 53B3 (▲), and the Rat IgG2a/*Kappa* (✕) and Rat IgG2b/*Kappa* (◆) controls.

Figures 3A-3D. The selected rat mAbs were tested for their ability to block the binding of canine IL-31 to canine IL-31RA by ELISA. The results indicated that some of the selected mAbs can block the binding of canine IL-31 to canine IL-31RA in a dose-dependent manner, whereas

10 others could not:

Figure 3A: 20B8 (●), 22B4 (■), 27A10 (▲); and the Rat IgG2a/*Kappa* (Δ) and Rat IgG2b/*Kappa* (▽) controls. Figure 3B: 38A6 (●), 49D3 (▲), 10A12(■); and the Rat IgG2a/*Kappa* (Δ) and Rat IgG2b/*Kappa* (▽) controls. Figure 3C: 53B3(■), 7D7(●); and the Rat IgG2a/*Kappa* (Δ) and Rat IgG2b/*Kappa* (▽) controls. Figure 3D: 51G4 (■), 47F3(●); and Rat

15 IgG2b/*Kappa* (▼) control.

Figure 4. Ba/f3-OI cells expressing the IL-31 receptor complex was tested for IL-31-induced STAT-3 phosphorylation. The results indicate that STAT-3 phosphorylation was induced by IL-31 in the Baf3-OI cells (■) in a dose-dependent manner, implying that: (i) the canine IL-31 receptor complex is successfully expressed on cell surface; (ii) that the binding of

20 canine IL-31 to the IL-31 receptor can stimulate the endogenous STAT3 phosphorylation; and (iii) then initiate its downstream signaling pathway. Ba/f3 cells (●) were used as the control.

Figure 5 Inhibition of IL-31-mediated STAT-3 phosphorylation in Ba/f3-OI cells. The results show the inhibition of IL-31-mediated STAT-3 phosphorylation by the antibodies 10A12(▼) and 47F3(■). The media with (✕) or without cIL-31 (+) were used as controls.

25 Figures 6A-6C show the binding of caninized anti-canine IL-31RA antibodies containing either lambda (L) or kappa (k) light chains as evaluated by ELISA.

Figure 6A depicts the binding of caninized anti-canine IL-31RA antibodies (cA12) containing lambda (L) light chains as evaluated by ELISA. Chimeric rat/caninized: chimeric 10A12 [●], caninized 10A12VH1VL5 [▽], caninized 10A12VH1VL6 [■], caninized

30 10A12VH2VL5[▲], and caninized 10A12VH2VL6 [▼].

Figure 6B depicts the binding of caninized anti-canine IL-31RA antibodies (28F12) containing kappa (k) light chains as evaluated by ELISA. Chimeric rat/caninized: chimeric

28F12 [●], caninized 28F12VH1VK3 [■], caninized 28F12VH1VK4 [▲], caninized 28F12VH2VK2 [▼], caninized 28F12VH2VK3[▽], and caninized 28F12VH2VK4 [Δ].

Figure 6C depicts the binding of caninized anti-canine IL-31RA antibodies (44E2) containing kappa (k) light chains as evaluated by ELISA. Chimeric rat/caninized: chimeric 44E2 [●], caninized 44E2VH2VK1[■], caninized 44E2VH2VK2 [▲], caninized 44E2VH5VK1[▼], caninized 44E2VH5VK2 [▽], and caninized 44E2VH4VK1[Δ]. The results show that caninized anti-canine IL-31RA antibodies bind to canine IL-31RA.

Figure 7A-7C are graphs showing the inhibition of cIL-31-mediated STAT-3 phosphorylation by cIL-31RA antibodies. Three different caninized monoclonal anti-canine IL-31RA antibodies designated caninized 10A12, caninized c28F12, and caninized 44E2 were evaluated for their ability to inhibit STAT-3 phosphorylation.

Figure 7A depicts the inhibition of cIL-31-mediated STAT-3 phosphorylation by cIL-31RA antibodies (c10A12). Chimeric rat/caninized: chimeric 10A12 [●] and caninized 10A12VH2VL6 [■].

Figure 7B depicts the inhibition of cIL-31-mediated STAT-3 phosphorylation by cIL-31RA antibodies (c28F12). Chimeric rat/caninized: chimeric 28F12 [●], caninized 28F12VH2K2[■] and caninized 28F12VH2VK3 [▲].

Figure 7C depicts the inhibition of cIL-31-mediated STAT-3 phosphorylation by cIL-31RA antibodies (c44E2). Chimeric rat/caninized: chimeric c44E2 [●], caninized 44E2VH2VK1[■], caninized 44E2VH2VK2 [▲], and caninized 44E2VH5VK2 [▼]. The data show that all three antibodies result in a dose dependent inhibition of STAT-3 phosphorylation in the presence of IL-31.

Figures 8A-8C show the epitopes on canine IL-31RA for the antibodies 10A12, 28F12 and 44E2, respectively. Figure 8A depicts the amino acid sequences of SEQ ID NO: 104 and SEQ ID NO: 105 respectively. Figure 8B depicts the amino acid sequence of SEQ ID NO: 101. Figure 8C depicts the amino acid sequences of SEQ ID NO: 102 and SEQ ID NO: 103, respectively.

## DETAILED DESCRIPTION OF THE INVENTION

In response to need for better therapies for atopic dermatitis, the present invention provides formulations and methodology that can achieve a significant effect on the skin inflammation associated with atopic dermatitis.

**ABBREVIATIONS**

Throughout the detailed description and examples of the invention the following abbreviations will be used:

|    |          |                                                                                                                                                         |
|----|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
|    | ADCC     | Antibody-dependent cellular cytotoxicity                                                                                                                |
| 5  | CDC      | Complement-dependent cytotoxicity                                                                                                                       |
|    | CDR      | Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system                                     |
|    | EC50     | concentration resulting in 50% efficacy or binding                                                                                                      |
|    | ELISA    | Enzyme-linked immunosorbant assay                                                                                                                       |
| 10 | FR       | Antibody framework region: the immunoglobulin variable regions excluding the CDR regions.                                                               |
|    | IC50     | concentration resulting in 50% inhibition                                                                                                               |
|    | IgG      | Immunoglobulin G                                                                                                                                        |
|    | Kabat    | An immunoglobulin alignment and numbering system pioneered by Elvin                                                                                     |
| 15 |          | A. Kabat [ <i>Sequences of Proteins of Immunological Interest</i> , 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)] |
|    | mAb      | Monoclonal antibody (also Mab or MAb)                                                                                                                   |
|    | V region | The segment of IgG chains which is variable in sequence between different antibodies.                                                                   |
| 20 | VH       | Immunoglobulin heavy chain variable region                                                                                                              |
|    | VL       | Immunoglobulin <i>lambda</i> light chain variable region                                                                                                |
|    | VK       | Immunoglobulin <i>kappa</i> light chain variable region                                                                                                 |

**DEFINITIONS**

25 So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, including the appended claims, the singular forms of words such as "a,"  
 30 "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

"Administration" and "treatment", as it applies to an animal, *e.g.*, a canine subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic,

diagnostic agent, or composition to the animal *e.g.*, a canine subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell.

"Administration" and "treatment" also mean *in vitro* and *ex vivo* treatments, *e.g.*, of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any organism, preferably an animal, more preferably a mammal (*e.g.*, canine, feline, or human) and most preferably a canine.

"Treat" or "treating" means to administer a therapeutic agent, such as a composition containing any of the antibodies of the present invention, internally or externally to *e.g.*, a canine subject or patient having one or more symptoms, or being suspected of having a condition, for which the agent has therapeutic activity. Typically, the agent is administered in an amount effective to alleviate and/or ameliorate one or more disease/condition symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease/condition symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease/condition state, age, and weight of the patient (*e.g.*, canine), and the ability of the pharmaceutical composition to elicit a desired response in the subject. Whether a disease/condition symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veterinarians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (*e.g.*, a treatment method or article of manufacture) may not be effective in alleviating the target disease/condition symptom(s) in every subject, it should alleviate the target disease/condition symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi<sup>2</sup>-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Treatment," as it applies to a human, veterinary (*e.g.*, canine), or research subject, refers to therapeutic treatment, as well as research and diagnostic applications. "Treatment" as it applies to a human, veterinary (*e.g.*, canine), or research subject, or cell, tissue, or organ, encompasses contact of the antibodies of the present invention to *e.g.*, a canine or other animal subject, a cell, tissue, physiological compartment, or physiological fluid.

As used herein, the term "canine" includes all domestic dogs, *Canis lupus familiaris* or *Canis familiaris*, unless otherwise indicated.

As used herein, the term "feline" refers to any member of the *Felidae* family. Members of this family include wild, zoo, and domestic members, including domestic cats, pure-bred and/or mongrel companion cats, show cats, laboratory cats, cloned cats, and wild or feral cats.

As used herein the term "canine frame" refers to the amino acid sequence of the heavy chain and light chain of a canine antibody other than the hypervariable region residues defined herein as CDR residues. With regard to a caninized antibody, in the majority of embodiments the amino acid sequences of the native canine CDRs are replaced with the corresponding foreign CDRs (*e.g.*, those from a mouse or rat antibody) in both chains. Optionally the heavy and/or light chains of the canine antibody may contain some foreign non-CDR residues, *e.g.*, so as to preserve the conformation of the foreign CDRs within the canine antibody, and/or to modify the Fc function, as exemplified below and/or disclosed in U.S. 10,106,607 B2, hereby incorporated by reference herein in its entirety.

The "Fragment crystallizable region" abbreviated as "Fc" corresponds to the CH3-CH2 portion of an antibody that interacts with cell surface receptors called Fc receptors. The canine fragment crystallizable region (cFc) of each of the four canine IgGs were first described by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001); *see also*, Bergeron *et al.*, *Vet. Immunol. Immunopathol.* 157: 31-41 (2014) and U.S. 10,106,607 B2].

As used herein the canine Fc (cFc) "IgG-Bm" is canine IgG-B Fc comprising two (2) amino acid residue substitutions, D31A and N63A, as in the amino acid sequence of SEQ ID NO: 78 of IgG-B (*see* below) and without the c-terminal lysine ('K'). Both the aspartic acid residue (D) at position 31 of SEQ ID NO: 77 and the asparagine residue (N) at position 63 of SEQ ID NO: 77, are substituted by an alanine residue (A) in IgG-Bm. These two amino acid residue substitutions serve to significantly diminish the antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) of the naturally occurring canine IgG-B [*see*, U.S. 10,106,607 B2, the contents of which are hereby incorporated by reference in their entirety]. Further amino acid substitutions to the IgG-Bm are also envisioned, which parallel those which can be made in IgG-B and may include amino acid substitutions to favor heterodimer formation in bispecific antibodies. The amino acid sequence of IgG-B, SEQ ID NO: 77 is:

|    |                                                                  |     |
|----|------------------------------------------------------------------|-----|
| 30 | 1                                                                | 50  |
|    | LGGPSVFIFP PKPKDTLLIA RTPEVTCVVV <u>D</u> LDPEDPEVQ ISWFVDGKQM   |     |
|    | ↳ CH2                                                            |     |
|    | 51                                                               | 100 |
|    | QTAKTQPREE QF <u>N</u> NGTYRVVS VLPIGHQDWL KGKQFTCKVN NKALPSPIER |     |

101 150  
TISKARGQAH QPSVYVLPPS REELSKNTVS LTCLIKDFFP PDIDVEWQSN  
└─ CH3  
5 151 200  
GQQEPESKYR TTPPQLDEDG SYFLYSKLSV DKSRWQRGDT FICAVMHEAL  
201 215  
HNHYTQKSLS HSPGK

10

The amino acid sequence of IgG-Bm, SEQ ID NO: 78, is provided below.

LGGPSVFIFPPKPKDTHLLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAG  
TYRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNT  
VSLTCLIKDFFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFI  
15 CAVMHEALHNHYTQESLSHSPG

As used herein, a “substitution of an amino acid residue” with another amino acid residue in an amino acid sequence of an antibody for example, is equivalent to “replacing an amino acid residue” with another amino acid residue and denotes that a particular amino acid residue at a specific position in the amino acid sequence has been replaced by (or substituted for) by a different amino acid residue. Such substitutions can be particularly designed *i.e.*, purposefully replacing an alanine with a serine at a specific position in the amino acid sequence by *e.g.*, recombinant DNA technology. Alternatively, a particular amino acid residue or string of amino acid residues of an antibody can be replaced by one or more amino acid residues through more natural selection processes *e.g.*, based on the ability of the antibody produced by a cell to bind to a given region on that antigen, *e.g.*, one containing an epitope or a portion thereof, and/or for the antibody to comprise a particular CDR that retains the same canonical structure as the CDR it is replacing. Such substitutions/replacements can lead to “variant” CDRs and/or variant antibodies.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. An antibody can be a monomer, dimer, or larger multimer. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific antibodies (*e.g.*, bispecific antibodies), caninized antibodies, fully canine antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by

exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as caninization of an antibody for use as a canine therapeutic antibody.

As used herein, antibodies of the present invention that "block" or is "blocking" or is "blocking the binding" of *e.g.*, a canine receptor to its binding partner (ligand), is an antibody that blocks (partially or fully) the binding of the canine receptor to its canine ligand and *vice versa*, as determined in standard binding assays (*e.g.*, BIACore<sup>®</sup>, ELISA, or flow cytometry).

Typically, an antibody or antigen binding fragment of the invention retains at least 10% of its canine antigen binding activity (when compared to the parental antibody) when that activity is expressed on a molar basis. Preferably, an antibody or antigen binding fragment of the invention retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the canine antigen binding affinity as the parental antibody. It is also intended that an antibody or antigen binding fragment of the invention can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

"Isolated antibody" refers to the purification status and in such context means the molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

As used herein, an antibody is said to bind specifically to a polypeptide comprising a given antigen sequence (in this case a portion of the amino acid sequence of canine IL-31RA) if it binds to polypeptides comprising the portion of the amino acid sequence of canine IL-31RA, but does not bind to other canine proteins lacking that portion of the sequence of canine IL-31RA. For example, an antibody that specifically binds to a polypeptide comprising canine IL-31RA, may bind to a FLAG<sup>®</sup>-tagged form of canine IL-31RA, but will not bind to other FLAG<sup>®</sup>-tagged canine proteins. An antibody, or binding compound derived from the antigen-binding site of an antibody, binds to its canine antigen, or a variant or mutein thereof, "with specificity" when it has an affinity for that canine antigen or a variant or mutein thereof which is at least ten-times greater, more preferably at least 20-times greater, and even more preferably at least 100-times greater than its affinity for any other canine antigen tested.

As used herein, a "chimeric antibody" is an antibody having the variable domain from a first antibody and the constant domain from a second antibody, where the first and second

antibodies are from different species. [U.S. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 6851-6855 (1984)]. Typically the variable domains are obtained from an antibody from an experimental animal (the "parental antibody"), such as a rodent, and the constant domain sequences are obtained from the animal subject antibodies, *e.g.*, human or canine so that the resulting chimeric antibody will be less likely to elicit an adverse immune response in a human or canine subject respectively, than the parental (*e.g.*, rodent) antibody.

As used herein, the term "caninized antibody" refers to forms of antibodies that contain sequences from both canine and non-canine (*e.g.*, rat) antibodies. In general, the caninized antibody will comprise substantially all of at least one or more typically, two variable domains in which all or substantially all of the hypervariable loops correspond to those of a non-canine immunoglobulin (*e.g.*, comprising 6 CDRs as exemplified below), and all or substantially all of the framework (FR) regions (and typically all or substantially all of the remaining frame) are those of a canine immunoglobulin sequence. As exemplified herein, a caninized antibody comprises both the three heavy chain CDRs and the three light chain CDRs from a rat anti-canine antigen antibody together with a canine frame or a modified canine frame. A modified canine frame comprises one or more amino acids changes as exemplified herein that further optimize the effectiveness of the caninized antibody, *e.g.*, to increase its binding to its canine antigen and/or its ability to block the binding of that canine antigen to the canine antigen's natural binding partner.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same. Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of *Sequences of Proteins of Immunological Interest*, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5<sup>th</sup> ed.; NIH Publ. No. 91-3242 (1991); Kabat, *Adv. Prot. Chem.* 32:1-75 (1978); Kabat, *et al.*, *J. Biol. Chem.* 252:6609-6616 (1977); Chothia, *et al.*, *J. Mol. Biol.* 196:901-917 (1987) or Chothia, *et al.*, *Nature* 342:878-883 (1989)].

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* LCDR1, LCDR2 and LCDR3 in the light chain variable domain and HCDR1, HCDR2 and HCDR3 in the heavy chain variable domain). [See Kabat *et al. Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), defining the CDR regions of an antibody by sequence; *see also* Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987) defining the CDR regions of an antibody by structure]. As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and *kappa*. In specific embodiments of the invention, besides binding canine IL-31RA, a canine or caninized antibody against its antigen of the present invention optimally has two attributes:

1. Lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and
2. be readily purified on a large scale using industry standard technologies such as that based on protein A chromatography.

None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has high level of ADCC activity. On the other hand, IgG-A binds weakly to protein A, but also displays ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D displays no ADCC activity. (IgG-C has considerable ADCC activity). One way the present invention addresses these issues is by providing modified canine IgG-B antibodies of the present invention specific to an antigen of the present invention that lack the effector functions such as ADCC and can be easily purified using industry standard protein A chromatography.

As used herein an "antipruritic agent" is a compound, macromolecule, and/or formulation that tends to inhibit, relieve, and/or prevent itching. Antipruritic agents are colloquially referred to as anti-itch drugs.

As used herein an "antipruritic antibody" is an antibody that can act as an antipruritic agent in an animal, including a mammal such as a human, a canine, and/or a feline, particularly with respect to atopic dermatitis. In particular embodiments, the antipruritic antibody binds to

specific proteins in the IL-31 signaling pathway, such as IL-31 or its receptor IL-31RA. The binding of the antipruritic antibody to its corresponding antigen (*e.g.*, IL-31 or IL-31RA) inhibits the binding of *e.g.*, IL-31 with IL-31RA, and interferes with and/or prevents the successful signaling of this pathway, and thereby inhibits, relieves, and/or prevents the itching that is otherwise caused by the IL-31 signaling pathway.

"Homology", as used herein, refers to sequence similarity between two polynucleotide sequences or between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same base or amino acid residue, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology is the number of homologous positions shared by the two sequences divided by the total number of positions compared  $\times 100$ . For example, if 6 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 60% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology. Sequence identity refers to the degree to which the amino acids of two polypeptides are the same at equivalent positions when the two sequences are optimally aligned. As used herein one amino acid sequence is 100% "identical" to a second amino acid sequence when the amino acid residues of both sequences are identical. Accordingly, an amino acid sequence is 50% "identical" to a second amino acid sequence when 50% of the amino acid residues of the two amino acid sequences are identical. The sequence comparison is performed over a contiguous block of amino acid residues comprised by a given protein, *e.g.*, a protein, or a portion of the polypeptide being compared. In particular embodiments, selected deletions or insertions that could otherwise alter the correspondence between the two amino acid sequences are taken into account. Sequence similarity includes identical residues and nonidentical, biochemically related amino acids. Biochemically related amino acids that share similar properties and may be interchangeable.

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (*e.g.* charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity of the protein. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity [*see, e.g.*, Watson *et al.*, *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.);

1987)]. In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity. Exemplary conservative substitutions are set forth in Table A directly below.

5

**TABLE A****Exemplary Conservative Amino Acid Substitutions**

| Original residue | Conservative substitution |
|------------------|---------------------------|
| Ala (A)          | Gly; Ser                  |
| Arg (R)          | Lys; His                  |
| Asn (N)          | Gln; His                  |
| Asp (D)          | Glu; Asn                  |
| Cys (C)          | Ser; Ala                  |
| Gln (Q)          | Asn                       |
| Glu (E)          | Asp; Gln                  |
| Gly (G)          | Ala                       |
| His (H)          | Asn; Gln                  |
| Ile (I)          | Leu; Val                  |
| Leu (L)          | Ile; Val                  |
| Lys (K)          | Arg; His                  |
| Met (M)          | Leu; Ile; Tyr             |
| Phe (F)          | Tyr; Met; Leu             |
| Pro (P)          | Ala; Gly                  |
| Ser (S)          | Thr                       |
| Thr (T)          | Ser                       |
| Trp (W)          | Tyr; Phe                  |
| Tyr (Y)          | Trp; Phe                  |
| Val (V)          | Ile; Leu                  |

Function-conservative variants of the antibodies of the invention are also contemplated by the present invention. "Function-conservative variants," as used herein, refers to antibodies or fragments in which one or more amino acid residues have been changed without altering a desired property, such as antigen affinity and/or specificity. Such variants include, but

10

are not limited to, replacement of an amino acid with one having similar properties, such as the conservative amino acid substitutions of Table A above.

"Isolated nucleic acid molecule" means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, or is linked to a polynucleotide to which it is not linked in nature. For purposes of this disclosure, it should be understood that "a nucleic acid molecule comprising" a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules "comprising" specified nucleic acid sequences may include, in addition to the specified sequences, coding sequences for up to ten or even up to twenty or more other proteins or portions or fragments thereof, or may include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or may include vector sequences.

The present invention provides isolated caninized antibodies of the present invention, methods of use of the antibodies in the treatment of a condition *e.g.*, the treatment of atopic dermatitis in canines. In canine, there are four IgG heavy chains referred to as A, B, C, and D. These heavy chains represent four different subclasses of dog IgG, which are referred to as IgG-A (or IgGA), IgG-B (or IgGB), IgG-C (or IgGC) and IgG-D (or IgGD). Each of the two heavy chains consists of one variable domain (VH) and three constant domains referred to as CH-1, CH-2, and CH-3. The CH-1 domain is connected to the CH-2 domain *via* an amino acid sequence referred to as the "hinge" or alternatively as the "hinge region".

The nucleic acid and amino acid sequences of these four heavy chains were first identified by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001)]. The amino acid and nucleic sequences for these heavy chains are also available from the GenBank data bases. For example, the amino acid sequence of IgGA heavy chain has accession number AAL35301.1, IgGB has accession number AAL35302.1, IgGC has accession number AAL35303.1, and IgGD has accession number (AAL35304.1). Canine antibodies also contain two types of light chains, *kappa* and *lambda*. The DNA and amino acid sequence of these light chains can be obtained from GenBank Databases. For example, the *kappa* light chain amino acid sequence has accession number ABY 57289.1 and the *lambda* light chain has accession number ABY 55569.1.

In the present invention, the amino acid sequence for each of the four canine IgG Fc fragments is based on the identified boundary of CH1 and CH2 domains as determined by Tang *et al, supra*. Caninized rat anti-canine antibodies that bind canine IL-31RA include, but are not

limited to: antibodies of the present invention that comprise canine IgG-A, IgG-B, IgG-C, and IgG-D heavy chains and/or canine *kappa* or *lambda* light chains together with rat anti-canine IL-31RA CDRs. Accordingly, the present invention provides caninized rat anti-canine antibodies of the present invention, including isolated caninized rat anti-canine antibodies, that bind to canine IL-31RA and that preferably also block the binding of that canine IL-31RA to canine IL-31.

Accordingly, the present invention further provides caninized rat antibodies and methods of use of the antibodies of the present invention in the treatment of a condition *e.g.*, the treatment of atopic dermatitis in canines.

The present invention further provides full length canine heavy chains that can be matched with corresponding light chains to make a caninized antibody. Accordingly, the present invention further provides caninized rat anti-canine antigen antibodies (including isolated caninized rat anti-canine antibodies) of the present invention and methods of use of the antibodies of the present invention in the treatment of a condition *e.g.*, the treatment of atopic dermatitis in canines.

The present invention also provides antibodies of the present invention that comprise a canine fragment crystallizable region (cFc region) in which the cFc has been genetically modified to augment, decrease, or eliminate one or more effector functions. In one aspect of the present invention, the genetically modified cFc decreases or eliminates one or more effector functions. In another aspect of the invention the genetically modified cFc augments one or more effector function. In certain embodiments, the genetically modified cFc region is a genetically modified canine IgGB Fc region. In another such embodiment, the genetically modified cFc region is a genetically modified canine IgGC Fc region. In a particular embodiment the effector function is antibody-dependent cytotoxicity (ADCC) that is augmented, decreased, or eliminated. In another embodiment the effector function is complement-dependent cytotoxicity (CDC) that is augmented, decreased, or eliminated. In yet another embodiment, the cFc region has been genetically modified to augment, decrease, or eliminate both the ADCC and the CDC.

In order to generate variants of canine IgG that lack effector functions, a number of mutant canine IgGB heavy chains were generated. These variants may include one or more of the following single or combined substitutions in the Fc portion of the heavy chain amino acid sequence: P4A, D31A, N63A, G64P, T65A, A93G, and P95A. Variant heavy chains (*i.e.*, containing such amino acid substitutions) are cloned into expression plasmids and are transfected into HEK 293 cells along with a plasmid containing the gene encoding a light chain. Intact

antibodies are expressed and purified from HEK 293 cells and then can be evaluated for binding to Fc $\gamma$ RI and C1q to assess their potential for mediation of immune effector functions. [See, U.S. 10,106,607 B2, the contents of which are hereby incorporated by reference in its entirety.]

The present invention also provides modified canine IgG-Ds which in place of its natural

5 IgG-D hinge region they comprise a hinge region from:

|        |                        |                   |
|--------|------------------------|-------------------|
| IgG-A: | FNECRCTDTPPCPVPEP      | SEQ ID NO: 79     |
| IgG-B: | PKRENGRVPRPPDCPKCPAPEM | SEQ ID NO: 80; or |
| IgG-C: | AKECECKCNCNNCPGCGGL    | SEQ ID NO: 81.    |

10 Alternatively, the IgG-D hinge region can be genetically modified by replacing a serine residue with a proline residue, *i.e.*, PKESTCKC**I**PPCPVPES, SEQ ID NO: 82 (with the proline residue (P) underlined and in bold substituting for the naturally occurring serine residue). Such modifications can lead to a canine IgG-D lacking fab arm exchange. The modified canine IgG-Ds can be constructed using standard methods of recombinant DNA technology [*e.g.*,  
15 Maniatis *et al.*, *Molecular Cloning, A Laboratory Manual* (1982)]. In order to construct these variants, the nucleic acids encoding the amino acid sequence of canine IgG-D can be modified so that it encodes the modified IgG-Ds. The modified nucleic acid sequences are then cloned into expression plasmids for protein expression.

The six complementary determining regions (CDRs) of a caninized rat anti-canine  
20 antibody, as described herein can comprises a canine antibody *kappa* (*k*) or *lambda* (*l*) light chain comprising a rat light chain LCDR1, LCDR2, and LCDR3 and a canine antibody heavy chain IgG comprising a rat heavy chain HCDR1, HCDR2, and HCDR3.

### Nucleic Acids

25 The present invention further comprises the nucleic acids encoding the antibodies of the present invention (*see e.g.*, Examples below).

Also included in the present invention are nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at  
30 least about 95% identical (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to the amino acid sequences of the caninized antibodies, with the exception of the CDRs which do not change, provided herein when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire

length of the respective reference sequences. The present invention further provides nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to any of the reference amino acid sequences when the comparison is performed with a BLAST algorithm, wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences, are also included in the present invention.

As used herein, nucleotide and amino acid sequence percent identity can be determined using C, MacVector (MacVector, Inc. Cary, NC 27519), Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search under the default filter conditions can be used, *e.g.*, using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

The following references relate to BLAST algorithms often used for sequence analysis: BLAST ALGORITHMS: Altschul, S.F., *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish, W., *et al.*, *Nature Genet.* 3:266-272 (1993); Madden, T.L., *et al.*, *Meth. Enzymol.* 266:131-141(1996); Altschul, S.F., *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang, J., *et al.*, *Genome Res.* 7:649-656 (1997); Wootton, J.C., *et al.*, *Comput. Chem.* 17:149-163 (1993); Hancock, J.M. *et al.*, *Comput. Appl. Biosci.* 10:67-70 (1994); ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, (1978); *Natl. Biomed. Res. Found.*, Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3." (1978), M.O. Dayhoff (ed.), pp. 353-358 (1978), *Natl. Biomed. Res. Found.*, Washington, DC; Altschul, S.F., *J. Mol. Biol.* 219:555-565 (1991); States, D.J., *et al.*, *Methods* 3:66-70(1991); Henikoff, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992); Altschul, S.F., *et al.*, *J. Mol. Evol.* 36:290-300 (1993); ALIGNMENT STATISTICS: Karlin, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:2264-2268 (1990); Karlin, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 90:5873-5877 (1993); Dembo, A., *et al.*, *Ann. Prob.* 22:2022-2039 (1994); and Altschul, S.F. "Evaluating the statistical significance of

multiple distinct local alignments." in *Theoretical and Computational Methods in Genome Research* (S. Suhai, ed.), pp. 1-14, Plenum, New York (1997).

### *Antibody Protein Engineering*

By way of example, and not limitation, the canine heavy chain constant region can be from IgGA, IgG-B, IgGC, IgGD, or a modified cFc, such as the IgG-Bm used herein [see, U.S. 10,106,607 B2, hereby incorporated by reference in its entirety] and the canine light chain constant region can be from *kappa* or *lambda*.

The antibodies can be engineered to include modifications to the canine framework and/or the canine frame residues within the variable domains of a parental (i.e., rat) monoclonal antibody, e.g. to improve the properties of the antibody.

The construction of caninized anti-canine IL-31 receptor *alpha* monoclonal antibodies can be performed by determining a DNA sequence that encodes the heavy and light chains of canine IgG were determined. The DNA and protein sequence of the canine heavy and light chains are known in the art and can be obtained by searching of the NCBI gene and protein databases. As indicated above, for canine antibodies there are four known IgG subtypes: IgG-A, IgG-B, IgG-C, and IgG-D, and two types of light chains, i.e., *kappa* and *lambda*.

A caninized rat anti-canine IL-31 antibody can be produced recombinantly by methods that are known in the field. Mammalian cell lines available as hosts for expression of the antibodies or fragments disclosed herein are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines.

Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion or fragment thereof, the light chain and/or antigen-binding fragment thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein purification methods. Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

In certain embodiments, the antibody or antigen binding fragment comprises a heavy chain constant region, *e.g.*, a canine constant region, such as IgG-A, IgG-B, IgG-C and IgG-D canine heavy chain constant region or a variant thereof. In certain embodiments, the antibody or antigen binding fragment comprises a light chain constant region, *e.g.*, a canine light chain constant region, such as *lambda* or *kappa* canine light chain region or variant thereof. By way of example, and not limitation, the canine heavy chain constant region can be from IgG-B and the canine light chain constant region can be from *kappa*.

#### *Epitope Mapping*

The interaction of antibodies with their cognate protein antigens is mediated through the binding of specific amino acids of the antibodies (paratopes) with specific amino acids (epitopes) of target antigens. An epitope is an antigenic determinant that causes a specific reaction by an immunoglobulin. An epitope consists of a group of amino acids on the surface of the antigen. A protein of interest may contain several epitopes that are recognized by different antibodies. The epitopes recognized by antibodies are classified as linear or conformational epitopes. Linear epitopes are formed by a stretch of a continuous sequence of amino acids in a protein, while conformational epitopes are composed of amino acids that are discontinuous (*e.g.*, far apart) in the primary amino acid sequence, but are brought together upon three-dimensional protein folding.

Epitope mapping refers to the process of identifying the amino acid sequences (*i.e.*, epitopes) that are recognized by antibodies on their target antigens. Identification of epitopes recognized by monoclonal antibodies (mAbs) on target antigens has important applications. For example, it can aid in the development of new therapeutics, diagnostics, and vaccines. Epitope mapping can also aid in the selection of optimized therapeutic mAbs and help elucidate their mechanisms of action. Epitope information on IL-31 receptor *alpha* can also elucidate unique epitopes and define the protective or pathogenic effects of vaccines. Epitope identification also

can lead to development of subunit vaccines based on chemical or genetic coupling of the identified peptide epitope to a carrier protein or other immunostimulating agents.

Epitope mapping can be carried out using polyclonal or monoclonal antibodies and several methods are employed for epitope identification depending on the suspected nature of the epitope (*i.e.*, linear *versus* conformational). Mapping linear epitopes is more straightforward and relatively, easier to perform. For this purpose, commercial services for linear epitope mapping often employ peptide scanning. In this case, an overlapping set of short peptide sequences of the target protein are chemically synthesized and tested for their ability to bind antibodies of interest. The strategy is rapid, high-throughput, and relatively inexpensive to perform. On the other hand, mapping of a discontinuous epitope is more technically challenging and requires more specialized techniques such as x-ray co-crystallography of a monoclonal antibody together with its target protein, Hydrogen-Deuterium (H/D) exchange, Mass Spectrometry coupled with enzymatic digestion as well as several other methods known to those skilled in the art.

#### *Epitope Binding and Cross-Blocking Antibodies*

An anti-canine IL-31RA antibody or antigen-binding fragment thereof of the present invention includes any antibody or antigen-binding fragment thereof that binds to the same epitope in canine IL-31RA as the one of the antibodies, disclosed herein, bind, *e.g.*, as the 28F12 antibody which binds to the epitope comprising the amino acid sequence of SEQ ID NO: 101, including caninized antibodies, and any antibody or antigen-binding fragment that cross-blocks (partially or fully) or is cross-blocked (partially or fully) by an antibody or fragment discussed herein for canine IL-31RA binding; as well as any variant thereof.

The cross-blocking antibodies and antigen-binding fragments can be identified based on their ability to cross-compete with *e.g.*, the 28F12 antibody in standard binding assays (*e.g.*, BIAcore<sup>®</sup>, ELISA, as exemplified below, or flow cytometry). For example, standard ELISA assays can be used in which a recombinant canine IL-31RA protein is immobilized on the plate, one of the antibodies is fluorescently labeled and the ability of non-labeled antibodies to compete off the binding of the labeled antibody is evaluated. Additionally or alternatively, BIAcore<sup>®</sup> analysis can be used to assess the ability of the antibodies to cross-compete. The ability of a test antibody to inhibit the binding of the 28F12 antibody, to canine IL-31RA demonstrates that the test antibody can compete with the 28F12 antibody for binding to canine IL-31RA and thus, may, in some cases, bind to the same epitope on canine IL-31RA as the 28F12 antibody binds.

Antibodies and fragments thereof that bind to the same epitope as any of the anti-canine IL-31RA antibodies or fragments of the present invention also form part of the present invention.

*Pharmaceutical Compositions and Administration*

5 To prepare pharmaceutical or sterile compositions comprising the antibodies of the present invention, these antibodies can be admixed with a pharmaceutically acceptable carrier or excipient. [See, e.g., *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984)].

Formulations of therapeutic and diagnostic agents may be prepared by mixing with  
10 acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions or suspensions [see, e.g., Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*,  
15 Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY]. In one embodiment, the antibodies of the present invention are diluted to an appropriate concentration in a sodium acetate solution pH 5-6, and  
20 NaCl or sucrose is added for tonicity. Additional agents, such as polysorbate 20 or polysorbate 80, may be added to enhance stability.

Toxicity and therapeutic efficacy of the antibody compositions, administered alone or in combination with another agent, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of  
25 the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD<sub>50</sub>/ ED<sub>50</sub>). In particular aspects, antibodies exhibiting high therapeutic indices are desirable. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in canines. The dosage of such compounds lies preferably within a range of circulating  
30 concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration.

The mode of administration can vary. Suitable routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal, or intra-arterial. In particular embodiments, the antibodies of the present invention can be administered by an invasive route such as by injection. In further embodiments of the invention, the antibodies of the present invention, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, or by inhalation, aerosol delivery. Administration by non-invasive routes (*e.g.*, orally; for example, in a pill, capsule or tablet) is also within the scope of the present invention.

Compositions can be administered with medical devices known in the art. For example, a pharmaceutical composition of the invention can be administered by injection with a hypodermic needle, including, *e.g.*, a prefilled syringe or autoinjector. The pharmaceutical compositions disclosed herein may also be administered with a needleless hypodermic injection device; such as the devices disclosed in U.S. Patent Nos.: 6,620,135; 6,096,002; 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556.

The pharmaceutical compositions disclosed herein may also be administered by infusion. Examples of well-known implants and modules for administering pharmaceutical compositions include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

Alternatively, one may administer the antibodies of the present invention in a local rather than systemic manner, often in a depot or sustained release formulation.

The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibodies, the level of symptoms, the immunogenicity of the therapeutic antibodies and the accessibility of the target cells in the biological matrix. Preferably, the administration regimen delivers sufficient therapeutic antibodies to effect improvement in the target disease/condition state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular

therapeutic antibodies and the severity of the condition being treated. Guidance in selecting appropriate doses of therapeutic antibodies is available [see, e.g., Wawrzynczak *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK (1996); Kresina (ed.) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY (1991); Bach (ed.) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY (1993); Baert, *et al. New Engl. J. Med.* 348:601-608 (2003); Milgrom *et al. New Engl. J. Med.* 341:1966-1973 (1999); Slamon *et al. New Engl. J. Med.* 344:783-792 (2001); Beniaminovitz *et al. New Engl. J. Med.* 342:613-619 (2000); Ghosh *et al. New Engl. J. Med.* 348:24-32 (2003); Lipsky *et al. New Engl. J. Med.* 343:1594-1602 (2000)].

Determination of the appropriate dose is made by the veterinarian, e.g., using parameters or factors known or suspected in the art to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of the symptoms.

Antibodies provided herein may be provided by continuous infusion, or by doses administered, e.g., daily, 1-7 times per week, weekly, bi-weekly, monthly, bimonthly, quarterly, semiannually, annually etc. Doses may be provided, e.g., intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, intraspinally, or by inhalation. A total weekly dose is generally at least 0.05 µg/kg body weight, more generally at least 0.2 µg/kg, 0.5 µg/kg, 1 µg/kg, 10 µg/kg, 100 µg/kg, 0.25 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 5.0 mg/ml, 10 mg/kg, 25 mg/kg, 50 mg/kg or more [see, e.g., Yang, *et al. New Engl. J. Med.* 349:427-434 (2003); Herold, *et al. New Engl. J. Med.* 346:1692-1698 (2002); Liu, *et al. J. Neurol. Neurosurg. Psych.* 67:451-456 (1999); Portielji, *et al. Cancer Immunol. Immunother.* 52:133-144 (2003)]. Doses may also be provided to achieve a pre-determined target concentration of antibodies of the present invention in the canine's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 µg/ml or more. In other embodiments, antibodies of the present invention is administered subcutaneously or intravenously, on a weekly, biweekly, "every 4 weeks," monthly, bimonthly, or quarterly basis at 10, 20, 50, 80, 100, 200, 500, 1000 or 2500 mg/subject.

As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with a disorder and/or a reduction in the severity of the symptoms of such disorder. The terms further include ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result has been

conferred on a vertebrate subject (*e.g.*, a canine) with a disorder, condition and/or symptom, or with the potential to develop such a disorder, disease or symptom.

As used herein, the terms "therapeutically effective amount", "therapeutically effective dose" and "effective amount" refer to an amount of antibodies of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, *e.g.*, canine, is effective to cause a measurable improvement in one or more symptoms of a disease or condition or the progression of such disease or condition. A therapeutically effective dose further refers to that amount of the antibodies sufficient to result in at least partial amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially, or simultaneously. An effective amount of a therapeutic will result in an improvement of a diagnostic measure or parameter by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%. An effective amount can also result in an improvement in a subjective measure in cases where subjective measures are used to assess severity of the condition.

## **EXAMPLES**

### **EXAMPLE 1**

#### **IL-31 RECEPTOR *alpha***

##### *Nucleotide Sequence*

The nucleotide sequence of SEQ ID NO: 1 encodes the extracellular domain of the canine IL-31 receptor *alpha* (cIL-31RA) fused to a HIS tag. Canine IL-31RA ECD HIS-tagged protein comprises the amino acid sequence of SEQ ID NO: 2. The nucleotide sequence was prepared by chemical synthesis and then cloned into expression plasmids that are suitable for production of the corresponding proteins in eukaryotic cells, either HEK-293 or CHO cells.

##### **Canine IL-31RA ECD-10His: [SEQ ID NO: 1]**

```
gtgctgccccgccaagccccgagaacatcagctgcatcttctactacgaggagaacttcacctgcacctgga
gccccgagaaggaggccagctacacctggtacaagggtgaagagaacctacagctacggctacaagagcga
catctgcagcaccgacaacagcaccagaggcaaccacgccagctgcagcttcttgcacccccaccatcacc
aaccgacaaactacaccatccaggtggaggccagaacgccgacggcatcatgaagagcgacatcacct
actggaacctggacgccatcatgaagatcgagcccccgagatcttcagcgtgaagagcgtgctgggcat
caagagaatgctgcagatcaagtggatcagaccgtgctggccccccacagcagcaccctgaagtacacc
ctgagattcagaacatcaacagcgcctactggatggagggtgaacttcaccaaggaggacatcgacagag
acgagacctacaacctgaccgagctgcaggccttcaccgagtagctgatgacctgagatgcgccccgc
```

cgagagcatgttctggagcggctggagccaggagaaggtgggcaccaccgaggaggagggccccctacggc  
 ctggacctgtggagagtgtgaagcccgccatggtggacggcagaagaccctgacagctgatgtggaaga  
 aggccaccggcgccccctgtgctggagaaggccctgggtacaacatctggtacttccccgagaacaacac  
 caacctgaccgagaccgtgaacaccaccaaccagaccacagagctgtacctgggcggaagacctactgg  
 5 gtgtacctgtggtgagctacaacagcctgggcgagagccccgtggccaccctgagaatccccgccctgaacg  
 agaagaccttccagtgcacgcagggccatgcaggcctgcctgaccaggaccagctggtggtggagtggca  
 gagcagcgcccccgaggtggacacctggatggtggagtgggttccccgacgtggacagcgagcccagcagc  
 ttacagctgggagagcgtgagccaggccagaaactggaccatccagaaggacgagctgaagccccctgtggt  
 10 gctacaacatcagcgtgtacccccgtgctgagagacagagtgggcccagccctacagcaccaggccctacgt  
 gcaggaggggcatccccagcgccggccccgtgaccaggccgacagcatcggcgtgaagaccgtgaccatc  
 acctggaaggagatccccaagagcaagagaaacggcttcatcaagaactacaccatcttctaccaggccg  
 aggacggcaaggagttcagcaagaccgtgaacagcaacatcctgcagtacagactggagagcctgaccag  
 aagaaccagctacagcctgcaggtgatggccagcaccaacgcggcgccaccaacggcaccaagatcaac  
 15 ttcaagaccctgagcatcagccaccaccaccaccaccaccaccaccaccac

## EXAMPLE 2

### EXPRESSION AND PURIFICATION OF IL-31 RECEPTOR *alpha* ECD

Plasmids comprising the nucleotide sequence of SEQ ID NO: 1 were transfected into  
 20 HEK-293 or CHO cells using electroporation *via* the MaxCyte instrument as per the  
 manufacturer's recommendation. Several days following transfection, the supernatants of  
 transfected cells and un-transfected controls were harvested and spun down to remove cellular  
 debris. IL-31RA with the HIS tag was purified from cell culture fluids by passing the clarified  
 harvested fluid from transfected cells over nickel columns as per the manufacturer's  
 25 recommendation. Purified proteins were quantified by measuring their absorbance of ultraviolet  
 light at 280 nm.

Canine IL-31RA ECD-10His: [SEQ ID NO: 2]

VLPAPENISCI FYEENFTCTWSPEKEASYTWYKVKRTYSYGYKSDICSTDNSTRGNHASCSE  
 30 LPPTITNPDNYTIQVEAQNADGIMKSDITYWNLDAIMKIEPPEIFSVKSVLGKIRMLQIKWIRP  
 VLAPHSSTLKYTLRFRTINSAYWMEVNFTKEDIDRDETYNLTELQAFTEYVMTLRCAPAESMF  
 SGWSQEKVGTTEEEAPYGLDLWRVLKPAMVDGRRPVQLMWKKATGAPVLEKALGYNIWYFPENN  
 TNLTETVNTTNQTHELYLGKTYWVYVVSNSLGESEPVATLRIPALNEKTFQCIAMQACLTQD  
 QLVVEWQSSAPEVDTWMVEWFPDVDSEPSFSWESVSQARNWTIQKDELKPLWCYNISVYPVLR  
 35 DRVGQPYSTQAYVQEGIPSAGPVTQADSIGVKTVTITWKEIPKSKRNGFIKNYTIIFYQAEDGKE  
 FSKTVNSNILQYRLESLTRRTSYSLQVMASSTNAGGTNGTKINFKTLSISHHHHHHHHHH

## EXAMPLE 3

### BINDING OF CANINE IL-31RA TO BIOTINYLATED CANINE IL-31:

#### Protocol

1. Coat immunoplate(s) with IL-31RA proteins by diluting to 1 µg/mL in phosphate-  
 buffered saline solution (PBS). Add 100µL/well. Incubate the plate(s) at 2-7° overnight.

2. Wash the plates 3 times with 275  $\mu$ L/well of phosphate-buffered saline solution plus TWEEN 20 (PBST).
3. Block the plates with 200  $\mu$ L/well of blocking buffer [1% nonfat dried milk (NFDM) in PBST] for 30-45 minutes at  $36 \pm 2^\circ\text{C}$  with gentle shaking ( $120 \pm 20$  RPM).
- 5 4. Wash the plates 3 times with 275  $\mu$ L/well of PBST.
5. 3-fold dilute biotinylated IL-31 (at 10  $\mu\text{g/mL}$ ) in 1% NFDM in PBST on a dilution plate, and transfer 100  $\mu$ L/well to the immunoplate(s). Incubate for 30-45 minutes at  $36 \pm 2^\circ\text{C}$  with gentle shaking ( $120 \pm 20$  RPM).
6. Wash the plates 3 times with 275  $\mu$ L/well of PBST.
- 10 7. Dilute horse raddish peroxidase-Streptavidin (HRP-Streptavidin) to a final dilution of 1:1000 in 1% NFDM in PBST.
8. Add 100  $\mu$ L/well of HRP-Streptavidin to the immunoplate(s) and incubate for 30-45 minutes at  $36 \pm 2^\circ\text{C}$  with gentle shaking ( $120 \pm 20$  RPM).
9. Wash the plates 3 times with 275  $\mu$ L/well of PBST.
- 15 10. Combine equal volumes of pre-warmed TMP 2-Component substrate immediately before use.
11. Add 100  $\mu$ L/well of prepared 3,3',5,5'-tetramethylbenzidine (TMP) substrate to the immunoplate(s) and incubate in the dark for 10 to 15 minutes at  $36 \pm 2^\circ\text{C}$  with gentle shaking ( $120 \pm 20$  RPM).
- 20 12. Stop the reaction by addition of 100  $\mu$ L/well of 1 M  $\text{H}_3\text{PO}_4$ .
13. Read the plates using a microplate reader at a wavelength of 450 nm with a reference wavelength of 540 nm.

#### EXAMPLE 4

##### 25 MONOCLONAL ANTIBODIES AGAINST CANINE IL-31 RECEPTOR *alpha*

Monoclonal antibodies (mAbs) against canine IL-31RA were produced by the immunization of two Lewis rats multiple times with canine IL-31RA ECD (using 10 $\mu\text{g}$  or 25 $\mu\text{g}$  of antigen/rat each time) over a 3 to 4 week period. Following immunization, sera was collected from each rat and tested against canine IL-31RA by ELISA. The lymph node cells of the rat

30 with the highest IL-31RA ECD reactivity were fused with the myeloma SP2/0 cell line to produce hybridomas. Approximately 10 days after the fusion, supernatants from growing hybridomas were screened on IL-31RA ECD protein coated plates by ELISA using the protocol described below. There were approximately 260 clones selected that showed potential binding to

IL-31RA in this ELISA, as exemplified in Figures 2A-2E below with the rat IgG2a/*Kappa* used as the negative control. The majority of clones had an OD450 > 1.

*The Procedure for the ELISA:*

- 5        1. Coat 96-well half area plates with IL-31RA (1 µg/mL in PBS buffer), 25 µL/well.
2. Incubate the plates at 4°C overnight.
3. Wash the plates 3 times with PBST (PBS +0.05% Tween 20)
4. Block the plates with blocking buffer (PBS with 5% fetal bovine serum (FBS)], 25ul/well  
             for 30 minutes at room temperature.
- 10       5. Transfer 25 ul/well hybridoma supernatant to the 96-well plates, incubate 60 minutes at  
             room temperature.
6. Wash the plates 3 times with PBST.
7. Add 25ul/well anti-rat HRP, 1:4000 dilution in blocking buffer, to the plates and incubate  
             60 minutes at room temperature.
- 15       8. Wash the plates 5 times by PBST.
9. Add TMB based reagent to the plates for colorimetric reaction for 2-3 minutes.
10. Stop the reactions with 0.16M sulfuric acid.
11. Read the plates by plate reader.

Fourteen rat antibodies raised against canine IL-31RA that bind IL-31 were selected.

- 20       The heavy and light chain variable regions of the rat antibodies are provided below. These  
             antibodies were further tested in Example 5 below for their ability to block the binding of canine  
             IL-31RA to canine IL-31.

49D3VH [SEQ ID NO: 49]

25       EVQLVESGGGLVQPGRSMKLSCAASGFTFSNYMAWVRQAPTKGLEWVASISTGGGNTYYRDSV  
             KGRFTISRDNASTLYLQMDSLRSEDATYYCARHGTLYFDYWGQGVMTVSS

49D3VL [SEQ ID NO: 50]

30       QFTLTQPKSVSGSLRSTITIPCERSSGDIGDSYVSWYQQHLGRPPINVIYADDQRPSEVSDRFS  
             GSIDSSSNSASLTITNLQMDDEADYFCQSYDSNIDGPVFVGGGTKLTVL

10A12VH [SEQ ID NO: 51]

         EVQLVESGGGLVKPGRSMKLSCAASGFTFSNYMAWVRQAPTKGLEWVASISTGGGNTYYRDSV  
             KGRFTISRDNAKRTLYLQMDSLRSEDATYYCGRHGTLYFDYWGQGVMTVSS

35       10A12VL [SEQ ID NO: 52]  
             QFTLTQPKSVSGSLRSTITIPCERSSGDIGDSYVSWYQQHLGRPPINVIYVDDQRPSEVSDRFS  
             GSIDSSSNSASLTITDLQMDDEADYFCQSYDSNIDGPVFVGGGTKLTVL

## 47F3VH [SEQ ID NO: 53]

EVQLVESGGGLVQPGRSMKLSCLVASGFTFSNYMAWVRQAPTKGLEWVASISTGGGNTYYRDSV  
KGRFTISRDNASTLYLQMDSLRSEDATYYCARHGTLYFDYWGQGMVTVS

## 5 47F3VL [SEQ ID NO: 54]

QFTLTQPKSVSGSLRSTITIPCERSSGDIGNTYVSWYQQHLGRPPINVIYADDQRPSEVSDRFS  
GSIDSSSNSASLTITNLQMDDEADYFCQSYDSNIDGPVFEGGKLTVL

## 51G4VH [SEQ ID NO: 55]

10 EVQLVESGGGLVQPGRSMKLSCAALGFTFSNYMAWVRQAPTKGLEWVASISTGGGNTYYRDSV  
KGRFTISRDNASTLYLQMDSLRSEDATYYCARHGTIAAMDYWGQGMVTVSS

## 51G4VL [SEQ ID NO: 56]

15 QFTLTQPKSVSGSLRSTITIPCERNNGDIGDSYVSWYQQHLGRPPINVIYADDQRPSEVSDRFS  
GSIDSSSNSASLTITNLQMDDEADYFCQSYDSNIDGPVFEGGKLTVL

## 53B3VH [SEQ ID NO: 57]

20 EVQLVESGGGLVQPGRSMKLSCAAFGFTFNYYMAWVRQAPTKGLEWVASISTGGGNTFYRDSV  
KGRFTISRDNVKSILSLQMDSLRSEDATYYCARHGTIAAMDYWGQGMVTVSS

## 53B3VL [SEQ ID NO: 58]

QFTLTQPKSVSGSLRSTITIPCERTSGDIGDNYVSWYQQHLGRPPINVIYADDQRPSEVSDRFS  
GSIDSSSNSASLTITNLQMDDEADYFCQSYDSNIDGPVFEGGKLTVL

## 25 27A10VH [SEQ ID NO: 59]

EVQLVESGGGLVQPGRSMKLSCTASGFTFSNYMAWVRQAPTKGLEWVASISTGGGNTYYRDSV  
KGRFTISRDNASTLYLQMDSLRAEDTATYYCARHTMGYFDYWGQGMVTVSS

## 27A10VL [SEQ ID NO: 60]

30 QFTLTQPKSVSGSLRSTITIPCERSSGDIGDNYVSWYQQHLGRPPINVIYADDQRPSEVSDRFS  
GSIDSSSNSASLTITNLQMDDEADYFCQSYDGKIEIPVFEGGKLTVL

## 44E2VH [SEQ ID NO: 61]

35 QVQLKESGPGVLQPSQTLSTCTVSGFSLTSNGVSWVRQPPGKGLEWIAAIISSGGSTYYNSVLK  
SRLSISRDTSKSQVFLKMNLSLQTEDTAIYFCTRRLSGYNYVPFAYWGQGLTVTVSS

## 44E2VK [SEQ ID NO: 62]

40 DIQMTQSPSLLSASVGDRTLTNCKASQNIYKHLAWCQQLGEPPNLLISNANSLQTGIPSRFSG  
SGSGTDFTLTISLQPEDVATYFCQQYYSGDTFGAGTKLELK

## 4G7VH [SEQ ID NO: 63]

EVQLQQYGAELGKPGTSVKLSCKVSGYNIRSTFMHWNQRPKGKLEWIGRIDPVNGNTIYSEKF  
KSKATLTADTSSNTAYMQLSQLKSDDTAIYFCAMFNYAGHSGDYWGQGMVTVSS

## 45 4G7VK [SEQ ID NO: 64]

DIQMTQSPSSMSVSLGDTVITICRASQDVGIIYVNWFFQQKPGKSPRRMIYRATNLADGVPSRFSG  
SRSGSDYSLTISLSESDVADYHCLQYDEYPYTFGAGTKLELK

50

## 28F12VH [SEQ ID NO: 65]

EVQLVESDGGGLAQPGRSCLKSCAASGFTFSDYYMAWVRQAPTKGLEWVATISYDGSSTYYRDSV  
RGRFTISRDNASTLYLQMDSLRS EDTATYYCARGPLTDWAPNWFAYWGQGLTVTVSS

## 5 28F12VK [SEQ ID NO: 66]

DIQMTQSPASLSASLG ETVTIQCQTS EDIYSGLAWYQQKPGKSPQFLIYGASRLEDGVPSRFSG  
SGSGTQYSLKISSMQTEDEGVYFCQQGLKYPNTFGAGTKLELK

## 38A6VH [SEQ ID NO: 67]

10 EVQLVESGGGLVQPGRSLKLSCVASGFTFN NYWMTWIRQAPGKGLEWVASITNTGGTTYYPDSV  
KGRFTISRDNASTLYLQMNSLRSEDTATYYCTRGP TTVVGWFAYWGQGLTVTVSS

## 38A6VK [SEQ ID NO: 68]

15 DIVMTQSPTSMSISVGDRVTMNC KASQNVGSNVDWYQQKTGQSPKVL IYRASSRSTGVPDRFTG  
SGSGTDFTFTISNMQAEDLAVYYCMQSN SYPPTFGGGTKLELK

## 20B8VH [SEQ ID NO: 69]

20 EVQLVESGGGLVQPGRSLKLSCVASGFTFN NYWMTWIRQAPGKGLEWVASITNTGGSTYYPDSV  
KGRFTISRDNASTLYLQMNSLRSEDTATYYCTRGP TTVVGWFAYWGQGLTVTVSS

## 20B8VK [SEQ ID NO: 70]

DIVMTQSPTSMSISVGDRVTMNC KASQNVGSNVDWYQQKTGQSPKLLIYRPSNRYTGVPDRFTG  
SGSGTDFTFTISNMQAEDLAVYYCMQSN SYPPTFGGGTKLELK

## 25 7D7VH [SEQ ID NO: 71]

EVQLVESGGGLVQPGRSLKLSCVASGFTFN NYWMTWIRQAPGKGLEWVASITNTGGSTFY PDSV  
KGRFTISRDNASTLYLQMNSLRSEDTATYYCTRGP DYGGHLNWFAYWGQGLTVTVSS

## 7D7VK [SEQ ID NO: 72]

30 DIVMTQSPTSMSISAGDRVTMNC KASQNVGSNVDWYQQKTGQSPKLLIYKASNRYTGVPDRCTG  
SGSGTDFTFTISNMQAEDLAVYYCMQSN SYPPTFGGGTKLELK

## 22B4VH [SEQ ID NO: 73]

35 EVQLVESGGGLVQPGRSLKLSCVASGFTFN KYWMTWIRQAPGKGLEWVASITNTGGSSYYSDSV  
KGRFTISRDNASTLYLQMNSLRSEETATYYCTRGP DYGGHLNWFAYWGQGLTVTVSS

## 22B4VK [SEQ ID NO: 74]

40 DIVMTQSPTSMSISVGDRVTMNC KASQNVGSNVDWYQ EKTGQSPKLVIYKASNRYTGVPDRFTG  
SGSGTDFTFTISNMQAEDLAVYYCMQSN SYPPTFGGGTKLELK

## 48B1VH [SEQ ID NO: 75]

EVQLVESGGGLVQPGRTLK LFCVASGFTFN NYWMTWIRQAPGKGLEWVASITNTGGSTYYPDSV  
KGRFTISRDNASTLYLQMNSLRSEDTATYYCTRGP DYGGHLNWFVYWGQGLTVTVSS

## 45 48B1VK [SEQ ID NO: 76]

DIVMTQSPTSMSISVGDRVTMNC KASQNVGSNVDWYQQKTGQSPKLLIYKASNRYTGVPDRFTG  
SGSGTDFTFTISNMQAEDLAVYYCMQSN SYPPTFGGGTKLELK

**EXAMPLE 5****BLOCKING ACTIVITY OF ANTI-IL-31 RECEPTOR *alpha* ANTIBODIES**

The ability of anti-canine IL-31RA hybridoma supernatants to block the binding of IL-31 to IL-31RA were evaluated in the blocking ELISA described below.

5

*Protocol*

1. Coat 96-well half area plates with IL-31RA (1 µg/mL in PBS buffer), 25 µL/well.
2. Incubate the plates at 4°C overnight.
3. Wash the plates 3 times by PBST (PBS +0.05% Tween 20)
- 10 4. Block the plates with blocking buffer (PBS with 5% FBS), 25ul/well, for 30 minutes at room temperature.
5. Transfer 25 ul/well hybridoma supernatant to the 96-well plates, incubate 60 minutes at room temperature.
6. Wash the plates 3 times with PBST.
- 15 7. Transfer 25 µL/well of biotinylated IL31 (0.5 µg/mL in blocking buffer,) incubate 60 minutes at room temperature.
8. Wash the plates 3 times with PBST.
9. Add 25 µl/well Streptavidin-HRP, 1:5000 dilution in blocking buffer, to the plates and incubate 60 minutes at room temperature.
- 20 10. Wash the plates five times with PBST.
11. Add TMB based reagent to the plates for colorimetric reaction for 2-3 minutes.
12. Stop the reactions with 0.16M sulfuric acid.
13. Read the plates by plate reader.

25 *Results:*

Out of the approximately 260 clones that showed binding to IL-31RA, only 20 to 25 clones also showed potential blocking of IL-31 binding to IL-31RA. Of these, a particular group of six rat anti-canine IL-31RA antibodies that both bind IL-31RA and block the binding of IL-31 to IL-31RA were identified as comprising sets of CDRs that have a striking amino acid sequence  
 30 similarity (*see* Figures 3A-3D below, in which rat immunoserum was used as a positive control and rat IgG2a was used as the negative control). These amino acid sequences also are provided in Table 3 below.

**HEAVY CHAIN:**

|    | <b><u>AB</u></b> | <b><u>HCDR1</u></b> | <b><u>SEQ ID</u></b> | <b><u>HCDR2</u></b>        | <b><u>SEQ ID</u></b> |
|----|------------------|---------------------|----------------------|----------------------------|----------------------|
| 5  | 49D3             | NYUMA               | NO: 31               | SISTGGGNTYYRDSVKG          | NO: 32               |
|    | 10A12            | NYUMA               | NO: 31               | SISTGGGNTYYRDSVKG          | NO: 32               |
|    | 47F3             | NYUMA               | NO: 31               | SISTGGGNTYYRDSVKG          | NO: 32               |
|    | 27A10            | NYUMA               | NO: 31               | SISTGGGNTYYRDSVKG          | NO: 32               |
|    | 51G4             | NYUMA               | NO: 31               | SISTGGGNTYYRDSVKG          | NO: 32               |
| 10 | 53B3             | NYUMA               | NO: 31               | SISTGGGNT <b>F</b> YRDSVKG | NO: 41               |

|    | <b><u>AB</u></b> | <b><u>HCDR3</u></b> | <b><u>SEQ ID</u></b> |
|----|------------------|---------------------|----------------------|
|    | 49D3             | HGTLYFDY            | NO: 33               |
|    | 10A12            | HGTLYFDY            | NO: 33               |
| 15 | 47F3             | HGTLYFDY            | NO: 33               |
|    | 27A10            | H <b>TMG</b> YFDY   | NO: 43               |
|    | 51G4             | HGT <b>IAAM</b> DY  | NO: 39               |
|    | 53B3             | HGT <b>IAAM</b> DY  | NO: 39               |

20 Indeed, all of the HCDR1's in this group of six antibodies have the identical amino acid sequence (SEQ ID NO: 31). Moreover, five of the six HCDR2's have the identical amino acid sequence (SEQ ID NO: 32), whereas the sixth, the HCDR2 of 53B3, has only a single amino acid substitution, that being the replacement of one aromatic amino acid, tyrosine, with its closely related aromatic amino acid, phenylalanine (SEQ ID NO: 41). And though the amino acid sequences of the six HCDR3's differ more than the corresponding amino acid sequences of the HCDR2 and HCDR1, still half of those HCDR3's have the identical amino acid sequence (SEQ ID NO: 33). Moreover, the amino acid sequences for all three HCDRs of antibodies 49D3, 10A12, and 47F3 are identical.

25 In addition, the amino acid sequences for two of the remaining three HCDR3's, *i.e.*, of 30 51G4 and 53B3, are identical to each other (SEQ ID NO: 39). Comparing the amino acid sequence of the HCDR3 of 51G4 and 53B3 to that of the HCDR3 of 49D3, 10A12, and 47F3, one finds the replacement of a central "LYF" of SEQ ID NO: 33, with "IAAM" (SEQ ID NO: 106) of SEQ ID NO: 39. Similarly, comparing the amino acid sequence of the sixth

HCDR3, *i.e.*, that of 27A10, to that of 49D3, 10A12, and 47F3, the “GTL” residues of SEQ ID NO: 33 appear scrambled relative to the “TMG” of SEQ ID NO: 43 of 27A10, and includes the replacement of a leucine residue by a methionine residue.

|    |                     |                                                     |               |              |               |
|----|---------------------|-----------------------------------------------------|---------------|--------------|---------------|
| 5  | <u>LIGHT CHAIN:</u> |                                                     |               |              |               |
|    | <u>AB</u>           | <u>LCDR1</u>                                        | <u>SEQ ID</u> | <u>LCDR2</u> | <u>SEQ ID</u> |
|    | 49D3                | ERSSGDIGDSYVS                                       | NO: 34        | ADDQRPS      | NO: 35        |
|    | 10A12               | ERSSGDIGDSYVS                                       | NO: 34        | VDDQRPS      | NO: 37        |
|    | 47F3                | ERSSGDIGN <del>T</del> YVS                          | NO: 38        | ADDQRPS      | NO: 35        |
| 10 | 27A10               | ERSSGDIGD <del>N</del> YVS                          | NO: 44        | ADDQRPS      | NO: 35        |
|    | 51G4                | ER <del>N</del> NGDIGDSYVS                          | NO: 40        | ADDQRPS      | NO: 35        |
|    | 53B3                | ERTSGDIGD <del>N</del> YVS                          | NO: 42        | ADDQRPS      | NO: 35        |
|    | <u>AB</u>           | <u>LCDR3</u>                                        | <u>SEQ ID</u> |              |               |
| 15 | 49D3                | QSYDSNIDGPV                                         | NO: 36        |              |               |
|    | 10A12               | QSYDSNIDGPV                                         | NO: 36        |              |               |
|    | 47F3                | QSYDSNIDGPV                                         | NO: 36        |              |               |
|    | 27A10               | QSYD <del>G</del> K <del>I</del> E <del>I</del> IPV | NO: 45        |              |               |
|    | 51G4                | QSYDSNIDGPV                                         | NO: 36        |              |               |
| 20 | 53B3                | QSYDSNIDGPV                                         | NO: 36        |              |               |

As the amino acid sequences of the sets of heavy chain CDRS for these six antibodies, the corresponding amino acid sequences of the sets of the light chains are also extremely similar. Interestingly, as opposed to the heavy chain CDR sets, it is the amino acid sequence of the LCDR1's that have the greatest diversity of the sets of light chain CDRs. Thus, whereas the amino acid sequences for the LCDR1 of 49D3 and 10A12 are identical to each other (SEQ ID NO: 34), the other four LCDR1's differ slightly. Accordingly, the amino acid sequence of the LCDR1 of 27A10 (SEQ ID NO: 44), has one amino acid residue substitution relative to that of 49D3 and 10A12, whereas the LCDR1's of the remaining three antibodies, 51G4, 47F3, and 53B3, all have two amino acid residue substitutions relative to 49D3 and 10A12, or a total of seven such substitutions in all. Six of the seven amino acid residues replaced in relation to the amino acid sequence of SEQ ID NO: 34 are serines, with the seventh being an aspartic acid

residue (compare SEQ ID NO: 34 with SEQ ID NO: 38). Notably, all of the replacement amino acid residues relative to SEQ ID NO: 34 are either asparagine residues or threonine residues.

Five of the six LCDR2's have the identical amino acid sequence (SEQ ID NO: 35), whereas the sixth, the LCDR2 of 10A12 (SEQ ID NO: 37), has only a single amino acid substitution, a valine residue replacing the N-terminal alanine residue. Similarly, five of the six amino acids of the LCDR3's are identical (SEQ ID NO: 36), whereas the sixth, 27A10, has the amino acid sequence of SEQ ID NO: 45, with two pairs of two amino acid substitutions joined by an invariant isoleucine, *i.e.*, "GKIEI" (SEQ ID NO: 107) replacing "SNIDG" (SEQ ID NO: 108) of SEQ ID NO: 36. Importantly, these relatively subtle amino acid differences in the heavy chain and light chain CDRs neither appear to affect the ability of the six individual antibodies to bind canine IL-31RA, nor hamper their ability to block the binding of canine IL-31RA to its ligand, canine IL-31.

A particular group of three rat anti-canine IL-31RA antibodies (44E2, 4G7, and 28F12) that both bind canine IL-31RA and block the binding of canine IL-31 to canine IL-31RA also were identified. The amino acid sequence of the CDRs of these antibodies are provided below. These amino acid sequences also are provided in Table 3 below.

#### HEAVY CHAIN CDRs:

|    | <u>AB</u> | <u>HCDR1</u> | <u>SEQ ID</u> | <u>HCDR2</u>      | <u>SEQ ID</u> |
|----|-----------|--------------|---------------|-------------------|---------------|
| 20 | 44E2      | SNGVS        | NO:13         | AISSGGSTYYNSVLKS  | NO:14         |
|    | 4G7       | STFMH        | NO:19         | RIDPVNGNTIYSEKFKS | NO:20         |
|    | 28F12     | DYYMA        | NO:25         | TISYDGSSTYYRDSVRG | NO:26         |

|    | <u>AB</u> | <u>HCDR3</u>  | <u>SEQ ID</u> |
|----|-----------|---------------|---------------|
| 25 | 44E2      | RLSGYNYVPFAY  | NO:15         |
|    | 4G7       | FNYAGHSGDY    | NO:21         |
|    | 28F12     | GPLTDWAPNWFAY | NO:27         |

#### LIGHT CHAIN CDRs:

|    | <u>AB</u> | <u>LCDR1</u> | <u>SEQ ID</u> | <u>LCDR2</u> | <u>SEQ ID</u> |
|----|-----------|--------------|---------------|--------------|---------------|
| 30 | 44E2      | KASQNIYKHLA  | NO:16         | NANSLQT      | NO:17         |
|    | 4G7       | RASQDVGIYVN  | NO:22         | RATNLAD      | NO:23         |
|    | 28F12     | QTSEDIYSGLA  | NO:28         | GASRLD       | NO:29         |

| <u>AB</u> | <u>LCDR3</u> | <u>SEQ ID</u> |
|-----------|--------------|---------------|
| 44E2      | QQYYSGDT     | NO: 18        |
| 4G7       | LQYDEYPYT    | NO: 24        |
| 28F12     | QQGLKYPNT    | NO: 30        |

5

**EXAMPLE 6****CANINIZED ANTIBODIES**

The overall process of producing caninized heavy and light chains that can be mixed in different combinations to produce caninized anti-canine IL-31 receptor *alpha* mAbs involves the following scheme:

- 10
- i) Identify the DNA sequence of VH and VL domains comprising the CDRs of desired anti-IL-31 receptor *alpha* mAbs
  - ii) Identify the H and L chain CDRs of desired anti-IL-31RA mAbs
  - iii) Identify a suitable sequence for H and L chain of canine IgG
  - 15 iv) Identify the DNA sequence encoding the endogenous CDRs of canine IgG H and L chains of the above sequence.
  - v) Replace the DNA sequence encoding endogenous canine H and L chain CDRs with DNA sequences encoding the desired anti-IL-31RA CDRs. In addition, optionally replace some canine framework residues with selected residues from
  - 20 the desired anti-IL-31 receptor *alpha* mAb framework regions.
  - vi) Synthesize the DNA from step (v), clone it into a suitable expression plasmid, and transfect the plasmids containing desired caninized H and L chains into HEK 293 cells.
  - vii) Purify expressed caninized antibody from HEK 293 supernatant.
  - 25 viii) Test purified caninized antibody for binding to canine IL-31 receptor *alpha* chain.

The application of the above outlined steps can result in a set of caninized H and L chain sequences provided below. The corresponding SEQ ID NOs. are listed in Table 5 below.

30 Figures 6A-6C show the binding of caninized anti-canine IL-31RA antibodies containing either lambda (L) or kappa (K) light chains as evaluated by ELISA. The results show that caninized anti-canine IL-31RA antibodies bind to canine IL-31RA.

Figures 7A-7C are plots show the inhibition of cIL-31-mediated STAT-3 phosphorylation by cIL-31RA antibodies employing the assay described in Example 6 below. Three different caninized monoclonal anti-canine IL-31RA antibodies designated c10A12, c28F12, and c44E2 were evaluated for their ability to inhibit STAT-3 phosphorylation. The data show that all three antibodies result in a dose dependent inhibition of STAT-3 phosphorylation in the presence of IL-31.

*Caninized Antibodies to Canine IL-31 Receptor alpha (cIL-31RA)*

**c10A12VL5-cCL [SEQ ID NO: 83]**

QPVLTQPPSLASLGTTARLTCERSSGDIGDSYVSWYQQKPGSPPRDLLYVDDQRP SGVSKSFS  
GSKDTSANAGLLLLISGLQPEDEADYYCQSYDSNIDGPVFGGGTHLTVLGQPKASPSVTLEFPSS  
EELGANKATLVCLISDFYPSGVTVAWKADGSPVTQGVETTKPSKQSNNKYAASSYLSLTPDKWK  
SHSSFSCLVTHEGSTVEKKVAPAECS

**c10A12VH1-cIgGBm [SEQ ID NO: 84]**

EVQLVESGGDLVKPGGSLRLSCVASGFTFSNYMAWVRQAPGKGLQWVASISTGGGNTYYRDSV  
KGRFTISRDNANTLYLQMNSLRAEDTAMYYCAKHGTLTYFDYWGGTGLTVTVSSASTTAPSVFPL  
APSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYLSLSSMVTVPSSR  
WPSETFTCNVAHPASKTKVDKPVKRENGRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDTHLI  
ARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWLKGK  
QFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDIDVEW  
QSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSH  
SPGK

**c10A12VH2-cIgGBm [SEQ ID NO: 85]**

EVQLVESGGDLVKPGGSLRLSCAASGFTFSNYMAWVRQAPGKGLQWVASISTGGGNTYYRDSV  
KGRFTISRDNANTLYLQMNSLRAEDTAMYYCARHGTLYFDYWGGTGLTVTVSSASTTAPSVFPL  
APSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYLSLSSMVTVPSSR  
WPSETFTCNVAHPASKTKVDKPVKRENGRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDTHLI  
ARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWLKGK  
QFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDIDVEW  
QSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSH  
SPGK

**c10A12VL4-cCL [SEQ ID NO: 86]**

QSVLTQPASVSGSLGQRVTISCERSSGDIGDSYVSWYQQLP GKAPSLLIYVDDQRP SGVPERFS  
GSKSGSSNSATLTITGLQAEDEADYYCQSYDSNIDGPVFGGGTHLTVLGQPKASPSVTLEFPSS  
EELGANKATLVCLISDFYPSGVTVAWKADGSPVTQGVETTKPSKQSNNKYAASSYLSLTPDKWK  
SHSSFSCLVTHEGSTVEKKVAPAECS

**c10A12VL6-cCL [SEQ ID NO: 87]**

QPVLTQPPSLASLGTTARLTCERSSGDIGDSYVSWYQQKPGSPPRDVIYVDDQRPSEVSKSFS  
GSKDTSANAGLLLLISGLQPEDEADYFCQSYDSNIDGPVFGGGTHLTVLGQPKASPSVTLEFPSS  
EELGANKATLVCLISDFYPSGVTVAWKADGSPVTQGVETTKPSKQSNNKYAASSYLSLTPDKWK  
SHSSFSCLVTHEGSTVEKKVAPAECS

**c28F12VH1-clgGBm [SEQ ID NO: 88]**

EVQLVESGGDLVKPGGSLRLSCVASGFTFSDYYMAWVRQAPGKGLQWVATISYDGSSTYYRDSV  
 RGRFTISRDNANTLYLQMNSLRAEDTAMYYCAKGPLTDWAPNWFAYWGQGLVTVSSASTTAP  
 SVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVT  
 5 VPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPK  
 DTLIARTPEVTCVVVALDPEDPEVQISWFDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQD  
 WLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPD  
 IDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQ  
 ESLSHSPGK

**c28F12VH2-clgGBm [SEQ ID NO: 89]**

EVQLVESGGDLVKPGGSLRLSCAASGFTFSDYYMAWVRQAPGKGLQWVATISYDGSSTYYRDSV  
 RGRFTISRDNANTLYLQMNSLRAEDTAMYYCARGPLTDWAPNWFAYWGQGLVTVSSASTTAP  
 SVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVT  
 15 VPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPK  
 DTLIARTPEVTCVVVALDPEDPEVQISWFDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQD  
 WLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPD  
 IDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQ  
 ESLSHSPGK

**c28F12VL1-cCK [SEQ ID NO: 90]**

DIVMTQTPLSLSVSPGETASISCTSEDIYSGLAWFRQKPGQSPQRLIYGASRLEDGVPDRFSG  
 SSGTDFTLRISTVEADDTGVYYCQQGLKYPNTFGAGTKVELKRNDAPAVYLFQPSPDQLHTG  
 SASVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYS  
 25 CEITHKSLPSTLIKS FQRSECQRVD

**c28F12VL2-cCK [SEQ ID NO: 91]**

EIVMTQSPASLSLSQEEKVTITCQTSEDIYSGLAWYQQKPGQAPKLLIYGASRLEDGVPSRFSG  
 SSGTDFSFSTISSLEPEDVAVYYCQQGLKYPNTFGAGTKVELKRNDAPAVYLFQPSPDQLHTG  
 30 SASVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYS  
 CEITHKSLPSTLIKS FQRSECQRVD

**c28F12VL3-cCK [SEQ ID NO: 92]**

DIVMTQSPASLSLSQEEKVTITCQTSEDIYSGLAWYQQKPGQAPKLLIYGASRLEDGVPSRFSG  
 35 SSGTDFSFSTISSLEPEDVAVYFCQQGLKYPNTFGAGTKVELKRNDAPAVYLFQPSPDQLHTG  
 SASVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYS  
 CEITHKSLPSTLIKS FQRSECQRVD

**c28F12VL4-cCK [SEQ ID NO: 93]**

DIVMTQTPLSLSVSPGETASISCTSEDIYSGLAWFRQKPGQSPQLLIYGASRLEDGVPDRFSG  
 40 SSGTDFTLRISTVEADDTGVYFCQQGLKYPNTFGAGTKVELKRNDAPAVYLFQPSPDQLHTG  
 SASVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYS  
 CEITHKSLPSTLIKS FQRSECQRVD

**c44E2VH1-clgGBm [SEQ ID NO: 94]**

EVQLVESGGDLVKPEGSLRLSCVVS GFTFSSNGVSWVRQAPGKGLQWVAAISSGGSTYYNSVLK  
 SRFTISRDNANTLYLQMNSLRTEDTAVYYCAKRLSGYNYVPFAYWGQGLVTVSSASTTAPSV  
 FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP  
 SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT  
 50 LLIARTPEVTCVVVALDPEDPEVQISWFDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWL  
 KGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDID

VEWQSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQES  
LSHSPGK

**c44E2VH4-cIgGBm [SEQ ID NO: 95]**

5 ELTLQESGPGLVKPSQTLSTLCVVSNGSVSWIRQRPGRGLEWMGAISSGGSTYYNSVLK  
SRISITADTAKNQFSLQLSSMTTEDTAVYYCARRLSGYNYPFAYWGQGTLTVTSSASTTAPSV  
FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP  
SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT  
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWL  
10 KGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDID  
VEWQSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQES  
LSHSPGK

**c44E2VH5-clgGBm [SEQ ID NO: 96]**

15 ELTLQESGPGLVKPSQTLSTCTVSGFSLTNGSVSWIRQRPGRGLEWMGAISSGGSTYYNSVLK  
SRISITADTAKNQFSLQLSSMTTEDTAVYYCARRLSGYNYPFAYWGQGTLTVTSSASTTAPSV  
FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP  
SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT  
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWL  
20 KGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDID  
VEWQSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQES  
LSHSPGK

**c44E2VL1-cCK [SEQ ID NO: 97]**

25 EIVMTQSPASLSLSQEEKVTITCKASQNIYKHLAWYQQKPGQAPKLLIYNANSLQTGVPSRFSG  
SGSGTDFSF TISSLEPEDVAVYYCQQYYSGDTFGAGTKVELKRNDAPAVYLFQPSPDQLHTGS  
ASVVCLLNSFYPKDINVWKVVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSC  
EITHKSLPSTLIKS FQRSECQRVD

**c44E2VL2-CcK [SEQ ID NO: 98]**

30 EIVMTQSPASLSLSQEEKVTITCKASQNIYKHLAWYQQKPGQAPKLLIYNANSLQTGIPSRFSG  
SGSGTDFSF TISSLEPEDVAVYFCQQYYSGDTFGAGTKVELKRNDAPAVYLFQPSPDQLHTGS  
ASVVCLLNSFYPKDINVWKVVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSC  
EITHKSLPSTLIKS FQRSECQRVD

**c44E2VL4-cCK [SEQ ID NO: 99]**

35 EIVMTQSPGSLAGSAGESVSINCKASQNIYKHLAWYQQKPGERPKLLIYNANSLQTGVPARFSS  
SGSGTDFTLTINNLAEDVDYQCQQYYSGDTFGAGTKVELKRNDAPAVYLFQPSPDQLHTGS  
ASVVCLLNSFYPKDINVWKVVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSC  
40 EITHKSLPSTLIKS FQRSECQRVD

**c44E2VH2-clgGBm [SEQ ID NO: 100]**

EVQLVESGGDLVKPEGSLRLSCVVSNGSVSWVRQAPGKGLQWIAAIISSGGSTYYNSVLK  
SRLTISRDNKNTLYLQMNSLRTEDTAVYYCARRLSGYNYPFAYWGQGTLTVTSSASTTAPSV  
45 FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP  
SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT  
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWL  
KGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDID  
VEWQSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQES  
50 LSHSPGK

## EXAMPLE 7 STAT-3 ASSAY

Stat-3 is known to be activated by IL-31 in cells comprising the the heterodimeric receptor for IL-31. In order to develop an assay to assess the activation of STAT-3 by canine IL-31, the nucleotide sequences encoding IL-31RA and OSMR, respectively, were prepared by chemical synthesis and then cloned into expression vectors pcDNA3.1. The vectors containing the IL-31RA and OSMR nucleotide sequences, respectively, were co-transfected into Ba/f3 cells and the transfected cells, denoted as “Ba/f3-OI”, were grown as a pool under antibiotic selection. The ability of canine IL-31 to induce STAT-3 activation was tested as follows.

### *Materials:*

Cell line: Ba/f3-OI stable pool cells

Growth medium with mouse IL-3 or with canine IL-31 (cIL-31):

RPMI 1640 435 ml (ThermoFisher, 12633-020)

FBS 50 mL (SAFC cat# 12003c-500mL)

2-Mercaptoethanol (50 mM) 0.5 mL (Gibco 31350-010)

100X Pen Strep 5 mL (Gibco 15140-122 Lot1734040)

200mM L-Glu 10 ml (Gibco 25030-081 Lot1677185)

500 ng/mL Geneticin G418 (from Gibco or Sigma)

5 ng/mL mIL-3 or 100 ng/mL cIL-31

Starvation medium: the growth medium without mIL-3 and cIL-31

p-STAT3 (Tyr705) Assay Kit: PerkinElmer, ALSU-PST3-A-HV

### *Procedure*

#### *Cell culture*

1. Thaw a vial of the Ba/f3-OI cells, and grow the cells in the growth medium with mIL-3 in 37°C CO2 shaker with 125 rpm.
2. Passage the cells 2 – 3 passages to have the cells with  $\geq 90\%$  viability before set a cell-based assay.
3. To setup assay, harvest and resuspend the cells in the starvation medium to  $1 \times 10^7$  viable cells/mL.
4. Dispense cells into 96 well plate, 50  $\mu$ L/well (about  $5 \times 10^5$  cells/well).
5. Three-fold dilute cIL-31 in starvation medium in a dilution plate, and then transfer 50  $\mu$ L of each of the serial diluted cIL-31 aliquots into the cell plate.

6. Incubate the cell plate for 15-30 min in 37°C CO<sub>2</sub> shaker with 125 rpm for 1-2 hrs.  
*AlphaLISA assay as per manufacturer's instruction:*
7. Spin down the cells, aspirate the supernatant, and add 1x lysis buffer of 50 – 100 µL/well. Incubate at RT for 10 min with 1000rpm shaking.
- 5 8. Remove 30 µL of the cell lysate into a ½ area plate or freeze and store at -80°C for future test.
9. *SureFire Assay:* add 15 µL /well acceptor mix to the cell lysate. Seal and agitate plate for 2 min at 1000 rpm and then incubate for 1-2 hours at RT.
- 10 10. Add 15 µL/well donor mix to the cell lysate. Seal and agitate for 2 min at 1000 rpm, and then incubate for 1-2 hours at RT (the plate can be stored at 4°C overnight. Incubate at room temp for 1 hr before reading the plate next day)
11. Read the plate on Alpha plate reader at 520 – 620 nm.

*Results:*

- 15 As shown in Figure 4, canine IL-31 stimulates activation of STAT-3 in Ba/f3-OI cells in a dose dependent manner.

## EXAMPLE 8

### BIOLOGICAL ACTIVITY OF ANTI-CANINE IL-31RA ANTIBODIES

- 20 The ability of the anti-canine IL-31RA mAbs to inhibit the activation of STAT-3 in Ba/f3-OI cells is assessed as follows:
  1. Thaw a vial of the Ba/f3-OI cells, and grow the Ba/f3-OI cells in the growth medium with mIL-3 in 37°C CO<sub>2</sub> shaker with 125 rpm.
  2. Passage the cells 2 – 3 passages to have the cells with  $\geq 90\%$  viability before set a cell-based assay.
  - 25 3. To setup assay, harvest and resuspend the cells in the starvation medium to  $1 \times 10^7$  viable cells/mL.
  4. Dispense cells into 96 well plate, 50 µL/well (about  $5 \times 10^5$  cells/well).
  5. Three-fold dilute the antibody in starvation medium in a row on a 96 well plate, starting concentration at 200 µg/mL. Then add 5-10 µL cIL-31 in each well to get final concentration of 100 ng/mL.
  - 30 6. Transfer 50 µL of the diluted antibody and cIL-31 mix into each well of the cell plate, gently mix.

7. Incubate the cell plate in 37°C CO<sub>2</sub> shaker with 125 rpm for 1-2 hrs.

AlphaLISA assay as per manufacturer's instruction: (refer to Example 7)

*Results:*

- 5 As exemplified in Figure 5, both antibody 47F3 and 10A12 inhibit the ability of canine IL-31 to stimulate activation of STAT-3 in Ba/f3-OI cells.

## EXAMPLE 9

### MAPPING OF CANINE IL-31 RECEPTOR *alpha* EPITOPES

#### 10 USING MASS SPECTROSCOPY

A method based on chemical crosslinking and mass spectrometry detection was employed to identify epitopes recognized by anti-canine IL-31 receptor *alpha* mAbs [CovalX Instrument Incorporated, located at 999 Broadway, Suite 305, Saugus, MA 01906-4510]. The application of this technology to epitope mapping of canine IL-31 receptor *alpha* chain resulted in identification of epitopes recognized by the mAbs listed in Table 6. The results from the epitope mapping of canine IL-31 receptor *alpha* with three antibodies disclosed herein indicates that the mAbs recognize specific peptide epitopes that are present within the extracellular domain of canine IL-31 receptor *alpha* (see, Table 6 below).

Notably, the epitopes identified for each of the three monoclonal antibodies (mAbs) tested were markedly different. As depicted in Table 6 below, the data indicates the 28F12 antibody binds to a single epitope comprising the amino acid sequence of SEQ ID NO: 101, where the antibody binds with arginine (R) residues at positions 215 and 225, and a lysine (K) residue at position 233 of the amino acid sequence SEQ ID NO: 2. The data further indicates the 44E2 antibody binds to two different epitopes: the first comprising the amino acid sequence of SEQ ID NO: 102, where the antibody binds with a threonine (T) residue at position 408 of the amino acid sequence SEQ ID NO: 2 and a second epitope comprising the amino acid sequence of SEQ ID NO: 103, which has two separate parts, one of which the antibody binds with serine (S) residues at positions 464 and 472, and a tyrosine (Y) residue at position 471 of the amino acid sequence SEQ ID NO: 2 and the other part, where the antibody binds with a threonine (T) residue at position 487 of the amino acid sequence SEQ ID NO: 2. The data further indicates the 10A12 antibody binds to two different epitopes the first comprising the amino acid sequence of SEQ ID NO: 104, where the antibody binds with

tyrosine (Y) residues at positions 31, 34, and 42, and a threonine (T) residue at position 39 of the amino acid sequence SEQ ID NO: 2 and a second epitope comprising the amino acid sequence of SEQ ID NO: 105, where the antibody binds with a lysine (K) residue at position 89, a serine (S) residue at position 90, a threonine (T) residue at position 93, and a tyrosine (Y) residue at position 94 of the amino acid sequence SEQ ID NO: 2.

### **SEQUENCE TABLES**

**TABLE 1**  
**CANINE IL-31RA EXTRACELLULAR DOMAIN -HIS TAG**

|                          | <b>SEQ ID<br/>NO:</b> | <b>Nucleic<br/>Acid</b> | <b>Amino<br/>Acid</b> |
|--------------------------|-----------------------|-------------------------|-----------------------|
| canine IL-31RA ECD-10His | 1                     | X                       |                       |
| canine IL-31RA ECD-10His | 2                     |                         | X                     |

**TABLE 2**  
**CDRs of Non-BLOCKING ANTIBODIES**

| <b>Amino Acid<br/>Sequence</b> |                   | <b>SEQ ID<br/>NO:</b> | <b>Amino Acid<br/>Sequence</b> | <b>SEQ ID<br/>NO:</b> |
|--------------------------------|-------------------|-----------------------|--------------------------------|-----------------------|
| <b>20B8</b>                    |                   |                       | <b>38A6</b>                    |                       |
| HCDR1                          | NYWMT             | 3                     | NYWMT                          | 3                     |
| HCDR2                          | SITNTGGSTYYPDSVKG | 4                     | SITNTGGSTYYPDSVKG              | 4                     |
| HCDR3                          | GPTTVVGGWFAY      | 5                     | GPTTVVGGWFAY                   | 5                     |
| LCDR1                          | KASQNVGSNVD       | 6                     | KASQNVGSNVD                    | 6                     |
| LCDR2                          | RPSNRYT           | 7                     | RASSRST                        | 9                     |
| LCDR3                          | MQSNSYPPT         | 8                     | MQSNSYPPT                      | 8                     |
| <b>7D7</b>                     |                   |                       | <b>48B1</b>                    |                       |
| HCDR1                          | NYWMT             | 3                     | NYWMT                          | 3                     |
| HCDR2                          | SITNTGGSTFYPSVKG  | 10                    | SITNTGGSTYYPDSVKG              | 4                     |
| HCDR3                          | GPDYGGHLNWFAY     | 11                    | GPDYGGHLNWFVY                  | 46                    |
| LCDR1                          | KASQNVGSNVD       | 6                     | KASQNVGSNVD                    | 6                     |
| LCDR2                          | KASNRYT           | 12                    | KASNRYT                        | 12                    |
| LCDR3                          | MQSNSYPPT         | 8                     | MQSNSYPPT                      | 8                     |
| <b>22B4</b>                    |                   |                       |                                |                       |
| HCDR1                          | KYWMT             | 47                    |                                |                       |

|       |                   |    |  |  |
|-------|-------------------|----|--|--|
| HCDR2 | SITNTGGSSYYSDSVKG | 48 |  |  |
| HCDR3 | GPDYGGHLNWFAY     | 11 |  |  |
| LCDR1 | KASQNVGSNVD       | 6  |  |  |
| LCDR2 | KASNRYT           | 12 |  |  |
| LCDR3 | MQSNSYPPT         | 8  |  |  |

**TABLE 3**  
**CDRs of BLOCKING ANTIBODIES**

| Amino Acid<br>Sequence |                   | SEQ ID<br>NO: | Amino Acid<br>Sequence | SEQ ID<br>NO: |
|------------------------|-------------------|---------------|------------------------|---------------|
|                        | <b>44E2</b>       |               | <b>4G7</b>             |               |
| HCDR1                  | SNGVS             | 13            | STFMH                  | 19            |
| HCDR2                  | AISSGGSTYYNSVLKS  | 14            | RIDPVNGNTIYSEKFKS      | 20            |
| HCDR3                  | RLSGYNYVPFAY      | 15            | FNYAGHSGDY             | 21            |
| LCDR1                  | KASQNIYKHLA       | 16            | RASQDVGIYVN            | 22            |
| LCDR2                  | NANSLQT           | 17            | RATNLAD                | 23            |
| LCDR3                  | QQYYSGDT          | 18            | LQYDEYPYT              | 24            |
|                        | <b>28F12</b>      |               |                        |               |
| HCDR1                  | DYYMA             | 25            |                        |               |
| HCDR2                  | TISYDGSSTYYRDSVRG | 26            |                        |               |
| HCDR3                  | GPLTDWAPNWFAY     | 27            |                        |               |
| LCDR1                  | QTSEDIYSGLA       | 28            |                        |               |
| LCDR2                  | GASRLED           | 29            |                        |               |
| LCDR3                  | QQGLKYPNT         | 30            |                        |               |
|                        | <b>49D3</b>       |               | <b>10A12</b>           |               |
| HCDR1                  | NYDMA             | 31            | NYDMA                  | 31            |
| HCDR2                  | SISTGGGNTYYRDSVKG | 32            | SISTGGGNTYYRDSVKG      | 32            |
| HCDR3                  | HGTLYFDY          | 33            | HGTLYFDY               | 33            |
| LCDR1                  | ERSSGDIGDSYVS     | 34            | ERSSGDIGDSYVS          | 34            |
| LCDR2                  | ADDQRPS           | 35            | VDDQRPS                | 37            |
| LCDR3                  | QSYDSNIDGPV       | 36            | QSYDSNIDGPV            | 36            |
|                        | <b>47F3</b>       |               | <b>51G4</b>            |               |

|       |                   |    |                   |    |
|-------|-------------------|----|-------------------|----|
| HCDR1 | NYYMA             | 31 | NYYMA             | 31 |
| HCDR2 | SISTGGGNTYYRDSVKG | 32 | SISTGGGNTYYRDSVKG | 32 |
| HCDR3 | HGTLYFDY          | 33 | HGTIAAMDY         | 39 |
| LCDR1 | ERSSGDIGNTYVS     | 38 | ERNNGDIGDSYVS     | 40 |
| LCDR2 | ADDQRPS           | 35 | ADDQRPS           | 35 |
| LCDR3 | QSYDSNIDGPV       | 36 | QSYDSNIDGPV       | 36 |
|       | <b>53B3</b>       |    | <b>27A10</b>      |    |
| HCDR1 | NYYMA             | 31 | NYYMA             | 31 |
| HCDR2 | SISTGGGNTFYRDSVKG | 41 | SISTGGGNTYYRDSVKG | 32 |
| HCDR3 | HGTIAAMDY         | 39 | HTMGYFDY          | 43 |
| LCDR1 | ERTSGDIGDNYVS     | 42 | ERSSGDIGDNYVS     | 44 |
| LCDR2 | ADDQRPS           | 35 | ADDQRPS           | 35 |
| LCDR3 | QSYDSNIDGPV       | 36 | QSYDGKIEIPV       | 45 |

**TABLE 4**  
**RAT ANTI-CANINE IL-31RA VARIABLE REGIONS**

| SEQ ID<br>NO: | Heavy Chain | SEQ ID<br>NO: | Light Chain |
|---------------|-------------|---------------|-------------|
| 49            | 49D3VH      | 50            | 49D3VL      |
| 51            | 10A12VH     | 52            | 10A12VL     |
| 53            | 47F3VH      | 54            | 47F3VL      |
| 55            | 51G4VH      | 56            | 51G4VL      |
| 57            | 53B3VH      | 58            | 53B3VL      |
| 59            | 27A10VH     | 60            | 27A10VL     |
| 61            | 44E2VH      | 62            | 44E2VK      |
| 63            | 4G7VH       | 64            | 4G7VK       |
| 65            | 28F12VH     | 66            | 28F12VK     |
| 67            | 38A6VH      | 68            | 38A6VK      |
| 69            | 20B8VH      | 70            | 20B8VK      |
| 71            | 7D7VH       | 72            | 7D7VK       |
| 73            | 22B4VH      | 74            | 22B4VK      |
| 75            | 48B1VH      | 76            | 48B1VK      |

**TABLE 5**  
**AMINO ACID SEQUENCES OF CANINIZED**  
**ANTIBODIES TO CANINE IL-31RA**

| SEQ ID NO: | IL-31 Receptor <i>alpha</i> | Heavy Chain | Light Chain |
|------------|-----------------------------|-------------|-------------|
| 83         | c10A12VL5-cCL               |             | √           |
| 84         | c10A12VH1-cIgGBm            | √           |             |
| 85         | c10A12VH2-cIgGBm            | √           |             |
| 86         | c10A12VL4-cCL               |             | √           |
| 87         | c10A12VL6-cCL               |             | √           |
| 88         | c28F12VH1-cIgGBm            | √           |             |
| 89         | c28F12VH2-cIgGBm            | √           |             |
| 90         | c28F12VK1-cCK               |             | √           |
| 91         | c28F12VK2-cCK               |             | √           |
| 92         | c28F12VK3-cCK               |             | √           |
| 93         | c28F12VK4-cCK               |             | √           |
| 94         | c44E2VH1-cIgGBm             | √           |             |
| 95         | c44E2VH4-cIgGBm             | √           |             |
| 96         | c44E2VH5-cIgGBm             | √           |             |
| 97         | c44E2VK1-cCK                |             | √           |
| 98         | c44E2VK2-cCK                |             | √           |
| 99         | c44E2VK4-CcK                |             | √           |
| 100        | c44E2VH2-cIgGBm             | √           |             |

5

**TABLE 6**  
**Epitope Sequences**

| Antibody | SEQ ID NO: | Amino Acid Sequences of Epitopes of cIL-31RA       |
|----------|------------|----------------------------------------------------|
| 28F12    | 101        | GLDLWRVLKPAMVDGRRPVQLMWKKATGAPV                    |
| 44E2     | 102        | GIPSAGPVTQADSIGVKTVTTITWKEI                        |
| 44E2     | 103        | SKTVNSNILQYRLESLTRRTSYSLQVMASTNAGGTNGTKINFKTLSSIS  |
| 10A12    | 104        | SYTWYKVKRTYSYGYKSDICS                              |
| 10A12    | 105        | EAQNADGIMKSDITYWNLDAIMKIEPPEIFSVKSVLGIKRLQIKWIRPVL |

## WHAT IS CLAIMED IS:

1. An isolated mammalian antibody or antigen binding fragment thereof that binds canine interleukin-31 receptor *alpha* (canine IL-31RA) comprising a set of three heavy chain complementary determining regions (HCDRs): a CDR heavy 1 (HCDR1), a CDR heavy 2 (HCDR2), and a CDR heavy 3 (HCDR3); wherein
- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 31;
- (ii) the HCDR2 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 32 and SEQ ID NO: 41;
- (iii) the HCDR3 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 33, SEQ ID NO: 39, and SEQ ID NO: 43.
2. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1, that when bound to canine IL-31RA the antibody binds to an epitope comprised by the amino acid sequence selected from the group consisting of SEQ ID NO: 104, SEQ ID NO: 105, and both SEQ ID NO: 104 and SEQ ID NO: 105.
3. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1 or 2, wherein
- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 31;
- (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 32; and
- (iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 33.
4. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1 or 2, wherein
- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 31;
- (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 32; and
- (iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 39.
5. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1 or 2, wherein
- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 31;
- (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 32; and

(iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 43.

6. The isolated mammalian antibody or antigen binding fragment thereof of Claim 1 or 2, wherein

- 5 (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 31;  
(ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 41; and  
(iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 39.

7. The isolated mammalian antibody or antigen binding fragment thereof of any one of Claims 1-6, further comprising a set of three light chain complementary determining regions (LCDRs): a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3); wherein

(iv) the LCDR1 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 34, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, and SEQ ID NO: 44;

(v) the LCDR2 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 35 and SEQ ID NO: 37; and

(vi) the LCDR3 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 36 and SEQ ID NO: 45.

20

8. The isolated mammalian antibody or antigen binding fragment thereof of any one of Claim 1 to 7, wherein

(iv) the LCDR1 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 34, SEQ ID NO: 38, and SEQ ID NO: 44;

25 (v) the LCDR2 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 35 and 37; and

(vi) the LCDR3 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 36.

30 9. The isolated mammalian antibody or antigen binding fragment thereof of any one of Claims 1 to 8, wherein the antibody and antigen binding fragment thereof bind canine IL-31RA and block the binding of canine IL-31RA to canine interleukin-31.

10. The isolated mammalian antibody or antigen binding fragment thereof of Claim 9, that is a caninized antibody or a caninized antigen binding fragment thereof.

11. The caninized antibody or antigen binding fragment thereof of Claim 10, that  
5 comprises a hinge region that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, and SEQ ID NO: 82.

12. The caninized antibody or antigen binding fragment thereof of Claim 10, that  
10 comprises a heavy chain comprising a modified canine IgG-B (IgG-Bm) comprising the amino acid sequence of SEQ ID NO: 78.

13. The caninized antibody or antigen binding fragment thereof of Claim 10, wherein the caninized IL-31RA antibody comprises a light chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 83, SEQ ID NO: 86, and SEQ ID NO: 87;  
15 and a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 84 and SEQ ID NO: 85.

14. The caninized antibody or antigen binding fragment thereof of Claim 13, wherein the caninized IL-31RA antibody comprises:  
20 a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 84; or  
a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 85; or  
a light chain comprising the amino acid sequence of SEQ ID NO: 86 and a heavy chain  
25 comprising the amino acid sequence of SEQ ID NO: 84; or  
a light chain comprising the amino acid sequence of SEQ ID NO: 86 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 85; or  
a light chain comprising the amino acid sequence of SEQ ID NO: 87 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 84; or  
30 a light chain comprising the amino acid sequence of SEQ ID NO: 87 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 85.

15. An isolated nucleic acid that encodes the heavy chain of the caninized antibody or antigen binding fragment thereof of Claims 1-14.

5 16. An isolated nucleic acid that encodes the light chain of the caninized antibody or antigen binding fragment thereof of any one Claims 1-14.

17. An expression vector comprising the isolated nucleic acid of Claim 16.

10 18. An expression vector comprising the isolated nucleic acid of Claim 15.

19. A host cell comprising the expression vector of Claim 17, Claim 18, or both Claims 17 and 18.

15 20. A pharmaceutical composition comprising the caninized antibody or antigen binding fragment thereof of Claims 10-14, and a pharmaceutically acceptable carrier or diluent.

20 21. A method of aiding in the blocking the pruritus associated with atopic dermatitis, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of Claim 20.

Figure 1

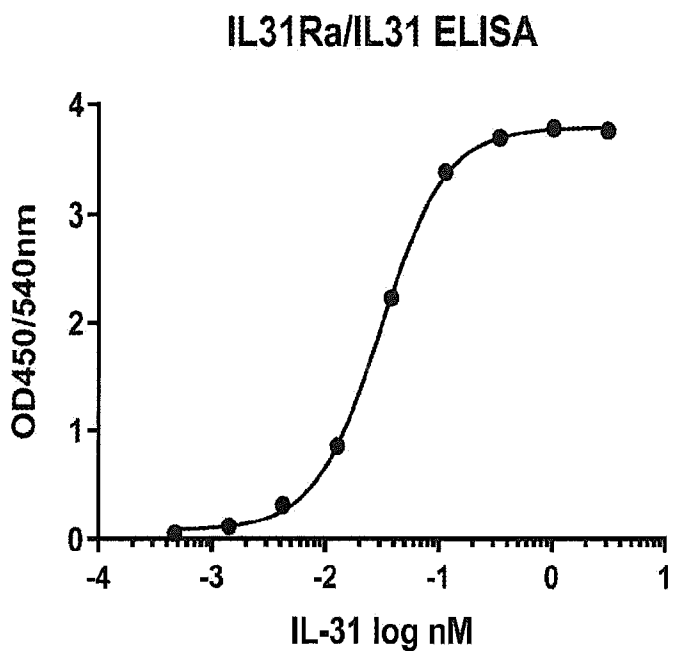


Figure 2A

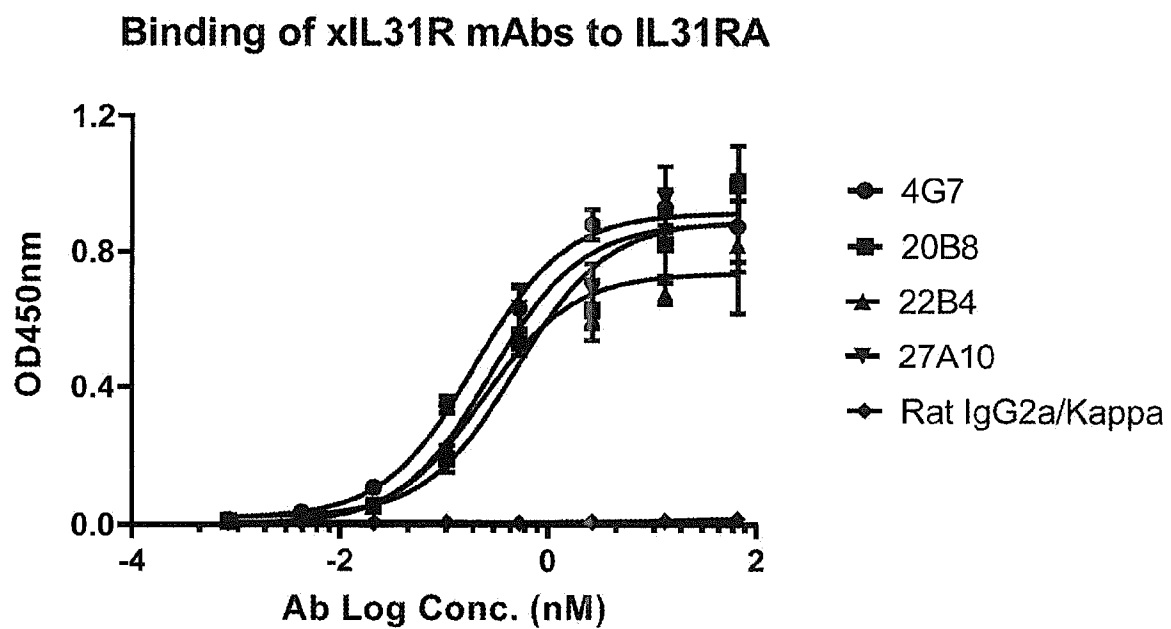


Figure 2B

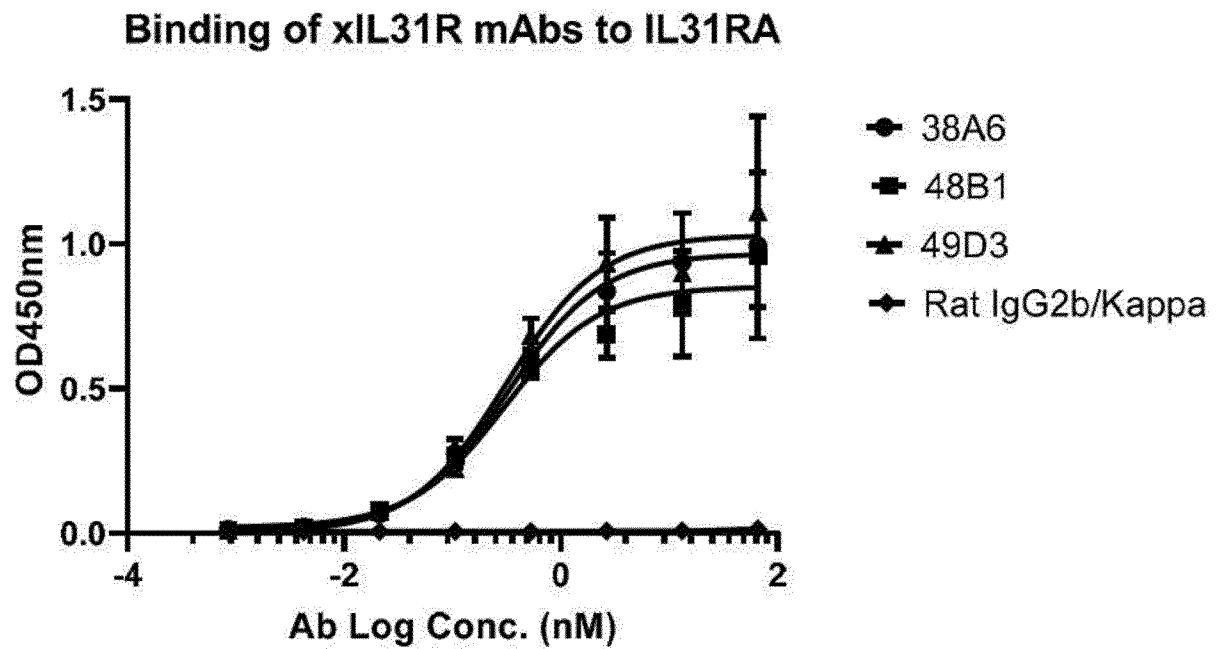


Figure 2C

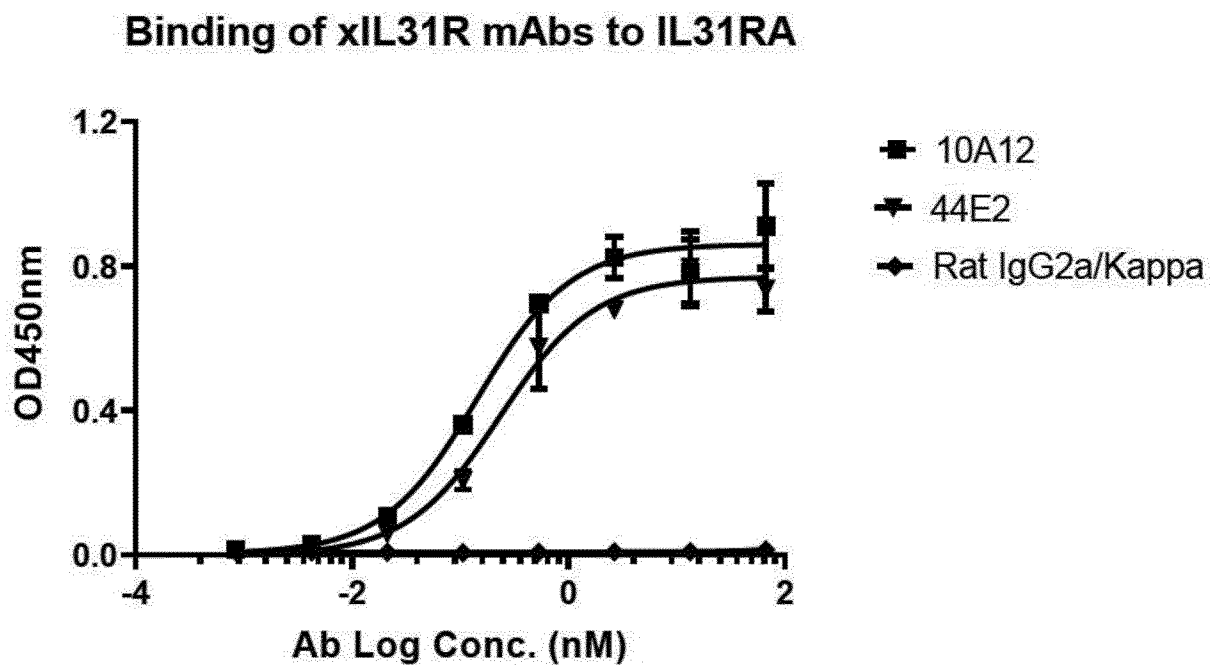


Figure 2D

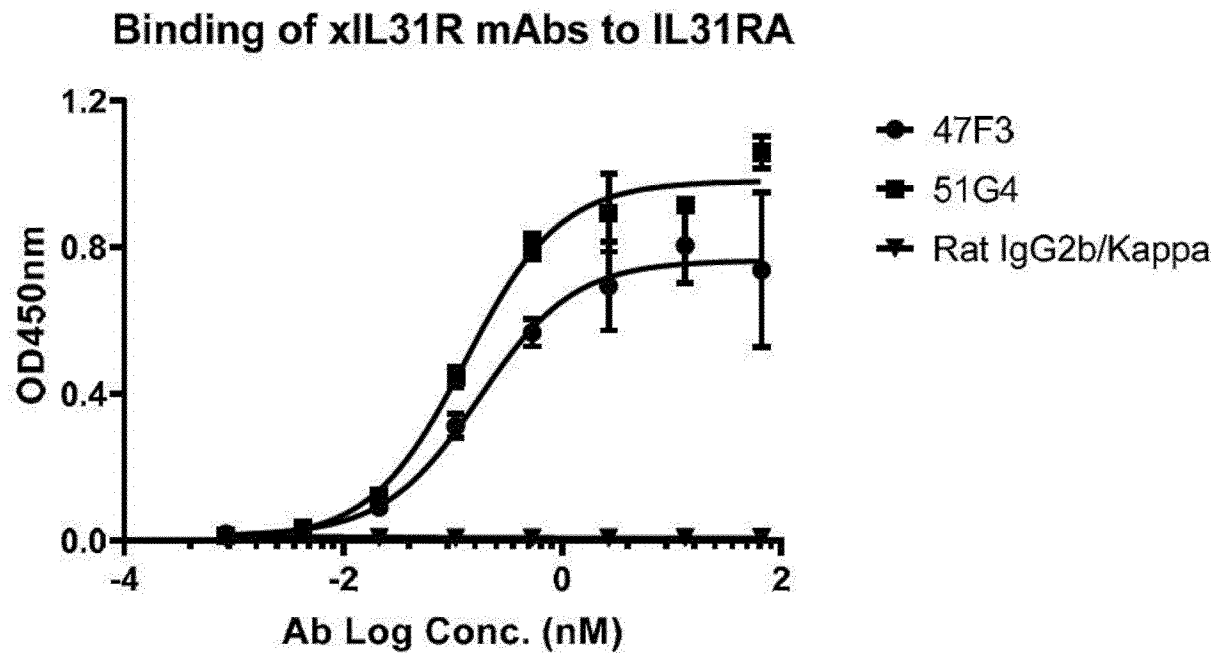


Figure 2E

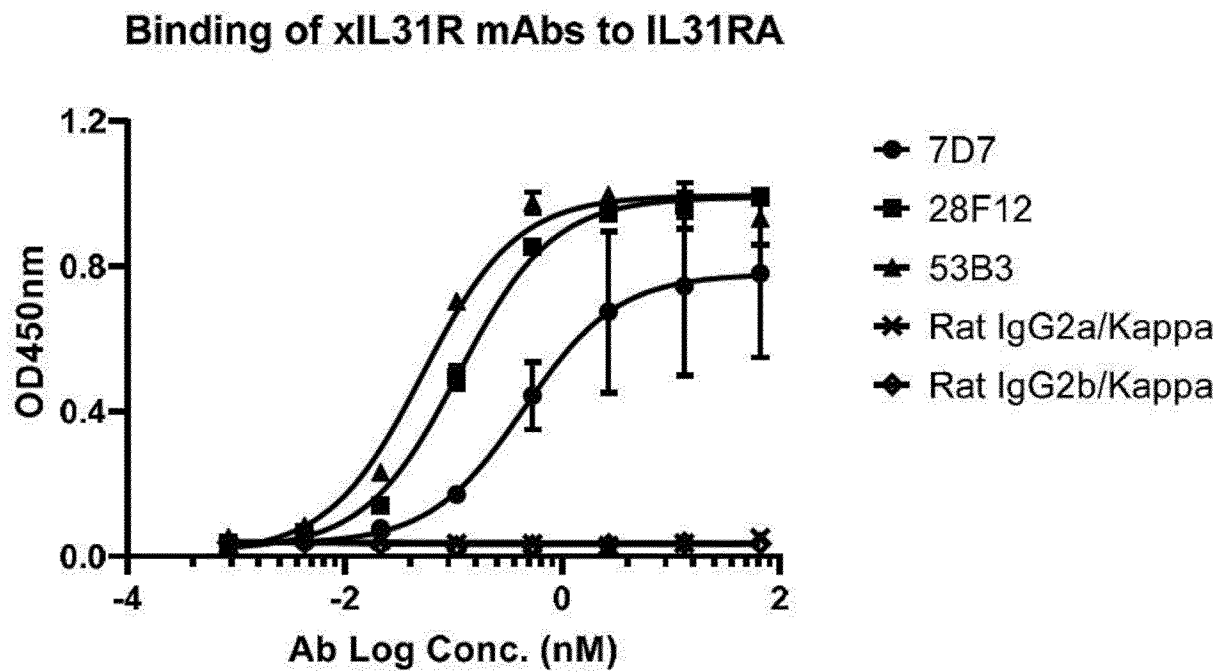


Figure 3A

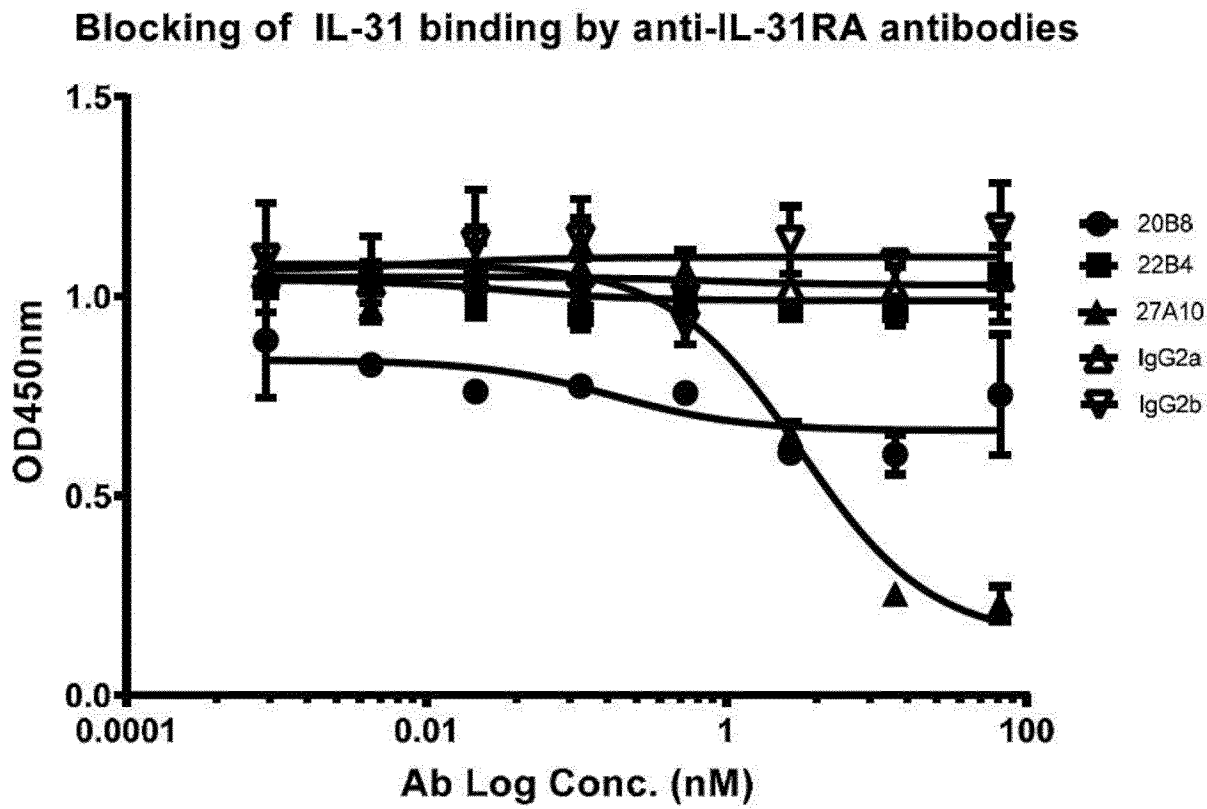


Figure 3B

Blocking of IL-31 binding by anti-IL-31RA antibodies

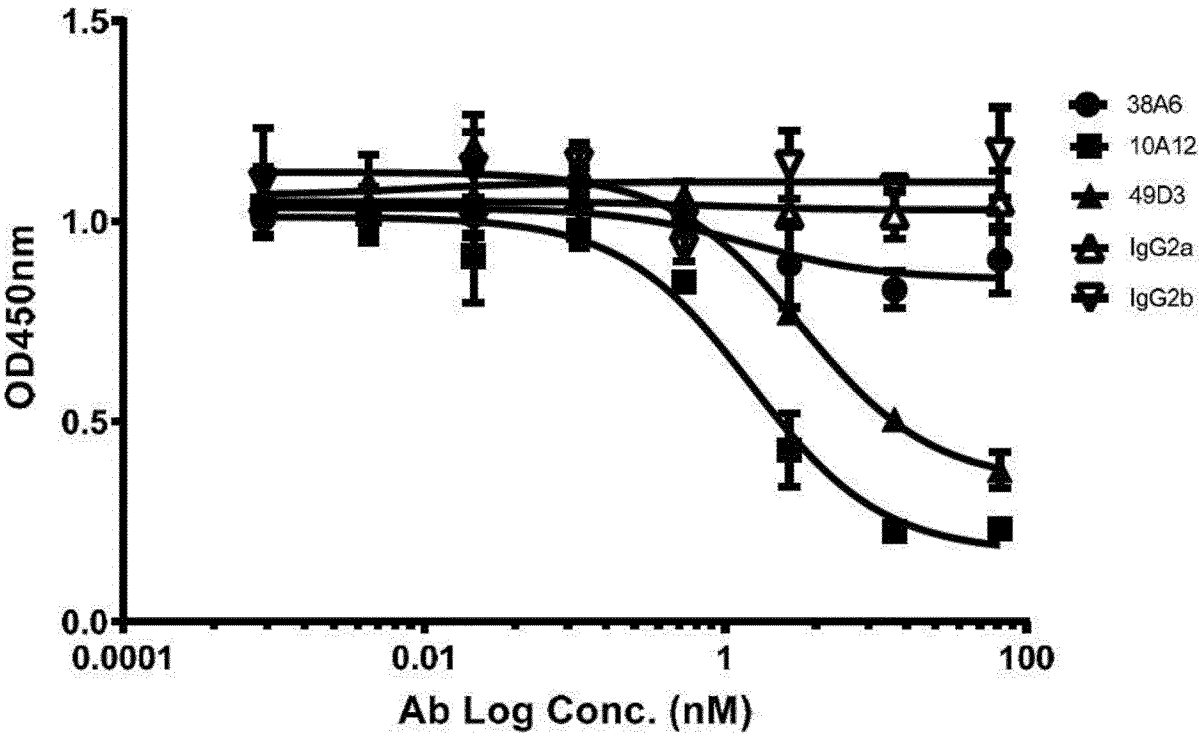


Figure 3C

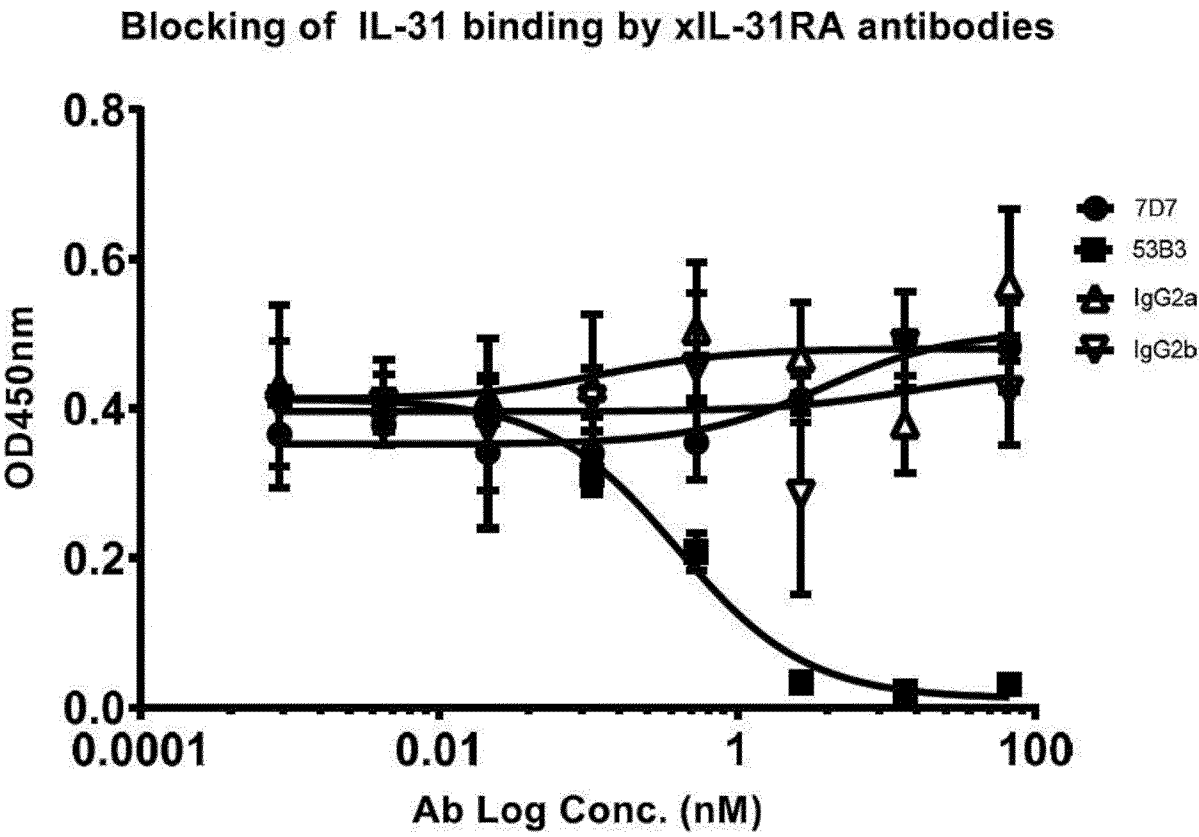


Figure 3D

Blocking of IL-31 binding by anti-IL-31RA antibodies

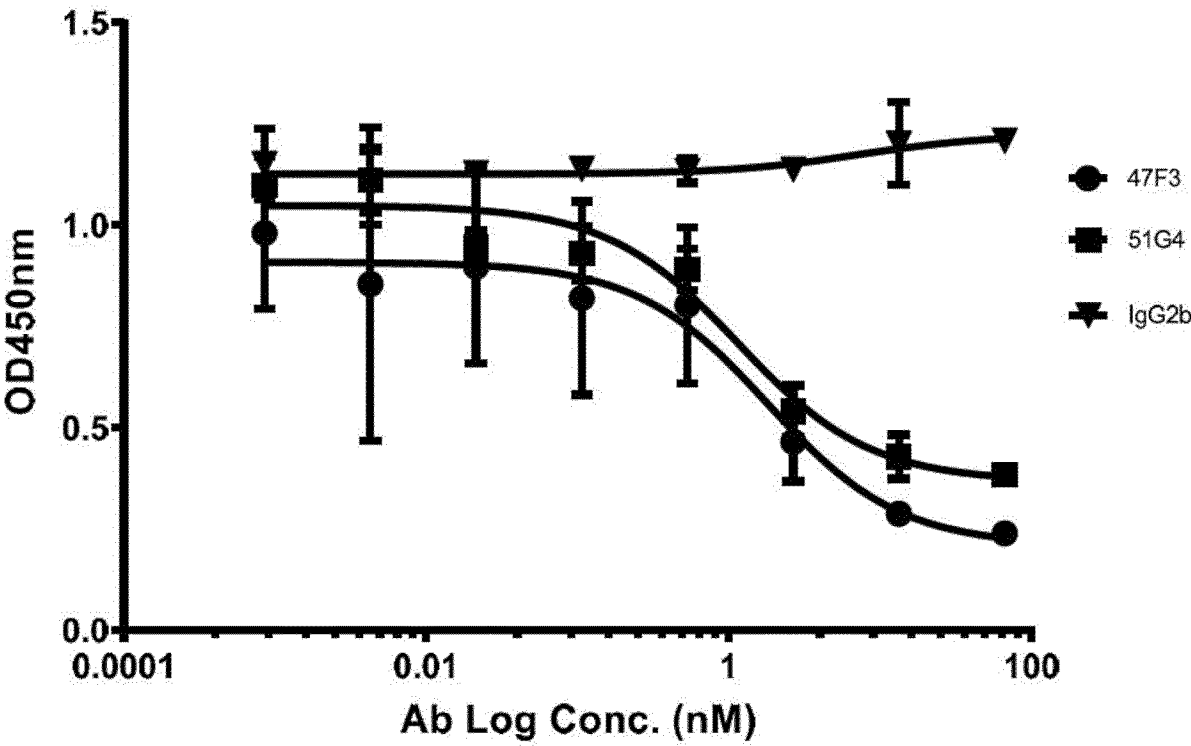


Figure 4

INDUCTION OF STAT-3 PHOSPHORYLATION BY IL-31

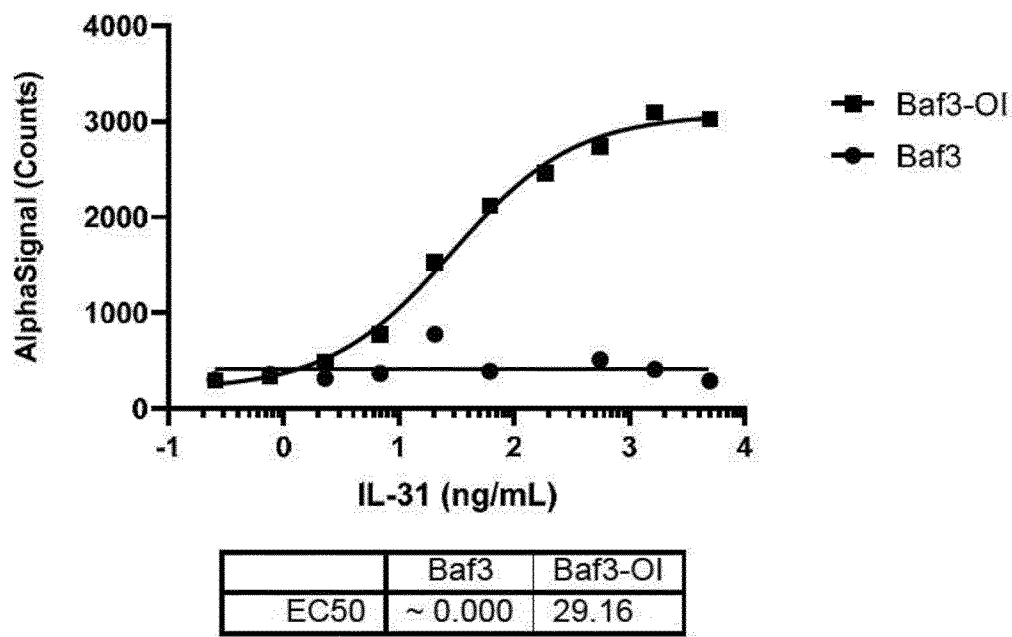


Figure 5

Inhibition of cIL-31 mediated STAT-3 phosphorylation in Ba/F3-OI cells by the selected xIL-31R1a antibodies

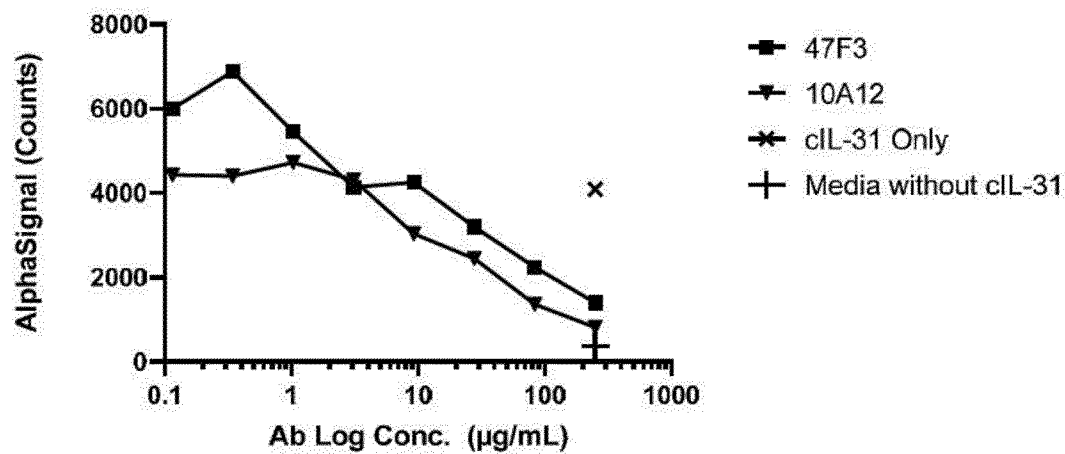


Figure 6A

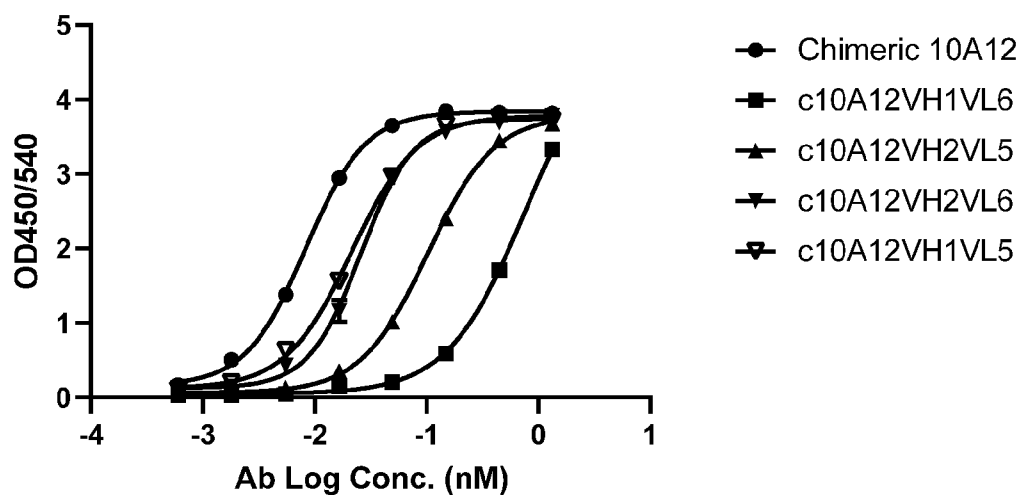
**Binding of caninized xIL-31R mAbs to cIL31-RA**

Figure 6B

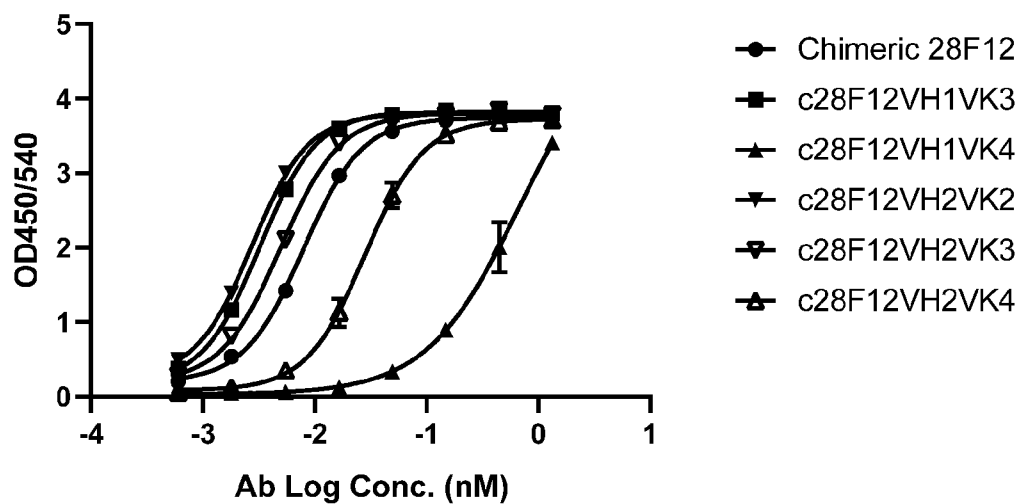
**Binding of caninized xIL-31R mAbs to cIL31-RA**

Figure 6C

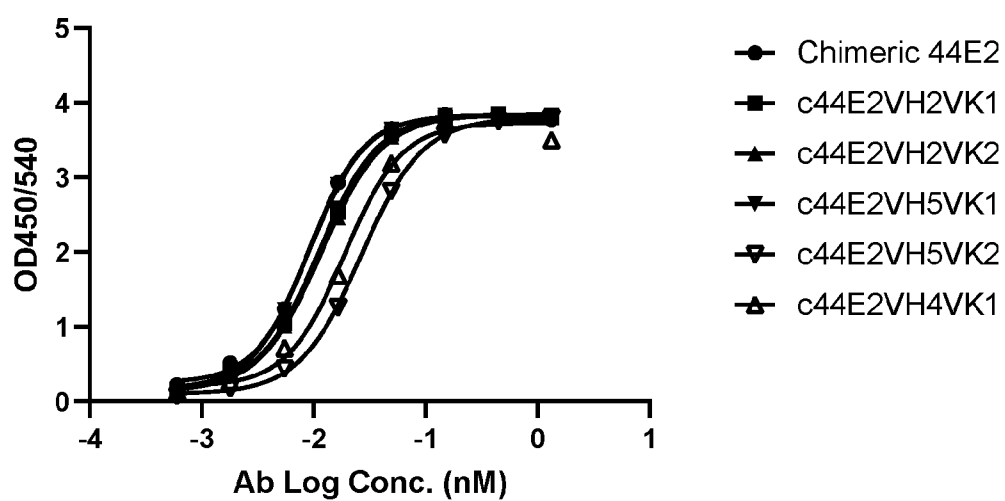
**Binding of caninized xIL-31R mAbs to cIL31-RA**

Figure 7A

**Inhibition of cIL-31 mediated STAT-3 phosphorylation  
by caninized xIL-31RA mAbs to cIL-31RA**

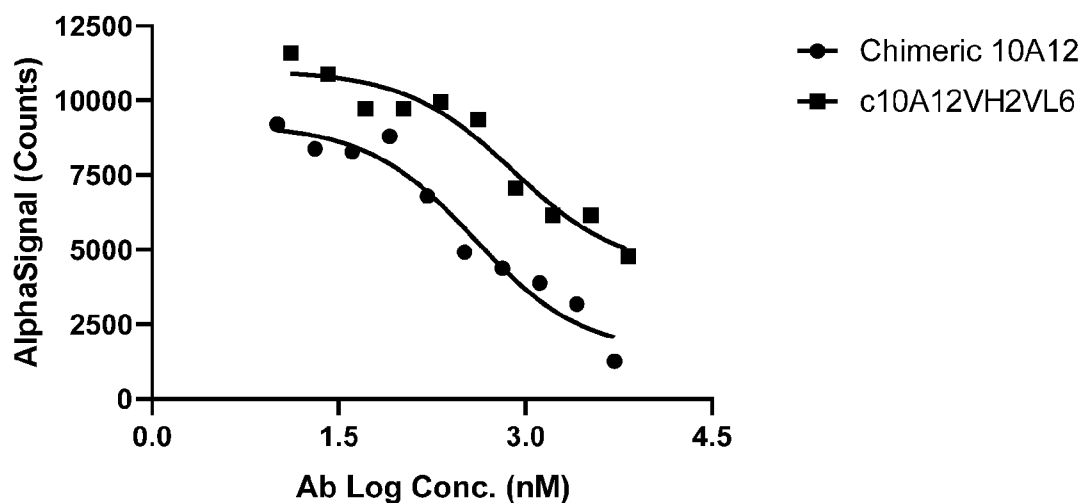


Figure 7B

**Inhibition of cIL-31 mediated STAT-3 phosphorylation  
by caninized xIL-31RA mAbs to cIL-31RA**

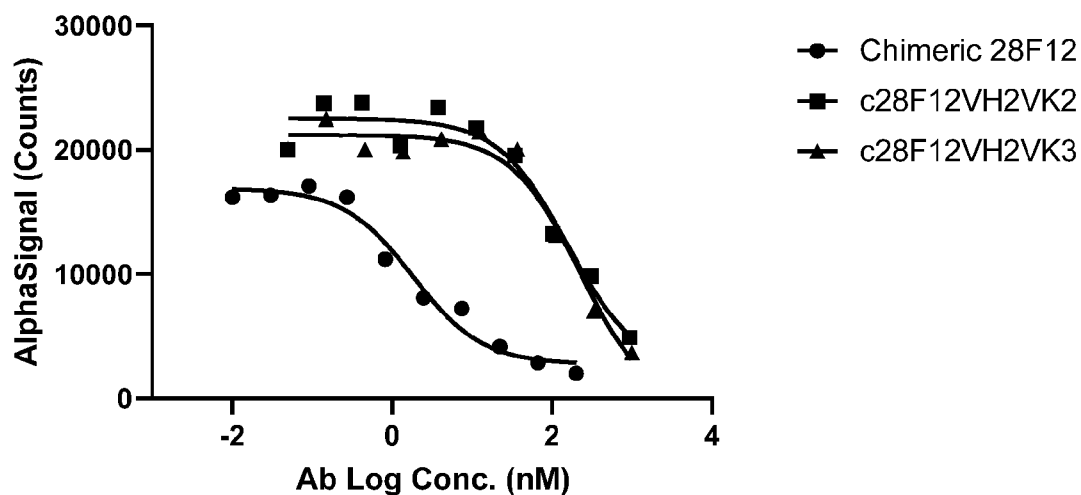
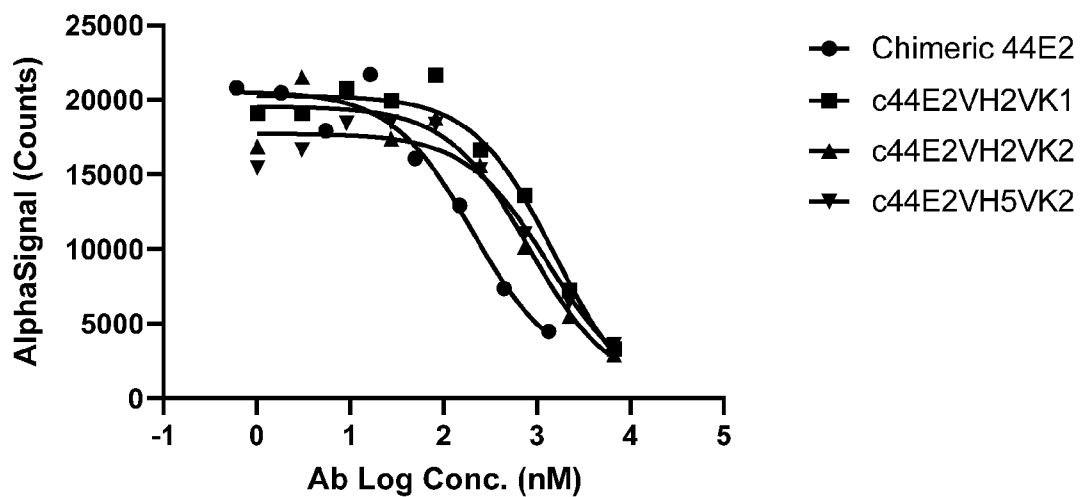


Figure 7C

**Inhibition of cIL-31 mediated STAT-3 phosphorylation  
by caninized xIL-31RA mAbs to cIL-31RA**



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Fig. 8A

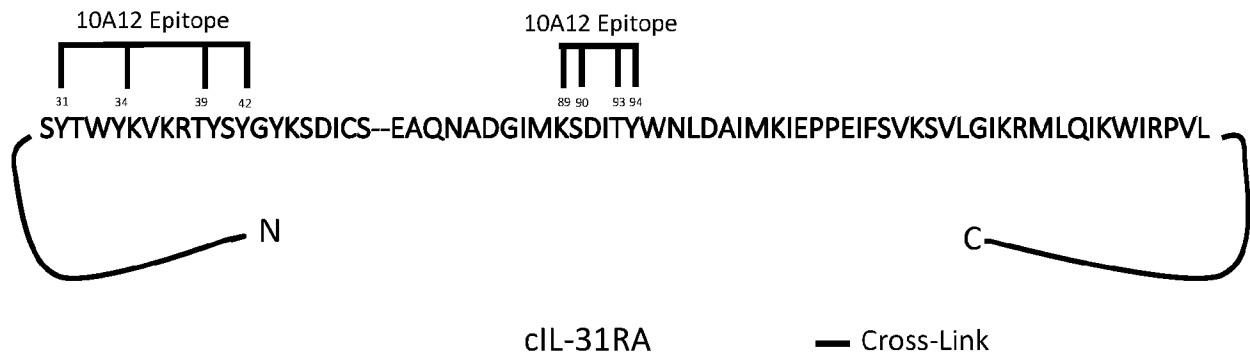


Fig. 8B

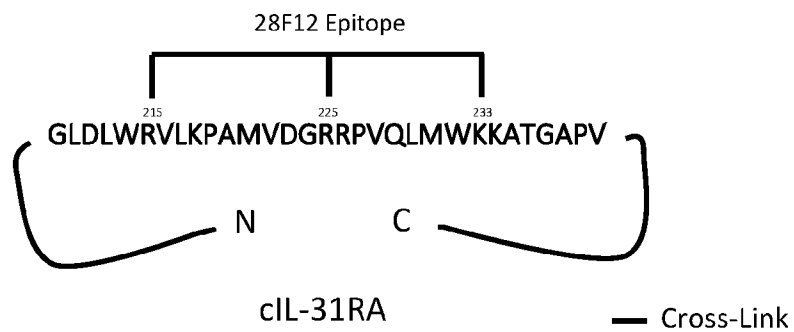
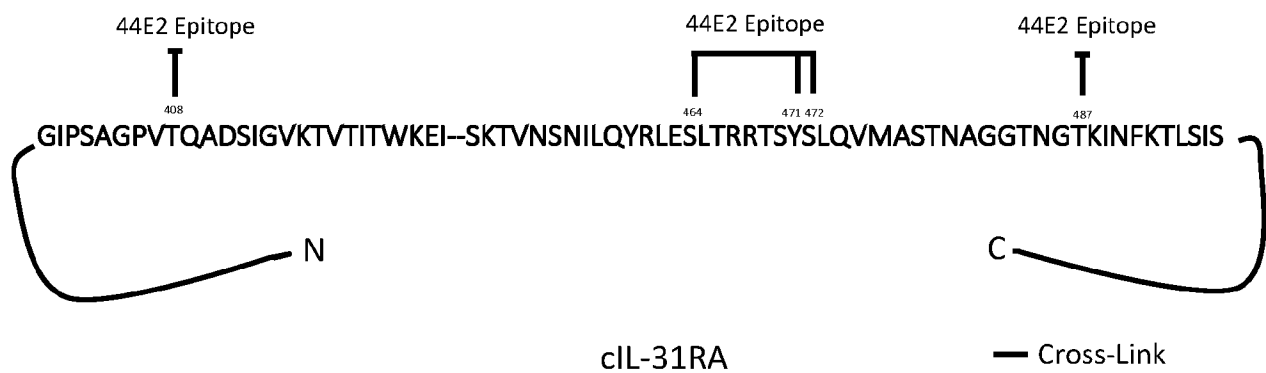


Fig. 8C



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/078385

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a. ☒ forming part of the international application as filed:
    - ☒ in the form of an Annex C/ST.25 text file.
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3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No  
**PCT/EP2021/078385**

**A. CLASSIFICATION OF SUBJECT MATTER**  
**INV. C07K16/18**  
**ADD.**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

**C07K A61P**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**EPO-Internal, BIOSIS, Sequence Search, WPI Data**

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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| <b>Y</b>  | <b>US 2020/239563 A1 (PIKETTY CHRISTOPHE [FR]) 30 July 2020 (2020-07-30) the whole document</b> | <b>1-21</b>           |

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Date of the actual completion of the international search

**21 January 2022**

Date of mailing of the international search report

**31/01/2022**

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International application No  
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## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No

PCT/EP2021/078385

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