Canine Atopic Dermatitis Immunotherapeutic* 
*First to Know Slides 
September 2015

*This product license is conditional. Safety and efficacy studies are in progress.
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PATHOGENESIS OF CANINE ATOPIC DERMATITIS
Cytokines: An Excellent Therapeutic Target

- Cytokines have a role in pathologic processes and development of clinical manifestations of allergic skin disease in dogs
  - Involved in **pruritus** of acute and chronic conditions
  - Role in **inflammation**, skin barrier deterioration and fueling of the cycle of itch seen in chronic atopic dermatitis

Cytokines: The Common Denominator in Allergic Skin Disease

Adaptive Immune Response
- IL-2 (JAK1, JAK3)
- IL-4 (JAK1, JAK3)\(^1,2\)
- IL-5 (JAK2)\(^1\)
- IL-9 (JAK1, JAK3)
- IL-10 (JAK1, Tyk2)
- IL-11 (JAK1)
- IL-12 (JAK2 and Tyk2)\(^1\)
- IL-13 (JAK1)\(^1,2\)
- IL-25
- IL-31 (JAK1, JAK2)\(^2\)
- INF-gamma (JAK1, JAK2)
- RANTES (JAK independent)

Innate Immune Response
- TNF-alpha (JAK independent)
- IL-1 (JAK independent)
- IL-6 (JAK1, JAK2, Tyk2)
- IL-18 (JAK independent)\(^1\)
- GM-CSF (JAK2)\(^1\)
- TSLP (JAK1, ?)\(^2\)

\(^1\) Genetic link shown in human atopic dermatitis to date
\(^2\) Overexpression in transgenic mice induces pruritus and skin lesions

Cytokines Currently Implicated in Pruritus

- **Interleukin (IL)-2**
  - Human cancer patients treated with high dose IL-2 therapy can experience severe pruritus
  - IL-2 injected into mice induces pruritus

- **IL-4**
  - IL-4 transgenic mice exhibit pruritic behaviors and skin changes similar to allergic skin disease

- **IL-13**
  - IL-13 transgenic mice exhibit pruritic behaviors and skin changes similar to allergic skin disease

- **IL-31**
  - Largest body of data implicating IL-31 in pruritic skin conditions

IL-31 is Associated with Pruritus and Atopic Dermatitis in Humans

- IL-31 mRNA is produced by Th2 cells
  - After binding to its receptor it induces JAK/STAT signaling, scratching behavior and dermatitis in IL-31 transgenic mice*
  - IL-31 mRNA is present in CLA+ skin homing T-cells in human AD patients†
  - IL-31 mRNA is over expressed in pruritic atopic skin conditions compared to nonpruritic psoriatic skin inflammation‡

IL-31 is Associated with Pruritus and Atopic Dermatitis in Humans

- Inhibition of canine IL-31 activity may be a viable therapeutic approach for the treatment of atopic dermatitis and pruritus associated with allergic skin disease
  - IL-31 serum levels correlate with severity of atopic dermatitis in adults and children\(^*\) †‡
  - IL-31 receptor subunits have been reported in a subset of small-sized nociceptive neurons of adult mouse and human dorsal root ganglia\(^*\) £

IL-31 is Produced by Th2 Polarized Cell Cultures

- PBMCs isolated from HDM sensitized dogs are polarized toward a Th2 phenotype and produce cytokines such as IL-4 and IL-31

Canine IL-31 was cloned, expressed and purified at Zoetis.

IL-31 binds the heterodimeric receptor consisting of IL-31RA and OSMR beta.

IL-31 binding activates the following pathways:
- JAK/STAT
- MAPK (ERK1/2)
- PI3K/Akt
Canine IL-31 Induces JAK/STAT and MAPK Pathways in Canine DH82 Cells

In Laboratory Model Studies Canine IL-31 Induces Pruritic Behaviors in Dogs

- cIL-31 was injected i.v. into laboratory beagle dogs
- Pruritic behavior scored over a 2 hour observation window

* T01 vs T03, \( p=0.0004 \)
** T02 vs T03, \( p=0.0003 \)

IL-31 is Detected in ~ 50% of Canine Patients with Naturally Occurring Atopic Dermatitis

- Cytokines tend to act locally so IL-31 may not be detected in serum

<table>
<thead>
<tr>
<th>Canine Populations</th>
<th>Number of Animals Evaluated</th>
<th>Number of Animals with Detectable IL-31 in Serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose-bred beagles</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Purpose-bred beagles sensitized to HDM</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Mixed breed dogs – no fleas</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Mixed breed dogs – infested with fleas</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Healthy client owned animals - multiple breeds</td>
<td>87</td>
<td>0</td>
</tr>
<tr>
<td>Naturally occurring atopic dermatitis in client owned animals – multiple breeds</td>
<td>224</td>
<td>128</td>
</tr>
</tbody>
</table>

*Less than 13 pg/mL is below limits of detection.

Caninized (c)IL-31 Induces Pruritus in Dogs Regardless of the Route of Injection

- Animal behavior recorded by video monitors
- Pruritic behavior displayed during a 4 hr window post-cIL-31 treatment-measured in actual time (seconds)

cIL-31-induced Pruritus Model Validated by Response to Prednisolone

Pruritic score

Day 0

Day 6

Placebo
Prednisolone @ 0.50 mg/kg BID

*\( p=0.0656 \)

**\( p=0.0003 \)

Reference: Study Report No. 7D61R-60-11-B68, Zoetis Inc.
Atopic Dermatitis – Sensitization
Atopic Dermatitis – Progression
Cutaneous Itch – Stimulation of Selective Neurons in the Skin
IL-31 Receptors Located on Many Different Cell Types
DIAGNOSIS OF ATOPIC DERMATITIS IN DOGS
Is the Dog Itching Because It’s Infected or Because It’s Allergic?

Infectious Dermatoses

1. Rule out scabies with history, physical examination, negative skin scraping and lack of response to treatment
   - Selamectin q 30 days × 2
   - Ivermectin 14 days × 3, lime sulfur q 14 days × 3, fipronil spray q 14 days × 3, milbemycin, organophosphate rinses
   - Make sure to treat all in-contact dogs

2. Rule out *Malassezia* colonization with physical examination, cutaneous cytology and lack of response to treatment
   - Ketoconazole 5-10 mg/kg qd × days
   - Also itraconazole, fluconazole or terbenifine
   - Antifungal shampoo—PRN, 10 minutes of contact time (chlorhexidine, miconazole, ketoconazole, selenium)
   - Leave-on rinse (25% vinegar, miconazole, ketoconazole, pH modifier)
3. Rule out *Staphylococcus* colonization with physical examination, cutaneous cytology and lack of response to treatment

- Identify and treat the underlying cause
  - Skin scrape to rule out demodicosis
  - Check thyroid status
  - Weight loss if infection involves intertriginous areas

- Systemic antibiotic for minimum of 14 days for superficial infections
  - Cefovecin, cefpodoxime, amoxicillin/clavulanic acid, cephalexin, clindamycin, lincomycin, ormetoprim/sulfadimethoxine
  - Antibacterial shampoo—PRN, 10 minutes of contact time (benzoyl peroxide, ethyl lactate, chlorhexidine, triclosan)
Allergic Dermatoses

4. Rule out flea bite hypersensitivity with history, physical examination, identification of fleas, flea feces and/or intradermal test with flea allergen
   - Treat or prevent fleas
     ▪ Selamectin, fipronil, imidacloprid, etc.
   - Treat secondary infections with antibiotics or anti-yeast products
   - Treat pruritus with a short course of corticosteroids

5. Rule out an adverse reaction to foods with history, physical examination and a minimum of 1 month of a novel protein and carbohydrate diet trial
6. Diagnose atopic dermatitis with history, physical examination, positive response to treatment with corticosteroids and exclusion of all other causes of pruritus
   - Treat with Apoquel® (oclacitinib tablet)
   - Treat with safe doses of corticosteroids
   - Control infections
   - Try antihistamines, ω-3 fatty acids or cyclosporin
   - Intradermal test or allergen-specific IgE serology to select allergens for allergen-specific immunotherapy
Atopic Dermatitis is a Diagnosis of Exclusion

“Illness Diseases”
1. Sarcoptes scabiei
2. Malassezia colonization
3. Staphylococcal pyoderma

Demodex
Hypothyroidism
Conformation (obesity)

4. Other (dermatophytosis, Cheyletiella, lice, etc)

Allergic Diseases
1. Flea allergy dermatitis
2. Atopic dermatitis

Food
Non-IgE (atopic-like)

Environmental allergens

3. Other allergic diseases (drug eruptions, contact allergy, etc)
Atopic Dermatitis is a diagnosis of Exclusion.
Diagnostic Approach to Pruritus in Dogs

**History**
- Age of onset
- Seasonality
- Acute or Chronic
- Skin lesions - timing of onset
- A rash that itches or An itch that exists?
- Response to treatment

**Physical Exam**
- Assess pattern of lesions
- Primary or Secondary
- Cost combing
- Hair plucks

**Cytology**
- NO
  - POSITIVE
    - CONSIDER OTHER PRURITIC DIAGNOSES
  - YES
    - LEARN PRURITUS TRIGGER
      - DOES NOT ELIMINATE PRURITUS TRIGGER
        - TREAT ACCORDING TO RESULTS
          - IDENTIFY UNDERLYING CAUSE & TREAT
            - Hypothyroidism
            - Demodicosis
            - Conformational folds (obesity)
            - Other

**Lesion**
- Dorsal thorax

**Physical Exam**
- Observe rashes/dirt

**Flea Allergy Dermatitis**
- Pruritus, lentil caudal elbows, ventrum, etc.

**Skin Scraping**
- Scurries

**Trial Therapy**
- Positive response

**Lesion**
- Papules, papules, crusting, serpentine, etc.

**Sarcoptes**
- Lesion
  - Personal, groin, face, pruritus, head, caudal elbow

**Historical Findings**
- GI signs (xantholipemia)

**Trial Therapy**
- Diet trial

**Cutaneous Adverse Reaction to Food**
- Lesion
  - Caudal carpus
  - Caudal tarsus
  - Pelvicol/perineal
  - NOT distalnumeric
  - NOT ear margins

**Atopic Dermatitis**
- YES
ELEMENTS OF MULTIMODAL THERAPY FOR CANINE ATOPIC DERMATITIS
# Treatment for Canine AD is Often Multimodal

<table>
<thead>
<tr>
<th>Options</th>
<th>Efficacy</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential Fatty Acids (ω3 and ω6) *</td>
<td>~ 25%</td>
<td>Improvement in haircoat and skin</td>
<td>Hi dose needed No evidence of efficacy</td>
</tr>
<tr>
<td>Antihistamines *</td>
<td>25 – 30%</td>
<td>Inexpensive Perceived safety</td>
<td>No evidence of efficacy No FDA-approved product for dogs</td>
</tr>
<tr>
<td>Glucocorticoids *</td>
<td>Very effective in treating itching &amp; inflammation</td>
<td>Rapid onset</td>
<td>Immediate annoying side effects Serious over time</td>
</tr>
<tr>
<td>Cyclosporin *</td>
<td>Shown to work in 74% of case of AD</td>
<td>FDA-approved</td>
<td>GI upset Delayed efficacy</td>
</tr>
<tr>
<td>ASIT *</td>
<td>Varying (~ 2/3 dogs show some improvement after 1 year)</td>
<td>May target the root of the condition</td>
<td>May take up to a year to work</td>
</tr>
<tr>
<td>Topicals (shampoos, rinses, sprays, ointments)</td>
<td>Varies</td>
<td>Removes allergen from the skin</td>
<td>Inconvenient for owners</td>
</tr>
<tr>
<td>Oclacinib +</td>
<td>Up to 67% reduction in pruritus ~ 48% reduction in skin lesions</td>
<td>Rapid onset Improvement in pruritus and skin condition No PU/PD</td>
<td>Daily oral administration</td>
</tr>
</tbody>
</table>

Fatty Acids

Allergens
Including staph and malassezia

Scratching

Neuronal Itch Stimulation

Inflammatory Process

Cytokines e.g. IL-17, TNF-α

Cytokines e.g. IFN-γ

Mast Cell

Neutrophil

Monocyte

Eosinophil

Sensory Nerve

Blood Vessel

Th1 Cell

Th2 Cell

Dendritic Cell

Langerhans cell

Allergen Presentation Th Cell

Cytokines e.g. aaTSLP
Antihistamines

Allergens
Including staph and malassezia

Scratching

Neuronal Itch Stimulation

Inflammatory Process

Ah

Langerhans cell

Dendritic Cell

Ah

Neutrophil

Monocyte

Th1 Cell

Th2 Cell

Eosinophil

Sensory Nerve

Blood Vessel

Ah

Cytokines e.g. IL-1, TNF-α

Cytokines e.g. IFN-γ

Cytokines e.g. aTSLP
Glucocorticoid

Allergens
Including staph and malassezia

Scratching

Neuronal Itch Stimulation

Inflammatory Process

Langerhans cell

Cytokines e.g. aaTSLp

Sensory Nerve

Blood Vessel

Dendritic Cell

Mast Cell

Neutrophil

Monocyte

Eosinophil

Th1 Cell

Th2 Cell

Cytokines e.g. IFN-γ

Cytokines e.g. IL-1, TNF-α
Glucocorticoid Activity

Changes happen in most every cell of the body since corticosteroid receptors are in almost every cell.

1. Corticosteroid diffuses into cell
2. Corticosteroid binds corticosteroid receptor, and...
3. Protein complex dissociates
4. RNA polymerase transcription of DNA
5. Translation of mRNA produces proteins
6. Cell function altered

Kochevar D. *Hill’s Symposium on Dermatology* 2006
Systemic glucocorticoids are indicated for treatment of pruritic dermatoses

- Commonly used therapeutic choices
- Highly effective
- Limited by high incidence of side effects
- Prednisone, prednisolone, methylprednisolone are commonly used oral agents
Side Effects of Glucocorticoid Therapy can be Bothersome and Medically Important

Common side effects in dogs

- Polyuria, Polydipsia, Polyphagia
- Weight gain
- GI disturbance
- Diarrhea, Melena
- Vomiting
- Possible behavioral changes (depression, hyperactivity, aggression)
- Panting
- Hyperlipidemia, Elevated liver enzymes
- Diabetes mellitus
- GI ulceration, Pancreatitis (high doses)
- Muscle wasting (high doses)
- Poor hair coat (long-term treatment)

Canine Organs Affected by Corticosteroids

CNS
• Polydipsia / Polyuria
• Mood changes

LYMPH NODES
• Suppression of the immune system
• Lymphopenia

HEART & BLOOD VESSELS
• Water retention
• Muscle weakening

MUSCLE
• Thinning
• Weakness
• Pendulous abdomen
• Temporal muscle atrophy

LIVER
• Fat accumulation
• Elevated liver enzymes

ADRENAL GLANDS
• Suppression or iatrogenic hyperadrenocorticism (Cushing’s disease)

KIDNEYS
• Polyuria
• Altered electrolyte balance
• Protein losing glomerulonephropathy

PANCREAS
• Predisposed for type II diabetes
• Predisposed for pancreatitis

SKIN & FUR
• Hair loss
• Thinning of the skin
• Increased susceptibility to infection
• Calcinosis cutis

BLADDER
• Increased susceptibility to infection
Activation of T Lymphocytes

1. Antigen presentation by antigen presenting cell

2. Increase in cytoplasmic calcium (Ca^{2+})

3. Calcineurin activated

4. NFAT (Nuclear factor of activated T-cells, a transcription factor) dephosphorylated and activated

5. RNA polymerase transcription of DNA

6. Translation of mRNA produces proteins

7. Cell function altered

Atopica FOI, 2003
Cyclosporin
1. Antigen *presentation* by antigen presenting cell

2. Increase in cytoplasmic calcium (Ca\(^{2+}\))

3. cyclosporin – cyclophilin complex *bind* and *inhibit* calcineurin

Inhibition works upstream blocking pro-inflammatory cytokines such as IL-2, and pro-inflammatory pathways

Atopica FOI, 2003
Matsuda S. et al. *Immunopharmacology* 2000
Atopica® (cyclosporin capsules) is indicated for control of atopic dermatitis in dogs

- Oral immunosuppressive agent
- Delayed action of efficacy
- Efficacy comparable to glucocorticoids after 3 weeks of therapy
- Evaluated for control of canine atopic dermatitis
- Capsules can’t be broken or split
Vomiting and Diarrhea are Reported in >20% of Dogs Treated with Atopica*

Cyclosporin side effects in dogs

- Vomiting
- Diarrhea
- Soft stools
- Bacterial pyoderma
- Anorexia
- Lethargy
- Gingival hyperplasia

Published reports of masked controlled clinical trials treating atopic dermatitis in dogs show that glucocorticoids decreased owner VAS measurement of pruritus 33%-81%

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Design</th>
<th>Efficacy</th>
<th>Safety</th>
</tr>
</thead>
</table>
| Atopic dermatitis* | Prednisolone 0.5 mg/kg SID for 6 weeks (double-masked; n=14) | • 69% (30-84%) mean reduction from baseline in CADESI-02 lesion scores  
• 81% (45-86%) mean reduction from baseline in owner VAS scores | • 43% (6/14) of dogs experienced an adverse event |
| Atopic dermatitis† | Methylprednisolone 0.5-1 mg/kg SID for 1 week, then EOD for 3 weeks; dose tapered at the end of the 4 week period (single-masked; n=59) | • 45% (35-56%) mean reduction from baseline in CADESI-02 lesion scores  
• 33% (23-43%) mean reduction from baseline in the owner VAS scores | • 15% (9/59) of dogs dropped out for inefficacy  
• 19% (11/59) did not complete study due to worsening clinical signs  
• Polyuria/polydipsia occurred in 25% (15/59) |

Successful Therapy for Canine Atopic Dermatitis: A Historical Perspective from the Literature

Published reports of masked controlled clinical trials treating atopic dermatitis in dogs show that cyclosporin decreased owner VAS measurement of pruritus 36%-78%

<table>
<thead>
<tr>
<th>Diagnosis</th>
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<th>Efficacy</th>
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<tbody>
<tr>
<td>Cyclosporin</td>
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<td></td>
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</tr>
<tr>
<td>Atopic dermatitis</td>
<td>cyclosporin 5 mg/kg SID for 6 weeks</td>
<td>• 58% (43-74%) mean reduction from baseline in CADESI-02 lesion scores</td>
<td>• 31% (4/13) of dogs experienced adverse events</td>
</tr>
<tr>
<td></td>
<td>(double-blinded; n=13)</td>
<td>• 78% (52-87%) mean reduction from baseline in owner VAS scores</td>
<td>• 20% of dogs on cyclosporin occasionally developed diarrhea or soft stools</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Atopic dermatitis †</td>
<td>cyclosporin 5 mg/kg SID for 4 months;</td>
<td>• 52% (44-59%) mean reduction from baseline in CADESI-02 lesion scores</td>
<td>• 9% (10/117) of dogs dropped out for inefficacy (increase CADESI-02 scores from baseline</td>
</tr>
<tr>
<td></td>
<td>dose tapered at the end of 4 weeks of</td>
<td>• 36% (27-43%) mean reduction from baseline in owner VAS scores</td>
<td>• Vomiting 37% (43/117)</td>
</tr>
<tr>
<td></td>
<td>treatment according to clinical response</td>
<td></td>
<td>• Diarrhea 18% (21/117)</td>
</tr>
<tr>
<td></td>
<td>(single-blinded; n=117)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>cyclosporin 5 mg/kg SID for 30 days</td>
<td>• 45% reduction in CADESI-02 lesion scores versus 9% in placebo-treated dogs</td>
<td>Of the 265 total number of dogs treated:</td>
</tr>
<tr>
<td></td>
<td>followed by up to 16 weeks at SID, EOD, or</td>
<td>• 74% of dogs showed an improvement in pruritus versus 24% in placebo-</td>
<td>• Vomiting 30.9%</td>
</tr>
<tr>
<td></td>
<td>twice per week (Phase 2; n=192)</td>
<td>treated dogs</td>
<td>• Diarrhea 20.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Persistent otitis externa 6.8%</td>
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<td></td>
<td></td>
<td></td>
<td>• UTI 3.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Anorexia 3.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Lethargy 2.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Gingival hyperplasia 2.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Lymphadenopathy 2.3%</td>
</tr>
</tbody>
</table>

Allergen-specific Immunotherapy
1. JAK inhibitors bind JAK
2. Cytokine binds receptor
3. Receptor dimerization

JAK (Janus kinase)

APOQUEL

3. JAK inhibitors block downstream activity in the cell

JAK inhibitors only work to block the activity in cells where activity is mediated by cytokines that work through JAK

APOQUEL® (oclacitinib tablet) Activity
APOQUEL® should not be used in dogs less than 12 months of age or in dogs with serious infections. APOQUEL may increase the susceptibility to infection and demodicosis and may exacerbate neoplastic conditions. APOQUEL has not been evaluated in combination with systemic immunosuppressive agents such as glucocorticoids or cyclosporine. APOQUEL should not be used in breeding dogs, or pregnant or lactating dogs. The most common side effects seen in dogs administered APOQUEL were vomiting and diarrhea. APOQUEL has been safely used in conjunction with other common medications including antibiotics and parasiticides and with vaccinations.

See full prescribing information at:
www.APOQUEL.com/APOQUEL_PI
INTRODUCTION TO MONOCLONAL ANTIBODY THERAPY
REVIEW OF THE IMMUNE SYSTEM
Organization of the Immune System

IMMUNE SYSTEM ORGANIZATION

INNATE IMMUNITY

ADAPTIVE IMMUNITY

ANTIBODY-MEDIATED

CELL-MEDIATED
Cells of the Innate Immune System

- **Myeloid cells**
  - Rapid Response (hours)
  - Orchestrates adaptive immune responses
Cells of the Adaptive Immune System

- Lymphoid cells
  - Slower responses
  - Highly specialized antigen receptors (e.g. TCR and BCR)
  - Memory develops, which may provide lifelong immunity to reinfection with the same pathogen
    - e.g. antibodies, cytotoxic T-cells
Innate and Adaptive Immune Systems Working Together

T-Cell

Antigen Presenting Cell

Naive B Lymphocyte (B Cell)

Antigen Processing

Cytokine Release (IL-4, IL-13)

Activation

B Cell – T Cell Interaction

Activation

Differentiation and Proliferation

Activated B Lymphocyte

Plasma Cells

Memory B Lymphocyte

Antibodies
HARNESSING THE BENEFICIAL EFFECTS OF THE IMMUNE SYSTEM

History of Antibodies and their Therapeutic Potential
History of Monoclonal Antibodies and their Therapeutic Potential (Video)

Click on the arrow to start the video
THERAPEUTIC USE OF ANTIBODIES
A HISTORICAL PERSPECTIVE

1760’s
COWPOX OBSERVED TO PROTECT AGAINST SMALLPOX
Edward Jenner

1760’s
COWPOX OBSERVED TO PROTECT AGAINST SMALLPOX
Edward Jenner

1879
FIRST LABORATORY VACCINE CREATED
Louis Pasteur

1879
FIRST LABORATORY VACCINE CREATED
Louis Pasteur

1890
FIRST USE OF ANTIBODIES TO TREAT DISEASE
Shibasaburo Kitasato, Emil von Behring

1890
FIRST USE OF ANTIBODIES TO TREAT DISEASE
Shibasaburo Kitasato, Emil von Behring

1920’s
PROTEINS IDENTIFIED AS THE BUILDING BLOCKS OF ANTIBODIES
Michael Hiedelberger, Oswald Avery

1920’s
PROTEINS IDENTIFIED AS THE BUILDING BLOCKS OF ANTIBODIES
Michael Hiedelberger, Oswald Avery

1940’s
ANTIBODY EXQUISITE SPECIFICITY DEMONSTRATED, LOCK-AND-KEY MECHANISM CONFIRMED
Linus Pauling

1940’s
ANTIBODY EXQUISITE SPECIFICITY DEMONSTRATED, LOCK-AND-KEY MECHANISM CONFIRMED
Linus Pauling

1975
MONOCLONAL ANTIBODIES FIRST DEVELOPED IN THE LABORATORY
César Milstein, Georges J. Köhler

1975
MONOCLONAL ANTIBODIES FIRST DEVELOPED IN THE LABORATORY
César Milstein, Georges J. Köhler

1990’s
THERAPEUTIC ANTIBODIES IMPROVED

1990’s
THERAPEUTIC ANTIBODIES IMPROVED

2000’s
HUMAN ANTIBODY THERAPIES PROGRESS

2000’s
HUMAN ANTIBODY THERAPIES PROGRESS

2010’s
ANTIBODY THERAPIES ARE BEING DEVELOPED FOR VETERINARY MEDICINE

2010’s
ANTIBODY THERAPIES ARE BEING DEVELOPED FOR VETERINARY MEDICINE

1000’s
DISCOVERING A PROTECTIVE INFECTION
China

1000’s
DISCOVERING A PROTECTIVE INFECTION
China

IMAGE CREDITS: Historical images: National Library of Medicine, History of Medicine; Antibody models: (top) Data from RCSB Protein Data Bank, (bottom) Zoetis, Inc.
1000’s

DISCOVERING A PROTECTIVE INFECTION

China

Material taken from smallpox (variola) infected patients (dried scabs) and put into another person to create a mild, but protective infection - called variolation.

Image: HISTORICAL IMAGE OF SMALLPOX LESIONS
Inset: MICROGRAPH OF SMALLPOX VIRUS

(image: National Library of Medicine, History of Medicine)
1760’s

COWPOX OBSERVED TO PROTECT AGAINST SMALLPOX / Edward Jenner

OBSERVED that dairy workers did not contract smallpox, because they had already been infected with the cowpox virus. Jenner inoculated pus from a cowpox blister into a boy. When infected with smallpox, the boy did not become ill.

Image: CHILDREN BEING INOCULATED WITH COWPOX (National Library of Medicine, History of Medicine)
1879

FIRST LABORATORY VACCINE CREATED

Louis Pasteur

Louis Pasteur made a series of important discoveries in the fields of vaccination and pasteurization. He produced the first laboratory-developed vaccine: the vaccine for chicken cholera in 1879.

Image: LOUIS PASTEUR
Inset: PHOTOMICROGRAPH OF CHOLERA
(image: National Library of Medicine, History of Medicine)
1890

FIRST USE OF ANTIBODIES TO TREAT DISEASE

Shibasaburo Kitasato, Emil von Behring

Kitasato and von Behring showed that they could cure diphtheria in pigs by injecting them with the blood of an immunized animal.

Image: PHOTOMICROGRAPH OF DIPHtheria
Inset: DR. KITASATO INJECTING A HORSE FOR IMMUNIZATION
(images: background - Centers for Disease Control; inset: National Library of Medicine)
1920’s

PROTEINS IDENTIFIED AS THE BUILDING BLOCKS OF ANTIBODIES

Michael Hiedelberger, Oswald Avery

Demonstrated that antibodies are comprised of proteins.

Inset: MOLECULAR STRUCTURE OF AN ANTIBODY
Image: LAB NOTEBOOK, MICHAEL HIEDELBÉRGER
1940’s

ANTIBODY EXQUISITE SPECIFICITY DEMONSTRATED – LOCK-AND-KEY MECHANISM CONFIRMED

Linus Pauling

Confirms the lock-and-key mechanism theory of Paul Ehrlich from the 1890s. Proving that binding was dependent upon the shape, rather than the chemical composition.
1975

MONOCLONAL ANTIBODIES FIRST DEVELOPED IN THE LABORATORY

César Milstein, Georges J. Köhler

The technique uses hybrid cell lines, which grow in culture, to produce single-specificity (to a specific antigen) monoclonal antibodies.

Milstein and Köhler jointly received the 1984 Nobel Prize in Medicine and Physiology for developing the hybridoma techniques of producing antibodies with single specificity.

(image: top - Link Studio, LLC)
1990’s

THERAPEUTIC ANTIBODIES IMPROVED

Antibody engineering leads to molecular modifications to enhance therapeutic potential of antibodies (longer half-life, tighter binding, decreased immunogenicity risks)

*Image: ANTIGEN – ANTIBODY BINDING*
2000's

HUMAN ANTIBODY THERAPIES PROGRESS

New technologies used for discovering antibodies for human therapy. Antibodies produced to neutralize disease causing proteins in multiple therapeutic areas.

EXAMPLES OF NEW MEDICINES

**Xolair®** (omalizumab) - developed for the control of moderate and severe asthma.

**Humira®** (adalimumab) - developed for the treatment of rheumatoid arthritis

(image: model from RCSB Protein Data Bank data)
ANTIBODY THERAPIES ARE BEING DEVELOPED FOR VETERINARY MEDICINE

Canine antibodies are engineered for various therapeutic applications in canine diseases.

Image: CANINE ANTIBODY
AN OVERVIEW
OF ANTIBODIES
Antibodies

- Y-shaped proteins
- Produced by mature B-cells (plasma cells)
- Found in the blood or other tissue fluids
- Used by the immune system to identify and neutralize foreign substances
- Isotypes in mammals
  - IgG, IgM, IgA, IgE, IgD
Antibody Structure

LOCK-AND-KEY
(CDR-Complementarity Determining Region)

A

Fragment antigen-binding (Fab) region

Light chains

Heavy chains

Antigen binding site

Fragment crystallizable (Fc) region

B

Antigen binding site

Fab region

Light chain

Fc region

Heavy chain
What is an Epitope?

A. Small protein antigen with one epitope, bound (lock-and-key) to an antibody

- **Antigen**
- **Epitope**
  
  *Region of an antigen that is specific to (and binds to) a certain antibody*

- **Antibody**

B. Large antigen containing multiple epitopes, bound (lock-and-key) to multiple antibodies

- **Large antigen (i.e., Parvovirus)**
- **Epitopes**
- **Antibody**

How Do Antibodies Work?

The body naturally produces antibodies in response to ‘foreign’ protein (antigen) as part of its normal response to disease.
How Do Therapeutic Antibodies Work?

Using the same principles, antibodies can now be administered by injection and used therapeutically.
Natural Antibodies and Therapeutic Antibodies Work in Similar Ways

A) Antibody binds and prevents binding of a protein to its receptor.

B) Antibody has an agonist/antagonist effect on a membrane receptor.

C) Antibody eliminates virus or malignant cell by complement or cytotoxicity. Complement initiates viral destruction cascade.
MONOCLONAL ANTIBODIES: THERAPEUTIC BY DESIGN
Types of Therapy

- **Pharmaceutical**
  - Low molecular weight, carbon-based chemical substances, synthesized using medicinal chemistry approaches or purified using organic chemistry approaches from natural sources, and developed to treat disease

- **Antibody Therapy**
  - Higher molecular weight protein-, DNA-, RNA- or cell-based products made from a living organism, or laboratory-produced versions of such substances, made to help treat disease
# Traditional Pharmaceuticals vs Antibody Therapy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Traditional Pharmaceuticals</th>
<th>Antibody Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition</strong></td>
<td>• Low MW organic chemicals, synthetic chemicals or plants</td>
<td>• Polyclonal or monoclonal antibodies</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td>• Small molecules (e.g. aspirin)</td>
<td>• Large MW protein macromolecules</td>
</tr>
<tr>
<td><strong>Route/Frequency</strong></td>
<td>• Mostly oral pills</td>
<td>• Injectable (SQ)</td>
</tr>
<tr>
<td></td>
<td>• Often daily administration</td>
<td>• Monthly or less often</td>
</tr>
<tr>
<td><strong>M.O.A. and Specificity</strong></td>
<td>• Drug-receptor interaction - size, shape</td>
<td>• Mimics natural interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Extreme specificity</td>
</tr>
<tr>
<td><strong>Targets</strong></td>
<td>• Intracellular targets (ex. bacterial cell walls, JAK enzymes)</td>
<td>• Extracellular and “self” targets (ex. cytokines, receptors)</td>
</tr>
<tr>
<td><strong>Metabolism, clearance</strong></td>
<td>• Hepatic, renal metabolism and elimination</td>
<td>• Protein catabolism; minimal hepatic, renal elimination</td>
</tr>
</tbody>
</table>
Why Develop Monoclonal Biotherapeutics Using Monoclonal Antibodies?

- Expand drug targets small molecules can’t reach
- More target-selective, less side-effects
- Mimic cellular interactions in the body
- Less frequent administration
- Large amounts produced in lab, can be frozen for future use
<table>
<thead>
<tr>
<th>Polyclonal Antibodies</th>
<th>Monoclonal Antibodies (mAbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A collection of antibodies secreted by different lineages of B-cells</td>
<td></td>
</tr>
<tr>
<td>- They will have different sequences from each other</td>
<td></td>
</tr>
<tr>
<td>- They will recognize different epitopes</td>
<td></td>
</tr>
<tr>
<td>A single pure homogeneous antibody preparation produced by a single lineage of B-cells</td>
<td></td>
</tr>
<tr>
<td>- They all have the same sequence</td>
<td></td>
</tr>
<tr>
<td>- They all recognize a single epitope</td>
<td></td>
</tr>
</tbody>
</table>
### Safety Considerations for Antibody Therapy

#### Augmented Pharmacology
- Consequences of the primary pharmacological effect
- Consequences of mAb effector function
- Cytotoxic properties on unintended cells or tissues

#### Immune Related
- Immunogenicity (animal develops antibodies to mAb)
- Injection site or infusion reactions
- Hypersensitivity reaction
- Effects of target-mAb complexes on organ systems
# Safety Evaluation 101: Where to Start?

<table>
<thead>
<tr>
<th>Contexts of Toxic Responses*</th>
<th>Illustrative Safety studies and tools...</th>
<th>Illustrative Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptor-driven / dose-titratable responses</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **Target-related**: interaction with the targeted receptor (incl. excess effect) | Drug:receptor binding  
Dose/Response, Overdose, Duration  
PK, ADME | Rimadyl® (carprofen) (COX inhibition) |
| **“Off-target”**-unintended & undesired receptor binding | Above plus *in vitro* screening studies of arrays of possible target receptors | Benadryl® (anti*cholinergic*) |
| **Metabolic Activation**: biotransformation to new active compound | Above plus special tests when indicated | Enalapril, Nitrofurazone |
| **Responses much less closely related to dose** | | |
| **Immunogenicity**—adaptive/acquired immunity | PV, follow-up studies; ADA & clinical consequences | Beta-lactams; Vaccines |
| **Idiosyncratic**—low frequency, difficult to predict or study | Large field studies; Pharmacovigilance | [Pharma’s] Vaccines |
| [Route of administration] | Clinical & pathology evaluations | [Injectables] |

* Guengerich, FP, *Drug Metab Pharmacokinet*, 2011
# Pre-Approval Evaluation for Adverse Effects: Classical Products

<table>
<thead>
<tr>
<th>Contexts of Toxic Responses(^1)</th>
<th>Classical Pharmaceutical “Small Molecule”</th>
<th>Classical Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptor-driven dose-titratable responses</strong>&lt;br&gt;Target related</td>
<td>Directly Evaluated</td>
<td>n/a (^2)</td>
</tr>
<tr>
<td>Off-Target</td>
<td>Directly Evaluated</td>
<td>n/a</td>
</tr>
<tr>
<td>Metabolic Activation</td>
<td>Dir. Eval. or Monitored (^3)</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Responses not closely related to dose</strong>&lt;br&gt;Immunogenicity</td>
<td>Monitored</td>
<td>Desired (^4)</td>
</tr>
<tr>
<td>Idiosyncratic</td>
<td>Monitored</td>
<td>Directly Evaluated (^4)</td>
</tr>
</tbody>
</table>

1 Guengerich, FP. *Drug Metab Pharmacokinet* 2011
2 n/a: potential for this context of toxic response is ~nil.
3 Monitored: response is low-frequency & unpredictable, difficult to methodically evaluate.
4 Cytokine-related or immune-related clinical signs can be more frequent for vaccines than other classes, yet still be considered acceptable.
### Contexts of Toxic Responses

<table>
<thead>
<tr>
<th>Contexts of Toxic Responses</th>
<th>Anticipated Adverse Effects¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target related effects (including unintended on-target)</td>
<td>Target pathway and downstream effects; Other, e.g., Drug:Target complex fate, effector function</td>
</tr>
<tr>
<td>“Off-target”-unintended &amp; undesired</td>
<td>Product-specific</td>
</tr>
<tr>
<td>Metabolized to new compound</td>
<td>Product-specific</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>ADA &amp; clinical consequences</td>
</tr>
<tr>
<td>Nonspecific effect (e.g. fever, malaise…)</td>
<td>Post-administration Rx, etc</td>
</tr>
<tr>
<td>[Route of administration]</td>
<td>Standard Injection site evaluations?</td>
</tr>
</tbody>
</table>

¹ These are “places to look” rather than known, definite adverse effects. The list is compiled from literature specific to the target and pathway, and from literature general to biopharma’s & pharma’s, immunology, dogs, the disease process, toxicology/pharmacology, etc.
## Current Applications in Human Health: mAbs You May be Familiar With…

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Target</th>
<th>Therapy Area</th>
<th>Approval Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Humira®</strong> <em>(adalimumab; AbbVie, Inc.)</em></td>
<td>TNF-α</td>
<td>Autoimmune/Gastrointestinal diseases</td>
<td>2002</td>
</tr>
<tr>
<td><strong>Xolair®</strong> <em>(omalizumab; Genentech/Novartis)</em></td>
<td>IgE</td>
<td>Respiratory-allergic asthma</td>
<td>2004</td>
</tr>
<tr>
<td><strong>Lucentis®</strong> <em>(ranibizumab; Genentech)</em></td>
<td>VEGF</td>
<td>Ophthalmology</td>
<td>2006</td>
</tr>
</tbody>
</table>
Antibody Therapy May Have Application to Many Therapeutic Areas in Companion Animal Medicine

- Osteoarthritis
  - Pain
- Atopic Dermatitis
- Chronic Kidney Disease
- Oncology
- Cardiac Disease

Biological Therapies
How are Antibody Therapies Created?

1. Immunize animals with target protein
2. Isolate B-cells from the spleen or lymph node
3. Identify cells that produce antibodies with desired binding properties to target

Optimize DNA sequences:
- ↑Affinity
- ↑Half-life
- ↓Immunogenicity
- Change effector function

Speciate the antibody
Identify key DNA sequences from the desired antibodies
Caninized Antibodies: Designed to be a Less Immunogenic Therapeutic Antibody for Dogs

Speciation is a key step to decreasing the potential immunogenicity of a therapeutic antibody

A Murine antibody
B Canine-murine chimeric antibody
C 90% Caninized antibody

Less Immunogenic
Absorption

- SC injection – therapeutic antibodies are injected into interstitial space
  - 50-100% bioavailability
- Therapeutic antibodies move from interstitial space into the bloodstream by several pathways
  - Transport via lymphatics to blood
  - Direct absorption into capillaries
  - Receptor-mediated cell uptake (endocytosis), transfer to the blood
Therapeutic antibodies stay in the blood
- Barriers to diffusion out of capillaries
- Lymphatic transport back to blood

Tissue distribution low, dictated by specific binding to receptors

Targets are circulating in blood or on cell surfaces – not inside cells.
Clearance mechanisms include:
- Binding to target
- Anti-mAb antibodies in circulation
- Flow out of capillaries into interstitial space, taken up by cells and catabolized

Therapeutic antibodies that attach to the FcRn within the endosome are protected from catabolization and are recycled back into the blood or lymph which extends half-life
Antibodies are catabolized to peptides and amino acids within cells

Antibodies are **NOT** metabolized by traditional metabolizing enzymes in the kidney or liver
- Drug-drug interactions rare
- Are not converted into reactive or toxic metabolites

Antibodies are **NOT** excreted in urine by the kidneys
Antibodies can be broken down by many cell types. Within the cells, antibodies are degraded into amino acids which are reused by the body.
Within endothelial cells, antibodies can be recycled and reused by the body.

A. Antibody is taken up via pinocytosis.

B. Within the acidic environment of the endosome, the antibody binds to the FcRn receptor.

C. The antibody is protected from degradation.

D. The antibody is recycled back into circulation.
Unlike Pharmaceuticals, Hepatic and Renal Elimination is Minimal with Therapeutic Antibodies

Therapeutic antibodies are degraded by normal pathways for protein catabolism, thus highly unlikely to induce liver or kidney toxicity

- Is the liver important for metabolism?
  - Radiolabeled mAb given to mice, at 24 h
    - 3.6% in liver

- Is the kidney important for metabolism?
  - Radiolabeled mAb given to mice, at 24 h
    - 2-3% in kidney
  - Renal filtration has a MW cutoff of approximately 30-50 kDa, mAbs (150 KD) not filtered

Hnatowich DJ, et al. Science 1983
OPPORTUNITY FOR TREATMENT OF CANINE ATOPIC DERMATITIS
Cytokines: The Common Denominator in Atopic Skin Disease
Cytokines Are Involved in Canine Allergic Skin Disease

Many cytokines implicated in allergic skin disease (e.g., Atopic Dermatitis) are secreted from activated T-lymphocytes.

- Langerhans cell
- T-lymphocyte

- IL-2: Activate other immune cells involved in allergy and inflammation
- IL-4
- IL-5: Induction of neuronal itch stimulation
- IL-6
- IL-13
- IL-31: Continued breakdown of skin barrier

Effective therapies for atopic dermatitis inhibit T-cell function. How they affect immune function or other organ systems may lead to differential safety profiles.
~ 50% of Dogs with Naturally Occurring Atopic Dermatitis Have Measurable Serum Levels of IL-31

<table>
<thead>
<tr>
<th>Canine Populations</th>
<th>Number of Animals Evaluated</th>
<th>Number of Animals with Detectable IL-31 in Serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose-bred beagles</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Purpose-bred beagles sensitized to HDM</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Mixed breed dogs – no fleas</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Mixed breed dogs – infested with fleas</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Healthy client owned animals-multiple breeds</td>
<td>87</td>
<td>0</td>
</tr>
<tr>
<td>Naturally occurring atopic dermatitis in client owned animals – multiple breeds</td>
<td>224</td>
<td><strong>128</strong></td>
</tr>
</tbody>
</table>

*Less than 13 pg/mL is below limits of detection.

In Laboratory Model Studies Canine IL-31 Induces Pruritic Behaviors in Dogs

- cIL-31 was injected i.v. into laboratory beagle dogs
- Pruritic behavior scored over a 2 hour observation window

*T01 vs T03, *p=0.0004. **T02 vs T03, *p=0.0003

IL-31 Plays a Major Role in Itch
Oclacitinib Potently Inhibits cIL-31-induced Itch in Laboratory Model

Fleck TJ, et al., NAVDF 2013
APOQUEL (oclacitinib tablet) Inhibits the Activity of Many Cytokines – Including IL-31

Many Cytokines Implicated in Allergic Skin Disease (e.g., Atopic Dermatitis) Are Secreted from Activated T-lymphocytes

APOQUEL Blocks the Activity of Pruritogenic and Pro-inflammatory Cytokines That Utilize JAK 1/JAK3
What Is the Role of IL-31 in the Animal?

- Regulates aspects of innate as well as adaptive immunity in tissues that are exposed to the environment.

- To date, IL-31 has only been identified to play a role in inflammatory diseases:
  - Skin disease – atopic dermatitis
  - Lung disease – allergic asthma
  - Bowel diseases – IBD

- Preliminary evidence that IL-31 controls the proliferation and differentiation of cells of non-hematopoietic origin, lending support to the concept that this cytokine plays an integrative role in the proper formation of epithelia.
Inhibiting IL-31 Using a Monoclonal Antibody Approach Holds Potential for Specificity and Low Toxicity

Corticosteroids bind corticosteroid receptors present in all cells
**Effects on:** immune system, CNS, metabolism, homeostasis

Atopica binds cyclophilin and inhibits calcineurin function
**Effects on:** NFAT signaling and antigen presentation

Apoquel inhibits JAK1 enzyme function
**Effects on:** JAK1 dependent cytokines driving clinical signs

Anti-IL-31 monoclonal antibody
**Effects on:** IL-31, a key JAK1-dependent cytokine driving clinical signs
Zoetis’ Target Product Profile for the Caninized Anti-cIL-31 Monoclonal Antibody Therapy

- A novel therapy for atopic dogs
  - Not a pharmaceutical therapy
  - Not a corticosteroid
  - Targets a single cytokine (IL-31)
- Injectable
- Duration of effect of one month
- Rapid onset of efficacy
  - Similar to prednisolone and APOQUEL in ability to reduce pruritus
  - Improvement in dermatitis/skin lesions within 7 days
- Unique Safety Profile
  - No immune suppression
  - No production of anti-mAb antibodies
  - No contraindications for other drugs or disease
  - Use in dogs without any limitation to age
So Zoetis Scientists Asked…

“Would inhibiting only IL-31 provide sufficient relief from itch and inflammation in canine patients with atopic dermatitis?”
CANINE ATOPIC DERMATITIS IMMUNOTHERAPEUTIC*

* Product license is conditional. Safety and efficacy studies are ongoing.
Monoclonal Antibody Generation
• Mouse immunization and characterization

In vitro evaluation/Speciation
• IL-31 binding evaluation
• In vitro inhibition of IL-31 signaling
• Conversion of mouse mAb to canine mAb

Laboratory Target Animal Safety

Field Safety and Efficacy in Target Patient Population
LABORATORY SAFETY STUDY
Laboratory Target Animal Safety Study in Dogs Demonstrates Margin of Safety Over 7 Month Study

**Objective**
- To demonstrate the margin of safety of Canine Atopic Dermatitis Immunotherapeutic in normal laboratory Beagles when administered SC at up to 10 mg/kg once monthly for 7 consecutive monthly doses

<table>
<thead>
<tr>
<th>Treatment (mg/kg)*</th>
<th>Administration</th>
<th>Route</th>
<th>Animals Enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Days 0, 28, 56, 84, 112, 140, 168</td>
<td>SC</td>
<td>6/sex/group (n=12)</td>
</tr>
<tr>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All dogs dosed according to the dosing chart

ZTS-00103289 was Safely Administered at up to 10 mg/kg for up to 7 Sequential Monthly Doses

- Randomized, placebo-controlled study
- 36 healthy, laboratory Beagle dogs (n=12 per group; 6 males/6 females)
- Doses tested were administered by subcutaneous (SQ) injection: 0 mg/kg (placebo), 3.3 mg/kg, 10 mg/kg ZTS-00103289 monthly for 7 months
- Dogs were approximately 4 months of age at Day 0
- Safety assessments included clinical signs, clinical pathology, complete histopathology, pharmacokinetics, and anti-ZTS-00103289 antibodies

ZTS-00103289 was Safely Administered at up to 10 mg/kg for up to 7 Sequential Monthly (con’t)

### Abnormal Clinical Observations (Data = Number of Dogs)

<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>Treatment</th>
<th>Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-7</td>
</tr>
<tr>
<td>Lameness</td>
<td>Placebo</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Placebo</td>
<td>0</td>
</tr>
<tr>
<td>Erythema ventral abdominal area</td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>3</td>
</tr>
<tr>
<td>Loss of condition</td>
<td>Placebo</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>3</td>
</tr>
<tr>
<td>Swollen urogenital area</td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>Thin</td>
<td>Placebo</td>
<td>0</td>
</tr>
<tr>
<td>Dose site mild localized erythema</td>
<td>Placebo</td>
<td>1</td>
</tr>
<tr>
<td>resembling a razor burn</td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>0</td>
</tr>
</tbody>
</table>

ZTS-00103289 was Safely Administered at up to 10 mg/kg for up to 7 Sequential Monthly (con’t)

<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>Treatment</th>
<th>0-7</th>
<th>28-35</th>
<th>56-63</th>
<th>84-91</th>
<th>112-119</th>
<th>140-147</th>
<th>168-175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased defecation</td>
<td>Placebo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 10 mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Placebo</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 10 mg/kg</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Estrus</td>
<td>Placebo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ZTS-00103289 3.3 mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
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ZTS-00103289 was Safely Administered at up to 10 mg/kg for up to 7 Sequential Monthly (con’t)

<table>
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<th>Clinical Sign</th>
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ZTS-00103289 was Safely Administered at up to 10 mg/kg for up to 7 Sequential Monthly (con’t)

### Abnormal Clinical Observations (Data = Number of Dogs)

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<tr>
<th>Clinical Sign</th>
<th>Treatment</th>
<th>Period (days)</th>
<th>0-7</th>
<th>28-35</th>
<th>56-63</th>
<th>84-91</th>
<th>112-119</th>
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<tr>
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<tr>
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<td>0</td>
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<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td></td>
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<td>1</td>
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<td>Emesis containing food</td>
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<td>0</td>
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<tr>
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<td>ZTS-00103289 10 mg/kg</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
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<td>Regurgitation</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

ZTS-00103289 was Safely Administered at up to 10 mg/kg for up to 7 Sequential Monthly (con’t)

- Results: no treatment effects over 6 months in any of the following safety evaluations
  - Body weight or food consumption
  - Hypersensitivity-related reactions or post-treatment fever
  - Anti-ZTS-00103289 antibodies
  - Clinical pathology or pathology evaluations
  - Special pathology evaluation of immune tissues
  - Clinical observations were normal background for lab dogs

- Injection sites: minor changes typical of any injected product

ZTS-00103289 was well tolerated for 7 consecutive monthly treatments at up to 10 mg/kg

- No apparent side effects from absence of normal constitutive function of IL-31 for 6 months
- Injection sites were normal
- No hypersensitivity-like responses to dose administration
- No anti-mAb antibodies

CLINICAL FIELD TRIALS

Safety and Efficacy in Client-owned Dogs with Atopic Dermatitis
Assessment of Efficacy in Client-Owned Dogs with Atopic Dermatitis

- **Objective**
  - To evaluate onset and duration of efficacy and safety of Canine Atopic Dermatitis Immunotherapeutic for reduction of clinical signs of atopic dermatitis (AD) in client-owned dogs
- All study personnel with the exception of the product dispenser were masked and unaware of treatment group assignments
- Dogs with worsening clinical signs of AD could drop out of the study and were counted as treatment failures from that point forward

<table>
<thead>
<tr>
<th>Canine Atopic Dermatitis Immunotherapeutic (mg/kg)</th>
<th>Dosing/Route</th>
<th>Days of Study Visits</th>
<th>Animals Enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>Day 0/ SC</td>
<td>0, 7, 14, 28, 42, 56</td>
<td>52</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td></td>
<td>50</td>
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</tbody>
</table>

Owner Assessment of Pruritus VAS

**Extremely severe itching.** Dog is scratching, chewing, licking almost continuously. Itching practically never stops regardless of what else is happening around the dog.

**Severe itching.** Prolonged episodes of itching when the dog is awake. Itching occurs at night and also when eating, playing, exercising, or when otherwise distracted.

**Moderate itching.** Regular episodes of itching when the dog is awake. Itching might occur at night and wake the dog. No itching when eating, playing, exercising, or when being distracted.

**Mild itching.** More frequent episodes of itching. May notice occasional episodes of itching at night. No itching when sleeping, eating, playing, exercising or when being distracted.

**Very mild itching.** Occasional episodes of itching. The dog is slightly more itchy than before the problem began.

**Normal Dog.** Itching is not a problem.

- 10 cm (100 mm) line
- Owner places mark on line that best represents the dog's level of pruritus (itch)
- Measurement from the bottom of the line (“normal dog”) to the owner’s mark on the line is recorded and analyzed

10 cm lines with text descriptors at 2 cm intervals

### Assessment of Skin Lesions/Dermatitis by Veterinary Specialists Using CADESI-03

<table>
<thead>
<tr>
<th>Site</th>
<th>E</th>
<th>L</th>
<th>X</th>
<th>A</th>
<th>Site</th>
<th>E</th>
<th>L</th>
<th>X</th>
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</thead>
<tbody>
<tr>
<td>Face</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Forelimb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hind Limb</td>
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<tr>
<td>- Periauricular</td>
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<td>- L Lateral</td>
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<td>- L Lateral</td>
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</tr>
<tr>
<td>- Perilabial</td>
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<td></td>
<td></td>
<td></td>
<td>- L Cubital Flexor</td>
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<td></td>
<td></td>
<td></td>
<td>- L Stifle Flexor</td>
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<td></td>
<td></td>
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<tr>
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<td>- R Dorsal Interdigital</td>
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</tbody>
</table>

- 248 observations recorded
  - 0 = None
  - 1 = Mild
  - 2, 3 = Moderate
  - 4, 5 = Severe

- Abbreviations
  - E = Erythema
  - L = Lichenification
  - X = Excoriation
  - A = Alopecia

- Maximum score of 1240

Dogs with Chronic Atopic Dermatitis Were Included in Field Trial of Efficacy and Safety

- Owner indicated a pruritus score of ≥30 (of 100) on a Visual Analog Scale
- Dermatologists assigned a CADESI-03 score of ≥30
- Dogs were ≥1 year of age, weighed 2.0-80.0 kg and were physically healthy other than their atopic disease
- Dogs had a ≥1 year documented history of chronic non-seasonal atopic dermatitis
  - Based on Favrot (2010): requires presence of ≥5 of the following
    1) Age at onset <3 years
    2) Non-affected dorso-lumbar area
    3) Corticosteroid-responsive pruritus
    4) Chronic or recurrent yeast infections
    5) Mostly indoor
    6) Affected ear pinnae
    7) Non-affected ear margins
    8) Affected front feet

Dogs with Chronic Atopic Dermatitis Were Included in Field Trial of Efficacy and Safety

- Dogs were flea free and received appropriate preventatives/treatments
- Dogs had been appropriately withdrawn from protocol specified medications and therapies that had the potential to confound efficacy assessments
  - Including but not limited to corticosteroids, cyclosporins, antimicrobials and antihistamines

Dogs with Non-atopic Pruritus Were Not Included

- Dogs with malignant neoplasia
- Dogs with evidence of immune suppression
- Dogs with evidence of demodicosis within the past year
  - If history of demodicosis, two or more negative skin scrapings required
- Lactating bitches or dogs intended for breeding
- Dogs receiving systemic antimicrobial therapy for treatment of bacterial or fungal skin infections

Response Based on Owner Assessed Pruritus and CADESI-03 Score: Study Design

Visit 1, Day 0, Randomization
- Physical examination
- Blood and Urine sample
- Inclusion/Exclusion criteria
- Owner pruritus assessment
- Investigator CADESI-03 scoring
- Investigator visual analog assessment
- Test article dispensing and administration
- Concomitant Treatment record

Visit 2, Day 7±3
- Physical examination
- Blood sample
- Investigator CADESI-03 scoring
- Investigator visual analog assessment
- Owner pruritus assessment

Visit 3, Day 14±3
- Physical examination
- Blood sample
- Investigator CADESI-03 scoring
- Investigator visual analog assessment
- Owner pruritus assessment

Visit 4, Day 28±3
- Physical examination
- Blood and Urine sample
- Owner pruritus assessment
- Investigator CADESI-03 scoring
- Investigator visual analog assessment

Visit 5, Day 42±3
- Physical examination
- Blood sample
- Investigator CADESI-03 scoring
- Investigator visual analog assessment
- Owner pruritus assessment

Visit 6, Day 56±7, Final Study Day
- Physical examination
- Blood and Urine sample
- Owner pruritus assessment
- Investigator CADESI-03 scoring
- Investigator visual analog assessment
- Investigator response to treatment VAS
- Concomitant Treatment record
- Study Completion form
- Owner response to treatment VAS

Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, Visit 6

Day 0 1 2 3 7 14 21 28 35 42 49 56

Owner Pruritus Assessment (VAS)

Safety was Assessed Multiple Ways

- Adverse events reported by Owners and Dermatologists were summarized
- Clinical pathology summary statistics were calculated by treatment and day of sample collection (Days 0, 28, 56)
- Body weight changes were summarized
- Concomitant medication usage throughout the study was summarized
- PK data were summarized (Days 0, 7, 14, 28, 42, 56)
- Anti-mAb antibody data were summarized (Days 0, 7, 14, 28, 42, 56)

Dogs with atopic dermatitis greater than 1 year of age of any weight and breed were eligible for the study.

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Number of Animals</td>
<td>102</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.5 – 12 years</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>2.2 – 76.5 kg</td>
</tr>
<tr>
<td>Sites</td>
<td>15 Boarded Dermatology Specialty Practices</td>
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</tbody>
</table>

Treatment Success in Pruritus VAS Observed at 2 mg/kg

US Field Dose Titration Results

Treatment Success:
Owner Assessment of Pruritus VAS
20 mm Reduction vs Baseline

N≈50 dogs/group; t₁/₂ ≈ 16 days

Percent of dogs with treatment success

Day of Study

Dose

Placebo
2 mg/kg Canine Atopic Dermatitis Immunotherapeutic


*Statistically significant (p≤0.05) compared to placebo
Significant Improvement in Pruritus Observed at 2 mg/kg

US Field Dose Titration Results

Owner Assessment of Pruritus VAS
Least Squares Means

Day 0 means are arithmetic mean values.


*Statistically significant (p≤0.05) compared to placebo
Treatment Success in Improvement of Skin Condition Observed at 2 mg/kg

US Field Dose Titration Results

Treatment Success:
Investigator Assessment of Skin Condition (CADESI-03)
50% Reduction vs Baseline


*Statistically significant (p≤0.05) compared to placebo
Improvement in Skin Condition Mirrored Decrease in Owner-Assessed Itch at 2 mg/kg

**Us Field Dose Titration Results**

**Investigator Assessment of Skin Condition (CADESI-03)**

Least Squares Means

Day 0 means are arithmetic mean values.


*Statistically significant (p≤0.05) compared to placebo
Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=52)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic 2 mg/kg (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet formulations for maintenance</td>
<td>65.4 (34)</td>
<td>76.0 (38)</td>
</tr>
<tr>
<td>Endectocides</td>
<td>75.0 (39)</td>
<td>74.0 (37)</td>
</tr>
<tr>
<td>Medicated shampoos</td>
<td>48.1 (25)</td>
<td>36.0 (18)</td>
</tr>
<tr>
<td>Diet formulations for treatment of food allergies</td>
<td>34.6 (18)</td>
<td>26.0 (13)</td>
</tr>
<tr>
<td>Ectoparasiticides, insecticides and repellants</td>
<td>21.2 (11)</td>
<td>24.0 (12)</td>
</tr>
<tr>
<td>Emollients and protectants</td>
<td>21.2 (11)</td>
<td>24.0 (12)</td>
</tr>
<tr>
<td>Antigen Specific Immunotherapy</td>
<td>21.2 (11)</td>
<td>18.0 (9)</td>
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<td>Omega 3 Fatty Acids</td>
<td>11.5 (6)</td>
<td>16.0 (8)</td>
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<tr>
<td>Antiseptics and disinfectants other than shampoos</td>
<td>23.1 (12)</td>
<td>14.0 (7)</td>
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<tr>
<td>Other anthelmintic agents, optional classification</td>
<td>17.3 (9)</td>
<td>12.0 (6)</td>
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<tr>
<td>Otic cleansers, non-medicated</td>
<td>11.5 (6)</td>
<td>8.0 (4)</td>
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<tr>
<td>Otic antifungals, topical</td>
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<td>8.0 (4)</td>
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<td>Non-medicated shampoos</td>
<td>5.8 (3)</td>
<td>6.0 (3)</td>
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<tr>
<td>Antibiotics for topical use</td>
<td>1.9 (1)</td>
<td>4.0 (2)</td>
</tr>
<tr>
<td>All other non-therapeutic products</td>
<td>0.0 (0)</td>
<td>4.0 (2)</td>
</tr>
<tr>
<td>Diet formulations – homemade</td>
<td>1.9 (1)</td>
<td>4.0 (2)</td>
</tr>
<tr>
<td>General nutrients</td>
<td>5.8 (3)</td>
<td>4.0 (2)</td>
</tr>
<tr>
<td>Antiemetics and antinauseants</td>
<td>0.0 (0)</td>
<td>4.0 (2)</td>
</tr>
<tr>
<td>Thyroid preparations</td>
<td>5.8 (3)</td>
<td>2.0 (1)</td>
</tr>
</tbody>
</table>

Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications (cont’d.)

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=52)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic 2 mg/kg (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other dermatologicales</td>
<td>1.9 (1)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Analgesics</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Antibiotics for systemic use</td>
<td>1.9 (1)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Anti-inflammatory and anti-inflammatory products</td>
<td>5.8 (3)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Ace inhibitors, plain</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Salicylic acid preparations</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Other anti-inflammatory and anti-rheumatic agents, non-steroids</td>
<td>5.8 (3)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Antidiarrheals, intestinal anti-inflammatory/anti-infective agents</td>
<td>1.9 (1)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Corticosteroid, dermatological preparations</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Combinations of opium alkaloids and derivatives</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Electrolyte solutions</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Methocarbamol</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Selective calcium channel blockers with mainly vascular effects</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>0.0 (0)</td>
<td>2.0 (1)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Artificial tears and other indifferent preparations</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Vitamins, other combinations</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Corticosteroid and anti-infective(s) in combination</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Anthelments</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
</tbody>
</table>

Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications (cont’d.)

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=52)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic 2 mg/kg (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loperamide</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Other antidiarrheals</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Protectives against UV-radiation</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Vaccines</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>H2-receptor antagonists</td>
<td>3.8 (2)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Atipamezole</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Corticosteroids, plain</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Dexmedetomidine</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Insulins and analogues</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Loratadine</td>
<td>1.9 (1)</td>
<td>0.0 (0)</td>
</tr>
</tbody>
</table>

No Immediate Hypersensitivity or Immunogenicity Reported

- There were no hypersensitivity-related reactions immediately post-dosing
- No treatment-induced or treatment-boosted anti-mAb antibodies developed in Canine Atopic Dermatitis Immunotherapeutic-treated animals
- $t_{1/2} \approx 16$ days; $T_{\text{max}} \approx 10$ days

### Commonly Reported Abnormal Health Event

*Abnormal health events ordered by descending frequency of occurrence in dogs administered Canine Atopic Dermatitis Immunotherapeutic*

<table>
<thead>
<tr>
<th>Abnormal Health Event</th>
<th>0.0 mg/kg (n=52) n (%)</th>
<th>2.0 mg/kg (n=50) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>1 (1.9)</td>
<td>4 (8.0)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0 (0.0)</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td>Pyoderma</td>
<td>2 (3.8)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>0 (0.0)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0 (0.0)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>2 (3.8)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Otitis Externa</td>
<td>2 (3.8)</td>
<td>1 (2.0)</td>
</tr>
</tbody>
</table>

1 Tabulated on a per animal basis (Days 0-56); occurred in ≥4% of any dog treated with Canine Atopic Dermatitis Immunotherapeutic.

Safety Assessment

- One dog had a possible injection site reaction that resolved by the end of the study without treatment.
- No dogs were withdrawn for abnormal clinical pathology results or possible adverse health event attributed to treatment.
- Overall means for RBC and WBC counts, clinical chemistry variables and urine protein:creatinine remained within reference range for each analyte; urinalysis results were unremarkable.
- A wide variety of concomitant medications were well tolerated.

Clinical Safety in Client-owned Dogs with Atopic Dermatitis Dosed Twice up to 3.3 mg/kg

- **Objective**
  - To evaluate the safety of Canine Atopic Dermatitis Immunotherapeutic in client owned dogs with atopic dermatitis
- **No restrictions on dog age, BW, concomitant medications; no minimum level of pruritus**
- **All study personnel with the exception of the product dispenser were masked and unaware of treatment group assignments**
- **Safety data (adverse events, clinical pathology) collected; no efficacy data**

### Canine Atopic Dermatitis Immunotherapeutic (mg/kg)

<table>
<thead>
<tr>
<th></th>
<th>Dosing/Route</th>
<th>Days of Study Visits</th>
<th>Animals Enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Days 0, 28 / SC</td>
<td>0, (14), 28, 42</td>
<td>75</td>
</tr>
<tr>
<td>Up to 3.3</td>
<td></td>
<td></td>
<td>150</td>
</tr>
</tbody>
</table>

Dogs of any age, weight and breed with atopic dermatitis were eligible for the study.

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Animals</td>
<td>245</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9 months – 14.5 years</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>2.4 – 88.6 kg</td>
</tr>
</tbody>
</table>

## US Field Safety

The Most Common AHEs (> 2.0%) In Either Group

<table>
<thead>
<tr>
<th>Abnormal Health Event Preferred Term</th>
<th>Placebo n=83</th>
<th>Canine Atopic Dermatitis Immunotherapeutic n=162</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otitis externa</td>
<td>12.0 (10)</td>
<td>13.0 (21)</td>
</tr>
<tr>
<td>Dermatitis and eczema</td>
<td>13.3 (11)</td>
<td>9.9 (16)</td>
</tr>
<tr>
<td>Bacterial skin infection</td>
<td>12.0 (10)</td>
<td>9.3 (15)</td>
</tr>
<tr>
<td>Erythema</td>
<td>4.8 (4)</td>
<td>8.0 (13)</td>
</tr>
<tr>
<td>Emesis</td>
<td>10.8 (9)</td>
<td>7.4 (12)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>4.8 (4)</td>
<td>6.2 (10)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>6.0 (5)</td>
<td>5.6 (9)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>19.3 (16)</td>
<td>4.9 (8)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4.8 (4)</td>
<td>3.7 (6)</td>
</tr>
<tr>
<td>Urine abnormalities NOS</td>
<td>2.4 (2)</td>
<td>3.7 (6)</td>
</tr>
<tr>
<td>Abnormal test result</td>
<td>3.6 (3)</td>
<td>3.1 (5)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>7.2 (6)</td>
<td>2.5 (4)</td>
</tr>
<tr>
<td>External parasite</td>
<td>2.4 (2)</td>
<td>2.5 (4)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>3.6 (3)</td>
<td>1.9 (3)</td>
</tr>
<tr>
<td>Anemia NOS</td>
<td>2.4 (2)</td>
<td>1.9 (3)</td>
</tr>
<tr>
<td>Fever</td>
<td>2.4 (2)</td>
<td>1.2 (2)</td>
</tr>
<tr>
<td>Lameness</td>
<td>2.4 (2)</td>
<td>1.2 (2)</td>
</tr>
<tr>
<td>Hair change</td>
<td>2.4 (2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Skin disorder NOS</td>
<td>2.4 (2)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

1 Occurrence was calculated on a per case basis - no matter how many observations of the same AHE a dog had it contributed one observation to the occurrence calculation.

Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications

Concomitant Medications Administered at Least Once on Days 0-42 (% (n))

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=83)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic up to 3.3 mg/kg (n=162)</th>
<th>All (n=245)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heartworm preventatives, orally administered</td>
<td>41.0 (34)</td>
<td>34.6 (56)</td>
<td>36.7 (90)</td>
</tr>
<tr>
<td>Oclacitinib</td>
<td>41.0 (34)</td>
<td>32.7 (53)</td>
<td>35.5 (87)</td>
</tr>
<tr>
<td>Ectoparasiticides, insecticides and repellants</td>
<td>37.3 (31)</td>
<td>28.4 (46)</td>
<td>31.4 (77)</td>
</tr>
<tr>
<td>Antibacterials for systemic use</td>
<td>26.5 (22)</td>
<td>25.3 (41)</td>
<td>25.7 (63)</td>
</tr>
<tr>
<td>Endectocides</td>
<td>13.3 (11)</td>
<td>24.7 (40)</td>
<td>20.8 (51)</td>
</tr>
<tr>
<td>Antihistamines for systemic use, excluding combinations with corticosteroids</td>
<td>22.9 (19)</td>
<td>23.5 (38)</td>
<td>23.3 (57)</td>
</tr>
<tr>
<td>Antiinfectives/antiseptics in combination with corticosteroids (topical skin and ear preparations)</td>
<td>30.1 (25)</td>
<td>22.8 (37)</td>
<td>25.3 (62)</td>
</tr>
<tr>
<td>Corticosteroids for systemic use, excluding combinations with antihistamines</td>
<td>24.1 (20)</td>
<td>21.6 (35)</td>
<td>22.4 (55)</td>
</tr>
<tr>
<td>Omega 3 fatty acids</td>
<td>16.9 (14)</td>
<td>16.7 (27)</td>
<td>16.7 (41)</td>
</tr>
<tr>
<td>Medicated shampoos</td>
<td>18.1 (15)</td>
<td>14.8 (24)</td>
<td>15.9 (39)</td>
</tr>
<tr>
<td>Antiinfectives and antiseptics, excluding combinations with corticosteroids (topical skin and ear preparations)</td>
<td>26.5 (22)</td>
<td>14.2 (23)</td>
<td>18.4 (45)</td>
</tr>
<tr>
<td>Antigen specific immunotherapy</td>
<td>9.6 (8)</td>
<td>14.2 (23)</td>
<td>12.7 (31)</td>
</tr>
<tr>
<td>Antifungals for systemic use</td>
<td>9.6 (8)</td>
<td>11.1 (18)</td>
<td>10.6 (26)</td>
</tr>
<tr>
<td>Ciclosporin</td>
<td>3.6 (3)</td>
<td>9.3 (15)</td>
<td>7.3 (18)</td>
</tr>
<tr>
<td>Glucosamine with/without chondroitin</td>
<td>3.6 (3)</td>
<td>8.6 (14)</td>
<td>6.9 (17)</td>
</tr>
</tbody>
</table>

Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications (cont’d.)

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=83)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic up to 3.3 mg/kg (n=162)</th>
<th>All (n=245)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines</td>
<td>7.2 (6)</td>
<td>7.4 (12)</td>
<td>7.3 (18)</td>
</tr>
<tr>
<td>Otic cleanser, non-medicated</td>
<td>8.4 (7)</td>
<td>6.8 (11)</td>
<td>7.3 (18)</td>
</tr>
<tr>
<td>Thyroid preparations</td>
<td>7.2 (6)</td>
<td>6.8 (11)</td>
<td>6.9 (17)</td>
</tr>
<tr>
<td>Trimeprazine with prednisolone</td>
<td>12.0 (10)</td>
<td>6.2 (10)</td>
<td>8.2 (20)</td>
</tr>
<tr>
<td>Antibiotics for topical use (topical skin)</td>
<td>2.4 (2)</td>
<td>5.6 (9)</td>
<td>4.5 (11)</td>
</tr>
<tr>
<td>Antiinflammatory agents and anti-infectives in combination (ophthalmicals)</td>
<td>4.8 (4)</td>
<td>5.6 (9)</td>
<td>5.3 (13)</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2.4 (2)</td>
<td>4.9 (8)</td>
<td>4.1 (10)</td>
</tr>
<tr>
<td>Drugs for peptic ulcer and gastro-esophageal reflux disease</td>
<td>2.4 (2)</td>
<td>4.9 (8)</td>
<td>4.1 (10)</td>
</tr>
<tr>
<td>Non-medicated shampoos</td>
<td>2.4 (2)</td>
<td>4.9 (8)</td>
<td>4.1 (10)</td>
</tr>
<tr>
<td>Vitamins, other combinations</td>
<td>2.4 (2)</td>
<td>4.9 (8)</td>
<td>4.1 (10)</td>
</tr>
<tr>
<td>Antiemetics and antinauseants</td>
<td>1.2 (1)</td>
<td>4.3 (7)</td>
<td>3.3 (8)</td>
</tr>
<tr>
<td>All other non-therapeutic products</td>
<td>2.4 (2)</td>
<td>3.7 (6)</td>
<td>3.3 (8)</td>
</tr>
<tr>
<td>Corticosteroids, dermatological preparations (shampoos, lotions, ear preparations such as “Synotic”)</td>
<td>1.2 (1)</td>
<td>3.7 (6)</td>
<td>2.9 (7)</td>
</tr>
<tr>
<td>Anthelmintics</td>
<td>2.4 (2)</td>
<td>3.1 (5)</td>
<td>2.9 (7)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>2.4 (2)</td>
<td>3.1 (5)</td>
<td>2.9 (7)</td>
</tr>
</tbody>
</table>

Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications (cont’d.)

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=83)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic up to 3.3 mg/kg (n=162)</th>
<th>All (n=245)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal anti-infectives</td>
<td>2.4 (2)</td>
<td>3.1 (5)</td>
<td>2.9 (7)</td>
</tr>
<tr>
<td>Other dermatologicals (emollients, oils, baby wipes)</td>
<td>1.2 (1)</td>
<td>3.1 (5)</td>
<td>2.4 (6)</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>1.2 (1)</td>
<td>3.1 (5)</td>
<td>2.4 (6)</td>
</tr>
<tr>
<td>Atipamezole</td>
<td>1.2 (1)</td>
<td>2.5 (4)</td>
<td>2.0 (5)</td>
</tr>
<tr>
<td>Dexmedetomidine</td>
<td>1.2 (1)</td>
<td>2.5 (4)</td>
<td>2.0 (5)</td>
</tr>
<tr>
<td>I.V. solutions administered intravenously or subcutaneously</td>
<td>1.2 (1)</td>
<td>2.5 (4)</td>
<td>2.0 (5)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>1.2 (1)</td>
<td>1.9 (3)</td>
<td>1.6 (4)</td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>4.8 (4)</td>
<td>1.9 (3)</td>
<td>2.9 (7)</td>
</tr>
<tr>
<td>Ace inhibitors, plain</td>
<td>0.0 (0)</td>
<td>1.2 (2)</td>
<td>0.8 (2)</td>
</tr>
<tr>
<td>Antifungals for topical use</td>
<td>1.2 (1)</td>
<td>1.2 (2)</td>
<td>1.2 (3)</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>1.2 (1)</td>
<td>1.2 (2)</td>
<td>1.2 (3)</td>
</tr>
<tr>
<td>Moxidectin</td>
<td>1.2 (1)</td>
<td>1.2 (2)</td>
<td>1.2 (3)</td>
</tr>
<tr>
<td>Other ophthalmologicals</td>
<td>1.2 (1)</td>
<td>1.2 (2)</td>
<td>1.2 (3)</td>
</tr>
<tr>
<td>Tramadol</td>
<td>0.0 (0)</td>
<td>1.2 (2)</td>
<td>0.8 (2)</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Blood and related products</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
</tbody>
</table>

Canine Atopic Dermatitis Immunotherapeutic was Safely Administered with Commonly-Used Concurrent Medications (cont’d.)

<table>
<thead>
<tr>
<th>Functional use term</th>
<th>Placebo (n=83)</th>
<th>Canine Atopic Dermatitis Immunotherapeutic up to 3.3 mg/kg (n=162)</th>
<th>All (n=245)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids (ophthalmic)</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Cyproheptadine</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>1.2 (1)</td>
<td>0.6 (1)</td>
<td>0.8 (2)</td>
</tr>
<tr>
<td>I.V. solution additives</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Metamizole sodium</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Mineralocorticoids</td>
<td>1.2 (1)</td>
<td>0.6 (1)</td>
<td>0.8 (2)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Salt solutions</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Staphylococcus vaccine</td>
<td>0.0 (0)</td>
<td>0.6 (1)</td>
<td>0.4 (1)</td>
</tr>
</tbody>
</table>

*Other treatments administered to only placebo dogs (one dog each) are not listed in this table.
Canine Atopic Dermatitis Immunotherapeutic was Safe When Used in Field Conditions

- No hypersensitivity-related reactions immediately post-dosing
- Frequency of injection pain responses similar between treatment groups
- Frequencies of the most common adverse events (e.g., vomiting, diarrhea, lethargy, etc.) similar between dogs receiving Canine Atopic Dermatitis Immunotherapeutic and placebo
- Wide variety of concomitant medications safely used, including parasiticides, antibiotics, antifungals, corticosteroids, vaccines, immunotherapy, antihistamines, oclacitinib and cyclosporin

LABELING
Veterinary biologics (such as a mAb like Canine Atopic Dermatitis Immunotherapeutic) are regulated by the Center for Veterinary Biologics (CVB) in the Animal and Plant Health Inspection Service (APHIS), which is a branch of the USDA.

In order for a veterinary biologic to be manufactured and sold, a firm must have 2 types of licenses issued by the USDA

- An establishment license
- A product license
USDA Conditional License

- The USDA will grant conditional licenses to meet an emergency condition, limited market, local situation, or other special circumstance.
- The product must demonstrate the same safety and purity requirements as fully licensed products but only needs to have a “reasonable expectation” of efficacy.
The firm must provide the following information in the application for a conditional license:

- evidence from scientific journals or experts that there is urgent need in the field for the product
- evidence of a “reasonable expectation” of efficacy with data from studies of the product
- all requirements for safety and purity (same as fully licensed products)
- testing that shows consistency between batches of manufactured product
Once a conditional license is granted, the firm is expected to make progress toward completion of the full efficacy, potency and safety data in order to obtain a full license.

While under a conditional license, products may need additional authorization for distribution in each state and in foreign countries. The product label must clearly state that the product license is conditional and no trade names may be used.

At this time, the USDA has communicated that all products for canine atopic dermatitis will be considered for conditional licensure only.
Canine Atopic Dermatitis Immunotherapeutic Label

- For Use in Dogs Only
- This product license is conditional. Safety and efficacy studies are in progress.
- Canine Atopic Dermatitis Immunotherapeutic aids in the reduction of clinical signs associated with atopic dermatitis in dogs.
- Canine Atopic Dermatitis Immunotherapeutic is a ready-to-use, sterile liquid containing a caninized monoclonal antibody (mAb) against canine interleukin-31 (IL-31). IL-31 has been shown to induce pruritus in dogs in laboratory studies.
- Canine Atopic Dermatitis Immunotherapeutic remains in circulation for several weeks. It exerts a therapeutic effect by binding to and neutralizing soluble cIL-31, thus inhibiting pruritus and reducing skin lesions. Like other naturally-occurring antibodies and antibody-antigen complexes, elimination is via normal protein degradation pathways.
Directions

- Canine Atopic Dermatitis Immunotherapeutic is available in 1-mL vials in four concentrations (10, 20, 30 or 40 mg). Administer Canine Atopic Dermatitis Immunotherapeutic by subcutaneous injection at a minimum dose of 2 mg/kg body weight according to the dosing table. Repeat administration monthly, as needed.

- The product does not contain a preservative. Each vial is for single use only, and should be discarded after puncture.
## Dosing Table

<table>
<thead>
<tr>
<th>Dog Body Weight</th>
<th>Presentations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pounds</td>
<td>Kilograms</td>
</tr>
<tr>
<td>&lt;5</td>
<td>&lt;2.3</td>
</tr>
</tbody>
</table>

* A single dose for dogs weighing less than 5 lb (less than 2.3 kg) requires a volume of 0.09 mL/lb or 0.2 mL/kg drawn from ONE 10-mg vial.

A single dose for dogs weighing 5-40 lb (2.3-18.1 kg) requires the full volume drawn from ONE 1-mL vial as indicated below.

| 5-10 | 2.3-4.5 | 1 | | |
| 10.1-20 | 4.6-9.0 | 1 |
| 20.1-30 | 9.1-13.6 | 1 |
| 30.1-40 | 13.7-18.1 | 1 |

A single dose for dogs weighing more than 40 lb (18.2 kg) requires the full volume of TWO OR MORE 1-mL vials as indicated below. Draw the entire dose into one syringe and administer as a single injection.

| 40.1-60 | 18.2-27.2 | 1 |
| 60.1-80 | 27.3-36.2 | 1 |
| 80.1-100 | 36.3-45.3 | 1 |
| 100.1-120 | 45.4-54.4 | 1 |
| 120.1-140 | 54.5-63.4 | 1 |
| 140.1-160 | 63.5-72.5 | 1 |
| 160.1-200 | 72.6-90.7 | 1 |

* A single dose for dogs weighing less than 3.0 kg (less than 6.6 pounds) requires a volume of 0.2 mL/kg from ONE 10 mg vial.
Precautions

1. The product does not have preservative. Each vial is for single use only, and should be discarded after puncture.
2. This product is intended for subcutaneous use only.
3. Store upright at 2°-8°C. Prolonged exposure to higher temperatures and/or direct sunlight may adversely affect potency. Do not freeze.
4. Use entire contents when first opened.
5. Sterilized syringes and needles should be used to administer this product. Do not sterilize with chemicals because traces of disinfectant may inactivate the product.
6. Burn containers and all unused contents.
7. This product has not been tested in pregnant, lactating or breeding animals.
• Technical inquiries should be directed to Zoetis, Inc. Veterinary Services, (888) 963-8471 (USA), (800) 461-0917 (Canada).

• This product has been shown to be efficacious in treating healthy dogs with canine atopic dermatitis. Canine Atopic Dermatitis Immunotherapeutic should be used under the supervision of a veterinarian.
References


References (cont’d.)

- Kochevar D. Glucocorticoids: new mechanisms for old drugs [presentation]. Presented at the Hill’s Symposium on Dermatology. April 2-4, 2006; Palm Springs, CA.


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McCall RB, Humphrey WR. Pilot Study: Evaluation of the anti-pruritic effect of anti-IL-31 monoclonal antibody CAN 34D03-65 (PF-6443537) in a canine model of IL-31-induced pruritus. Zoetis Study 7D61W-60-11-B00. 16 October 2012.


References (cont’d.)


- Ramsey DS, Dunham SA, Martinon O, Hoevers JD, Mahabir S. Proof of efficacy and safety of an anti-IL-31 monoclonal antibody (IL-31 mAb) for the treatment of atopic dermatitis in client-owned dogs. Zoetis Study 4962R-60-11-277. 15 February 2013.


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