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Attached is an Abstract of a Zoetis Presentation at the

7TH WORLD CONGRESS OF VETERINARY DERMATOLOGY, 2012

TITLE: DEVELOPMENT OF A MODEL OF IL-31 INDUCED PRURITUS IN BEAGLE DOGS

IMPORTANT SAFETY INFORMATION:

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DEVELOPMENT OF A MODEL OF IL-31 INDUCED PRURITUS IN BEAGLE DOGS

W.HUMPHREY, T.FLECK, M.ALEO, E.COSCARELLI, B.GALVAN, M.MULLINS, B.HUMMEL, J.SHELLY, J.MESSAMORE, S.MAHABIR, A.GONZALES, R.MCCALL

Zoetis, Kalamazoo, MI, USA

REFERENCE: *Veterinary Dermatology*, **23** (Suppl. 1), 35. FC-30.

ABSTRACT: Skin biopsies from pruritic and non-pruritic lesions in cases of human and mouse atopic dermatitis (AD) have revealed an up-regulation in interleukin-31 mRNA levels implicating a contributory role of this cytokine in the development of pruritus. Using recombinant canine IL-31 (cIL-31 or IL-31), we have developed an anti-pruritic screening model in dogs using exogenous IL-31 to induce episodes of pruritus in the presence/absence of test article treatments. In this model, dogs were acclimated to single-housing runs equipped with ceiling cameras. Pruritic behaviors displayed by the dogs such as licking, scratching, head-shaking, and body-rubbing were observed by video surveillance (in real-time) over pre-set time-intervals (usually ≤ 2h) before and/or after intravenous injection of IL-31. A categorical scoring system (yes/no determination of displayed pruritus made over consecutive discrete 1 minute intervals) was used to provide a measure of pruritic reactivity of each animal during these pre-set time intervals. IL-31 produced significant pruritus compared to mock protein or saline injections.The model was validated by demonstrating that administered prednisolone significantly decreased IL-31 induced pruritus. Additionally, the janus kinase inhibitor, oclacitinib, reduced IL-31 induced pruritus in the dog. These data indicate that IL-31 produces pruritus in the dog and that this can be used as a basis for a model to identify anti-pruritic compounds.

FUNDING: Zoetis.

CONFLICT OF INTEREST: Oclacitinib is a Zoetis investigational drug.

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TITLE: EFFICACY OF AN ANTI-IGE MONOCLONAL ANTIBODY IN AN ALLERGEN INDUCED TYPE I HYPERSENSITIVITY MODEL

EFFICACY OF AN ANTI-IGE MONOCLONAL ANTIBODY IN AN ALLERGEN INDUCED TYPE I HYPERSENSITIVITY MODEL

S.DUNHAM, D.WHEELER, M.ALEO, M.DUPUIS, J.BEAM, R.WALTERS, W.DUNKLE, Y.ZHU, C.RUGG, L.BERGERON, C.STRIETZEL, B.LEONE, D.DUNYAK, W.HUMPHREY, J.MESSAMORE, J.SHELLY, P.AEED, P.CLARE, G.BAINBRIDGE, K.GREENWOOD, J.HARDHAM, R.MCCALL, A.GONZALES, O.MARTINON

Zoetis, Kalamazoo, MI, USA

REFERENCE: *Veterinary Dermatology,* **23** (Suppl. 1), 35. FC-31

ABSTRACT: Atopic dermatitis (AD) is one of the most common pruritic skin diseases in dogs presenting to veterinarians. The disease appears to have a genetic component and is often correlated with exposure to environmental allergens followed by an increase in allergen-specific IgE. To inform the development of new therapeutic alternatives we have characterized fully caninized anti-IgE monoclonal antibodies (mAb) in a disease model of IgE-mediated type I hypersensitivity. Antibodies evaluated in these studies retain the necessary characteristics of an efficacious therapeutic anti-IgE mAb including: (i) the ability of the mAb to inhibit IgE from binding its high affinity receptor FCεR1, on mast cells and (ii) lack of ability to cross link IgE already bound to the surface of mast cells. To evaluate the in vivo efficacy of lead candidates we have sensitized beagles to house dust mite (HDM) allergens and assessed how subcutaneous administration of the mAb affects circulating free IgE levels and allergen-specific wheal and flare. The results of these studies demonstrated a dose dependent reduction in sensitivity to HDM extract in sensitized dogs for up to 5 weeks post mAb administration and that this wheal and flare reduction correlated with a reduction of free IgE. Taken together these findings suggest that these mAbs have great promise to simultaneously evaluate the role of IgE in the pathogenesis of canine atopic dermatitis and to define the utility of these molecules in the treatment of atopic disease.

FUNDING: Privately funded.

CONFLICT OF INTEREST: All authors are current or past employees of Zoetis

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Attached is an Abstract of a Zoetis Presentation at the

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TITLE: A MULTICENTER CLINICAL TRIAL TO EVALUATE THE EFFICACY AND FIELD SAFETY OF OCLACITINIB

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A MULTICENTER CLINICAL TRIAL TO EVALUATE THE EFFICACY AND FIELD SAFETY OF OCLACITINIB

S. COSGROVE, J. WREN, V. KING, D. WHEELER, M. STEGEMANN

Zoetis Incorporated, New York, NY, USA

REFERENCE: *Veterinary Dermatology,* **23** (Suppl. 1), 38. FC-35

ABSTRACT: The objective of this study was to demonstrate the efficacy and safety of a novel janus kinase inhibitor, oclacitinib, for control of atopic dermatitis (AD) in client-owned dogs.

Twenty-three veterinary dermatologists enrolled 341 dogs, randomly allocated in a 1:1 ratio, to either oclacitinib (0.4mg/kg twice daily orally for 14 days) or a matched placebo. Dogs had a documented history of chronic non-seasonal AD. Minimum enrolment criteria included: an owner's survey description of 'moderate itching/dermatitis' and a dermatologist's score of 25 using the Canine Atopic Dermatitis Extent and Severity Index (CADESI-02). Owner visual analog scale (VAS) pruritus measurements (100mm scale) and dermatologist CADESI-02 scores were analyzed with linear mixed models for repeated measures with a covariate. Baseline VAS means were 74 (oclacitinib) and 75 (placebo). VAS least squares mean (lsmean) measurements for oclacitinib were significantly reduced compared with placebo ($P < 0.0001$) for all observation days; D1: 55 vs. 66; D2: 43 vs. 65; D7: 30 vs. 68 and D14: 24 vs. 70. Oclacitinib treatment produced a significant ($P < 0.0001$) decrease in the CADESI-02 lsmean scores relative to placebo. On D0, CADESI-02 mean scores for oclacitinib and placebo were 56 and 59. By D14, lsmean scores were 26 and 57 respectively. Diarrhoea and/or emesis were the most frequently reported abnormal health events.

In this study, oclacitinib used at 0.4mg/kg twice daily for 14 days was effective and safe for control of canine AD. Owners observed significant improvement in VAS compared to placebo from D1 to D14. The dermatologists D14 CADESI-02 scores mirrored these findings.

FUNDING: Zoetis

CONFLICT OF INTEREST: All authors are employees and shareholders of Zoetis Incorporated

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TITLE: COMPARISON OF THE JANUS KINASE (JAK) INHIBITOR, OCLACITINIB, AND PREDNISOLONE IN CANINE MODELS OF PRURITUS

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COMPARISON OF THE JANUS KINASE (JAK) INHIBITOR, OCLACITINIB, AND PREDNISOLONE IN CANINE MODELS OF PRURITUS

T.FLECK, W.HUMPHREY, E. COSCARELLI, B.GALVAN, M.ALEO, A.GONZALES, J.SHELLY, S.MAHABIR, R.MCCALL

Zoetis, Kalamazoo, MI, USA

REFERENCE: *Veterinary Dermatology*, **23** (Suppl. 1), 38. FC-36.

ABSTRACT: Using recombinant canine IL-31 we have developed a model of pruritus in the dog. The objective of this study was to compare the efficacy and onset of action of the janus kinase (JAK) inhibitor oclacitinib and prednisolone in our IL-31 model and a flea allergic dog model.

Oclacitinib pretreatment significantly reduced pruritus induced by IL-31 in a dose-related manner. Significant reductions in pruritus were noted at single oral doses ranging between 0.05 and 0.4 mg/kg. The maximum reduction in pruritis was 80% compared to placebo controls. The onset and duration of effect of an oral dose of 0.4 mg/kg oclacitinib was determined. Oclacitinib significantly reduced pruritus when given 1, 6, 10, and 16 h prior to IL-31 injection but not 22h prior to injection. Oclacitinib reduced pruritus within 1h in a flea allergic dog model. Prednisolone (0.25 and 0.5mg/kg) failed to significantly reduce pruritus when given at 1h prior to IL-31 injection, but when a 0.5mg/kg dose was given 10h prior to IL-31 challenge, a significant 37% reduction in pruritic response was observed. In a separate study 1mg/kg oral prednisolone failed to significantly reduce pruritus when administered 2 and 6h prior to IL-31, reinforcing the time-dependency of the anti-pruritic response. Prednisolone (1mg/kg) had an onset of anti-pruritic activity of 8-12h in flea allergic dogs. These data indicate that the JAK inhibitor oclacitinib produced a greater suppression of pruritus and had a faster onset of action than prednisolone in the IL-31 canine model of pruritus.

FUNDING: Zoetis.

CONFLICT OF INTEREST: Oclacitinib is a Zoetis investigational drug.

Attached is an Abstract of a Zoetis Presentation at the

7TH WORLD CONGRESS OF VETERINARY DERMATOLOGY, 2012

TITLE: OCLACITINIB FOR THE TREATMENT OF PRURITUS AND LESIONS ASSOCIATED WITH CANINE FLEA-ALLERGIC DERMATITIS

IMPORTANT SAFETY INFORMATION:

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OCLACITINIB FOR THE TREATMENT OF PRURITUS AND LESIONS ASSOCIATED WITH CANINE FLEA-ALLERGIC DERMATITIS

D. WHEELER*, J. CIVIL, M**. PAYNE-JOHNSON**, M. R. STEGEMANN**, S.COSGROVE*

**Veterinary Medicine Research and Development, Zoetis, Kalamazoo, Michigan, USA*
***Veterinary Medicine Research and Development, Zoetis, Zaventem, Belgium.*

REFERENCE: *Veterinary Dermatology*, **23** (Suppl. 1), 38-39. FC-37.

ABSTRACT: A novel drug, Oclacitinib, was tested for its ability to reduce clinical signs associated with canine flea-allergic dermatitis.

Thirty six flea-allergic dogs were repeatedly infested with unfed fleas over a 29 day period. Dogs were treated orally twice daily with oclacitinib during the last 2 weeks of the study. Treatments included: T01: placebo, T02: 0.4 mg/kg oclacitinib, and T03: 0.8 mg/kg oclacitinib. Nocturnal pruritic behavior (licking, chewing, scratching, etc.) was videotaped twice before, and three times during, drug administration. Erythema and skin lesions (other than erythema) were evaluated using a ten cm visual analog scale (VAS) prior to flea infestation, prior to oclacitinib administration, and following oclacitinib administration.

Within 24 hours dogs in T02 and T03 had >58% decrease in pruritus compared to T01. After 2 weeks, oclacitinib-treated dogs had >76% decrease in pruritus. Dogs in T02 and T03 had significantly less pruritus than dogs in T01 ($P \leq 0.0120$) for all 3 days post-treatment. Mean VAS scores for erythema and lesions prior to and after flea infestation were similar for all treatments. After treatment with oclacitinib, dogs in T02 and T03 had VAS scores that were 31.6% to 55.6% of T01 scores. VAS scores were significantly reduced for both erythema ($P < 0.0001$) and lesions ($P \leq 0.0025$) compared to T01, whereas there were no significant differences between T02 and T03.

Under the conditions of this study, oclacitinib administered at 0.4mg/kg twice daily for 14 days, rapidly, safely, and significantly reduced pruritus, erythema, and lesions in flea-allergic dogs.

FUNDING: Veterinary Medicine Research and Development, Zoetis, Kalamazoo, Michigan, USA.

CONFLICT OF INTEREST: All authors are current or former employees of Zoetis. Oclacitinib is a Zoetis investigational drug.

Attached is an Abstract of a Zoetis Presentation at the

7TH WORLD CONGRESS OF VETERINARY DERMATOLOGY, 2012

TITLE: PRODUCTION OF IL-31 BY CANINE TH2 CELLS AND IDENTIFICATION OF INFLAMMATORY AND NEURONAL TARGET CELLS

PRODUCTION OF IL-31 BY CANINE TH2 CELLS AND IDENTIFICATION OF INFLAMMATORY AND NEURONAL TARGET CELLS

E.E. MCCANDLESS, J.MESSAMORE, C.RUGG, G.J.FICI, A.J. GONZALES

Zoetis, Kalamazoo, MI, USA

REFERENCE: *Veterinary Dermatology*, **23** (Suppl. 1), 52. FC-65.

ABSTRACT: Recent reports have suggested that the cytokine IL-31 may have a role in atopic dermatitis. Although IL-31 has been implicated in inflammatory responses and pruritus, very little is still understood around its interactions with canine cells. The purpose of the following work was to investigate the mechanism by which IL-31 may be involved in the propagation of disease by identifying a cellular source and a responder.

Differential stimulation of canine peripheral blood mononuclear cells (PBMCs) and immunoassay analysis suggested T cells as a source of IL-31. Further evaluation showed that costimulation of house dust mite specific T helper type 2 (Th2) polarized cells with antigen and the bacterial component Staphylococcus enterotoxin B (SEB) produced relatively high levels of IL-31 compared to either stimulant alone. Using real-time polymerase chain reaction (PCR), the canine monocytic line DH82 cells were shown to express the IL-31 receptor alpha chain. These cells were confirmed to be IL-31-responsive via induction of the MAP kinase signal cascade upon IL-31 treatment. Dorsal root ganglia were also examined by PCR and were found to express the IL-31 receptor alpha chain. These results indicate that canine Th2 cells are a source of IL-31 and suggest that both a monocytic cell line and neuronal cells express the IL-31 receptor.

In a multifaceted disease such as canine atopic dermatitis, the combination of Th2 polarization and microbial presence may lead to IL-31 mediated effects driving inflammation and pruritus via induction of signaling in macrophages and direct neuronal interaction.

FUNDING: Zoetis

CONFLICT OF INTEREST: All authors are employees and shareholders of Zoetis Incorporated

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TITLE: *DERMATOPHAGOIDES FARINAE*-SENSITIZED BEAGLES ARE TH2-POLARIZED AND HAVE ANTIGEN-SPECIFIC IGE CONSISTENT WITH DOGS WITH ATOPIC DERMATITIS.

DERMATOPHAGOIDES FARINAE-SENSITIZED BEAGLES ARE TH2-POLARIZED AND HAVE ANTIGEN-SPECIFIC IGE CONSISTENT WITH DOGS WITH ATOPIC DERMATITIS.

M ALEO, C RUGG, E MCCANDLESS, L SLY, B GALVAN, T FLECK, W HUMPHREY, E COSCARELLI, S MAHABIR, R MCCALL, A GONZALES

Zoetis, Kalamazoo, MI, USA

REFERENCE: *Veterinary Dermatology*, **23** (Suppl. 1), 57. P-008.

ABSTRACT: Canine atopic dermatitis (AD) has been reported to be associated with increased levels of allergen-specific IgE, as well as a Th1/Th2 cytokine imbalance (Th2 dominant) characterized by a low IFN-γ/IL-4 ratio. A relevant model of this condition should demonstrate immunological changes similar to those seen in clinical AD cases. To establish such a model, normal healthy beagle dogs were sensitized to house dust mites (HDM, Dermatophagoides farinae) over a 6 week period with a series of three subcutaneous injections of HDM antigen. Sensitization to HDM was confirmed with intradermal testing. The intradermal testing included six serial dilutions of D. farinae to determine the degree of sensitivity, as well as positive (Histamine, Sigma-Aldrich; St.Louis, MO, USA) and negative (phosphate buffered saline) controls. Sensitized dogs had at least a two-fold increase in circulating free and HDM-specific IgE for at least 7 weeks post-sensitization. IFN-γ and IL-4 protein levels were also measured pre- and post-sensitization in isolated peripheral blood mononuclear cells by ELISpot. The HDM-sensitized dogs had increased antigen-specific IL-4 levels and reduced IFN-γ/IL-4 ratios post-sensitization, thus confirming a cytokine profile polarized to Th2. Therefore, this model possesses the immunological imbalances frequently seen in AD dogs and may be a useful tool to investigate potential restorative therapies.

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TITLE: IL-31: ITS ROLE IN CANINE PRURITUS AND PREVALENCE IN NATURALLY OCCURRING CANINE ATOPIC DERMATITIS

IL-31: ITS ROLE IN CANINE PRURITUS AND PREVALENCE IN NATURALLY OCCURRING CANINE ATOPIC DERMATITIS

A. GONZALES, W. HUMPHREY, J. TEEL, G. FICI, J. MESSAMORE, C. CHIO, J. SHELLY, M. ALEO, E. COSCARELLI, T. FLECK, B. GALVAN, J. CURRY, D. MANN, D. WHEELER, S. COSGROVE, P. AEED, P. CLARE, G. BAMMERT, S. DUNHAM, S.MAHABIR, G. BAINBRIDGE, T. FULLER, O. MARTINON, K. GREENWOOD, R. MCCALL

Veterinary Medicine Research & Development, Zoetis, Kalamazoo, MI, USA

REFERENCE: *Veterinary Dermatology*, **23** (Suppl. 1), 6. Supporting Original Study 5.

Cytokines are secreted signaling proteins that play a key role in cell-to-cell communication; however, their dysregulation can contribute to a variety of chronic diseases. Interleukin (IL)-31 is a recently identified member of the gp130/IL-6 cytokine family that is produced by a variety of human cell types including Th2 lymphocytes and CLA+ skin homing T cells. When over-expressed in transgenic mice, IL-31 induces severe pruritus, alopecia, and skin lesions. In humans, IL-31 serum levels correlate with severity of atopic dermatitis (AD) in adults and children. As with humans, atopic dermatitis in dogs is believed to be a multifactorial disease determined by a combination of genetic and environmental factors affecting the skin barrier, immune system, and neurological responses. Because of the complexity of this disease, there remains a need for novel therapeutic approaches that provide safer and more effective control of atopic dermatitis throughout the lifetime of the animal.

To facilitate improvements in the treatment and management of canine atopic dermatitis, investments in expanding our understanding of the pathobiology of the disease must be made. Therefore, investigational studies were performed to evaluate the role of IL-31 in canine pruritus and its prevalence in dogs diagnosed with atopic dermatitis.

Recombinant canine IL-31 was generated by polymerase chain reaction (PCR) amplification of canine IL-31 (cIL-31) sequences using cDNA prepared from canine testis tissue. PCR products were then cloned into expression constructs that were transfected into mammalian cells for protein production followed by purification. To evaluate the biological function of purified cIL-31, the canine DH82 histiocytoma line was stimulated with cIL-31in vitro, and activation of signal transduction pathways were evaluated using western blotting and immunoassay techniques. The role of IL-31 in canine pruritus was evaluated in a laboratory model using purpose-bred beagles. Beagles were administered cIL-31 (10-40 ug) via several routes (intravenous, subcutaneous, intradermal), and pruritic behavior was observed/quantitated via video monitoring and use of a categorical scoring system. Finally, quantitative immunoassay techniques were employed to measure serum levels of IL-31 in samples obtained from non-diseased client-owned dogs, purpose-bred beagles and client owned dogs diagnosed with naturally occurring atopic dermatitis.

Purified cIL-31 protein was able to activate JAK/STAT and MAPK signal transduction pathways in the canine DH82 histiocytoma cell line in a dose dependent manner. Injection of cIL-31 into beagles caused

transient episodes of pruritic behavior such as licking, scratching, head-shaking and body-rubbing, regardless of administered route. When quantitated over a 2 h period, dogs receiving cIL-31 exhibited a significant increase in pruritic behaviour compared to placebo-administered controls. Finally, cIL-31 levels were detectable in over 50% of dogs with naturally occurring atopic dermatitis (≥ 13 pg/mL) but were below limits of quantitation (< 13 pg/mL) in normal, non-diseased animals.

These findings suggest that cIL-31 is functionally active against canine cells and can induce pruritus in dogs. Moreover, cIL-31 was detectable in the majority of dogs with atopic dermatitis, suggesting an important role for this cytokine in pruritic skin conditions such as atopic dermatitis in this species.

FUNDING: Zoetis, Kalamazoo, Michigan.

CONFLICT OF INTEREST: All authors are employees and shareholders of Zoetis Incorporated.