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A masked, randomised clinical trial evaluating the efficacy and safety of lokivetmab compared to saline control in client-owned dogs with allergic dermatitis

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Background – Interleukin (IL)-31 is an important mediator in canine atopic dermatitis (cAD) and also may be dysregulated in other allergic diseases.

Hypothesis/Objectives – To demonstrate the efficacy and safety of lokivetmab (canine anti-IL-31 monoclonal antibody) for treatment of pruritus associated with allergic dermatitis in dogs.

Animals – Dogs that were at least moderately pruritic with a presumptive diagnosis of allergic dermatitis were enrolled in Portugal, Hungary, France and Germany by 12 primary care practitioners and two veterinary dermatology referral specialists.

Methods and materials – Dogs were randomised to receive either placebo (saline) or lokivetmab (1.0–3.3 mg/kg) by subcutaneous injection on Day (D)0. Owners evaluated pruritus using a validated Visual Analog Scale (pVAS) daily until D7 and then weekly until D28. The severity of dermatitis was assessed by the investigators using a modified VAS on D0, D7, D14 and D28.

Results – Beginning at D1, owner-assessed pVAS least square means were significantly reduced in the treatment group versus the placebo group (57.7% versus 21.8% reduction on D28). For all time points, investigator-assessed VAS means were significantly reduced in the lokivetmab group versus the placebo group (57.1% versus 20.5% reduction on D28). Overall, the occurrence of adverse health events during the evaluation period was comparable between the two groups.

Conclusions and clinical importance – Lokivetmab is a safe and efficacious treatment for dogs with allergic dermatitis.

Introduction

The pro-inflammatory cytokine interleukin (IL)-31 is produced by activated T lymphocytes in multiple species and its pruritogenic nature has been well-described.[1,2] By binding to receptors on neurons (receptor complex consisting of IL-31 receptor A and oncostatin M receptor β), IL-31 triggers the activation of Janus kinases, which in turn signal to the brain, triggering an itch response and inducing scratching behaviour. In addition, IL-31 can stimulate the production of inflammatory mediators by promoting epithelial cell responses as demonstrated in transgenic mice overexpressing IL-31, a model in which severe pruritus as well as alopecia and skin lesions are induced.[3,4] Anti-IL-31 antibodies have been demonstrated in NC/Nga mice (a mouse model for human atopic dermatitis) to reduce or eliminate the pruritic effects of IL-31.[5]

A comprehensive global programme has been conducted and reported to demonstrate both safety and efficacy of lokivetmab in dogs with atopic dermatitis (AD), which led to its registration for treatment of clinical manifestations of canine AD (cAD).[6,7,8]The mode of action of lokivetmab combined with postmarketing studies on its use in pruritic skin diseases other than cAD, have suggested that it could be a valuable treatment alternative for dogs suffering from other allergic dermatoses.[9,10] The objective of the study, therefore, was to demonstrate the efficacy and safety of lokivetmab for the treatment of pruritus associated with allergic dermatitis of various aetiologies in dogs.

Methods and materials

The main procedures for the study were similar to previous studies of oclacitinib and lokivetmab, and summarised below.[6,11–13]

All data were collected in compliance with the principles of the International Cooperation on Harmonisation for Veterinary Medicines (VICH) Good Clinical Practice (GCP) Guideline 9.[14] The protocol was

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Conflicts of Interest: All authors are employees of Zoetis Inc.

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reviewed and approved before study initiation by the Sponsor Ethical Review Board, the Hungarian authorities (Budapest, Hungary; authorisation nos. 5300/1431-2/2019 and 5300/2336-1/2019), Portuguese authorities (Lisboa, Portugal; authorisation no. 62/ECVPT/2019), as well as ANSES (Fougères, France; authorisation no. EC-19-229) and German National Competent Authorities (authorisation no. Cytopoint-001-A). The owners gave written informed consent for their dogs to participate in the study.

In a masked, placebo-controlled field trial, dogs diagnosed with allergic pruritus were recruited from veterinary practices in France, Germany, Hungary and Portugal. Most of the participating investigators were veterinarians with experience in canine primary care medicine, whereas two were veterinary dermatology referral specialists. Animals were allocated randomly to one of two treatment groups in a 1:1 ratio of placebo (saline control) or lokivetmab at 1.0–3.3 mg/kg body weight. Dogs received one subcutaneous injection of either saline or lokivetmab on Day (D)0 and were followed for 28 days after treatment. Pruritus was assessed by the owner on a 10.0 cm long validated pruritus Visual Analog Scale (pVAS), and the condition of the skin was assessed by investigators using a severity of dermatitis VAS scale with descriptors adjusted to reflect different severities of the skin lesions (Figure S1).[12,15] Blood and urine samples were collected at enrolment as well as at the time of study completion.

Sample size estimates were derived from power calculations based on variance and effect sizes observed in a multicentre noninferiority field study with ciclosporin as the control product.[6] With ≥45 lokivetmab-treated dogs and ≥45 controls, there was ≥80% power to show a 32.72% difference in means for the primary efficacy endpoint of percentage change from baseline owner-assessed pVAS at D28. This outcome assumed mean responses for percentage change from baseline of 16.81% and 49.53% for placebo and lokivetmab, respectively, using a two-sided 5% significance level for the comparison of treatment means at D28.[6]

Inclusion criteria

Dogs were client-owned, weighed between 3 and 80 kg on D0, and were in overall good health apart from their diagnosis of allergic dermatitis. A presumptive diagnosis was made to include one or more of the following: adverse reaction to food, flea allergy, contact allergy, suspected AD or unspecified allergic dermatosis. The diagnoses were made at the discretion of the investigators (i.e. specific tests were not imperative) and several presumptive causes could be identified at the same time. A diagnosis of cAD was only allowed in combination with one of the other aetiologies. To be eligible for enrolment, owners had to assess their dogs as having at least moderate pruritus on a categorical assessment form at the initial (D0) assessment. Categories included: normal dog, very mild, mild, moderate, severe and extremely severe pruritus.

All dogs had to be on flea control for a minimum of four weeks before enrolment with no presence of fleas on D0 [no more than a mild infestation (i.e. flea faeces or debris present at most), no actual fleas visible], and continued use of this flea preventative during the study period was mandatory. Dogs diagnosed with cutaneous adverse reactions to food and that were consuming a hypoallergenic diet were required to have been on this diet for at least six weeks before D0. Regardless of food allergy status, all dogs had to remain on the same diet for the entire duration of the study. Allergen-specific immunotherapy was allowed if the dog had been on therapy for at least eight months before D0, or if the unsuccessful treatment had been discontinued for at least eight weeks before D0.

Prohibited and conditionally allowed medications and therapies

Withdrawal times for prohibited medications such as (and not limited to) oral and injectable corticosteroids, oclacitinib, ciclosporin and long-acting injectable antibacterials, were as described by Moyaert *et al.*[6] (Table S1). Some treatments were allowed, under the condition that the owners, investigators and other study personnel adhered to minimal use and frequency of use guidelines for the concomitant medication (Table S1).

Exclusion criteria included prior treatment with lokivetmab, signs of uncontrolled disease unrelated to allergic dermatitis, signs of immune suppression, and presence of active skin infection or infestation including (and not limited to) demodectic and sarcoptic acariasis, bacterial pyoderma and *Malassezia* dermatitis. Lactating bitches and any dog intended for use as a breeding animal also were excluded.

Randomisation and masking

Each animal was randomly allocated to either placebo (saline) or lokivetmab (Cytopoint, Zoetis Belgium SA; Louvain-la-Neuve, Belgium) treatment in a 1:1 ratio. Animals were randomised on D0 according to a randomised complete block design with one-way treatment structure replicated in multiple clinics. Within each study site the dogs were blocked on order of enrolment. Within each block, dogs were allocated at random to treatment groups, with each treatment occurring at least once. Investigators and all site personnel, with the exception of the treatment dispenser, were masked to the treatment group assignments as were owners and the laboratory personnel. The treatment dispenser drew up the correct dose of injectable treatment (lokivetmab or saline, with identical appearance) into a syringe and provided it to the investigator for administration.

The demographic dataset on D0 was not analysed statistically because animals were blocked on order of enrolment within a study site and treatment groups were assigned randomly. Any potential differences for demographics on D0 between treatment groups could occur by chance and would mimic normal field conditions.

Treatment administration

Lokivetmab was provided as per commercial formulation in single-use vial containing 1 mL that contained no preservative. Vials provided contained solution in one of four concentrations (10, 20, 30 and 40 mg/mL). Based on the dosing chart, each dog was administered 1.0–3.3 mg/kg of lokivetmab depending upon the dog's body weight.

Study schedule and variables measured

Baseline data (demographic, physical examination, initial assessment of pruritus and adherence to inclusion criteria) were collected at enrolment on D0. Owners performed an assessment of the severity of their dog's itch using the pVAS on D0 (before treatment and repeated approximately 4 h post-treatment), and D1–D7, D14, D21 and D28. This 10 cm long scale had descriptors along its side at 2 cm intervals to help the owners assess the severity of the dog's pruritus during the past 24 h.[15] The overall condition of the dog's skin was evaluated by the investigator on a similar 10 cm VAS scale with descriptors at 2 cm increments adjusted to reflect different severities of skin condition: no (normal dog), very mild, mild, moderate, severe and extremely severe dermatitis (Figure S1).[12] Combined with a general physical examination, these investigator-driven assessments were performed on D0, D7, D14 and D28. Dogs were observed for 30 min following administration for signs of immediate adverse reactions to treatment. Abnormal health events (AHE) and/or concomitant treatment reported by owners or identified on physical examination were recorded throughout the study.

On the final day of study (D28, or earlier for dogs withdrawn before D28), the dog's overall response to treatment (RTT) was assessed by both the owner and the investigator by drawing a vertical line on a horizontal 10 cm scale ranging from "no improvement" to "excellent results".

A physical examination including body weight was performed at each clinic visit. Any signs of ill health that were not pre-existing (or any change in severity) were reported on an AHE form. The condition of eyes and ears, and skin and appendages attributable to the pre-existing disease of allergic dermatitis was captured on the investigator severity of dermatitis VAS form and treatment differences between both groups reported as such. The investigators were instructed only to report a worsening of the associated clinical signs as an AHE.

Blood samples (haematological and serum chemical parameters) and urine samples (urinalysis and protein creatinine ratio) were collected at enrolment and study completion. Blood and urine were

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collected again at the discretion of the investigator if the dog presented for an AHE. All samples for haematological, serum chemical, urinalysis and urine protein creatinine ratio investigations were sent to the same laboratory.

In cases of suspected secondary bacterial infections, it was recommended to collect a swab sample for standard bacteriological investigation, including antibiogram, through standard veterinary procedures.

Efficacy outcome measures

The efficacy dataset excluded those dogs that were considered to have had a protocol deviation that affected the collection or integrity of their efficacy data, such as treatment with prohibited medications or visits performed outside the allowed visit windows. Dogs withdrawn from the study before D28 as a consequence of worsening signs of allergic dermatitis (lack of efficacy) were included in the analysis as failing to achieve 50% or 75% reduction from baseline for all subsequent time points after their withdrawal.

For the owner pVAS, data were summarised for D0, D0+4 h, D1–D7 (±1 day), D14 (±2 days), D21 (±2 days) and D28 (±3 days). For investigator VAS, data were summarised for D0, D7 (±1 day), D14 (±2 days) and D28 (±3 days).

The primary efficacy end-point was defined as the reduction from baseline of the owner-assessed pruritus as measured by pVAS, on D28.

Secondary efficacy end-points included percentage reduction from baseline of owner pVAS and investigator VAS at each time point, proportion of dogs achieving 50% and 75% decrease of owner-assessed pruritus compared at each time point compared to D0, percentage of dogs achieving a "normal range" on the pVAS on each of the study time points, and assessment of overall RTT from the owner and the investigator at study completion or withdrawal. Using the pVAS, a score of 0–19 mm was assumed to be the best approximation of a "normal range".[16]

Safety outcome measures

Data from all animals were included in the dataset used for the assessment of safety, independent of the occurrence of protocol deviations. Similar to the study reported by Moyaert *et al.*,[6] frequencies of dogs reported to show at least one AHE were summarised by clinical signs, and frequencies of dogs receiving concomitant medication over the course of the study were summarised by an Anatomical Therapeutic Chemical classification system for veterinary medicinal products (ATC) functional use term.[6]

Summary statistics (means with standard deviations or medians with ranges) were calculated by treatment and intended day of sampling for each haematological, serum chemical and quantitative urinalysis value, reporting the number of dogs that fell below, within or above the normal range (provided by the laboratory) at each day of sampling for haematological and serum chemical parameters specifically. In addition, shift tables provided the number of dogs that had an increased or decreased shift compared to baseline at each day of sampling.

Data analysis

Data analysis was performed using SAS v9.4 (SAS Institute; Cary, NC, USA) as described previously.[6,11–13] Mixed linear models were fitted using PROC MIXED. Where appropriate, transformations were applied to end-points before statistical analysis as a remedial measure to address violations in the assumptions for the statistical models. The level of significance was set at $\alpha = 0.05$ (two-sided).

Results

Sixty animals per treatment group were targeted for enrolment to allow for drop-outs and retention of 45 evaluable cases per treatment on D28. Withdrawals were allowed for missed assessments resulting from owner/investigator oversight and exclusions of data resulting from protocol deviations that could have biased the efficacy assessment.

Demographic data

A total of 123 dogs (62 saline controls; 61 lokivetmab-treated) were enrolled from 14 veterinary practices in France (n = 40), Germany (n = 25), Hungary (n = 35) and Portugal (n = 23). Their demographic details are summarised in Table 1. In the control group, the most common breeds were German shepherd dog (9.7%), Labrador retriever (8.1%) and French bulldog (6.5%). Among the lokivetmab-treated animals, the three most common breeds were French bulldog (13.1%), Labrador retriever (8.2%) and beagle (6.6%). All dogs were free of fleas (flea infestation was recorded as absent) at enrolment. One lokivetmab-treated animal was observed with a mild flea infestation (presence of flea faeces and identification of some flea bites) on D14, and returned to normal by the next visit (D28).

Aetiology

The most common presumptive diagnosis was food allergy (41.5%), followed by AD (33.3%, in combination with other aetiologies) and contact allergy (33.3%). Flea allergy was least reported, with only 13.8% of the enrolled cases represented. Distribution of the different aetiologies was similar in both treatment groups (Table S2).

For 29.3% of the animals, an allergic component was identified that could not be assigned to any of the predefined categories with the information at hand at the time of enrolment; these were assigned as unspecified allergic dermatitis (in combination or not with any of the other causes).

Treatment administration

On D0, the lokivetmab dose ranged between 1.0 and 2.9 mg/kg. Nearly half of the dogs received a dose of 1–1.2 mg/kg (n = 30), 30% (n = 18) received a dose of 1.3–1.5 mg/kg, and only one animal received a dose >2 mg/kg.

Assessment of effectiveness

The primary effectiveness dataset at D28 comprised 99 dogs in the owner pVAS dataset (44 control, 55 lokivetmab-treated animals). Eighteen dogs were excluded/missing from the analysis of owner assessments in the control group, and six in the lokivetmab-treated group on D28. The datasets for owner pVAS and investigator VAS changed at each time point as a result of early withdrawals, missing assessments because of owner/investigator oversight (e.g. missed assessment at home or visit skipped) or data excluded from the analysis as a result of protocol deviations that could have biased the efficacy assessment (e.g. forbidden medications given or assessment performed outside the allowed window).

Four animals (one placebo, three lokivetmab-treated dogs) received forbidden medication during the study period and as a result their efficacy data were excluded from the D28 dataset. Data from one additional placebo-treated animal were excluded because the D28 assessment was performed outside of the allowed window (D28 ± 3 days). Data from 16 control animals and three

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Table 1. Demographics of enrolled 123 dogs at Day 0

	Placebo n = 62 [%(n)]	Lokivetmab n = 61 [%(n)]
Breed distribution		
Pure-bred	77.4 (48)	77.0 (47)
Mixed breed	22.6 (14)	23.0 (14)
Sex distribution		
Male	54.8 (34)	45.9 (28)
Female	45.2 (28)	54.1 (33)
Neutered/spayed	56.4 (35)	57.4 (35)
Mean age at study start, months (range)	71.7 (5.0 to 228.0)	66.1 (8.0 to 180.0)
Mean weight at study start, kg (range)	21.4 (3.7 to 54.2)	19.7 (3.5 to 38.0)
Median owner pruritus VAS mm (range)	66.0 (52.0 to 98.0)	70.0 (43.0 to 96.0)
Median investigator dermatitis VAS mm (range)	65.0 (21.0 to 95.0)	66.0 (19.0 to 96.0)

n number of animals (all animals enrolled, irrespective of whether they were excluded from the analysis due to protocol deviations).

lokivetmab-treated dogs were not available because they were withdrawn from the study before D28. All withdrawals were to the result of worsening or lack of improvement of clinical signs of allergic dermatitis.

Owner-assessed pVAS

On D0, the pre-treatment mean pruritus score was 69 in the control group versus 70 in the lokivetmab-treated group. On D28, the least-square (LS) mean percentage reduction from baseline was significantly higher in the lokivetmab-treated animals (57.7%) compared to the placebo group (21.8%; $P < 0.0001$).

For all other time points (beginning with D1), the percentage reduction from baseline of owner-assessed pVAS LS means was significantly greater ($P \leq 0.0109$) in the group of animals treated with lokivetmab versus the group of animals treated with saline, ranging from a 14.5% difference between treatment groups on D1 to a maximum of 37.2% on D14 (Figure 1).

At every study time point beyond D1 (for 50% reduction) and/or D2 (for 75% reduction), the proportion of animals achieving ≥50% or 75% reduction in pVAS was significantly higher in the treatment group than the control group ($P \leq 0.0213$ for 50%; $P \leq 0.0451$ for 75%). Treatment success defined as ≥50% reduction in pVAS, achieved a maximum 26% on D6 in the control group, and decreased thereafter. In the lokivetmab-treated group, a maximum of 73% was reached on D14 and averaged between 66 and 70% during the last two weeks of the study period (Figure 2). In the control group, ≤1% of the dogs achieved 75% reduction at any of the time points, while in the lokivetmab-treated group, the percentage of dogs achieving 75% reduction varied between 2% on D0 (4 h post-dosing) to 32% of the animals on D21 (Figure 3).

At all time points after D0, the percentage of dogs achieving a "normal" VAS score was numerically higher in the lokivetmab-treated group compared to the control group. By D28, 45.5% of the lokivetmab-treated dogs were scored as "normal" in terms of level of pruritus versus 6.8% of the saline-treated dogs (Figure 4).

Investigator severity of dermatitis VAS

Similar to the pruritus assessment, a reduction in the VAS score reflected an improvement in the condition of the skin. For all time points, the percentage reduction from baseline of investigator-assessed skin condition VAS LS means was significantly higher ($P < 0.0001$) in the lokivetmab-treated group compared to the control group. In the control group, dogs achieved 14.1%, 20.6% and 20.5% reduction on D7, D14 and D28, respectively, compared to D0. In the lokivetmab-treated dogs, the corresponding reductions in percentages were 41.3%, 55.8% and 57.1%.

Response to treatment (RTT)

Owner and investigator RTT were significantly higher ($P < 0.0001$) in the treatment group than the control group. Means were 67.8% and 70.1%, respectively, for treated dogs, and 33.1% and 29.9%, respectively, for controls.

Adverse health events and concomitant medications:

Overall, the occurrence of AHEs was comparable between both treatment groups: 14.5% in the control group (n = 9) versus 11.5% in the lokivetmab-treated group (n = 7; Table S3).

Two dogs in each group were sampled for bacteriological culture owing to a suspected skin or ear infection, and one in each group subsequently was treated with systemic antimicrobials. For a control dog, a skin infection resulted in withdrawal before D28. Because this animal was withdrawn due to worsening signs of allergic dermatitis (lack of efficacy), it was treated as failing to achieve 50% and 75% reduction from baseline for all subsequent time points. One dog in the treatment group also received systemic antimicrobial therapy and was excluded from the efficacy analysis at all subsequent time points.

Overall, use of concomitant medication was comparable between both treatment groups. In the control group, 15 dogs were treated with oclacitinib at the time of early withdrawal due to lack of efficacy. In the lokivetmab treatment group, three dogs received oclacitinib as rescue treatment at the time of early withdrawal.

Haematological and serum chemical investigation, and urinalysis

For various serum chemical parameters, increasing and/or decreasing shifts were observed, yet these were not clinically significant and generally occurred in both groups. The mean serum chemical values remained within reference ranges at all study time points from D0 onwards.

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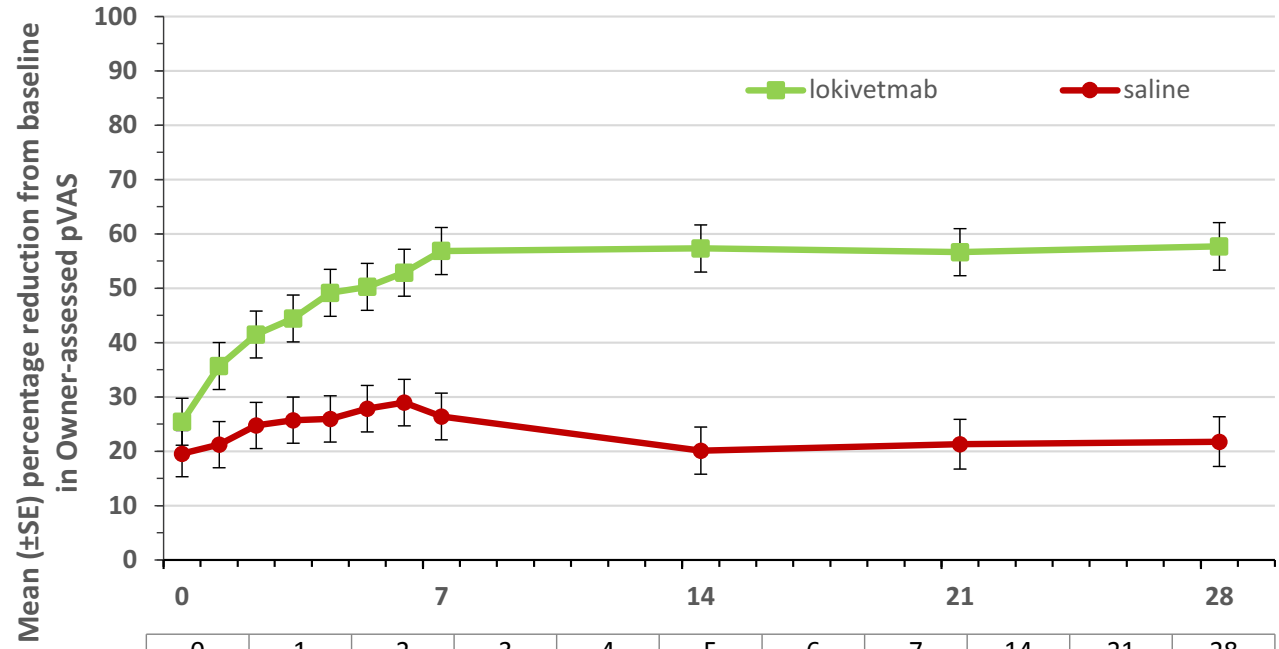
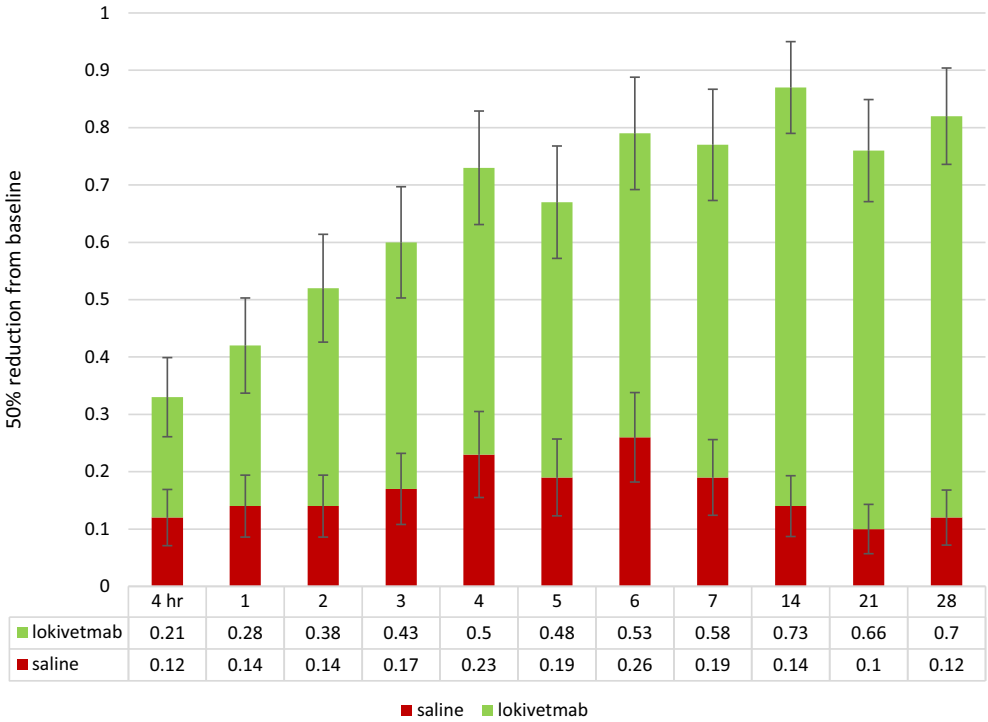


	0	1	2	3	4	5	6	7	14	21	28
lokivetmab	25.44	35.69	41.49	44.44	49.16	50.26	52.86	56.85	57.32	56.64	57.71
saline	19.57	21.22	24.75	25.74	25.96	27.84	28.96	26.41	20.13	21.31	21.78

Figure 1. Percentage reduction from baseline of owner-assessed pruritis Visual Analog Scale (pVAS) (plot of treatment least-square means).



	4 hr	1	2	3	4	5	6	7	14	21	28
lokivetmab	0.21	0.28	0.38	0.43	0.5	0.48	0.53	0.58	0.73	0.66	0.7
saline	0.12	0.14	0.14	0.17	0.23	0.19	0.26	0.19	0.14	0.1	0.12

Figure 2. Plot of 50% reduction from baseline of owner-assessed pruritis Visual Analog Scale (pVAS) (back-transformed treatment least-square means).

Increasing and/or decreasing shifts were observed equally in both treatment groups for haematological parameters. Overall, mean values remained within reference range at all study time points from D0 onwards, except for MCHC (mean corpuscular haemoglobin concentration) where the mean value was below the

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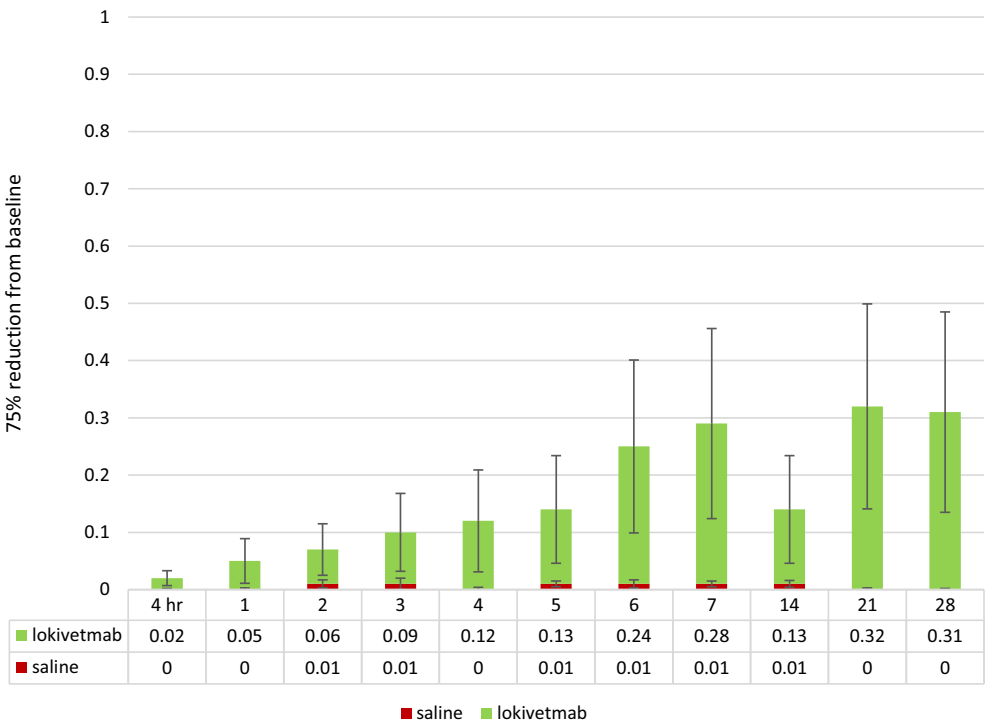

Figure 3. Plot of 75% reduction from baseline of owner assessed pruritis Visual Analog Scale (pVAS) (back-transformed treatment least-square means).

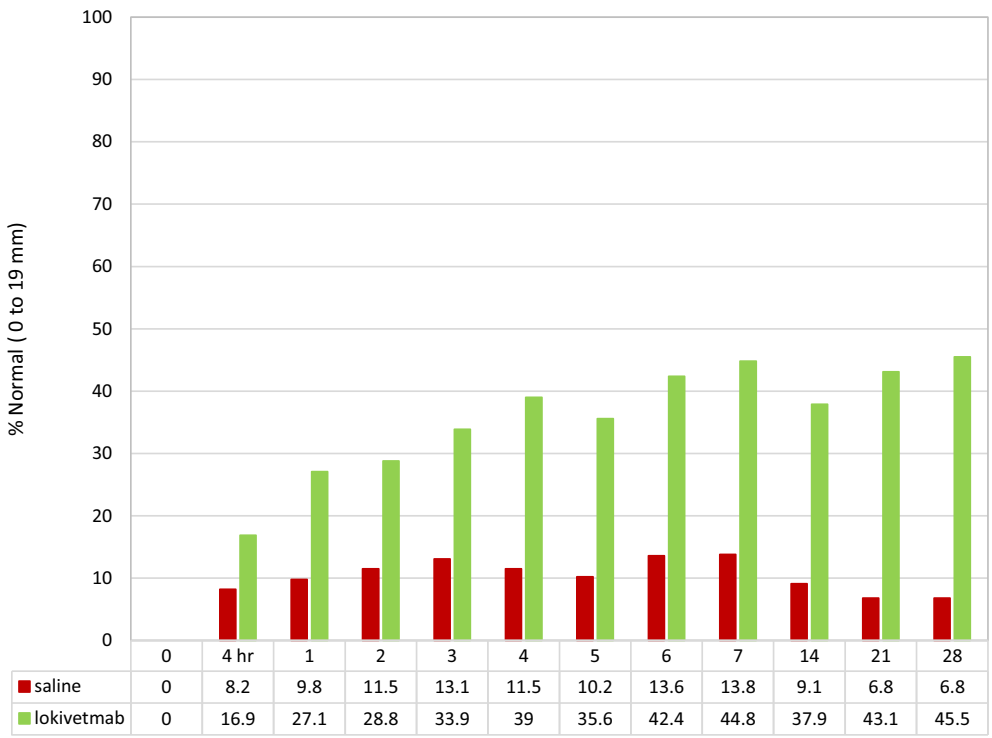

Figure 4. Plot of the percentage of animals with pruritis Visual Analog Scale (pVAS) in "normal" range (0–19 mm) by treatment on each day of evaluation.

reference range (32.6–39.2 g/dL) for both control animals (32.3 g/dL) and lokivetmab-treated dogs (32.5 g/dL) on D0, and in the control group (32.5 g/dL) on D28. This was not considered clinically relevant.

Urinalysis did not reveal any concerns for potential treatment-related abnormalities.

Discussion

The objective of this study was to demonstrate the efficacy and safety of lokivetmab for the treatment of pruritus associated with an allergic dermatitis in dogs. The targeted population included dogs with presumptive

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Table 2. Summary of the different efficacy outcomes after treatment with lokivetmab in atopic and allergic animals for owner assessment of pruritus Visual Analog Scale (pVAS) on Day 28

Efficacy outcome	Atopic dermatitis (Moyaert *et al.*, 2017)	Allergic dermatitis
% reduction in pVAS compared to baseline LSM	51.9	57.7
50% reduction in pVAS LSM proportion of dogs	0.57	0.70
75% reduction in pVAS LSM proportion of dogs	0.23	0.31
% normal pVAS (0–19 mm)	39.3	45.5

LSM least-square mean.

diagnoses of food allergy, flea allergy, contact allergy and AD in any combination, or unspecified allergic pruritus. Diagnoses were established at the discretion of the investigators, who were mainly primary care veterinarians. This approach was deliberately selected to reflect field conditions in general practice, in which lokivetmab might be prescribed and administered. In a recent study following a similar design (with presumptive diagnoses), a comparable distribution of disease aetiology was reported, with approximately 45% of the dogs diagnosed as suffering from contact allergy.[13] It is likely that more rigorous diagnostic criteria would have altered the distribution of the diagnoses,[17,18] as the prevalence of allergic contact dermatitis is much lower when assessed exclusively by dermatology specialists.[19]

Results of this study showed significant reduction of owner-assessed pruritus after treatment with lokivetmab compared to placebo (saline) control. The results are comparable to a prior study which enrolled only dogs with a diagnosis of AD (Table 2).[6]

In this study, a 53% reduction in pruritus was observed 6 days after the start of lokivetmab treatment which is in line with what was previously reported for oclacitinib (55%).[13] Equally, a peak in pruritus reduction was observed 14 days after injection of lokivetmab treatment The antipruritic activity of lokivetmab remained unchanged after D14, and on D28 there was still a 58% reduction in pruritus in the treated animals. In a nine month field study in dogs with AD, the pruritus score decreased further with subsequent injections and reached a plateau after four consecutive monthly injections.[6] A 50% reduction from baseline of mean pruritus score represents a clinically relevant threshold above which owners are satisfied with treatment.[20]This percentage has been used subsequently as a standard for assessing the efficacy of treatments for pruritus.[21–23] The proportion of animals exhibiting a 50% pruritus decrease on D14 (73%) in this study is nearly identical to the percentage reported for oclacitinib when treating a very similar population of animals diagnosed with allergic dermatitis.[13]

Another mechanism for determining efficacy is implementation of a threshold for what is considered to be a "normal" animal, where "normal" is defined as obtaining a pVAS score of <2 cm as proposed by Rybnicek *et al.*[16] The percentage of dogs with pruritus scores in the "normal" range on D28 was 45.5% for lokivetmab-treated dogs compared with 6.8% for placebo-treated dogs. This is in line with what has been reported for atopic dogs treated with lokivetmab, where 39% of the animals was assessed as "normal" on D28.[6] Therefore, it appears that neutralisation of IL-31 has an antipruritic effect in a broader population of pruritic dogs than just those with a confirmed diagnosis of AD. The data therefore suggest an association between allergic dermatoses in general and IL-31 dysregulation, as reported previously in humans.[24,25]

In addition to owner-assessed pruritus, the investigators assessed the severity of skin lesions using an unvalidated VAS, as has been described previously.[12,13] Validated scales which have been developed for scoring cAD were not employed, because they are specific for cAD. The results of the investigator-assessed dermatitis VAS mirrored the findings of the owners' pVAS scores, similar to what was observed with oclacitinib.[13] This also is consistent with results obtained in dogs with cAD, where lokivetmab treatment had a positive effect on cutaneous inflammation as assessed by reduction of Canine Atopic Dermatitis Extent and Severity Index (CADESI)-03 scores.[6,7]

In total, 19 dogs were withdrawn from the study before D28: 16 in the control group and three in the treatment group. All were withdrawn owing to progressive or static clinical signs. Treatment failures are to be expected when managing dogs with allergic skin disease, and even drugs with broader modes of action – such as methylprednisolone and ciclosporin – failed to control clinical signs of some dogs with cAD in a trial reported previously.[26]

The occurrence of abnormal health events was low and comparable between the treatment and placebo groups, and the nonremarkable haematological and serum chemical data also support the safety of lokivetmab, even when used with a wide variety of medicines and vaccines commonly used in canine practice. It is acknowledged that the duration of this clinical trial was limited to 28 days. However, the long-term safety and efficacy of lokivetmab has been demonstrated previously in field trials where dogs with cAD were treated and evaluated for up to nine months.[6]

In conclusion, the results of this study demonstrate that neutralisation of IL-31 has an antipruritic effect in a broader population of dogs than just those with a confirmed diagnosis of cAD. In addition to alleviating pruritus, lokivetmab also reduces inflammatory skin lesions. It therefore is a safe and efficaceous therapy for the treatment of dogs with various allergic dermatitides.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Investigator VAS for the assessment of dermatitis severity.
Table S1. Prohibited and conditionally allowed medications and therapy.
Table S2. Frequency distribution of the presumptive nature of allergic dermatitis.
Table S3. Adverse events; summary table by frequency of occurrence of system organ class and then by clinical sign as from D0.

Résumé
Contexte – L'interleukine (IL)-31 est un médiateur important de la dermatite atopique canine (cAD) et peut aussi être dérégulée dans d'autres dermatoses allergiques.
Hypothèses/Objectifs – Démontrer l'efficacité et l'innocuité du lokivetmab (anticorps monoclonal canin anti-IL-31) pour le traitement du prurit associé aux dermatites allergiques chez le chien.
Sujets – Les chiens qui étaient au moins modérément prurigineux avec un diagnostic présumé de dermatose allergique ont été enrôlés au Portugal, Hongrie, France, Allemagne par 13 praticiens généralistes et un vétérinaire spécialiste en dermatologie.
Matériels et méthodes – Les chiens ont été randomisés pour recevoir soit le placebo (solution saline) soit du lokivetmab (1.0-3.3 mg/kg) par injection sous cutanée à Jour (D) 0. Les propriétaires ont évalué le prurit

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à l'aide d'une échelle visuelle analogue (pVAS) validée une fois par jour jusqu'à D7 puis une fois par semaine jusqu'à D28. La sévérité de la dermatite a été évaluée par les investigateurs à l'aide d'une VAS modifiée à D0, D7, D14 et D28.
Résultats – Au départ à D1, la moyenne de pVAS des propriétaires étaient significativement diminuée dans le groupe traitement contre le groupe placebo (57.7% contre 21.8% de réduction à D28). A tous les moments, les moyennes de VAS par les investigateurs étaient significativement diminuées dans le groupe lokivetmab contre le groupe placebo (57.1% versus 20.5% de réduction à D28). En outre, les effets indésirables au cours de la période d'évaluation étaient comparables entre les deux groupes.
Conclusions et importance clinique – Le lokivetmab est un traitement sûr et efficace pour les chiens avec dermatite allergique.

Resumen

Introducción – la interleuquina (IL) -31 es un mediador importante en la dermatitis atópica canina (cAD) y también puede estar disregulada en otras enfermedades alérgicas.
Hipótesis/objetivos – Demostrar la eficacia y seguridad de lokivetmab (anticuerpo monoclonal anti-IL-31 canino) para el tratamiento del prurito asociado con la dermatitis alérgica en perros.
Animales – En Portugal, Hungría, Francia y Alemania, 13 veterinarios generalistas y un especialista en dermatología veterinaria inscribieron perros que tuviesen al menos prurito moderado con un diagnóstico presuntivo de dermatosis alérgica.
Métodos y materiales – los perros se asignaron al azar para recibir placebo (solución salina) o lokivetmab (1,0–3,3 mg/kg) por inyección subcutánea el día (D) 0. Los propietarios evaluaron el prurito usando una escala análoga visual validada (pVAS) diariamente hasta el D7 y luego semanalmente hasta el D28. Los investigadores evaluaron la gravedad de la dermatitis utilizando una VAS modificada en D0, D7, D14 y D28.
Resultados – a partir de D1, las medias de mínimos cuadrados de pVAS evaluadas por el propietario se redujeron significativamente en el grupo de tratamiento frente al grupo de placebo (una reducción del 57,7% frente al 21,8% en el D28). Para todos los puntos temporales, las medias de la VAS evaluadas por el investigador se redujeron significativamente en el grupo de lokivetmab frente al grupo de placebo (reducción del 57,1% frente al 20,5% en D28). En general, la ocurrencia de eventos adversos para la salud durante el período de evaluación fue comparable entre los dos grupos.
Conclusiones e importancia clínica – Lokivetmab es un tratamiento seguro y eficaz para perros con dermatitis alérgica.

Zusammenfassung

Hintergrund – Interleukin (IL)-31 ist ein wichtiger Mediator bei der atopischen Dermatitis des Hundes (cAD) und kann auch bei anderen allergischen Erkrankungen eine Dysregulation aufweisen.
Hypothese/Ziele – Es sollte die Wirksamkeit und die Sicherheit von Lokivetmab (caniner anti-IL-31 monoklonaler Antikörper) zur Behandlung von Juckreiz im Zusammenhang mit allergischer Dermatitis beim Hund gezeigt werden.
Tiere – Hunde mit zumindest moderatem Juckreiz und der vermeintlichen Diagnose einer allergischen Dermatose wurden in Portugal, Ungarn, Frankreich und Deutschland von 13 HaustierärztInnen und einem veterinärdermatologischen Überweisungstierarzt ausgewählt.
Methoden und Materialien – Die Hunde wurden zufällig ausgewählt, um entweder Plazebo (Kochsalzlösung) oder Lokivetmab (1.0-3-3 mg/kg) mittels subkutaner Injektion am Tag (D) 0 zu erhalten. Die BesitzerInnen evaluierten den Juckreiz mittels Visual Analog Scale (pVAS) bis zum D7 täglich und dann wöchentlich bis zum D28. Die Schwere der Dermatitis wurde von den UntersucherInnen mittels modifizierter VAS am D0, D7, D14 und D28 bestimmt.
Ergebnisse – Beginnend am D1 war das wenigste quadratische Mittel der durch BesitzerInnen erfassten pVAS in der Behandlungsgruppe im Gegensatz zur Plazebogruppe signifikant reduziert (57,7% versus 21,8% Reduktion am D28). Zu allen Zeitpunkten war der durch die UntersucherInnen erfasste VAS Durchschnitt in der Lokivetmab Gruppe im Vergleich zur Plazebogruppe signifikant reduziert (57,1% versus 20,5% Reduktion am D28). Insgesamt war das Auftreten von Nebenwirkungen auf die Gesundheit während der Untersuchungsphase in beiden Gruppen vergleichbar.
Schlussfolgerungen und klinische Bedeutung – Lokivetmab ist eine sichere und wirksame Behandlung bei Hunden mit einer allergischen Dermatitis.

要約

背景 – インターロイキン (IL)-31は、犬アトピー性皮膚炎 (cAD) において重要なメディエーターであり、他のアレルギー疾患においても調節不能である可能性がある。
仮説/目的 – 本研究の目的は、犬アレルギー性皮膚炎に伴う掻痒症の治療に対するlokivetmab (犬用抗IL-31モノクローナル抗体) の有効性および安全性を実証することであった。

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被験動物 – ポルトガル、ハンガリー、フランス、ドイツにおいて、アレルギー性皮膚炎と推定診断された少なくとも中等度の掻痒を伴う犬を、13人のプライマリーケア開業医および1人の獣医皮膚科紹介専門医が登録した。
材料と方法 – 犬は、プラセボ (生理食塩水) またはlokivetmab(1.0~3.3 mg/kg) のいずれかをDay(D)0に皮下注射するように無作為に割り付けられた。飼い主は、有効なVisual Analog Scale(pVAS) を用いて、D7までは毎日、その後D28までは毎週、掻痒を評価した。皮膚炎の重症度は, D0,D7,D14,D28に調査員が修正VASを用いて評価した。
結果 – D1以降、飼い主が評価したpVASの最小二乗平均値は、プラセボ群に対して治療群で有意に減少した (D28で57.7%対21.8%減少) 。すべての時点で、治験責任獣医師が評価したVASの平均値は、lokivetmab群がプラセボ群に対して有意に減少した (D28で57.1%対20.5%の減少) 。全体的に、評価期間中の健康上の有害事象の発生は両群間で同等であった。
結論と臨床上の重要性 – Lokivetmabはアレルギー性皮膚炎の犬に対する安全で効果的な治療法である。

摘要
背景 — 白细胞介素(IL)-31是犬特应性皮炎(cAD)的重要介质, 在其他过敏性疾病中也可能发生失调。
假设/目的 — 证明洛基维特单抗 (犬抗IL-31单克隆抗体) 治疗犬过敏性皮炎相关瘙痒的有效性和安全性。
动物 — 葡萄牙、匈牙利、法国和德国的13名初级保健医生和1名兽医皮肤病转诊专家招纳推定诊断为过敏性皮肤病的犬, 瘙痒程度至少为中度。
方法和材料 — 第(D)0天, 犬随机皮下注射给予安慰剂 (生理盐水) 或洛基维特单抗(1.0-3.3 mg/kg)。犬主人使用经验证的视觉模拟量表(pVAS)评价瘙痒, 每天一次, 直至D7, 然后每周一次, 直至D28。研究者在D0、D7、D14和D28使用改良VAS评估皮炎的严重程度。
结果 — 从D1开始, 与安慰剂组相比, 治疗组主人评估的pVAS最小均方值显著降低 (D28降低57.7%vs 21.8%) 。对于所有时间点, 与安慰剂组相比, 洛基维特单抗组中研究者评估的VAS平均值显著降低 (第28天降低57.1%vs 20.5%) 。总之, 在评价期间两组副反应发生率相当。
结论和临床重要性 — 对于患有过敏性皮炎的犬, 洛基维特单抗是一种安全有效的治疗方法。

Resumo
Contexto – A interleucina (IL)-31 é um importante mediador na dermatite atópica canina (DAC) e também pode estar desregulada em outras doenças alérgicas.
Hipótese/Objetivos – Demonstrar a eficácia e a segurança do lokivetmab (anticorpo monoclonal canino anti-IL-31) para o tratamento do prurido associado a dermatites alérgica em cães.
Animais – Cães com prurido ao menos moderado com diagnóstico presuntivo de dermatose alérgica foram inclusos em Portugal, Hungria, França e Alemanha por 13 veterinários clínicos gerais e um especialista em dermatologia veterinária.
Métodos e materiais – Os cães foram randomizados para receber placebo (solução salina) ou lokivetmab (1,0–3,3 mg/kg) por via subcutânea no Dia (D)0. Os proprietários avaliaram o prurido diariamente usando uma escala visual analógica (pVAS) validada até o D7 e, a seguir, semanalmente até o D28. A gravidade da dermatite foi avaliada pelos investigadores nos D0, D7, D14 e D28 usando uma VAS modificada.
Resultados – Começando em D1, as médias dos mínimos quadrados de pVAS avaliadas pelos proprietários apresentaram redução significativa no grupo de tratamento *versus* o grupo placebo (57,7% *versus* 21,8% de redução no D28). Para todos os tempos experimentais, as VAS médias avaliadas pelos pesquisadores estavam significativamente reduzidas no grupo lokivetmab *versus* o grupo placebo (57,1% *versus* 20,5% de redução no D28). No geral, a ocorrência de eventos adversos durante o período de avaliação foi comparável entre os dois grupos.
Conclusões e importância clínica – Lokivetmab é um tratamento seguro e eficaz para cães com dermatite alérgica.

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Table S1. Prohibited and conditionally allowed medications and therapy

Drug class	Withdrawal time (before Day 0)	Minimal use	Frequency of use	Examples (not all inclusive)
PROHIBITED				
Monoclonal antibodies	Naïve animals	NA	NA	Lokivetmab *(Cytopoint®)*
Ciclosporin or Tacrolimus (injectable, oral, topical)	4 weeks	NA	NA	Ciclosporin (*Atopica®*) Tacrolimus (*Protopic Ointment®*)
Injectable corticosteroids	6 weeks	NA	NA	Triamcinolone (*Triamolone Forte*) Methylprednisolone (*Depo-Medrol®*) Dexamethasone (*Dexafort®*)
Oral corticosteroids	4 weeks	NA	NA	Prednisolone (*Macrolone*)
Janus kinase inhibitors	2 weeks	NA	NA	Oclacitinib (*Apoquel®*)
Topical steroids, topical NSAIDs, DMSO, (e.g. shampoos, creams, ointments, sprays, including aural and ophthalmic products)	3 weeks	NA	NA	DMSO (*Domoso Roll On®*) Phenylbutazone (*Phenylzone® Cream*) Prednisolone (*Amacin® Ointment, Neocort Cream*) Hydrocortisone (*Cortavance™ Cutaneous Spray*)
Antihistamines (injectable, oral, topical)	2 weeks	NA	NA	Chlorpheniramine (Histamil Injection, Niralone Capsules)
Long-acting injectable antibacterials	4 weeks	NA	NA	Cefovecin (*Convenia®*)
Systemic antibacterials	1 week	NA	NA	Numerous products
Systemic azole antifungals	2 weeks	NA	NA	Ketoconazole (*Nizoral®*)
Miscellaneous compounds with known antipruritic activity	4 weeks	NA	NA	Staphage Lysate Gabapentin (*Neurontin®*) Pregabalin (*Lyrica®*) Tramadol (*Tramal®*)
Amantadine	6 weeks	NA	NA	*Symmetrel®*
Topical anaesthetics	1 week	NA	NA	Lignocaine (*Cort-Hex Cream, Neotopic H Lotion*)
CONDITIONALLY ALLOWED				
Essential fatty acids (EFA), Probiotics, Ceramides	NA	Past 6 weeks	No change in dosing regimen	EFA *(DermaPet EicosaDerm™)* Probiotics *(Protexin®)*
Nutraceuticals/DMOADs	NA	Past 6 weeks	No change in dosing regimen	Glucosamine/chondroitin (*Cosequin®*) Pentosan polysulphate (*Cartrophen*)
Vitamins	NA	Past 6 weeks	No change in dosing regimen	*Nutrigel, Anaplex, Vitamax™*
Anticonvulsants	NA	Past 6 weeks	No change in dosing regimen	Potassium bromide (*Epibrom*) Phenobarbital (*Phenomav*)
Selegiline, Fluoxetine	NA	Past 6 weeks	No change in dosing	*Anipryl®* *Reconcile™*

Drug class	Withdrawal time (before Day 0)	Minimal use	Frequency of use	Examples (not all inclusive)
			regimen	
Systemic NSAIDS	NA	Past 6 weeks	No change in dosing regimen	Carprofen (*Rimadyl*®) Meloxicam (*Metacam*®) Firocoxib (*Previcox*™)
Thyroid supplementation	NA	Past 6 weeks	No change in dosing regimen	Thyroxine (*Thyroxine*)
Medicated shampoos such as antiseborrhoeic, antimycotic, antiseptic or moisturising (no steroids/antihistamines)	NA	Past 6 weeks	Maximum of twice weekly	*Malaseb Shampoo*™ *Pyohex Shampoo*™ *Selederm Shampoo* *Pyoderm® S Shampoo* Phytosphingosine (*Douxo*®)
Colloidal oatmeal soaks, bathes, shampoos	NA	Past 6 weeks	Maximum of twice weekly	*Aloveen Shampoo*™ *Epi-Soothe® S* *Epi-Soothe® Cream Rinse*
Benzoyl peroxide	NA	Past 6 weeks	Maximum of twice weekly	*Pyoben® Shampoo*
Topical antibacterials, antifungals, antiseptics including sprays, lotions, ointments, wipes, ear drops and ophthalmic products	NA	Past 6 weeks	Maximum of every other day	*Imaverol*™ *Fungicidal Wash* *Tricin® Eye and Ear Ointment* *Baytril® Otic Drops* Phytosphingosine (*Douxo*®)
Menthol, Zinc oxide	NA	Past 6 weeks	No change in regimen	Zinc oxide (*Filta-Bac Cream*®) Menthol (*Isogel, Rapigel*®)
Phenylpropanolamine	NA	Past 6 weeks	No change in dosing regimen	Phenylpropanolamine (*Propalin® Syrup*)
Sedatives/tranquilisers	NA	NA	Occasional for situational anxiety only	Acepromazine (*Promex*) Diazepam (*Valium*®) Alprazolam (*Xanax*®)
Analgesics/ Tricyclic antidepressants	NA	NA	Occasional use as required	Opioids Clomipramine (*Clomicalm*®)
Antidiarrhoeals	NA	NA	Occasional use as required	*Immodium®* *Diarrhoea Capsules*
Anti-emetics/Anti-nausea drugs*	NA	NA	Occasional use as required	Maropitant (*Cerenia*®) Metoclopramide (*Metomide*®)

* These products were <u>not to be used two days before assessments</u> of pruritus.

Table S2. Frequency distribution of the presumptive nature of allergic dermatitis.

Treatment	Food allergy		Flea allergy		Contact allergy		Atopic dermatitis (in combination)		Unspecified allergic dermatitis	
	n	%	n	%	n	%	n	%	n	%

Treatment	Food allergy		Flea allergy		Contact allergy		Atopic dermatitis (in combination)		Unspecified allergic dermatitis	
	n	%	n	%	n	%	n	%	n	%
placebo (n = 62)	23	*37.1*	7	*11.3*	23	*37.1*	21	*33.9*	16	*25.8*
lokivetmab (n = 61)	28	*45.9*	10	*16.4*	18	*29.5*	23	*37.7*	20	*32.8*
Total (n = 123)	51	*41.5*	17	*13.8*	41	*33.3*	44	*35.8*	36	*29.3*

Diagnosis could be a combination of different aetiologies.

Table S3. Adverse events: Summary table by frequency of occurrence of system organ class and then by clinical sign as from Day 0.

		Placebo (n = 62)	**Lokivetmab (n = 61)**
System organ class	**Clinical sign**	**n (%)**	**n (%)**
Ear and labyrinth disorders		2 (3.2)	5 (8.2)
	Otitis externa	1 (1.6)	5 (8.2)
	Otorrhoea	1 (1.6)	0 (0.0)
Investigations		0 (0.0)	2 (3.3)
	Elevated serum alkaline phosphatase (SAP)	0 (0.0)	1 (1.6)
	Increased blood urea nitrogen (BUN) or creatinine	0 (0.0)	1 (1.6)
	Leucopenia	0 (0.0)	1 (1.6)
Skin and appendages disorders		5 (8.1)	2 (3.3)
	Dermatitis	2 (3.2)	2 (3.3)
	Bacterial skin infection	1 (1.6)	0 (0.0)
	Erythema	1 (1.6)	0 (0.0)
	Papule	1 (1.6)	0 (0.0)
Digestive tract disorders		0 (0.0)	1 (1.6)
	Stomatitis	0 (0.0)	1 (1.6)
Blood and lymphatic system disorders		1 (1.6)	0 (0.0)
	Lymphadenitis	1 (1.6)	0 (0.0)
Eye disorders		3 (4.8)	0 (0.0)
	Conjunctivitis	1 (1.6)	0 (0.0)
	Corneal ulcer	1 (1.6)	0 (0.0)
	Epiphora	1 (1.6)	0 (0.0)
	Any adverse event	**9 (14.5)**	**7 (11.5)**

Occurrence was calculated on a per animal basis: the results are presented by the number of animals with a specific observation (n) and the percentage of animals impacted (%) per treatment group. For total

number of occurrences, take into account that a single adverse event report might have consisted of a combination of two or more individual clinical signs.