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

Gina M. Michels*, Kelly F. Walsh*, Kristina A. Kryda*, Sean P. Mahabir*, Rodney R. Walters†, Jacquelien D. Hoevers* and Olivier M. Martinon†

*Global Development and Operations and †Global Therapeutics Research, Zoetis Inc., 333 Portage Street, Kalamazoo, MI 49007, USA
Correspondence: Gina M. Michels, Global Development and Operations, Zoetis Inc., 333 Portage Street, Kalamazoo, MI 49007, USA.
E-mail: gina.m.michels@zoetis.com

Introduction

Lokivetmab is a caninized anti-canine interleukin-31 (IL-31) monoclonal antibody (mAb) that binds to and neutralizes the soluble inflammatory mediator IL-31 in dogs. Field and laboratory studies show IL-31 is a critical cytokine involved in pruritus in dogs with atopic dermatitis (AD). The current report details results of a randomized clinical trial that evaluated the safety of lokivetmab in dogs with AD under field conditions where there were minimal restrictions on co-morbidities and no restrictions on concomitant medications.

Materials and methods

The study was conducted in support of product registration by the United States Department of Agriculture. The protocol was reviewed 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 enrollment within clinic. Dog was the experimental unit. Dog owners, laboratory personnel, clinicians and all site personnel, with the exception of the treatment dispenser, were masked to treatment group assignments. The treatment dispenser utilized a treatment randomization file that was unique to the site to determine the treatment group assignment, drew up the correct dose of treatment into a syringe and then provided it to study personnel for administration.

Lokivetmab was provided in ready-to-use one mL single-use (no preservative) vials containing 10, 20, 30 or 40 mg/mL in a histidine buffer. Dogs in the placebo group were given the same volume as dogs in the lokivetmab group; the placebo was identical in appearance to the lokivetmab and contained all of the same excipients except for lokivetmab. Placebo and lokivetmab were stored refrigerated (2–8°C) before use. Following randomization, dogs were

Methods – Dogs were randomized at a 2:1 ratio to receive either lokivetmab (1.0–3.3 mg/kg) or placebo administered subcutaneously on days 0 and 28. Clinicians examined dogs, and collected blood and urine for assessment of clinical pathology and immunogenicity (days 0, 28 and 42).

Results – There were no immediate hypersensitivity reactions (e.g. wheals, vomiting). Discomfort at administration occurred in 5.1% of dogs and was similar in frequency and severity between lokivetmab- and placebo-treated groups. Pruritus was reported as an adverse event during the study less frequently in the lokivetmab-treated group (4.9% and 19.3%, respectively); otherwise, adverse events occurred at a similar frequency between treatment groups. 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 diarrhoea 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

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

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

<table>
<thead>
<tr>
<th>Adverse Reactions*</th>
<th>Lokivetmab N = 162</th>
<th>Placebo N = 83</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Otitis externa</td>
<td>13.0 (21)</td>
<td>12.0 (10)</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>9.9 (16)</td>
<td>13.3 (11)</td>
</tr>
<tr>
<td>Bacterial skin infection</td>
<td>9.3 (15)</td>
<td>12.0 (10)</td>
</tr>
<tr>
<td>Erythema</td>
<td>8.0 (13)</td>
<td>4.8 (4)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7.4 (12)</td>
<td>10.8 (9)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>6.2 (10)</td>
<td>4.8 (4)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>5.6 (9)</td>
<td>6.0 (5)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>4.9 (8)</td>
<td>19.3 (16)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3.7 (6)</td>
<td>4.8 (4)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>2.5 (4)</td>
<td>7.2 (6)</td>
</tr>
<tr>
<td>Fleas</td>
<td>2.5 (4)</td>
<td>2.4 (2)</td>
</tr>
</tbody>
</table>

*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.

Acknowledgements
We would like to thank the following veterinarians who enrolled dogs in this study and performed the clinical investigations: Ryan Carpenter, Terry Cleiks, Karen Farver, Sam Geller, Robert Jackson, Carolyn Kidney, Thomas Lewis, Andrew Pickering, Chris Reeder, Jason St. Romain, Kristi Rowland, Jay Schweizer, Roger Sifferman and Timothy Strauss. The test article (lokivetmab or placebo) was provided at no cost to the clinic and clinicians were compensated for the costs associated with each dog’s clinic visits. We thank the following current and former Zoetis colleagues for their contributions: Aniket Badkar, Patrick Birkholz, Joseph F. Boucher, Anne E. Daniels, Clifford Ferree, Matthew Krautmann, David Garcia-Tapia, Mary Pat Gorman, Jessica Harfst, Nicole Honsberger, Ben Hummel, Cari LaGrow, Sunil Narisetty, Candace Sousa and Rostyslav Tsapyak.

References

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.

© 2016 Zoetis LLC. Veterinary Dermatology published by John Wiley & Sons Ltd on behalf of the ESVD and ACVD
Hypóteses/Objectifs – Esta investigación evaluó la innocuidad del lokivetmab en un ensayo controlado, doble ciego, en perros seleccionados aleatoriamente y con restricciones mínimas acerca de medicamentos concomitantes y otras enfermedades concurrentes.

Sujetos – Los propietarios de 14 clínicas veterinarias de 162 perros de propietarios privados con AD con restricciones mínimas acerca de medicamentos concomitantes y otras enfermedades concurrentes (n = 245) fueron seleccionados aleatoriamente.

Métodos – Los perros fueron asignados al azar en una proporción de 2:1 para recibir lokivetmab (1,0 a 3,3 mg/kg) por vía subcutánea o placebo administrado en los días 0 y 28. Los veterinarios examinaron los cambios clínicos y la inmunogenicidad en los grupos de tratamiento.

Resultados – No hubo reacciones de hipersensibilidad inmediata en los perros tratados con lokivetmab. El prurito fue controlado en el grupo tratado con lokivetmab (4,9% y 19,3% respectivamente).

Conclusión e importancia clínica – En un período de 42 días, el tratamiento con dosis mensuales de lokivetmab fue seguro, basado en los eventos adversos observados y los resultados de patología clínica durante un período de 42 días.
Lokivetmab is a monoclonal anti-IL-31 antibody that has demonstrated efficacy in the reduction of pruritus associated with dermatitis atopica (DA) in cães, in experiments of campo. This study aimed to evaluate the safety of lokivetmab (1.0–3.3 mg/kg) or placebo in 162 dogs with chronic dermatitis atopica. The treatment was given in a randomized way in two groups: lokivetmab (n=82) and placebo (n=80). The main observed adverse reactions were a dry cough and decreased appetite in 9.8% of cases in the lokivetmab group and 12.5% in the placebo group. The study demonstrated the efficacy of lokivetmab in reducing pruritus in dogs with chronic dermatitis atopica.

Resumo

Contexto – Lokivetmab (ZTS-00103289) é um anticorpo monoclonal caninizado anti-IL-31 canina que tem demonstrado eficácia na redução do prurido associado com dermatite atópica (DA) em cães, em pesquisas de campo.

Objetivos – Este estudo avaliou a segurança de lokivetmab em um ensaio clínico randomizado, duplo-cego, placebo controlado em cães com DA, pertencentes a clientes, e com mínimas restrições em relação a medicações concomitantes e comorbidades.

Animais – Vinte e quatro cães atendidos com DA crónica (n=245).

Métodos – Os cães foram randomizados em uma razão de 2:1 para receber lokivetmab (1,0–3,3 mg/kg) ou placebo administrados por via subcutânea nos dias 0 e 28. Os clínicos examinaram os animais e coleta-ram sangue e urina para avaliação clínico-patológica e de imunogeneticidade (dias 0, 28 e 42).

Resultados – Não houve nenhuma reação de hipersensibilidade imediata (papulas, vómito). Desconforto na administração ocorreu em 5,1% dos cães e foi simétrico em frequência e gravidade em ambos os grupos lokivetmab e placebo. Prurido foi menos frequentemente reportado como uma reação adversa no grupo tratado com lokivetmab que no placebo (4,9% e 19,3%, respectivamente); entretanto, efeitos adversos
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 amplo 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 crónica clinicamente diagnosticada foi seguro, baseado em reações adversas e análises clínicas, em um período de 42 dias.