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Original article

Correlation between clinical efficacy on pruritus and serum interleukin-31 levels in dogs with atopic dermatitis treated with lokivetmab

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Abstract

Studies on serum interleukin (IL)-31 levels in dogs with atopic dermatitis (AD) and their correlation with disease severity are limited. To the author's knowledge, there are no studies that measured serum IL-31 in dogs treated with lokivetmab injections, a selective inhibitor of this key cytokine in pruritus. The aim of the study was to evaluate serum IL-31 levels in dogs treated with lokivetmab and correlate it with the severity of canine atopic dermatitis using the pruritus visual analog scale (pVAS) and canine atopic dermatitis extent and severity index (CADESI-04). Ten client-owned dogs diagnosed with AD received two injections of lokivetmab four weeks apart. Disease severity was assessed using the pVAS and CADESI-04 scores before and after both injections. In addition, canine serum IL-31 levels were measured at the same moments. Serum IL-31 was detected in all dogs in the study. There was a significant reduction in pVAS scores and serum IL-31 after administrations. However, there was no difference in CADESI-04 scores, and there was no significant correlation between CADESI-04 scores and serum IL-31 in dogs diagnosed with AD. Nonetheless, a significant positive correlation was observed between the pVAS scores and serum IL-31 levels with lokivetmab therapy, which reinforces the role of IL-31 in the pathogenesis of pruritus in dogs with AD. The data presented here provide further evidence that IL-31 is directly involved in pruritus pathogenesis in dogs with AD. In addition, blocking IL-31 has a significant antipruritic effect, but has no influence on skin lesion severity and extension.

Keywords: atopic dermatitis, interleukin-31, lokivetmab, monoclonal antibody, pruritus

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Introduction

Canine atopic dermatitis (AD) affects 20-30% of the canine population (Marsella and De Benedetto 2017). It is defined as a genetically predisposed pruritic inflammatory cutaneous disease with clinical signs associated with the production of immunoglobulin E (IgE) in response to environmental allergens (Olivry et al. 2001, Halliwell 2006). Its pathogenesis is multifactorial and not completely elucidated (Saridomichelakis and Olivry 2016, Santoro 2019). The most relevant clinical sign is pruritus, which usually occurs before lesions, and is aggravated by the development of secondary infections, which are intense to severe, continuous, and perennial. Initial lesions included erythema, papules, alopecia, excoriations, and ulcerations. Pustules, crusts and desquamation are present in secondary infections, and signs such as lichenification and hyperpigmentation are related to the chronicity of the disease (Miller et al. 2013). Clinical signs are distributed in the perilabial and periocular regions, auricular pavilions, flexural areas of the radioulnar and tibiotarsal joints, interdigital areas, ventral abdomen, perineum, ventral part of the tail (Saridomichelakis and Olivry 2016), and armpits (Marsella and Samuelson 2019).

IL-31 has been previously described as a cytokine produced mainly by activated T helper 2 lymphocytes (Th2), mast cells, and keratinocytes, which in elevated conditions causes pruritus in dogs (Gonzales et al. 2013). It is known that IL-31 plays an essential role in pruritus induction and is critically involved in the pathogenesis of AD (Gonzales et al. 2013, Michels et al. 2016, Marsella et al. 2018). However, the correlation between disease severity and serum IL-31 levels in veterinary medicine has not been fully established, since there are few studies in the literature on serum IL-31 levels (Gonzales et al. 2013, Marsella et al. 2018, Ribeiro 2018).

Lokivetmab (Cytopoint®, Zoetis) is a canine monoclonal antibody (mAb) that selectively binds and neutralizes IL-31 (Souza et al. 2018), rendering it unavailable for binding to its receptor, thereby preventing the pruritic cascade (Santoro 2019).

Therefore, this prospective study aimed to evaluate serum levels of IL-31 correlating with the disease severity in ten dogs with AD using the pruritus visual analog scale (pVAS) and canine atopic dermatitis severity and extension index, version 04 (CADESI-04), before and after two lokivetmab injections 4 weeks apart between then.

Materials and Methods

The study was approved by the Ethics Committee on Animal Use of Federal University of Minas Gerais (protocol number 147/2019). All owners of the animals selected for this study signed an informed consent form.

Animals

Ten dogs with AD were selected from the dermatology service of a Veterinary Teaching Hospital and the definitive diagnosis was established based on anamnesis, clinical signs, adequacy in 5/8 of Favrot's Criteria included in criteria group 1 (Favrot et al. 2010), and exclusion of other pruritic and inflammatory skin diseases. Before the final diagnosis, all dogs received treatment for secondary infections and ectoparasites control. All dogs underwent a hypoallergenic restrictive diet for at least eight weeks for exclusion of food allergy. Dogs that showed partial improvement with diet were excluded from the study. Therefore, the dogs included in this study showed no improvement following a hypoallergenic diet. Moreover, all the dogs received lokivetmab for the first time and for the five dogs that had already received oclacitinib (Apoquel®, Zoetis) the withdrawal time was two weeks.

Ten dogs received two doses of lokivetmab subcutaneously, as recommended by the manufacturer. The injections occurred at two moments: moment 0 (M0) and moment 1 (M1), four weeks after the first dose. Moment 2 (M2), eight weeks after the first dose, was included for clinical and laboratory evaluation. Injectable, oral or topical glucocorticoids, cyclosporine, and allergen-specific immunotherapy, were not allowed during the study. In addition to lokivetmab treatment, all dogs received therapeutic baths once or twice a week according to their individual needs, with 3% chlorhexidine-based shampoo (Hexadene Spherulites®, Virbac) or associated with miconazole (2% chlorhexidine, 2.5% miconazole, Cloresten®, Agener União). Demographic data of all dogs were computed, and they routinely underwent general and specific clinical examinations.

The mean age and weight of the dogs were 4.7 years (± 2.7) and 8.1 kg (± 1.6), respectively. Pure breeds were more affected, and Shih Tzu was the most frequent breed in the study. The demographic data of the dogs with AD are shown in Table 1. The values obtained from the hemogram and biochemical profiles of all dogs were within the normal range for the species.

Table 1. Demographic data of ten dogs with atopic dermatitis from the dermatology service of a Veterinary Teaching Hospital.

Dogs	Sex	Reproductive *status*	Breed	Age (years)	Weight (kg)
1	F	Spayed	Shih tzu	2	6.6
2	M	Spayed	Pug	3	9.25
3	F	Spayed	Yorkshire terrier	3	3.9
4	M	Spayed	Mixed breed	1	9.4
5	F	Spayed	French bulldog	4	8.6
6	F	Not spayed	Pug	6	8
7	M	Spayed	Shih tzu	3	8
8	F	Spayed	Lhasa apso	6	9.6
9	M	Not spayed	Shih tzu	6	8.8
10	M	Spayed	Shih tzu	11	8.6

Pruritus visual analog scale (pVAS)

To evaluate pruritus, the owners were asked to mark, on adapted (Hill et al. 2007) pVAS, the point where the animal was at that moment, according to their perception of pruritus. Subsequently, another scale, numbered from zero to ten centimeters (Rybníček et al. 2009), was superimposed on the one marked by the owner and the value was recorded. The pVAS scores were evaluated in centimeters and classified as follows: 1) normal dog: 0-1.9 cm; 2) mild pruritus: 2-4 cm; 3) moderate pruritus: >4-6 cm; 4) severe pruritus: >6-8 cm; and 5) very severe pruritus: >8-10 cm.

Canine atopic dermatitis extent and severity index (CADESI)

CADESI scores have also been used to classify disease severity in dogs and is commonly used to quantify the clinical effects of canine AD. CADESI-04 is based on the assessment of erythema, a marker of acute inflammation, lichenification, a marker of chronic disease and a combination of excoriation and alopecia, markers of pruritus in various body regions (Olivry et al. 2014).

A four-point severity scale comprising scores indicating absent (score 0), mild (score 1), moderate (score 2), and severe (score 3) was established for all characteristics in the 20 regions evaluated. Disease severity based on these scores was classified as: 1) mild: scores between 10-35; 2) moderate: >35-60; and 3) severe: >60-180. Three trained raters performed CADESI-04. The individual score values were recorded and summed, and the final value was considered as the average of the three scores.

Blood samples

To evaluate their general health, all dogs underwent jugular or cephalic venous puncture to collect 5 mL of blood for blood cell counts (2 mL) and biochemical profiles (3 mL) before the study. At M0, M1, and M2, 4 mL of blood was collected in a tube with a clot activator to obtain 2 mL of serum for the subsequent measurement of IL-31. All serum samples were stored at -20°C until IL-31 measurement.

Measurement of canine IL-31

To specifically detect and measure canine serum IL-31, a commercially available kit (Canine Interleukin-31 ELISA Kit; MyBioSource®, San Diego, CA, USA) was used with a sensitivity of 1 pg/mL. Measurements were performed at all time points following the manufacturer's recommendations. The assay was based on double antibody sandwich technology, and the kit was refrigerated (2-8°C) until sample processing.

Statistical analyses

All statistical analyses were performed using R 3.6.1 software (R Core Team, 2019). Generalized estimating equation models for each response variable were used to test the differences between the three treatment times regarding the quantitative variables of pVAS scores, CADESI-04 scores, and IL-31. Differences between times were tested globally, and mean values and their respective 95% confidence intervals were calculated for each time point.

Multiple comparison tests (pairwise comparisons) were performed. Tukey's correction was used for these tests. The pVAS and CADESI-04 score variables presented a normal distribution in the normality test

Table 2. Visual analog scale scores of pruritus (pVAS), extension and severity index of canine atopic dermatitis (CADESI-04), and serum concentrations of canine interleukin-31 (pg/mL) at the three-time points evaluated in each dog with atopic dermatitis.

Dog	CADESI-04			pVAS			Serum IL-31		
	M0	M1	M2	M0	M1	M2	M0	M1	M2
1	28.33	55	34.33	7.6	5.5	4.8	544.83	129	277.33
2	32.33	32.66	33.66	8.2	6.1	5.9	188.16	169.83	159
3	21.66	14.66	15.33	6.7	4	5.8	188.16	135.66	121.5
4	17.33	5	6.66	6.2	2.8	2.9	268.16	169	126.5
5	30	29.33	29.33	9.8	5.5	6.2	182.33	156.5	196.5
6	15.33	19.66	16.66	9.6	6.6	5.8	279	144	137.33
7	14.33	12.33	26	5.9	2	3.8	188.16	245.66	237.33
8	31.66	32	40	5.7	3.8	5.8	261.5	146.16	192.33
9	16.33	24.33	24.66	7.8	7.5	5.5	249	251.5	229.83
10	25.33	32	29.66	6.8	3.5	3.7	209.83	164	175.66

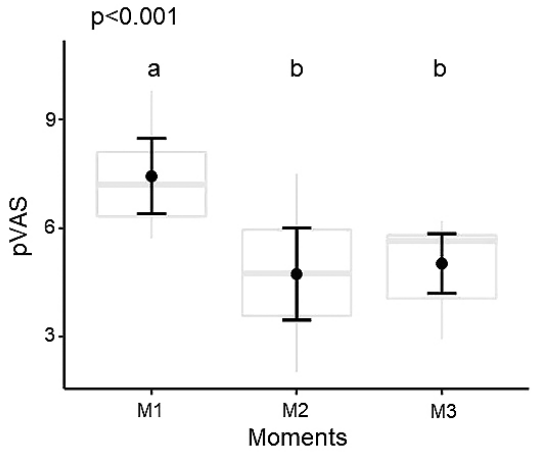

Fig. 1. Scatter plot of pVAS scores at each moment of treatment (M0, M1 and M2). The letters above each dog group represent the multiple comparisons. Groups with at least one letter in common have no significant differences between them at a significance level of 5%.

(Shapiro-Wilk), whereas the IL-31 variable presented an outlier. The study assumed a significance level of 5%, and the data were considered significant at $p<0.05$. Spearman's non-parametric correlation coefficient was calculated for each pair of variables.

Results

The pVAS and CADESI-04 scores and serum IL-31 levels are shown in Table 2. Before treatment (M0) the mean pVAS was 7.4 (±1.4), in M1 was 4.7 (± 1.8) and in M2 5.0 (± 1.2). Most dogs in the study (80%) had very severe or severe pruritus before therapy (M0). After the first injection of mAb (M1), no dogs had very severe pruritus, and the number of dogs with severe pruritus was reduced to 30%. Additionally, 70% of the dogs showed moderate or mild pruritus (20% and 50 %, respectively). After two injections (M2), none of the dogs had very severe pruritus and only 10% of dogs had severe pruritus, while 60% and 30% had moderate and mild pruritus, respectively.

pVAS values were statistically different ($p<0.001$) between M0 and M1 and between M0 and M2, but not between treatment time points M1 and M2 (Fig. 1), showing that the most significant reduction in pruritus occurred four weeks after the first injection.

The response to lokivetmab on pruritus was considered satisfactory when a reduction of ≥2 cm in the pVAS scores was observed (Souza et al. 2018). An outstanding response was defined as a reduction of ≥50% (Souza et al. 2018), which was detected in only 20% of the dogs (2/10) after the first injection. No dog reached pruritus values considered normal (0-1.9 cm), that is, the level at which owners would not seek veterinary care (Rybníček et al. 2009). However, the mean pVAS score decreased to 2.7 cm between M0 and M1 and 2.4 cm between M0 and M2.

All dogs in the study had mild disease severity, according to their baseline CADESI-04 scores. There was no statistically significant difference in the CADESI-04 scores ($p=0.515$) among the three time points (Fig. 2).

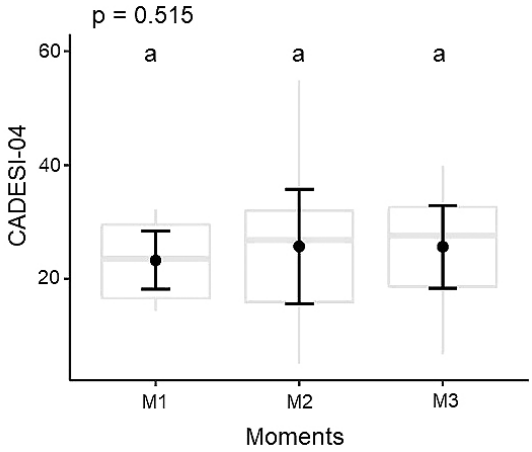

Fig. 2. Scatter plot of the CADESI-04 scores at each moment of treatment (M0, M1, and M2). The letters above each dog group represent multiple comparisons. Groups with at least one letter in common have no significant differences between them at a significance level of 5%.

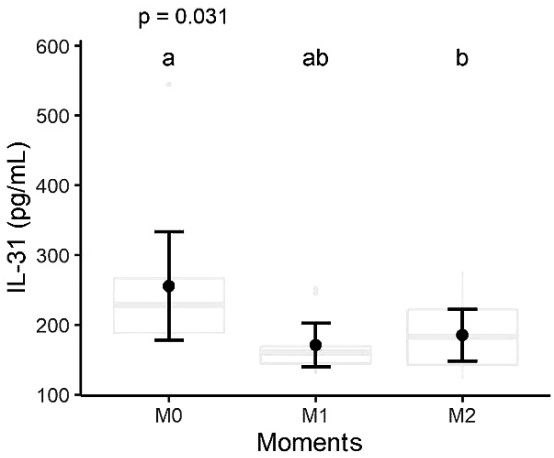

Fig. 3. Scatter plot of serum levels of canine IL-31 at each moment of treatment (M0, M1, and M2). The letters above each dog group represent multiple comparisons. Groups with at least one letter in common have no significant differences between them at a significance level of 5%.

IL-31 was detected in all dogs with AD, with a mean value of 255.91 pg/mL (±108.3) before lokivetmab treatment. The serum level of this cytokine was reduced in eight of the ten dogs after the first and second injection of lokivetmab. Between moments M0, M1, and M2, serum IL-31 levels gradually decreased in four dogs. The other four showed a reduction between M0 and M1, increasing again in M2. Only one dog (7) showed an increase in M1 and M2 compared to M0. There was a statistically significant difference (p=0.031) in serum IL-31 levels between M0 and M2. However, this difference was not observed between moments M0 and M1 and M1 and M2 (Fig. 3). Moreover, although considered statistically, weak, there was a significant positive correlation (p=0.047, R=0.37) between pVAS scores and serum IL-31 levels (Fig. 4).

Discussion

Currently, pVAS and CADESI-04 are excellent tools for enhancing studies on canine AD. An important role in canine pruritus has been attributed to IL-31 (Gonzales et al. 2013, Marsella et al. 2018); however, studies correlating its serum levels to pVAS and CADESI-04 in client-owned dogs are lacking in the veterinary literature, especially when a treatment is also addressed to evaluate these correlations. Furthermore, studies on these serum levels in patients treated with lokivetmab are non existent. In our study, a correlation between pVAS and IL-31 levels was found but not between CADESI-04 and this cytokine.

The greatest reduction in pruritus occurred four weeks after the first injection, with a 36.4% reduction in the pVAS scores, as previously reported (Moyaert et al. 2017, Szczepanik et al. 2019, Szczepanik et al. 2020). This finding reinforces the hypothesis that after

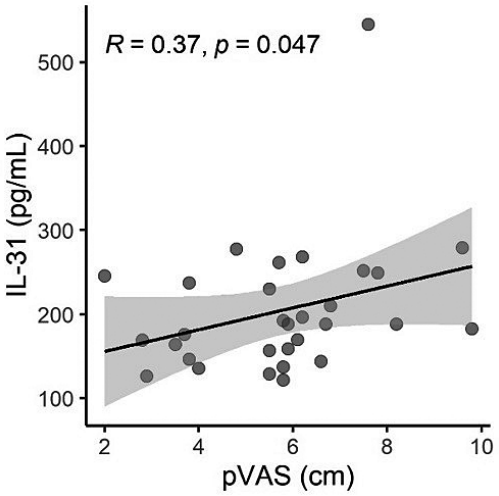

Fig. 4. Scatter plot of the correlation between serum IL-31 levels and pVAS scores at all treatment times (M0, M1, and M2).

the second mAb administration, pruritus remains mild to moderate and that lokivetmab serves as a maintenance therapy agent for this clinical sign in dogs with AD. Improvement in pruritus was achieved in 80% of the dogs after lokivetmab administration, consistent with previous results (Souza et al. 2018). An outstanding response (a reduction of ≥50%) was detected in only 20% of the dogs (2/10) in the study after the first injection, different from that found by Szczepanik et al. (2020), where 67.4% of dogs experienced a more than 50% reduction in pruritus after four weeks and 84.26% after eight weeks.

There was no difference (p=0.515) in CADESI-04 scores between the three time points evaluated. This corroborates the finding that lokivetmab does not prevent the development of skin lesions (Olivry and Banovic 2019). Nonetheless, our results differ from previous data showing a significant drop in scores four weeks after lokivetmab injection (Moyaert et al. 2017, Szczepanik et al. 2019, Szczepanik et al. 2020). In an earlier study, the greatest reduction in CADESI scores was observed in dogs treated with a higher dose compared with those treated with a lower dose (Michels et al. 2016). This suggests that the higher the dose of mAb, the more significant could be the reduction in the clinical signs.

Significant clinical remission of skin lesions is likely not achieved because of the broad Th2 response. Although mAb entirely blocks the action of IL-31, several cytokines and other inflammatory mediators participate in the development of inflammation, such as IL-4, IL-5, and IL-13, in addition to IL-25, IL-17, and TSLP (Tamamoto-Mochizuki et al. 2019), which contributes to clinical signs such as erythema, excoriation and alopecia, markers of acute inflammation and pruritus.

A previously published study showed that serum IL-31 levels increased in 57% (127/223) of dogs diagnosed with spontaneous AD, suggesting that the cytokine was deregulated in these dogs (Gonzales et al. 2013). Serum IL-31 levels significantly contribute to the pathogenesis of the disease and the manifestation of clinical signs, especially pruritus. However, in the same study, 43% of the animals with AD did not present detectable serum IL-31 levels (<13 pg/mL). The authors suggested that some dogs have low circulating IL-31 levels, acting locally in the target tissues and not being released into the bloodstream (Gonzales et al. 2013).

Alternatively, IL-31 may not play a significant role in canine AD pathogenesis in animals with undetectable IL-31 levels. Another hypothesis is that AD is a multifactorial disease involving complex interactions among susceptibility genes, skin barrier dysfunction, immune dysfunction, and neuroimmune interactions that collectively produce hypersensitivity to environmental allergens and pruritus (Gonzales et al. 2013). Owing to the complexity of the disease, not all dogs exhibit the same molecular or cellular changes. As mentioned previously (Gonzales et al. 2013) further observed that 52% of dogs (117/223) had serum IL-31 levels between 13 and 1000 pg/mL. Additionally, 4% (10/223) had levels of >1000 pg/mL. Nonetheless, the correlation between serum IL-31 levels and disease severity was not assessed at this time.

In another previous study, the authors investigated the correlation between serum IL-31 and severity of AD in an experimental model of beagle dogs percutaneously sensitized with *Dermatophagoides farina* (Marsella et al. 2018). The dogs were sensitized twice weekly for four weeks, and disease severity was assessed using the CADESI-03 before and after four weeks. Concurrent with the clinical evaluation, serum samples were collected to measure canine IL- 31 levels.

No positive correlation was found between CADESI-03 scores and serum IL-31 levels at moment zero. However, a correlation was observed after four weeks. Furthermore, in 50% of the dogs, the CADESI-03 scores increased from mild to moderate or severe. The authors hypothesized that continuous exposure to allergens (chronic disease) and an active crisis might be necessary to detect a significant correlation between disease severity and IL- 31 levels.

The sensitivity of the assay for the measurement of canine IL-31 was 1 pg/mL, which was consistent with a previous study (Marsella et al. 2018). However, the levels of IL-31 in our study varied between 182.33 and 544.83 pg/mL, differing from the lower levels observed previously by Marsella et al. (2018) before (8 to 121 pg/mL). In another study, the detection interval varied between 8,6 and 430 pg/mL (Ribeiro 2019). Nevertheless, the results cited above did not differ significantly, nor did they have levels as high as those initially reported (Gonzales et al. 2013). Other authors also observed discrepant serum IL-31 concentrations (Michels et al. 2016), but its levels were detectable in only 17.5% (37/211) of the dogs, with a baseline mean serum IL-31 level of 270 pg/mL. This mean corroborates with the mean of the present study before the administration of lokivetmab (255.91 pg/mL, ± 108.3).

In another previous study, the author also studied ten dogs with AD. However, the patients were treated with oclacitinib for 30 consecutive days (Ribeiro 2019). pVAS and CADESI-04 scores and serum concentrations of several cytokines involved in the pathogenesis of canine AD, including IL-31, were also measured using the enzyme-linked immunosorbent assay method (Ribeiro et al. 2019). At the end of the treatment, oclacitinib reduced all the cytokines studied, especially IL-31, in addition to a reduction in both clinical scores. Before oral administration of oclacitinib the median serum IL-31 concentrations were 59.2 pg/mL and, 30 days later, 40.9 pg/mL. These values were much lower than those detected in the present study, where a median of 229.415 pg/mL was observed at M0, 160.25 pg/mL at M1 and 183.995 pg/mL at M2. Nonetheless, the author observed no statistically significant correlations between cytokine concentrations, pVAS and CADESI-04 scores.

In the present study, no statistically significant correlation was observed between the CADESI-04 scores and serum IL-31 levels ($p=0.856$, $R=0.03$). However, although considered statistically weak, there was a significant positive correlation between pVAS scores and serum IL-31 levels ($p=0.047$, $R=0.37$). The higher the circulating IL-31 level, the higher is the pVAS score. This finding highlights the role of IL-31 in pruritus pathogenesis in dogs with AD.

Moreover, since lokivetmab acts selectively on IL-31, disease severity in dogs treated with this biological therapy should be evaluated through pVAS scores and not through CADESI-04 scores, since this index includes a marker of chronic disease (lichenification) and clinical signs of Th2 cytokine phenotype, which are secreted together with IL-31, contributing to the emergence of primary clinical lesions beyond pruritus. Notably, the dogs included in this study had mild CADESI-04 scores; lokivetmab is indicated in the proactive or maintenance phase, when the signs of inflammation are already under control (Olivry and Banovic 2019). For those dogs with significant chronic skin changes, lokivetmab is not an ideal therapy due to its lack of anti-inflammatory action (Jackson and Forsythe 2020).

Furthermore, because its reduction of pVAS scores, it is also possible to propose that lokivetmab be used in the reactive phase of the disease, as part as an integrative therapy to reduce pruritus more significantly in patients with AD and reduce the need for broader drugs.

Our data provide further evidence that IL-31 is involved in pruritus pathogenesis in dogs with canine AD. In addition, blocking IL-31 has a significant antipruritic effect, but has no influence on skin lesion severity and extension.

Acknowledgements

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