

A randomized, double-blind, placebo-controlled study to evaluate the effect of EFF1001, an *Actinidia arguta* (hardy kiwi) preparation, on CADESI score and pruritus in dogs with mild to moderate atopic dermatitis

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Conflict of Interest

None declared.

Abstract

Canine atopic dermatitis (AD) is common and new therapies are beneficial. This multicentric, randomized, double-blind, placebo-controlled study tested the efficacy of *Actinidia arguta* (hardy kiwi) (EFF1001) in dogs with mild/moderate AD. The study was divided into two stages. Stage 1 lasted 6 weeks. In the first 2 weeks prednisolone [days 1–3: 0.2 mg/kg twice daily (BID), days 4–14: 0.2 mg/kg every other day (EOD)] was administered. Responsive dogs were placed on prednisolone 0.2 mg/kg EOD + assigned test article [either placebo or EFF1001 (30 mg/kg)] once daily for 4 weeks. Stage 1 responders were advanced to stage 2, which involved 4 weeks of just EFF1001. Clinicians scored lesions using Canine Atopic Dermatitis Extent and Severity Index (CADESI) and owners scored pruritus using a Pruritus Visual Analogue Scale. Seventy-seven dogs were enrolled, 76 were randomized on day 14, and 57 (57/76 = 75%) completed stage 1 (27 in EFF1001 and 30 in placebo). At the end of stage 1, 35 of 57 dogs (35/57 = 61%) responded (18 in EFF1001 and 17 in placebo) and advanced to stage 2. At completion of stage 1, CADESI scores did not significantly differ between groups while pruritus decreased in EFF1001 group and approached significance. At completion of stage 2, 19 dogs (19/35 = 54%) responded (15/19 = 79% had received EFF1001 and 4/19 = 21% placebo in stage 1).

After completing stage 2, dogs placed on EFF1001 throughout the study were 3.5 times more likely to either maintain or improve scores than those that started it in stage 2. It is concluded that EFF1001 is beneficial adjunctive therapy after prolonged use.

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Introduction

Atopic dermatitis (AD) is the second most common allergy in dogs occurring in 10% of the canine population.¹ AD presents with pruritus and skin lesions that typically affect face, feet and ears, and can easily become generalized. Affected animals suffer from recurrent skin and ear infections that greatly decrease their quality of life. Despite the fact that AD is a very common disease, the understanding of the pathogenesis of this disease and the therapeutic options available for affected animals are limited. Systemic treatments like glucocorticoids and ciclosporin may be effective² but have the potential for adverse effects.³ Glucocorticoids are reasonably priced but tend to be less effective with chronic use¹ and oral ciclosporin may be cost-prohibitive in large breed dogs. Antihistamines and essential fatty acids may be tried in mild to moderate cases but the success rate is often unsatisfactory.¹ Allergen-specific immunotherapy is used in dogs with a long allergic season; however, clinical improvement is usually not seen for the first 6–9 months of therapy. Immunotherapy is delivered in the form of subcutaneous injections, which makes compliance challenging for some owners. Currently available topical agents, such as oatmeal and pramoxine, typically provide only partial and temporary relief. Thus, the identification of a safe and effective oral treatment for canine AD would be of tremendous benefit.

Preparations of *Actinidia arguta* have been used for years in East Asian traditional medicine to improve general health, and were recently demonstrated to decrease clinical signs of AD in a mouse model.⁴ The mechanism of action is unknown and further work is necessary to identify the active components present in the mixture. *Actinidia arguta* fruit is green, oblong in shape (2–2.5 cm), and grows in clusters. The fruit has edible smooth skin and the inside of the fruit looks much like the common fuzzy kiwi (*A. deliciosa*) but is usually sweeter. No adverse effects have been documented in people with

the consumption of this fruit, even when ingested in large quantities.

EFF1001 is a powdered formulation of dried *A. arguta* fruit that is boiled in water, filtered and concentrated. EFF1001 has been reported to decrease scratching and dermatitis index in mouse models of AD (NC/Nga mice). It also decreased IgE and IL-4, and increased IgG2a and IL-12 in these mice⁴. A hardy kiwi concentrate was also demonstrated to be promising as adjunctive treatment in human patients with AD⁵. Thus, EFF1001 has the potential of being an effective treatment for the management of canine AD. Therefore, the purposes of the present study were to investigate:

- 1 the efficacy of daily EFF1001 at 30 mg/kg to decrease clinical signs of mild/moderate AD over a 4-week period when used as an adjunct therapy in combination with prednisolone (0.2 mg/kg) every other day;
- 2 the efficacy of EFF1001 as monotherapy to decrease the need for steroid use in 'stabilized' AD dogs; and
- 3 possible adverse effects of this kiwiberry fruit concentrate in dogs.

Materials and methods

Experimental design

This study was prospective and multicentric designed in two phases with the first one being a randomized, double-blind, placebo-controlled study and the second phase being open (not placebo controlled). Privately owned dogs with nonseasonal AD were enrolled at four sites between December 2005 and September 2006. The study sites were selected to cover different geographical areas within the United States. These centres included the University of Florida, the Animal Dermatology Clinic (San Diego, CA), Colorado State University (Fort Collins, CO) and the Veterinary Referral Center of Colorado (VRCC, Englewood, CO). All procedures used in this study were approved by the respective Institutional Animal Care and Use Committees.

Inclusion criteria

The following inclusion criteria were used when selecting clinical cases for this study:

- 1 All dogs were judged healthy on physical examination aside from skin disease.
- 2 Diagnosis of nonseasonal AD (history for 1–6 years) of mild to moderate severity. Mild to moderate AD was defined as disease with no signs of microbial infection and CADESI score greater than 25. Diagnosis of AD was based on suggestive history, compatible clinical signs, exclusion of other pruritic skin diseases, and at least three positive reactions in an intradermal skin test using the 57–70 most common allergens of the region. Reactions scored as 2 or higher on subjective evaluation 15–20 min after injection were considered positive. Saline as negative control scored as 0 and histamine as positive control scored as 4.
- 3 To minimize the potential for clinical improvements due to flea control, all dogs were on a flea control programme. This programme included an adulticide (Advantage®) at least monthly, starting at least 1 month prior to inclusion in the study.
- 4 All dogs had undergone a food trial to rule out the possibility of food involvement in the pruritic dermatitis, or had a history of adequate food trial in their clinical work-up. Adequate food trial included a minimum of 2 months on a novel protein and carbohydrate diet to be selected based on the dietary history of the

individual patient. This diet was home cooked or commercially prepared, and prescribed and monitored. All flavoured medications and supplements were discontinued during the trial.

- 5 Dogs were on the same diet for the entire study and no dietary changes were allowed once dogs were included in the study.
- 6 Dogs with clinical signs or history potentially suggestive of scabies underwent treatment to rule out the possibility of sarcoptic mange. This treatment varied depending on the preference of the clinician and the specific needs of the dog. Acceptable scabies treatments used in the study included: weekly lime sulphur dips (5–7 times); oral or injectable ivermectin at doses ranging from 0.25 to 0.3 mg/kg, weekly or every 2 weeks for a minimum of three doses; or selamectin application once every 2 weeks (three times).
- 7 In order to be considered for the study all eligible dogs also needed to be off the following medications:
 - (i) All antihistamines for a minimum of 2 weeks
 - (ii) Long-acting injectable steroids (e.g. Depomedrol®) for a minimum of 2 months
 - (iii) Topical steroids for a minimum of 2 weeks
 - (iv) Oral steroids for a variable time (5–14 days) depending on the type and duration of treatment. Withdrawal time was left up to the discretion of the clinician enrolling the case.
 - (v) Essential fatty acid supplements for a minimum of 2 months
 - (vi) Oral ciclosporin for a minimum of 1 month

- 8 Animals were clear of any secondary infections prior to enrolment. This decision was based on physical examination and cytology.

A client consent form was signed prior to inclusion in the study in all cases. No medicated shampoos were allowed during the trial.

Exclusion criteria

The following criteria were followed for exclusion of dogs:

- 1 History of methicillin-resistant *Staphylococcus aureus* or *S. intermedius* (MRSA/MRSI), or of *S. schleiferi*.
- 2 Pre-existing systemic disease, with the exception of controlled hypothyroidism.
- 3 Exceptional severity of AD that did not allow discontinuation of previous treatments. Exceptional severity was not measured by a maximum CADESI and was left up to the determination of the clinician and the owner. If the owner determined that the dog was not doing well enough to be off other medications, that was considered severe AD regardless of the CADESI scores.
- 4 Nonresponsiveness to steroid therapy during the 2-week lead-in period.
- 5 Inability to discontinue medicated shampoos.
- 6 The need for concurrent allergen-specific immunotherapy, unless treatment had stabilized the disease and had been continued for at least a year.

Stage 1 of the study (days 0–42)

Stage 1 included 2 weeks of glucocorticoid therapy to initially stabilize the disease and four additional weeks of combination therapy.

Stabilization of disease (days 0–14): All dogs were stabilized solely with a standard, low-dose, oral steroid (prednisolone) from study day 1 to study day 14. Steroid dosing was done as follows: from days 1 to 3 prednisolone was administered at 0.2 mg/kg twice daily (BID); from days 4 to 14 prednisolone was administered at 0.2 mg/kg every other day. On day 14 a reassessment was scheduled to determine whether the dog was eligible to continue. At study day 14 the investigator assessed the clinical signs of AD through the CADESI score and the owner's pruritus diary. In order for the dog to continue to the 4-week adjunctive therapy period, the investigator's evaluation had to reflect the dog's improvement, but not the complete resolution of AD clinical signs.

Stage 1, combination therapy (days 15–42): If criteria were met, the dog continued stage 1 of the study and was placed on combina-

tion therapy, and continued to receive prednisolone (0.2 mg/kg) on alternate days with the assigned test article, either EFF1001 or placebo.

Selected dogs were randomized into two equal-sized groups of test article or placebo. Randomization in stage 1 was 1 : 1 in blocks of four and stratified by site. The grouping was randomized and blinded to owners and investigators. One treatment arm received EFF1001 at a dose of 30 mg/kg daily, while the placebo arm received the same dosing frequency and number of capsules. EFF1001 capsules contained the active *A. arguta* preparation, mixed in a 1 : 1 ratio with microcrystalline cellulose (MCC). The placebo capsules contained pure MCC.

Clinical assessment: Investigator's assessment was based on CADESI evaluation in conjunction with the assessment of cytology. The new and validated version of the CADESI was used for this study⁶. In this scoring system, the body was divided into regions, and each region evaluated and scored for a variety of clinical signs. The severity of each sign was evaluated on a scale from 0 to 3 corresponding to absent (0), mild (1), moderate (2) and severe (3). The total CADESI was then calculated by adding the scores for each clinical sign and each body region. In stage 1, CADESI scores were collected on days 1, 14, 28, 35 and 42. Photographs were taken of representative body areas at each study time point as deemed appropriate by the clinician.

Cytological assessment: Cytology from lesional areas was undertaken at recheck evaluations based on clinician's discretion. Scotch tape (3M, St Paul, MN, USA) was applied on the lesional skin and stained with Diff Quik (Baxter Diagnostics, McGraw Park, IL, USA). The tape was placed on a glass slide with the sticky side down and evaluated for the presence of bacteria and yeasts. As the number of bacteria or yeasts required for infection is variable and highly debated among clinicians, the diagnosis of relapsing secondary infection was based on cytology and clinical assessment by each clinician. This ensured that the cytology results were always correlated with the clinical presentation. Dogs relapsing with infections were eliminated from the trial and treated appropriately.

Owner's assessment: Owners were asked to fill in a weekly pruritus diary and provide this diary at each appointment. Pruritus was scored with a visual analogue scale (PVAS) from 0 to 5, with 5 as the most severe pruritus. Figure 1 provides details of the scoring system.

Laboratory evaluations: Laboratory evaluations, including complete blood count (CBC) and chemistry panels, were collected on days 1, 14 and 42. Figure 2 provides details of these procedures used in stage 1.

Stage 2 of the study

Dogs that demonstrated improved CADESI scores, and were deemed by the investigator as responders, were eligible for stage 2 (EFF1001 monotherapy). Blinding as to the stage 1 treatment assignment was retained during the open-label portion of the study (stage 2), thus investigators were still blinded to the treatment assignments of stage 1. Dogs that advanced to stage 2 began a 4-week, open-label, monotherapy period with test article only. As all dogs received EFF1001 during stage 2, there was no re-randomization required, and no attempt was made to achieve equal representation from the dif-

Score	Definition
0	No pruritus
1	Mild (<10% of the time)
2	Mild to moderate (10–30% of the time)
3	Moderate (30–50% of the time)
4	Moderate to severe (50–75% of the time, still able to sleep at night)
5	Severe (75–100% of the time, even at night and during a meal)

Figure 1. Definitions used in the pruritus scoring system: 0–5 with higher scores indicating more severe pruritus.

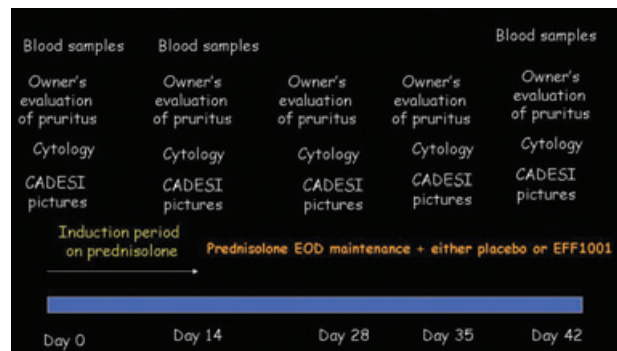


Figure 2. Study outline for stage 1. At each time point the CADESI, cytology and clinical photographs were taken and owners completed a weekly pruritus diary.

ferent stage 1 treatment arms into stage 2. In stage 2, CADESI and pruritus scores were recorded weekly on days 1, 7, 14, 21 and 28. Cytology and photographs were taken at each study time point. Laboratory evaluations, including CBC and chemistry panels, were collected on days 1, 14 and 28.

End points of stage 1

The primary end points of stage 1 were the overall investigator's evaluation of treatment response. Treatment responders were defined as 'dogs with a clinically appreciable CADESI score decrease from day 14 to 42 and the lack of need for rescue medications'. Treatment failures were defined as 'dogs with either no change in CADESI score, a return to baseline (day 14) CADESI score, or a worsening score due to a flare-up of AD, or to the development of a secondary infection'. Secondary efficacy end points included the pruritus ratings and evaluation of laboratory results for safety.

End points of stage 2

The main end points of stage 2 were the investigator's evaluation of treatment response. Treatment responders were dogs that had maintained improvement in stage 2 (e.g. no increase in CADESI and PVAS scores and no need for rescue medications), while treatment failures were dogs that had relapsed or needed rescue medications prior to the end of the 4-week period.

Data analysis

Primary efficacy analysis

The primary efficacy analysis was the proportion of subjects with positive response to the treatment based on investigator's evaluation (e.g. number of responders). For analysis of stage 1 results the chi-squared test was used. For analysis of stage 2 results, given the reduced sample size, Fisher's exact test was used. Relative risk was calculated as a measure of strength of association between treatment and the sustained benefits of the treatment.

Secondary efficacy analyses

The change from baseline on CADESI total score was analysed with an ANOVA for the intent-to-treat and per-protocol populations. Changes in pruritus rating and laboratory evaluations between days 1 and 14 were analysed by ANOVA for the intent-to-treat and per-protocol populations. $P < 0.05$ was considered significant.

Results

Stage 1 ($n = 77$)

Seventy-seven dogs were enrolled in stage 1 (see flow chart in Figure 3). Of the 77 dogs enrolled, 76 were randomized after the 2 weeks of prednisolone and 57 (57/76 = 75%) completed stage 1.

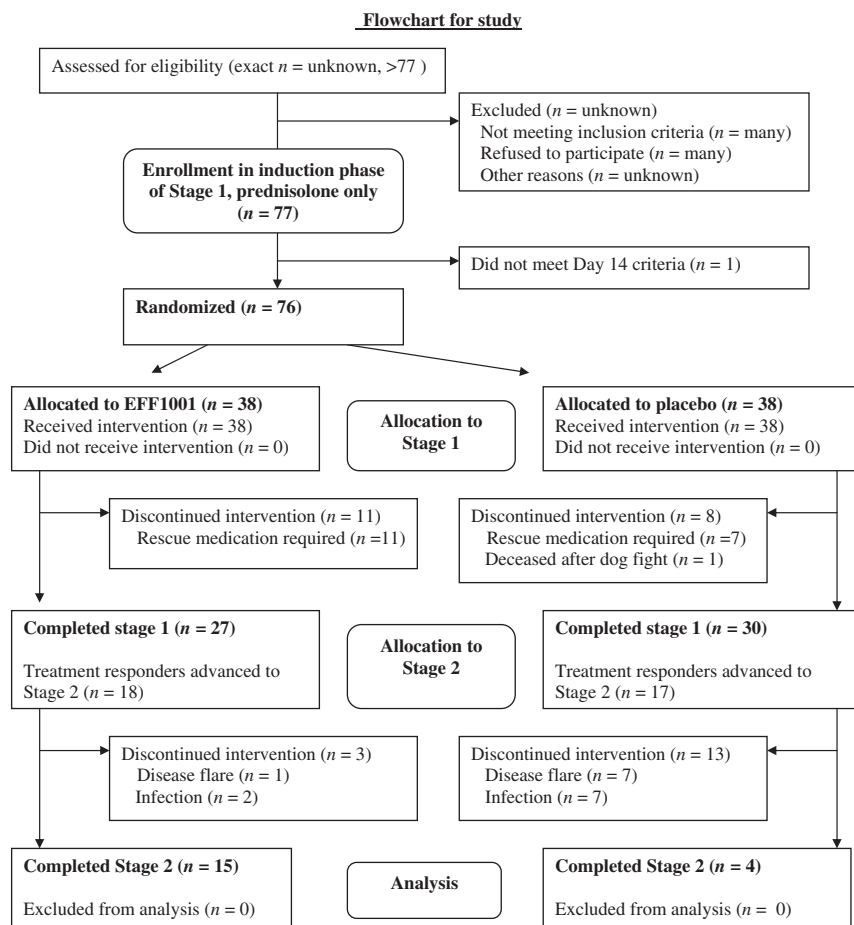


Figure 3. Flow chart outlining the outcome, number and details of dogs at various steps of the study.

At the end of stage 1 (day 42), the CADESI scores in the EFF1001 group improved more than the placebo group (−6.9 versus −4.0), although the between-group difference was not statistically significant ($P = 0.4371$; ANOVA). At the end of stage 1, an improvement in pruritus (a partial primary end point) was observed in those who

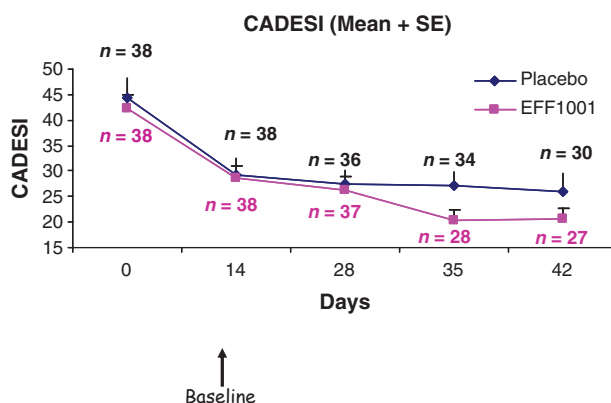


Figure 4. Mean Canine Atopic Dermatitis Extent and Severity Index (CADESI) scores and standard errors (SE) during stage 1. The arrow indicates the beginning of the clinical trial (baseline point) after the first 2 weeks of glucocorticoid therapy. No statistically significant differences between groups were detected at each time point. The number of dogs enrolled at each recheck visit is indicated.

received EFF1001, with this difference approaching significance on day 42 ($P = 0.0594$; ANOVA). The CADESI and pruritus scores from stage 1 are shown in Figures 4 and 5 respectively.

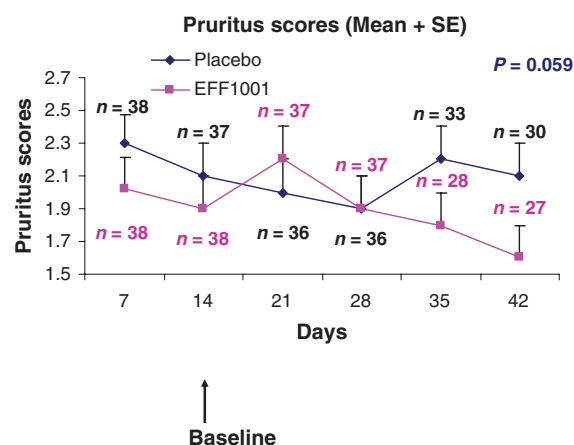


Figure 5. Mean pruritus scores and standard errors (SE) during stage 1. The arrow indicates the beginning of the clinical trial (baseline point) after the first 2 weeks of glucocorticoid therapy. No statistically significant differences between groups were detected at each time point. The largest difference in scores was detected at the end of stage 1 (day 42) when the scores almost reached significance ($P = 0.059$). The number of dogs enrolled at each recheck visit is indicated.

Thirty-five (35/57 = 61%) of the dogs completed stage 1 and advanced to stage 2. Of these, 18 had received EFF1001 in stage 1 and 17 had received placebo. Thus, there were nearly equal numbers of responders in both groups at the end of stage 1. The suitability to advance to stage 2 was determined by the investigators who remained blinded to their treatment assignment. The investigators relied on the dog's CADESI scores, pruritus scores, overall condition and the owners' assessments.

Stage 2 (n = 35)

Of the 18 dogs that received EFF1001 for a total of 8 weeks, 12 (~67%) were considered responders and six (~33%) were considered nonresponders (see Figure 3). Of the 17 dogs that received placebo for 4 weeks followed by EFF1001 for 4 weeks, only one (~6%) was considered a responder, while 16 (~94%) were nonresponders. The difference in response between the two groups was significant ($P = 0.0002$, Fisher's exact test). The CADESI and pruritus scores from stage 2 are shown in Figures 6 and 7 respectively.

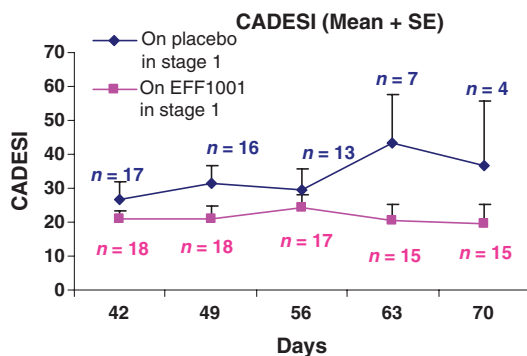


Figure 6. Mean Canine Atopic Dermatitis Extent and Severity Index (CADESI) scores and standard errors (SE) for stage 2. Stage 2 was a 4-week monotherapy trial for dogs that were considered responders in stage 1. Investigators were still blinded as far as the content of the capsules dispensed to the dogs and as far as previous allocation to groups. EFF1001 indicates the dogs that had been allocated to the EFF1001 group in stage 1. No statistically significant differences were detected between groups at each time point.

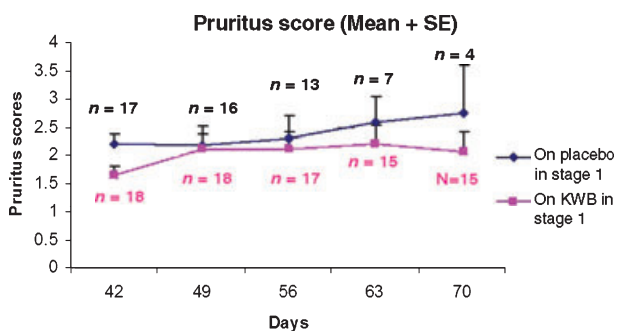


Figure 7. Mean pruritus scores and standard errors (SE) in stage 2. Stage 2 was a 4-week monotherapy trial for dogs that were considered responders in stage 1. Investigators were still blinded as far as the content of the capsules dispensed to the dogs and as far as previous allocation to groups. EFF1001 indicates the dogs that had been allocated to the EFF1001 group in stage 1. No significant differences between groups at each time point. There were 17 dogs that came from the placebo group, but only data from 16 dogs were available because one owner did not fill in the evaluations.

Of the 19 dogs that completed stage 2 and maintained or improved CADESI and pruritus scores, 15 (79%) were EFF1001 recipients in stage 1. Thus, dogs that had received EFF1001 in stage 1 were three and a half times more likely to sustain the benefit compared with placebo recipients ($RR = 3.54$, $P = 0.0006$, Fisher's exact test). In terms of relapses (either AD flare-up or the development of secondary infections), 81% occurred for dogs that had been placebo recipients in stage 1 and 19% occurred for dogs that were EFF1001 recipients in stage 1.

Adverse effects and laboratory results

No adverse events were considered related to test article administration. One dog assigned to placebo was hospitalized and died subsequent to injuries sustained in a dogfight. Nonserious adverse events were reported for one dog assigned to placebo (vaginitis) and two dogs assigned to EFF1001 (dew claw infection, lumbar muscle spasm). There were no clinical laboratory abnormalities reported (Appendix S1, Table S1, Table S2, Table S3).

Discussion

In this randomized, placebo-controlled study, the *A. arguta* preparation, EFF1001 was found to be a promising adjunctive treatment for canine AD when used for 8 weeks. Clinical improvement was most noticeable after prolonged use of EFF1001, suggesting that this treatment modality should be tried for two or more months before completely assessing the clinical benefit in the individual case. The reason for the need for longer use may be due to the fact that this natural concentrate is less potent than conventional therapies like steroids or ciclosporin. There may be an aspect of the mechanism of action that requires a slow accumulation or incorporation of one or more of the components of the mixture into key biological structures or pathways. A direct comparison between the efficacies of this extract with the efficacy of essential fatty acids is unfortunately not possible due to the different experimental designs and the different scoring system used.

The need for a prolonged administration was also observed in a previous study of *A. arguta* preparations that were administered to NC/Nga mice, an experimental model for AD⁴. In that study, the preparations were administered for and beneficial effects were most noticeable after several weeks of therapy. The dose in that study was in the $\mu\text{g/mL}$ range and, unfortunately, this is not directly relatable to the test dose in this study. It is interesting to note that the suppression of dermatitis appeared to be accompanied by decreases in plasma levels of IgE, IgG1 and IL-4, and increases in levels of IgG2a and IL-12. The number of eosinophils and the expression of eotaxin and thymus and activation-regulated chemokine were also decreased. Histological findings indicated that the thickening of the epidermis/dermis and the dermal infiltration of inflammatory cells, including mast cells, were greatly inhibited. In our clinical trial, blood samples were taken with the intent of measuring various immunological parameters but, unfortunately, resources were unavailable for the analysis of these samples. As a result, it is unknown whether dogs undergo biological effects

similar to those described above. The use of privately owned dogs also precluded the taking of sequential biopsies in some of the centres involved in this trial.

The molecular mechanism underlying the therapeutic effects of EFF1001 remains to be elucidated. *Actinidia arguta* preparations similar to EFF1001 suppress GATA-binding protein 3, and phosphorylated signal transducer and activator of transcription-6 in the skin lesions of NC mice⁷. Thus, it is possible that EFF1001 may act in a similar manner on these cellular transcription factors, and subsequently on the expression of Th2 cytokines such as IL-4 and IL-5, initiating the cascade reaction that may lead to a decrease in the level of IgE and respective chemokines.^{4,7}

The active components of EFF1001 are unknown. Analysis of the volatile constituents of fresh *A. arguta* fruit revealed more than 240 simple organic compounds, including a variety of monoterpenes; however, it is likely that many of these components are lost in the processing steps that lead to EFF1001.⁸ An unpublished analysis by Efficas, Inc. of the phenolic constituents of EFF1001 by HPLC-UV revealed the presence of the ubiquitous flavonoids quercetin, isorhamnetin, kaempferol, in approximate amounts ranging from 20 to 80 µg/mL. There are numerous reports in the literature on the anti-inflammatory effects of these and similar flavonoids;^{9–12} however, the degree to which these compounds contribute to the overall activity of EFF1001 is unknown.

This clinical trial was limited to the use of privately owned patients. Although the inclusion and exclusion criteria aimed to control as much as possible for confounding factors, dogs with variable intensity, clinical duration and number of allergens were included in the trial. The number of dogs was rather large for a veterinary clinical trial, and it is conceivable that this was necessary to fully observe the benefits of this type of therapy. It is interesting to note that the more challenging climates used in this study (Florida and California) reported higher dropout rates, and the drier cooler climate of Colorado reported better retention and treatment responses. In the humid climates, the main reason for dropping out of the study was the development of secondary infections. This suggests that the best clinical response would be observed in areas of the country where AD is less severe and less complicated by secondary infections. Future studies should be considered to investigate the immunological mechanisms of EFF1001 both by examination of skin biopsies and investigation of cytokine profiles. For this purpose, experimental models of canine AD would be more suitable than the use of privately owned patients with naturally occurring disease so that neither seasonal variations nor variability in the number of relevant allergens would make interpretation of the results challenging.

Future studies should also be carried out to evaluate the long-term benefits of this treatment modality. Variation in allergen exposure could have been responsible for improvement in some of the dogs in this trial but this is considered an unlikely explanation as nonseasonal disease was an inclusion criteria for this study. Additionally, many dogs were enrolled during summer months,

therefore improvement due to decreased allergen load is considered improbable.

In this clinical trial, the test article, EFF1001, was well tolerated and no clinical adverse effects were reported. The only complaint from owners was related to the size of the capsules, which was reported to be inconvenient for smaller dogs, or for dogs that required multiple capsules. It is important to note that this fruit product is considered safe even in human patients that may have food allergy to the fuzzy kiwi, an allergy that is common in people but quite rare in dogs. In a human study, it was found that the heat processing of the hardy kiwi fruit alters allergenic protein structure, dramatically reducing *in vitro* IgE binding.¹³ Processing likely reduces the risk of eliciting an allergic response in those with allergies to raw kiwi fruit.

In summary, in this double-blind clinical trial, EFF1001, an *A. arguta* preparation, was found to be a promising treatment for mild to moderate cases of AD. The efficacy was most noticeable after 2 months of continued use, and prolonged therapy is recommended when evaluating the clinical benefit of this type of treatment in individual clinical cases.

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Supporting Information

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

Appendix S1. Supporting information Regarding Safety of Eff1001, an *Actinidia arguta* (hardy kiwi) preparation.

Table S1. Hematology parameters.

Table S2. Clinical chemistry parameters.

Table S3. Shifts in parameters.

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Résumé La dermatite atopique canine (DAC) est fréquente et la découverte de nouvelles thérapeutiques est utile. Cette étude multicentrique, randomisée, en double aveugle, contre placebo a testé l'efficacité d'*Actinidia arguta* (kiwi d'été) (EFF1001) chez des chiens atteints de formes faibles à modérées de DAC. L'étude était divisée en deux parties. La première étape a duré 6 semaines. Les deux premières semaines, de la prednisolone [Jours 1–3: 0.2 mg/kg deux fois par jour, jours 4–14: 0.2 mg/kg un jour sur deux] a été administrée. Les chiens répondant à la corticothérapie ont été mis sous 0.2 mg/kg de prednisolone tous les deux jours et inclus dans l'étude (soit le placebo, soit EFF1001 (30 mg/kg) une fois par jour pendant 4 semaines). Les chiens répondant à l'étape 1 ont été inclus dans l'étape 2 qui consistait en l'administration d'EFFA001 seul pendant 4 semaines. Les investigateurs ont évalué les lésions cliniques à l'aide du CADESI et les propriétaires ont scoré le prurit à l'aide d'une échelle de prurit visuelle. Soixante dix sept chiens ont été inclus, soixante seize ont été randomisés à J14 et cinquante sept (57/76 = 75%) ont finalisé l'étape 1 (27 avec EFF1001 et 30 avec le placebo). A la fin de la première étape, les scores CADESI n'ont pas révélé de différences significatives entre les deux groupes alors que le prurit diminuait dans le groupe recevant EFF1001. A la fin de la deuxième étape, 19 chiens (19/35 = 54%) ont répondu (15/19 = 79% recevaient EFF1001 et 4/19 = 21% le placebo à l'étape 1). A la fin de la deuxième étape, les chiens recevant EFF1001 tout au long de l'étude étaient 3.5 fois plus enclins à maintenir ou à améliorer leurs scores que ceux ayant reçu EFF1001 à partir de la deuxième étape. EFF1001 apparaît donc comme une thérapeutique additionnelle bénéfique après utilisation prolongée.

Resumen La dermatitis atópica canina (AD) es una enfermedad común y nuevos tratamientos son beneficiosos. Este estudio multicéntrico, al azar, doble ciego, y controlado por placebo analizó la eficacia de *Actinidia arguta* (kiwiño) (EFF1001) en perro con leve a moderada AD. El estudio se dividió en dos fases. La primera fase duró 6 semanas. En las primeras dos semanas se administró prednisolona [días 1–3: 0.2 mg/kg dos veces al día (BID), días 4–14: 0.2 mg/kg en días alternos (EOD)]. Los perros que respondieron se trataron con prednisolona 0.2 mg/kg EOD + el artículo en estudio (placebo o EFF1001 (30 mg/kg) una vez al día durante 4 semanas. Los animales que respondieron en la fase uno pasaron a la fase 2, que abarcó cuatro semanas sólo con EFF1001. Los clínicos evaluaron las lesiones usando el índice de extensión y severidad de la dermatitis atópica canina (CADESI) y los dueños valoraron el prurito utilizando una escala análoga visual. Se utilizaron 77 perros, de los cuales 76 fueron distribuidos al azar en el día 14 y 57(57/76 = 75%) completaron la fase inicial (27 en EFF1001 y 30 en placebo). Al final de la fase 1, 35/57 perros (35/57 = 61%) respondieron (18 en EFF1001 y 17 en placebo) y avanzaron a la fase 2. Al final de la fase 1, los valores CADESI no variaron significativamente entre los grupos, mientras que el prurito disminuyó en el grupo tratado con EFF1001 casi con valor significativo. Al final de la fase 2, 19 perros (19/35 = 54%) respondieron (15/19 = 79% habían recibido EFF1001 y 4/19 = 21% placebo en la fase 1). Tras completar la fase 2, los perros tratados con EFF1001 a lo largo del estudio tuvieron 3,5 veces mayor probabilidad de mantener o mejorar los valores comparados con perros que iniciaron la fase 2. Se concluye que EFF1001 es beneficioso como terapia adjunta en uso prolongado.

Zusammenfassung Die canine atopische Dermatitis (AD) ist eine häufige Erkrankung und neue Therapien sind nützlich. Diese randomisierte, doppelblinde, Placebo-kontrollierte Multicenterstudie prüfte die Wirksamkeit von *Actinidia arguta* (Minikiwi) (EFF1001) bei Hunden mit milder/moderater AD. Die Studie war in zwei Phasen eingeteilt. Phase 1 dauerte 6 Wochen. In den ersten 2 Wochen wurde Prednisolon verabreicht [Tag 1–3: 0,2 mg/kg zweimal täglich (BID), Tag 4–14: 0,2 mg/kg jeden zweiten Tag (EOD)]. Hunde, die darauf ansprachen, bekamen 0,2 mg/kg Prednisolon EOD + den zugewiesenen Testartikel (entweder Placebo oder EFF1001 (30 mg/kg) einmal täglich vier Wochen lang). Jene, die in Phase 1 angesprochen hatten, wurde in Phase 2 vorgerückt, was die Verabreichung von EFF1001 alleine für vier Wochen bedeutete. Die Kliniker beurteilten die Hautveränderungen mittels „Canine Atopic Dermatitis Extent und Severity Index“ (CADESI) und die Besitzer bewerteten den Juckreiz mit einer visuellen Pruritus Analogskala. Siebenundsiebzig Hunde wurden in die Studie aufgenommen, sechsundsiebzig wurden am Tag 14 zufällig verteilt, und siebenundfünfzig (57/76 = 75%) beendeten Phase 1 (27 in der EFF1001 und 30 in der Placebo Gruppe). Am Ende von Phase 1 hatten 35/57 (35/57 = 61%) Hunden auf die Behandlung angesprochen (18 in der EFF1001 und 17 in der Placebo Gruppe) und wurden in die 2. Phase vorgerückt. Am Ende von Phase 1 unterschieden sich die CADESI Werte zwischen den einzelnen Gruppen nicht

signifikant, während der Juckreiz in der EFF1001 Gruppe weniger wurde und Signifikanz erreichte. Am Ende von Phase 2 hatten 19 Hunde ($19/35 = 54\%$) auf die Behandlung angesprochen ($15/19 = 79\%$ hatten EFF1001 und $4/19 = 21\%$ hatten Plazebo in der ersten Phase erhalten). Nach Beendigung von Phase 2 war die Wahrscheinlichkeit die Werte entweder zu erhalten oder zu verbessern bei Hunden, die EFF1001 während der ganzen Studie erhalten hatten, 3,5 Mal höher als bei den jenen, die es erst in Phase 2 begonnen hatten. Es wird daraus geschlossen, dass EFF1001 nach längerem Einsatz eine heilsame zusätzliche Therapie darstellt.

Supporting Information Regarding Safety of EFF1001, an *Actinidia arguta* (hardy kiwi) preparation

Each investigator used his or her judgment as to the appropriate haematology and clinical chemistry tests to conduct, so there are differences between sites. Also, clinical chemistry methodologies were not identical for all of the tests at all sites, as evidenced from the somewhat different reference ranges reported. In order to discover potential impacts of the test articles on haematology and clinical chemistry values, mean and standard errors were calculated only for those study participants with data for both baselines (Day 1 and Day 14) and the first post-baseline sample (Day 42). Day 1 represents a Baseline value for all study interventions. Day 14 data represent the impact of 14 days of prednisolone in both treatment arms, and also represent a Baseline for test articles. Day 42 shows the impact of 42 days of prednisolone plus 28 days of administration of test article EFF1001 or Placebo. Once prednisolone was stopped at Day 42, many of the dogs experienced a flare of their dermatitis symptoms and were withdrawn from the study, leading to a significantly reduced N. Day 56 represents a 14-day washout of prednisolone, 42 days of EFF1001 in the Active arm and 14 days of EFF1001 in the Placebo arm. Day 70 represents a 28-day washout of prednisolone, 56 days of EFF1001 in the Active arm, and 28 days of EFF1001 in the placebo arm. When a group size was ≤ 4 a mean was not calculated (indicated in the tables as "ND").

Haematology results are presented in Table S1. No effects were evident.

Clinical chemistry results are presented in Table S2. Albumin, alkaline phosphatase (ALP), lipase, and triglycerides increased transiently during prednisolone administration, but decreased again after prednisolone administration stopped. Mean triglycerides exceeded reference ranges on Days 14 and 42 in the placebo group but the other parameters remained within normal ranges in both treatment groups. No effects on clinical chemistry parameters due to EFF1001 administration were evident in any of the dogs tested.

Table S1. Haematology parameters. Mean (SE), N

		Day				
		1	14	42	56	70
WBC 4.0-15.5 10 ³ /mL	EFF1001	9.3 (0.9), 12	8.4 (0.7), 12	10.3 (1.5), 12	ND	6.6 (0.7), 5
	Placebo	8.3 (0.4), 19	8.7 (0.6), 19	9.3 (0.8), 19	8.5 (0.9), 6	ND
RBC 4.8-9.3 10 ⁶ /mL	EFF1001	6.8 (0.1), 19	6.8 (0.1), 19	6.8 (0.1), 19	6.9 (0.2), 6	6.9 (0.2), 6
	Placebo	6.9 (0.1), 27	6.8 (0.1), 27	6.7 (0.1), 27	6.8 (0.2), 8	ND
Haemoglobin 12.1-20.3 g/dL	EFF1001	16.3 (0.3), 19	16.6 (0.3), 19	16.5 (0.3), 19	16.7 (0.5), 6	16.4 (0.4), 6
	Placebo	16.6 (0.3), 27	16.6 (0.3), 27	16.3 (0.3), 26	16.3 (0.3), 8	ND
Haematocrit 36-60	EFF1001	49 (0.8), 12	50 (1.2), 12	49 (1.2), 12	ND	50 (1.2), 5
	Placebo	49 (1.0), 19	49 (0.9), 19	48 (1.1), 19	48 (1.2), 6	ND
MCV 58-79 fL	EFF1001	70 (0.7), 19	71 (0.7), 19	70 (0.7), 19	72 (1.1), 6	71 (1.1), 6
	Placebo	71 (0.6), 27	71 (0.7), 27	71 (0.6), 27	70 (1.3), 8	ND
MCH 19-28 pg	EFF1001	24 (0.3), 12	24 (0.3), 12	24 (0.3), 12	ND	24 (0.6), 5
	Placebo	24 (0.2), 19	24 (0.3), 19	25 (0.3), 18	24 (0.3), 6	ND
MCHC 30-38 %	EFF1001	34 (0.3), 19	34 (0.3), 19	35 (0.2), 19	34 (0.7), 6	34 (0.4), 6
	Placebo	34 (0.2), 27	35 (0.3), 27	34 (0.4), 26	34 (0.2), 8	ND
RDW 12-15%	EFF1001	13 (0.2), 10	13 (0.2), 10	13 (0.2), 10	ND	ND
	Placebo	13.5 (0.3), 11	13.3 (0.3), 11	13.4 (0.2), 11	ND	ND
Platelets 170-400 10 ³ /mL	EFF1001	277 (27), 18	287 (24), 17	257 (17), 19	313 (19), 5	331 (38), 5
	Placebo	258 (20), 24	269 (18), 26	266 (18), 25	316 (31), 8	ND
MPV 7.7-14.6 fL	EFF1001	10.7 (0.6), 9	10.2 (0.3), 9	10.8 (0.4), 9	ND	ND
	Placebo	11.0 (0.4), 10	11.6 (0.6), 9	10.8 (0.3), 9	ND	ND
Segmented Neutrophils %	EFF1001	68 (2), 19	68 (2), 19	77 (3), 19	66 (4), 6	73 (4), 6
	Placebo	71 (1.8), 27	69 (1.8), 27	71 (1.9), 27	72 (3.3), 8	ND
Segmented Neutrophils	EFF1001	5877 (528), 19	5349 (438), 19	8068 (996), 19	4268 (624), 6	4907 (529), 6

Table S1. Haematology parameters. Mean (SE), N						
		Day				
		1	14	42	56	70
2060-10600/mL	Placebo	5659 (304), 27	6045 (432), 27	6420 (582), 27	5923 (671), 8	ND
Lymphocytes %	EFF1001	21 (2), 19	29 (7), 19	15 (2), 19	25 (3), 6	20 (3), 6
	Placebo	18.4 (1.4), 27	19.7 (14), 27	17.4 (1.6), 27	19 (2.1), 8	ND
Lymphocytes 690-4500/mL	EFF1001	1748 (153), 19	1583 (132), 19	1333 (159), 19	1518 (154), 6	1303 (172), 6
	Placebo	1444 (137), 27	1632 (134), 27	1419 (149), 27	1526 (170), 18	1534 (234), 4
Monocytes %	EFF1001	5.8 (0.6), 19	6.1 (0.5), 19	4.7 (0.6), 19	4.5 (0.6), 6	4.5 (1.1), 6
	Placebo	6.1 (0.5), 27	6.0 (0.5), 27	6.4 (0.7), 27	4.3 (0.7), 8	ND
Monocytes 0-840/mL	EFF1001	461 (40), 19	479 (49), 19	463 (66), 19	276 (31), 6	298 (68), 6
	Placebo	464 (40), 27	504 (47), 27	538 (57), 27	345 (68), 8	ND
Eosinophils %	EFF1001	5.3 (1.1), 16	4.9 (1.2), 16	2.9 (0.7), 16	4.2 (1.0), 5	2.6 (1.2), 5
	Placebo	3.8 (0.6), 26	4.7 (0.6), 26	4.6 (0.8), 26	4.9 (1.2), 7	ND
Eosinophils 0-1200/mL	EFF1001	540 (138), 16	383 (89), 16	260 (46), 16	246 (50), 5	202 (103), 5
	Placebo	289 (41), 26	385 (51), 26	356 (54), 26	385 (97), 7	ND

MCV = Mean corpuscular volume; RBC = Red blood cell count; WBC = White blood cell count.

Table S2. Clinical chemistry parameters. Mean (SE), N

		Day				
		1	14	42	56	70
Albumin 2.5 – 4.0 g/dL	EFF1001	3.5 (0.1), 20	3.7 (0.1), 20	3.9 (0.1), 20	3.7 (0.1), 6	3.7 (0.1), 6
	Placebo	3.7 (0.1), 27	3.9 (0.1), 27	4.0 (0.1), 27	3.6 (0.1), 9	ND
Albumin/Globulin 0.8 – 1.6 g/dL	EFF1001	1.4 (0.1), 20	1.4 (0.1), 20	1.5 (0.1), 20	1.6 (0.1), 6	1.6 (0.1), 6
	Placebo	1.6 (0.1), 27	1.8 (0.3), 27	1.6 (0.1), 26	1.6 (0.1), 9	ND
ALP 20 – 142 IU/L	EFF1001	70 (19), 20	122 (38), 20	117 (49), 20	54 (10), 6	60 (14), 6
	Placebo	75 (13), 27	130 (24), 27	103 (17), 27	100 (25), 9	ND
ALT 10 – 110 IU/L	EFF1001	42 (5), 20	37 (4), 20	36 (4), 20	31 (4), 6	35 (5), 6
	Placebo	44 (5), 27	46 (7), 27	41 (4), 27	88 (42), 9	ND
AST 15 – 66 IU/L 0 – 9 IU/L	EFF1001	37 (6), 20	31 (5), 20	25 (2), 20	27 (2), 6	25 (2), 6
	Placebo	29 (1), 27	23 (2), 27	26 (2), 27	35 (2), 9	ND
Amylase 290 – 1125 IU/L	EFF1001	520 (43), 9	436 (35), 9	467 (52), 9	ND	504 (77), 5
	Placebo	624 (46), 16	551 (43), 16	557 (47), 16	650 (84), 6	ND
Anion Gap 13 – 22	EFF1001	17 (1), 11	21 (2), 11	21 (2), 11	ND	ND
	Placebo	18 (1), 11	20 (1), 11	19 (1), 11	ND	ND
Bilirubin 0 – 0.3 mg/dL	EFF1001	0.1 (0.01), 20	0.1 (0.02), 20	0.1 (0.01), 20	0.1 (0.02), 6	0.1 (0.02), 6
	Placebo	0.1 (0.01), 27	0.15 (0.03), 27	0.16 (0.03), 27	0.1 (0.01), 9	ND
BUN 7 – 32 mg/dL	EFF1001	15.2 (1), 20	17.1 (2), 20	17.6 (2), 20	14.3 (1), 6	15 (1), 6
	Placebo	15.9 (1), 27	16.8 (1), 27	18.1 (1), 27	15.8 (1), 9	ND
BUN/Creatinine 4 – 27	EFF1001	14 (1), 20	18 (3), 20	17 (2), 20	13 (1), 6	13 (1), 6
	Placebo	15 (1), 27	17 (2), 27	19 (2), 27	13 (1), 9	ND
Calcium 9.2 – 11.7 mg/dL	EFF1001	10.1 (0.1), 20	10.3 (0.3), 20	10.7 (0.2), 20	10.3 (0.1), 6	10.3 (0.2), 6
	Placebo	10.5 (0.1), 27	10.5 (0.1), 27	10.5 (0.1), 27	10.4 (0.1), 9	ND
Chloride 108 – 120 mEq/L	EFF1001	113 (0.8), 20	112 (0.8), 20	111 (0.7), 20	114 (0.8), 6	114 (0.9), 6
	Placebo	113 (0.6), 27	110 (0.5), 27	111 (0.7), 27	113 (1.0), 9	ND

Table S2. Clinical chemistry parameters. Mean (SE), N

		Day				
		1	14	42	56	70
Cholesterol 130 – 300 mg/dL	EFF1001	207 (12), 20	214 (12), 20	233 (13), 20	227 (18), 6	230 (11), 6
	Placebo	234 (11), 27	245 (12), 27	268 (13), 27	248 (27), 9	ND
CPK (CK) 50 – 275 IU/L	EFF1001	138 (20), 17	114 (28), 17	100 (19), 17	139 (34), 6	118 (14), 6
	Placebo	124 (16), 24	117 (20), 24	117 (16), 24	190 (38), 9	ND
Creatinine 0.4 – 1.5 mg/dL	EFF1001	1.1 (0.1), 20	1.0 (0.1), 20	1.1 (0.1), 20	1.2 (0.1), 6	1.2 (0.1), 6
	Placebo	1.1 (0.1), 27	1.0 (0.1), 27	1.0 (0.1), 27	1.3 (0.1), 9	ND
GGT 1 – 12 IU/L	EFF1001	5.8 (1.6), 17	3.8 (0.8), 17	3.7 (0.6), 17	2.7 (0.6), 6	2.5 (0.7), 6
	Placebo	3.5 (0.5), 24	3.5 (0.6), 24	4.0 (0.7), 24	3.6 (1.1), 9	ND
Globulin 2 – 3.8 g/dL	EFF1001	2.6 (0.1), 20	2.7 (0.1), 20	2.6 (0.1), 20	2.4 (0.2), 6	2.4 (0.2), 6
	Placebo	2.5 (0.1), 27	2.5 (0.1), 27	2.4 (0.1), 27	2.3 (0.1), 9	ND
Glucose 75 – 130 mg/dL	EFF1001	94 (2), 20	95 (5), 20	94 (3), 20	100 (3), 6	102 (1), 6
	Placebo	98 (2), 27	100 (2), 27	98 (2), 27	96 (4), 9	ND
Lipase 77 – 695 IU/L	EFF1001	362 (48), 9	407 (44), 9	438 (56), 9	391 (86), 4	332 (61), 5
	Placebo	359 (48), 16	382 (36), 16	428 (49), 16	377 (93), 6	ND
Magnesium 1.8 -2.5 mg/dL	EFF1001	1.9 (0.1), 20	2.2 (0.1), 20	2.2 (0.1), 20	1.9 (0.1), 6	2.1 (0.1), 6
	Placebo	2.1 (0.1), 27	2.2 (0.1), 27	2.3 (0.2), 27	2.0 (0.1), 9	ND
Osmolality 227 – 311 mOsm/kg	EFF1001	300 (3), 17	303 (3), 17	300 (3), 15	306 (6), 5	306 (5), 5
	Placebo	302 (2), 24	303 (2), 24	300 (3), 19	301 (6), 6	ND
Phosphorus 2.1 – 6.0 mg/dL	EFF1001	4.2 (0.2), 20	4.4 (0.3), 20	4.3 (0.2), 20	4.1 (0.1), 6	4.1 (0.2), 6
	Placebo	4.2 (0.2), 27	5.4 (1.0), 27	4.2 (0.1), 27	4.1 (0.3), 9	ND
Potassium 3.5 – 5.2 mEq/L	EFF1001	4.5 (0.1), 20	4.4 (0.2), 20	4.2 (0.1), 20	4.5 (0.1), 6	4.6 (0.1), 6
	Placebo	4.4 (0.1), 27	4.3 (0.1), 27	4.3 (0.1), 27	4.5 (0.1), 9	ND
Total Protein 5.3 – 7.2 g/dL	EFF1001	6.1 (0.1), 20	6.4 (0.1), 20	6.5 (0.1), 20	6.1 (0.2), 6	6.1 (0.1), 6
	Placebo	6.2 (0.1), 27	6.4 (0.1), 27	6.4 (0.1), 27	5.9 (0.1), 9	ND
Sodium 142 – 152 mEq/L	EFF1001	147 (1), 20	150 (1), 20	149 (1), 20	150 (1), 6	149 (1), 6
	Placebo	148 (1), 27	148 (1), 27	148 (1), 27	149 (1), 9	ND

Table S2. Clinical chemistry parameters. Mean (SE), N						
		Day				
		1	14	42	56	70
Sodium/Potassium 27 - 38	EFF1001	32 (0.6), 9	33 (1.7), 9	35 (0.9), 9	ND	32 (0.6), 5
	Placebo	34 (0.7), 16	34 (0.7), 16	35 (0.9), 16	33 (1.2), 6	ND
Thyroxine (T4) 1 – 4 mg/dL	EFF1001	1.6 (0.2), 8	1.5 (0.2), 8	1.6 (0.2), 8	ND	1.4 (0.4), 5
	Placebo	1.8 (0.1), 12	1.7 (0.2), 12	1.7 (0.2), 12	1.3 (0.2), 5	ND
Triglycerides 29 – 291 mg/dL	EFF1001	80 (14), 9	256 (95), 9	217 (112), 9	ND	64 (9), 5
	Placebo	212 (87), 16	703 (240), 16	621 (193), 16	209 (88), 6	ND

ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; BUN = Blood urea nitrogen; CPK (CK) = Creatine kinase; GGT = Gamma glutamyltransferase.

Shift Tables (Table S3) show qualitative changes (relative to reference ranges) in specific parameters from Day 14 to Day 42. Day 14 represents a baseline for test article administration while also providing results for impacts after 14 days of oral prednisolone administration. At Day 42 the dogs had received 42 days of prednisolone administration and 28 days of test article administration. Shift tables were generated for ALP and triglycerides due to their sensitivity to prednisolone therapy and for AST because the reference ranges varied significantly between clinical sites making interpretation of mean (SE) data difficult. The shift tables confirm that EFF1001 had no significant impact on these clinical chemistry parameters in any of the dogs tested.

Table S3. Shifts in Parameters							
Active Day 1 4	EFF1001 Day 42			Placebo Day 14	Placebo Day 42		
	Normal	High	Low		Normal	High	Low
Alkaline Phosphatase (ALP)							
Normal	17			Normal	19		
High	1	2		High	1	6	
Low				Low	1		
Aspartate aminotransferase (AST)							
Normal	17		2	Normal	23	1	
High	1			High			
Low				Low	2		1
Triglycerides (measured at sites 2 and 4 only)							
Normal	4	1	1	Normal	5	5	
High	2	1		High	2	4	
Low				Low			

In conclusion, EFF1001 had no significant impact on any haematology or clinical chemistry parameter in any of the dogs tested during four to eight weeks of oral administration at 30 mg/kg body weight.