A blinded, randomized, placebo-controlled trial of the safety of lokivetmab (ZTS-00103289), a caninized anti-canine IL-31 monoclonal antibody in client-owned dogs with atopic dermatitis

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Materials and methods

The study was conducted in support of product registration by the United States Department of Agriculture. The protocol was reviewed by and approved before study initiation by the Zoetis Ethical Review Board. The owners gave written informed consent for each dog to participate in the study.

Dogs were client-owned of any age and body weight, overall healthy, apart from AD, based on the initial (Day 0) physical examination and were diagnosed with AD based on clinical signs and compatible history, according to the veterinarian. Exclusion criteria included dogs with evidence of malignant neoplasia or immune suppression (e.g. hyperadrenocorticism) and lactating bitches or dogs intended for use as breeding animals.

Enrolled dogs were randomized to treatment with lokivetmab or placebo in a 2:1 ratio at each clinic using SAS v9.3 (SAS Institute Inc.; Cary, NC, USA). Blocking was based on order of enrolment within clinic. Dogs were examined and were treated with two monthly doses of lokivetmab or placebo. There were no clinically important differences between groups in clinical pathology results. Treatment-induced immunogenicity was found in 2.5% of lokivetmab treated dogs. A wide variety of concomitant medications were used with no clinically apparent adverse interactions.

Conclusions and clinical importance—Among a diverse population of 162 client owned dogs with a clinical diagnosis of AD, treatment with two monthly doses of lokivetmab was safe, based on observed adverse events and clinical pathology results over a 42 day period.
assigned to receive either placebo or lokivetmab (1.0–3.3 mg/kg) subcutaneously on days 0 and 28 (±3 days). A dose of 1.0 mg/kg represented a nominal dose.

Baseline data (demographic, physical examination) were collected on enrolment at Day 0. Owners returned dogs to the clinic on days 28 (±3) and 42 (±3) for physical examination. Clinicians recorded adverse events reported by owners or identified on physical examination throughout the study.

Blood samples [complete blood count, serum chemistry, anti-drug antibodies (ADAs) and lokivetmab concentrations] and urine samples for urinalysis and urine protein creatinine ratio were collected on days 0 and 28 (before dosing) and Day 42. The samples were sent to one laboratory (Heska Corp.; Loveland, CO, USA). Serum samples at each time point were analysed for lokivetmab and ADAs using validated methods at Zoetis Inc., Kalamazoo, MI, USA.

Data were summarized using SAS v9.3 (SAS Institute). No hypothesis testing was conducted.

For each continuous haematology and serum chemistry measure, summary statistics (mean, median, standard deviation, minimum and maximum) were calculated by treatment and time point. Frequencies of dogs reported to experience at least one adverse event were summarized by clinical sign for all unique terms. Frequencies of dogs receiving each concomitant medication over the course of the study were summarized.

Results

Two hundred and forty five dogs were enrolled from 14 veterinary clinics (Table 1). All enrolled dogs were included in the summaries. The same percentage (1.2%) of cases in both treatment groups were withdrawn from study or lost to follow-up before Day 42.

Table 2 provides a comparison of adverse events that occurred in >2% of lokivetmab-treated dogs. A similar proportion of vomiting, anorexia, lethargy and diarrhea adverse events in both groups resolved spontaneously or with supportive care. Adverse events involving skin infection (e.g. pyoderma) were followed post-study until resolution or considered by the clinician to be a chronic condition. Discomfort associated with injection persisting beyond the immediate post-injection period was reported once and involved scratching at the site of lokivetmab administration for 15 min following the first dose only. There were no hypersensitivity reactions (e.g. wheals, vomiting) immediately post-dosing and no reports of injection site reactions (e.g. injection site swelling or redness). The remaining adverse events occurred in <2.0% of the lokivetmab-treated group. Arithmetic mean values for all clinical pathology analytes in both treatment groups fell within the laboratory’s normal reference ranges at all visits (days 0, 28 and 42) except serum alkaline phosphatase (placebo group) which was slightly above reference range throughout the study.

Two dogs in each treatment group showed serious adverse events. The first case was a 4-year-old neutered female English cocker spaniel; significant findings on Day 0 before treatment with lokivetmab included fever (39.8°C), mild regenerative anaemia, slight polychromasia and three nucleated red blood cells per 100 white blood cells; platelets were clumped and an accurate count was, therefore, unavailable. Treatment with cefpodoxime proxetil was initiated (Day 8) to treat a cough associated with tracheobronchitis of one day duration. Immune-mediated haemolytic anaemia and immune-mediated thrombocytopenia were diagnosed (Day 12); remission was achieved by Day 43 with immunosuppressive treatment. Serious adverse events in the remaining three dogs included a placebo-treated dog diagnosed with diabetes mellitus after initiating a corticosteroid and two dogs, one placebo-treated and one lokivetmab-treated, that had pre-existing conditions (well-controlled hypoadrenocorticism and moderate regenerative anaemia, respectively) and were diagnosed with lymphoma while on study.

Of the >200 concomitant medications administered during this study, those most frequently used (i.e. ≥6%) of lokivetmab-treated group) are summarized in Supplementary Table 1.

Four (2.5%) of the lokivetmab-treated dogs were categorized as having treatment-induced immunogenicity; anti-lokivetmab titres in these dogs were <10 at Day 0, remained low on Day 28 (<10 to 10) and increased on Day 42 (32–315). Average day 28 and 42 serum lokivetmab concentrations were ~90% lower than the remaining treated animals.

Discussion

The lack of restrictions on concomitant medications in the current trial likely contributed to a similar proportion of placebo- and lokivetmab-treated dogs completing the study, thus allowing a direct comparison of adverse event frequencies. The cases with serious adverse events reported would not have been eligible to enrol in a traditionally designed field efficacy study with restrictions on concomitant conditions at enrolment, and where corticosteroids and systemic antibiotics are not permitted during study or shortly before enrolment.

Table 1. Demographics of enrolled dogs at Day 0

| Variable                  | Lokivetmab N = 162 | Placebo N = 83 |
|---------------------------|--------------------|---------------|
| Breeding distribution     |                    |               |
| Purebred                  | 72.8 (118)         | 72.3 (60)     |
| Mixed breed               | 27.2 (44)          | 27.7 (23)     |
| Sex distribution          |                    |               |
| Male                      | 51.9 (84)          | 59.0 (49)     |
| Female                    | 48.1 (78)          | 41.0 (34)     |
| Age at study onset, years (range) | 6.8 (1.0–14.5) | 6.2 (0.8–13.0) |
| Weight at study onset, kg (range) | 26.0 (2.4–88.6) | 20.7 (4.6–63.6) |

Table 2. Adverse events occurring at least once in >2% of lokivetmab-treated group over the course of the 42 day study

| Adverse Reactions* | Lokivetmab N = 162 | Placebo N = 83 |
|--------------------|--------------------|---------------|
| N                  | % (r)              | % (r)         |
| Otis externa       | 13.0 (21)          | 12.0 (10)     |
| Dermatitis         | 9.9 (16)           | 13.3 (11)     |
| Bacterial skin infection | 9.3 (15)   | 12.0 (10)     |
| Erythema           | 8.0 (13)           | 4.8 (4)       |
| Vomiting           | 7.4 (12)           | 10.8 (9)      |
| Anorexia           | 6.2 (10)           | 4.8 (4)       |
| Lethargy           | 5.6 (9)            | 6.0 (5)       |
| Pruritus           | 4.9 (8)            | 19.3 (16)     |
| Diarrhoea          | 3.7 (6)            | 4.8 (4)       |
| Alopecia           | 2.5 (4)            | 7.2 (6)       |
| Fleas              | 2.5 (4)            | 2.4 (2)       |

*Adverse reactions were tabulated per animal.
There were no clinically apparent adverse interactions between lokivetmab and any of the concomitantly administered medications, although this study was not designed specifically to detect such interactions. Xenobiotic metabolizing enzymes, such as cytochrome P450 enzymes, are not involved in elimination of mAbs; therefore, metabolic drug–drug interactions, caused by inhibition or induction of cytochrome P450 enzymes, are not expected. However, other types of interactions are possible, notably cytokine-mediated changes in expression of drug-metabolizing enzymes. In chronic inflammatory diseases, elevated levels of cytokines such as IL-6 and TNF-α lead to downregulation of cytochrome P450 enzymes. Treatment with a mAb that blocks the action of pro-inflammatory cytokines can result in normalization of cytochrome P450 levels and thus affect the levels of other concomitantly administered small molecule drugs. If pro-inflammatory cytokines downregulate cytochrome P450 enzymes in dogs with AD, a decrease in these cytokines following administration of a therapeutic mAb could be expected to lead to a decrease in circulating concentrations of concomitantly administered CYP450-metabolized drugs (e.g. ciclosporin), although such an effect is most relevant for drugs with a narrow therapeutic range (e.g. antineoplastic drugs).

Lokivetmab is a “caninized” monoclonal antibody, such speciation decreases immunogenicity in the target species, even though all therapeutic mAbs remain immunogenic to some extent. ADAs may bind to therapeutic mAbs leading to neutralization or increased clearance and potentially result in decreased efficacy. ADAs have been associated with a higher risk of hypersensitivity reactions although such reactions have not been observed in dogs treated with lokivetmab in laboratory or clinical field trials thus far. The current study was not designed to compare safety of doses within the range administered (e.g. 1.0 mg/kg compared to 3.3 mg/kg). However, laboratory dog studies identified no treatment-related adverse effects following repeat administration of lokivetmab at the highest dose tested (10 mg/kg) (data on file). Results of this study demonstrated that under field conditions two consecutive monthly doses of lokivetmab (1.0–3.3 mg/kg) were safe in a diverse population of 162 client-owned dogs, diagnosed with AD, based on observed adverse events and clinical pathology results. Further studies are needed to evaluate the safety of lokivetmab following long-term use in dogs with AD.

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**Supporting Information**

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

**Table S1.** Concomitant medications and therapies administered at least once to at least 6% of lokivetmab-treated group over the course of the 42 day study.
Hypótesis/Objetivos – Este estudio evaluó la inocuidad del lokivetmab en un ensayo doble ciego, al azar, con placebo administrado en los días 0 y 28. Los veterinarios examinaron los perros en pruebas de campo.

Sujetos – Los clínicos de 14 clínicas veterinarias, que eran perros de dueños privados con AD con restricciones mínimas en las restricciones concomitantes y las comorbididades.

Métodos – Los perros fueron asignados al azar en una proporción de 2:1 para recibir lokivetmab (1,0 a 3,3 mg/kg) o placebo a lo largo de los días 0 y 28. Los veterinarios examinaron los perros en pruebas de campo.

Resultados – No hubo reacciones de hiperreactividad inmediata (por ejemplo, erupciones, vómitos). Se observó malestar tras la administración en un 5,1% de los perros que fue similar en frecuencia y gravedad entre lokivetmab y los grupos tratados con placebo. Prurito fue reportado como un efecto adverso durante el estudio en un 5,1% de los perros que fue similar en frecuencia y gravedad entre lokivetmab y los grupos tratados con placebo. Pandemia no hubo diferencias clínicamente importantes entre las dosis de tratamiento críticas. Los perros con AD crónica fueron utilizados durante el estudio sin interacciones adversas clínicamente aparentes.

Conclusiones e importancia clínica – En una población diversa de 162 perros de dueños privados con un diagnóstico clínico de AD, el tratamiento con dos dosis mensuales de lokivetmab fue seguro, basado en los eventos adversos observados y los resultados de patología clínica durante un periodo de 42 días.

Zusammenfassung

Hintergrund – Lokivetmab (ZTS-00103289) ist ein kaninchenisiertes Anti-Hunde IL-31 monoklonaler Antikörper, der bei der Reduzierung des Juckreizes, der durch eine atopische Dermatitis (AD) bei Hunden in Feldstudien bedingt war, Wirksamkeit gezeigt hat.

Hypothese/Ziele – Diese Studie erfasste die Sicherheit von Lokivetmab in einer randomisierten, doppelblindigen, Placebo-kontrollierten Studie mit Hunden in privatbesitz mit AD mit minimalen Einschränkungen von begleitenden Medikamenten und Begleiterkrankungen.

Tiere – KlinikerInnen in 14 Veterinärkliniken nahmen Privathunde (n = 245) mit chronischer AD in die Studie auf.

Methoden – Die Hunde wurden zufällig im Verhältnis 2:1 eingeteilt, um entweder Lokivetmab (1,0-3,3 mg/kg) oder Placebo, welches subkutan verabreicht wurde, an den Tagen 0 und 28 zu erhalten. Die KlinikerInnen untersuchten die Hunde und nahmen Blut- und Urinproben, um die klinische Pathologie und die Immunogenität (an den Tagen 0, 28 und 42) zu erfassen.

Ergebnisse – Es bestanden keine Immunreaktionen vom Soforttyp (z.B. Blasen, Vomitus). Bei 5,1% der Hunde bestand ein Unbehagen bei der Verabreichung, welches ähnlich in Frequenz und Schweregrad in
Lokivetmab(ZTS-00103289)はイヌ化抗IL-31モノクローナル抗体であり、臨床治験において、犬のアトピー性皮膚炎による痒みを減少させることが確認されている。

仮説/目的 — アトピー性皮膚炎の飼い犬を対象に、無作為抽出、二重盲検、プラセボ対照試験を実施し、lokivetmabの安全性を評価すること。患者を抽出する際の併用薬および併発疾病に対する制限は最小限とした。

供与動物 — 慢性のADに罹患した犬75例(n=245)を1:1の動物病院の臨床医師によって選出された。

方法 — 患者は2:1の割合で、無作為にlokivetmab群(1.0-3.3 mg/kg)あるいはプラセボ群に組み入れられ、day0およびday28にそれぞれ異なる薬剤を皮下投与された。患者は臨床医師による身体検査を受け、採取された血液および尿の臨床検査および免疫原性検査が実施された(day0, 28, 42)。

結果 — 急性の過敏反応(風疹、嘔吐)は認められなかった。投与時の不快感が1%の患者で認められたが、lokivetmab群およびプラセボ群とても同程度の頻度および重症度であった。治療中の有害事象としての副作用は、lokivetmab群でより低頻度に認められ(それぞれ1.4%および19.3%)、それ以外の有害事象は治療群間で同程度の頻度であった。臨床病理学者の評価において、2群間に臨床的意義のある差は認められなかった。治療による免疫原性の誘導がlokivetmab群の2.5%に認められた。様々な薬物使用が使用されが、臨床的に明らかに有害な薬物相互作用は認められなかった。

結論および臨床的意義 — アトピー性皮膚炎を診断された多種多様な飼い犬245例を対象に行ったlokivetmabの一月毎に2回の治療は安全であること、42日間の観察期間総数における有害事象および臨床病理学的検査の結果に基づいて示された。

要約

背景 — Lokivetmab(ZTS-00103289)はイヌ化抗IL-31モノクローナル抗体であり、臨床治験において、犬のアトピー性皮膚炎による痒みを減少させることが確認されている。

目的/目的 — アトピー性皮膚炎の対象犬を対象に、無作為抽出、二重盲検、プラセボ対照試験を実施し、lokivetmabの安全性を評価すること。患者を抽出する際の併用薬および併発疾病に対する制限は最小限とした。

動物 — 收集自14家獣医診療所の臨床医師の記録では慢性ADの犬の対象犬245例を対象とした。

方法 — 0-28日、犬10例/2例の比例で組み分け、プラセボあるいはlokivetmab(1.0-3.3 mg/kg)を投与した。毎日臨床検査を実施し、 lokivetmab治療群とプラセボ治療群の治療の有無を評価した（day0，28，42）。

結果 — 副作用は認知されなかった。臨床的に、 Lokivetmab治療群とプラセボ治療群とには有意な差を認められなかった。 Lokivetmab治療群で2.5%の犬に免疫原性の誘導が認められたが、臨床的には明らかに有害な薬物相互作用は認められなかった。

結論 — Lokivetmabの臨床試験は安全であることが示されたが、42日に観察期間中における有害事象および臨床病理学的検査の結果に基づいて示されている。
ocorreram em frequência similar entre os dois grupos. Não houve nenhuma alteração significativa entre os grupos nos resultados de patologia clínica. Imunogenicidade induzida pelo tratamento foi encontrada em 2,5% dos cães tratados com lokivetmab. Um ampla variedade de medicações concomitantes foi usada sem interações adversas aparentes.

**Conclusões e importância clínica** – O tratamento com lokivetmab em duas aplicações mensais em 162 cães com DA crônica clinicamente diagnosticada foi seguro, baseado em reações adversas e análises clínicas, em um período de 42 dias.