Date of Approval: September 19, 2024

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-585

Zenrelia™

(ilunocitinib tablets)

Dogs

Zenrelia™ is indicated for control of pruritus associated with allergic dermatitis and control of atopic dermatitis in dogs at least 12 months of age.

Sponsored by:

Elanco US Inc

Executive Summary

Zenrelia™ (ilunocitinib tablets) is approved for the control of pruritus associated with allergic dermatitis and control of atopic dermatitis in dogs at least 12 months of age. The drug is an immunosuppressant that is administered orally, once daily, with or without food.

Safety and Effectiveness

The sponsor conducted two field studies in client-owned dogs to demonstrate that Zenrelia™ is effective at controlling atopic dermatitis and pruritus associated with allergic dermatitis. The studies included mixed breed and purebred dogs of both sexes, with a range of ages and weights. To be enrolled in the atopic dermatitis field study, a dog had to be diagnosed with atopy by a veterinarian, have mild skin lesions as assessed by the veterinarian, and have moderate pruritus as assessed by the owner. To be enrolled in the allergic dermatitis field study, a dog had to be diagnosed with allergic dermatitis by a veterinarian and have moderate pruritus as assessed by the owner. In both studies in a 2:1 ratio, dogs received either Zenrelia™ or placebo tablets once daily beginning on Day 0. Treatment was given with or without food, and the placebo tablets were identical to the Zenrelia™ tablets but did not contain the active ingredient, ilunocitinib.

In the atopic dermatitis field study, a dog was considered a treatment success if, on Day 28, either the pruritus or the skin lesions were reduced by ≥50% from baseline (Day 0). Compared to the placebo group, a higher proportion of dogs in the Zenrelia™ group were treatment successes. Adverse reactions related to treatment with Zenrelia™ included gastrointestinal signs (vomiting or nausea, diarrhea, and anorexia), lethargy, otitis externa, and hematology and serum chemistry abnormalities, including leukopenia and increased hepatobiliary and renal values. One Zenrelia™-treated dog was diagnosed with metastatic splenic and hepatic hemangiosarcoma. Another Zenrelia™-treated dog had traumatic tendonitis and a puncture wound four days before the study was completed, which progressed to a serious infection and eventual limb amputation.

In the allergic dermatitis field study, a dog was considered a treatment success if the pruritus was reduced by ≥50% from baseline (Day 0) on at least 5 of the first 7 days of treatment. Compared to the placebo group, a higher proportion of dogs in the Zenrelia™ group were treatment successes. Adverse reactions related to treatment with Zenrelia™ included gastrointestinal signs (vomiting or nausea, diarrhea, and anorexia), lethargy, urinary tract infections, and hematology and serum chemistry abnormalities, including leukopenia and increased hepatobiliary and renal values.

The sponsor conducted a laboratory margin of safety study in young, healthy, male and female beagles. The dogs were administered Zenrelia™ once daily at 0X, 1X, 3X, or 5X the maximum intended dose of 0.8 mg/kg per day for 182 consecutive days. Dogs in the 0X group were sham dosed. The tablets were given to dogs in the fed state for maximum drug exposure. Treatment with Zenrelia™ was associated with interdigital papillomas and/or dermatitis/furunculosis, localized demodicosis, and decreased prostate gland weights. Clinical pathology findings included a dose-dependent decrease in hematocrit, hemoglobin, and red blood cell counts without a corresponding increase in absolute reticulocytes.

The sponsor conducted a 6-month pilot safety study in young, healthy, male and female

beagles using a non-final formulation of ilunocitinib as an oral suspension. The dogs were administered 0X, 1X, 3X, and 4.5X the maximum intended dose of 0.8 mg/kg per day via gavage once daily through Day 64. Dogs in the 0X group were sham dosed. Due to serious adverse reactions in the higher dose groups, the dose for the 4.5X group was decreased to 2X through Day 185. Three dogs (two in the 3X group and one in the 4.5X group) had acute onset of necrotizing hemorrhagic pneumonia, which was considered secondary to both ilunocitinib-induced immunosuppression and gavage. Clinical pathology findings were similar to the margin of safety study described above.

The sponsor also conducted a vaccine response study to evaluate how Zenrelia™ affects the response to vaccination. Young, healthy, male and female beagles that had never been previously vaccinated were administered Zenrelia™ once daily at 0X or 3X the maximum intended dose of 0.8 mg/kg per day for 89 consecutive days. Dogs in the 0X group received a placebo. The tablets were given to dogs in the fed state for maximum drug exposure. A canine multivalent modified live virus (MLV) vaccine was administered on Days 28 and 60. A single dose of a killed rabies virus (RV) vaccine was administered on Day 60. Starting on Day 89, there was an 84-day recovery period in which all dogs did not receive Zenrelia™ or placebo.

The results of the study demonstrate that it is not safe to administer vaccines in dogs that are concurrently receiving Zenrelia™. Dogs in the 3X group had drug-induced immunosuppression, which resulted in the emergence of fatal vaccine-induced adenoviral hepatitis and pancreatitis in one dog, infectious enteritis that potentially contributed to a fatal intussusception in one dog, and an inadequate immune response to canine distemper virus and RV vaccinations in one (of six) and four (of six) dogs, respectively. In addition to the animal safety concerns, the failure of four of the six treated dogs to mount an adequate immune response to the killed rabies vaccine raises a public health concern, given the serious zoonotic risk of rabies. These animal and public health concerns can be mitigated by withholding Zenrelia™ for a certain amount of time before and after vaccination (see below). Zenrelia™-induced immunosuppression also resulted in secondary clinical infections with *Cystoisospora canis*, interdigital cysts, and thickening and crusting of the ear margins. Clinical pathology findings were similar to the two safety studies described above, with additional findings of decreased total serum protein, albumin, and globulin.

Due to the risks in immunocompromised dogs of MLV vaccine-induced disease and an inadequate immune response to any vaccine, Zenrelia™ should be discontinued at least 28 days to 3 months before any vaccination and should not be administered for at least 28 days afterward. The 28-day to 3-month time period to discontinue Zenrelia™ before vaccination is based on data from the vaccine response study that showed evidence of recovery from drug-induced immunosuppression 27 to 83 days after stopping Zenrelia™. A 3-month washout period for immunosuppressants before vaccination is supported by veterinary and human vaccination guidelines. The 28-day time period to withhold Zenrelia™ after vaccination is based on published and unpublished data on the duration of viral shedding after administration of a MLV vaccine.

Conclusion
Based on the data submitted by the sponsor for the approval of Zenrelia™, FDA determined that the drug is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-585

B. Sponsor

Elanco US Inc.
2500 Innovation Way
Greenfield, IN 46140

Drug Labeler Code: 058198

C. Proprietary Name

Zenrelia™

D. Drug Product Established Name

ilunocitinib tablets

E. Pharmacological Category

Immunosuppressant

F. Dosage Form

Tablet

G. Amount of Active Ingredient

4.8, 6.4, 8.5, or 15 mg of ilunocitinib per tablet

H. How Supplied

Zenrelia™ is available in scored tablets in four strengths: 4.8 mg, 6.4 mg, 8.5 mg, and 15 mg. Each tablet strength is available in 10 and 30 count blister packages and 90 count bottles.

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

The dose of Zenrelia™ (ilunocitinib tablets) is 0.27 to 0.36 mg ilunocitinib/lb (0.6 to 0.8 mg ilunocitinib/kg) body weight, administered orally, once daily, with or without food.

K. Route of Administration

Oral

L. Species

Dogs

M. Indication

Zenrelia™ is indicated for the control of pruritus associated with allergic dermatitis and control of atopic dermatitis in dogs at least 12 months of age.

II. EFFECTIVENESS

A. Dosage Characterization

1. Pharmacological Properties of Zenrelia™

   Ilunocitinib is a non-selective Janus kinase (JAK) inhibitor that inhibits the function of a variety of pruritogenic, pro-inflammatory, and allergy related cytokines that are dependent upon these enzymes. Ilunocitinib has a high potency for JAK1, JAK2, and tyrosine kinase 2 (TYK2) inhibition. Ilunocitinib is not a corticosteroid or an antihistamine.

   Ilunocitinib is rapidly and well absorbed and excreted via the biliary/fecal route after oral administration in dogs. Following a single oral or intravenous administration of ilunocitinib at 0.8 mg/kg, the oral bioavailability based on area under the curve from the time of dosing to the last quantifiable plasma concentration (AUC_{last}) was 80% in the fed state and 60% in the fasted state. The systemic clearance following intravenous administration was 399 mL/h/kg with a terminal half-life of 3.6 h. The volume of distribution was 1390 mL/kg (n=8). The maximum plasma concentration (C_{max}) and AUC_{last} were 120% and 45% higher, respectively, in the fed state as compared to the fasted state (n=16). Pharmacokinetic parameters are presented as geometric means.

2. Dose Selection

   A once daily oral dose of 0.6-0.8 mg/kg (0.27-0.36 mg/lb) Zenrelia™ was selected based on the results of laboratory effectiveness studies conducted with varying doses given to dogs once daily. Laboratory studies investigated doses up to 2 mg/kg administered once daily. These studies demonstrated effectiveness with improvement of lesions and pruritus scores and longer duration of effect in the higher dose range of 0.6-0.8 mg/kg daily compared to lower doses tested (0.1-0.6 mg/kg/day).

   A masked, randomized, placebo-controlled, multi-site pilot field study was conducted in 169 client-owned dogs (127 Zenrelia™, 42 placebo) presenting with pruritus associated with allergic dermatitis. Dogs were administered Zenrelia™ once daily at three dose ranges (0.25-0.4 mg/kg, 0.4-0.6 mg/kg, and 0.6-0.8 mg/kg) or placebo for 28 days. Effectiveness was assessed by owner scores of pruritus utilizing a validated pruritus visual analog scale (PVAS), investigator scores for the extent and severity of skin lesions using the Canine Atopic Dermatitis Extent and Severity Index version 4 (CADESI-4), and both owner and investigator assessments for the overall response to treatment (RTT) using an RTT visual analog scale (VAS). Dogs treated with the dose range of 0.6-0.8

mg/kg once daily (n=42) achieved the most effective control of pruritus and improvement of skin lesions associated with allergic dermatitis.

B. Substantial Evidence

1. Field Study for Control of Atopic Dermatitis

Title: Efficacy and Field Safety of Once Daily Oral Administration of LY3411067 for Control of Atopic Dermatitis in Client-Owned Dogs. (Study No. ELA1900313)

Study Date: May 19, 2020 to June 19, 2023

Study Locations: Investigators included board-certified diplomates of the American College of Veterinary Dermatology (DACVD) and general veterinary practitioners.

Amarillo, TX
Bartlesville, OK
Bozeman, MT
Calgary, AB, Canada
Charlotte, NC
Dallas, TX
Eagle Point, OR
Frederick, CO
Gilbert, AZ
Houston, TX
Kirkland, WA
Lacey, WA
Mount Vernon, WA
Navarre, FL
Neosho, MO
Phoenix, AZ
Pittsburgh, PA
Portland, ME
Quakertown, PA
Raleigh, NC
San Antonio, TX
Springfield, MO
West Palm Beach, FL
Wheat Ridge, CO
Zachary, LA

Study Design:

Objective: To evaluate the effectiveness and safety of once daily oral administration of Zenrelia™ for the control of pruritus and skin lesions in dogs diagnosed with atopic dermatitis.

Study Animals: This study enrolled 268 client-owned dogs,147 male and 121 female dogs, definitively diagnosed with atopic dermatitis. Of the enrolled dogs, 144 were mixed-breed and 124 were pure-bred dogs, ranging in age from 1 to 17 years old and weighing between 3.1 to 67.3 kg. Commonly represented breeds enrolled were the German Shepherd Dog (9.7%), American Pit Bull Terrier (8.9%), Labrador Retriever (8.1%), French Bulldog (6.5%), Shih Tzu (6.5%), and Golden Retriever (5.6%). Enrollment required a minimum pruritus visual analog scale (PVAS) score of 6 (corresponding to moderate itching) as assessed by the owner, and a minimum Canine Atopic Dermatitis Extent and Severity Index version 4 (CADESI-4) score of 25 (representing a mild severity of atopic dermatitis) as assessed by the investigator. Dogs could be enrolled with concurrent health conditions if they were stable with their current treatment regimen and determined healthy enough to participate, as evaluated by the investigator. Dogs were required to be on a flea treatment or preventative

throughout the study. Concomitant use of therapies which may interfere with the evaluation of effectiveness (e.g., glucocorticoids, cyclosporine, topical anesthetics, or other JAK inhibitors) were not allowed on study and required a wash-out period prior to enrollment.

Experimental Design: This study was a masked, randomized, placebo-controlled, multi-site field study to assess the effectiveness and safety of Zenrelia™ for the control of pruritus and skin lesions in dogs diagnosed with atopic dermatitis. The study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Effectiveness was determined on Day 28 by the reduction from baseline of pruritus as assessed by the owner on the PVAS and reduction from baseline in skin lesion scores (CADESI-4) assessed by the investigator. Dogs enrolled in the study were randomized to receive once daily Zenrelia™, at 0.6-0.8 mg/kg, or placebo for up to 112 days.

Dogs were randomly assigned within each site, in a 2:1 ratio of Zenrelia™ to placebo, in order of enrollment. The treatment dispenser was the only unmasked study participant at each study site. The investigator, owner(s), and all other site personnel were masked to treatment assignments.

Table II.1. Treatment Groups

Treatment Group	Daily Dose Administered	Number of Dogs
Zenrelia™	0.6-0.8 mg/kg	181
Placebo*	0.0 mg/kg	87

* Placebo was the formulated tablet without the active ingredient, ilunocitinib

Drug Administration: Dogs were administered Zenrelia™ or placebo tablets once daily, at approximately the same time each day, beginning on Day 0. The appropriate combination of tablet strengths (or half tablets) or the equivalent number of placebo tablets was administered to each dog based on their body weight. Placebo tablets were identical in appearance to the Zenrelia™ tablets, containing all the same ingredients except for ilunocitinib. The assigned treatment could be given with or without food.

Measurements and Observations: Physical examination, hematology, serum chemistry, urinalysis, baseline assessments of pruritus on PVAS (by owner), and baseline assessments of the extent and severity of skin lesions on CADESI-4 (by the investigator) were recorded at enrollment on Day 0.

Owner assessments of pruritus on the PVAS were recorded daily at home through Day 7 and prior to scheduled clinic visits thereafter. The PVAS is a vertical line scale with text descriptors of severity and behavior corresponding to the severity of pruritus, with 0 being a normal dog and 10 indicating extremely severe itching. The investigator assessments of skin lesions on the CADESI-4 were recorded at scheduled clinic visits (Days 14, 28, 56, 84, and 112). CADESI-4 is a 180-point severity scale for assessing skin lesions of atopic dermatitis in

dogs, with a score of 10, 35, and 60 indicating mild, moderate, and severe atopic dermatitis skin lesions, respectively.

Owners and investigators recorded the dog's response to treatment (RTT) on a scale of 0 to 10, with 0 being no improvement and 10 being excellent improvement, starting on Day 14 and at defined study time points (Days 28, 56, 84, and 112). Owner diaries recording treatment administration and owner observations were completed daily while the dog was in the study.

Physical examinations were conducted at each clinic visit, with hematology and serum chemistry conducted monthly throughout the study (Day 28, 56, 84, and 112). Urinalysis was performed twice during the study (Day 28 and 112).

Treatment success for each dog was defined as at least a 50% reduction from baseline (Day 0) in owner-assessed PVAS score for pruritus or at least a 50% reduction from baseline in investigator assessed CADESI-4 score for skin lesions on Day 28. Dogs that did not achieve at least 50% reduction in one of these two assessments on Day 28 were considered treatment failures. Dogs that were electively withdrawn from the study due to perceived lack of effectiveness, or due to an adverse event on or before Day 28, were considered treatment failures.

Statistical Methods: Treatment success on Day 28 was assessed as a comparison of treatment success in each group, using a generalized linear mixed model (GLMM) with a logit link and binomial error. The statistical model included treatment as a fixed effect, and site and the site-by-treatment interaction as random effects. Estimated success proportions, their standard errors, and corresponding 95% confidence intervals (CI) were obtained by back-transformations from the GLMM least square estimates.

Effectiveness was established if the proportion of dogs achieving treatment success in the Zenrelia™ group was statistically significantly different from ($p<0.05$) and numerically greater than those in the placebo group.

Results:

Primary Effectiveness at Day 28:

The proportion of dogs that were treatment successes in the Zenrelia™ group was significantly different from ($p<0.001$) and greater than the placebo group. See Table II.2 below.

Table II.2. Estimated Proportion of Dogs Achieving Treatment Success on Day 28

Treatment Group	Number of Dogs with Treatment Success	Estimated Proportion of Success*	95% Confidence Interval
Zenrelia™ (N=172)	141	0.83	(0.74, 0.89)
Placebo (N=77)	25	0.31†	(0.17, 0.50)

* Based on back-transformed least squares means
† Placebo vs. Zenrelia™ p<0.001
N=Number of dogs

Study Withdrawals:

Fifty-nine dogs (14 Zenrelia™, 45 placebo) exited the study early due to perceived lack of effectiveness, of which 47 (10 Zenrelia™, 37 placebo) were considered treatment failures, due to exiting on or before Day 28. Seventeen dogs (12 Zenrelia™, 5 placebo) exited early due to an adverse event that interfered with study evaluations (see Adverse Reactions below).

Secondary Effectiveness Variables assessed for PVAS Scores:

The estimated mean PVAS scores for the Zenrelia™ group were lower than the placebo group at all time points.

Table II.3. Mean Owner-Assessed PVAS Scores by Day

Day	Zenrelia™ Group Mean* (N)	Placebo Group Mean* (N)
0	7.6 (174)	7.8 (81)
1	6.2 (168)	6.9 (79)
2	5.5 (168)	6.9 (79)
3	4.9 (168)	6.7 (78)
4	4.7 (166)	6.6 (78)
5	4.4 (167)	6.6 (77)
6	4.2 (168)	6.4 (77)
7	4 (162)	6.3 (73)
14	3.4 (171)	6.1 (73)
28	3 (168)	5.4 (54)
56	2.5 (153)	4.9 (37)
84	1.8 (135)	3.9 (28)
112	1.8 (133)	3.3 (28)

* The arithmetic mean of owner-assessed PVAS scores
N=Number of dogs with data for that time point

The Zenrelia™ group had a higher proportion of dogs with a ≥50% reduction from baseline in owner-assessed PVAS at every time point through the study, compared to the placebo group.

Table II.4. Proportion of Dogs with ≥50% Reduction from Baseline in Owner-Assessed PVAS Scores by Day

Day	Proportion of Zenrelia™ Group Dogs* (N)	Proportion of Placebo Group Dogs* (N)
1	0.11 (168)	0.05 (79)
2	0.19 (168)	0.06 (79)
3	0.29 (168)	0.08 (78)
4	0.33 (166)	0.08 (78)
5	0.39 (167)	0.08 (78)
6	0.41 (168)	0.10 (77)
7	0.48 (162)	0.12 (73)
14	0.55 (171)	0.14 (73)
28	0.64 (168)	0.25 (54)
56	0.77 (153)	0.23 (37)
84	0.85 (135)	0.38 (28)
112	0.82 (133)	0.58 (28)

* Based on back-transformed least squares means
N=Number of dogs with data for that time point

Secondary Effectiveness Variables Assessed for CADESI-4 Scores:

The estimated mean CADESI-4 scores for the Zenrelia™ group were lower than the placebo group at all timepoints.

Table II.5. Mean Investigator-Assessed CADESI-4 Scores by Day

Day	Zenrelia™ Group Mean* (N)	Placebo Group Mean* (N)
0	57.4 (174)	58.7 (81)
14	27.3 (172)	47.2 (72)
28	17.4 (168)	43.1 (54)
56	11.7 (153)	42.7 (37)
84	11.2 (135)	40 (28)
112	9.4 (133)	38.2 (28)

* The Day 0 values are the arithmetic mean investigator-assessed CADESI-4 scores, and the Day 14 to 112 values are the least squares means.
N=Number of dogs with data for that time point

The Zenrelia™ group had a higher proportion of dogs with a ≥50% reduction from baseline in investigator-assessed CADESI-4 scores at every time point through the study, compared to the placebo group.

Table II.6. Proportion of Dogs with ≥50% Reduction from Baseline in Investigator-Assessed CADESI-4 Scores by Day

Day	Proportion of Zenrelia™ Group Dogs* (N)	Proportion of Placebo Group Dogs* (N)
14	0.60 (172)	0.13 (72)
28	0.83 (168)	0.33 (54)
56	0.91 (153)	0.37 (37)
84	0.93 (135)	0.50 (28)
112	0.95 (133)	0.60 (28)

* Based on back-transformed least squares means
N=Number of dogs with data for that time point

Secondary Variables Assessed for Response to Treatment:

Owner's assessment of their dog's response to treatment (ORTT) was performed on a 10-unit visual analog scale (VAS) with 0=no improvement and 10=excellent improvement. The mean ORTT-VAS scores were higher in the Zenrelia™ group compared to the placebo group at every time point.

Table II.7. Mean Owner Assessment of Response to Treatment (ORTT-VAS) Scores by Day

Day	Zenrelia™ Group Mean* (N)	Placebo Group Mean* (N)
14	6.5 (172)	2.7 (72)
28	7.2 (167)	3.4 (54)
56	7.6 (153)	3.9 (37)
84	8.2 (134)	4.1 (28)
112	8.3 (132)	4.8 (28)

* Least squares means
N=Number of dogs with data for that time point

The investigator's assessment of the dog's response to treatment (IRTT) was performed on a 10-unit VAS with 0=no improvement and 10=excellent improvement. The mean IRTT-VAS scores were higher in the Zenrelia™ group compared to the placebo group at every time point.

Table II.8. Mean Investigator Assessment of Response to Treatment (IRTT-VAS) Scores by Day

Day	Zenrelia™ Group Mean* (N)	Placebo Group Mean* (N)
14	6.3 (172)	2.2 (72)
28	8.1 (153)	3.1 (37)
56	8.1 (153)	3.5 (37)
84	8.3 (134)	4.3 (28)
112	8.7 (133)	4.7 (28)

* Least squares means
N=Number of dogs with data for that time point

Adverse Reactions: All 268 (181 Zenrelia™, 87 placebo) enrolled dogs received at least one dose of their assigned treatment and were evaluated for safety. By Day 112, 66.7% of placebo-treated dogs and 22.1% of Zenrelia-treated dogs exited the study. Adverse reactions seen during the field study are summarized in Table II.9 below.

Table II.9. Adverse Reactions through Day 112

Adverse Reaction	Zenrelia™ (N=181) Number of dogs (%)	Placebo (N=87) Number of dogs (%)
Vomiting or nausea	40 (22.1%)	14 (16.1%)
Diarrhea	36 (19.9%)	9 (10.3%)
Lethargy	22 (12.2%)	9 (10.3%)
Otitis externa	19 (10.5%)	20 (23%)
Anorexia	17 (9.4%)	7 (8%)
Dermal growth (e.g., cyst, papilloma)	16 (8.8%)	4 (4.6%)
Epiphora or ocular discharge	14 (7.7%)	1 (1.1%)
Coughing or wheezing, including respiratory infections	12 (6.6%)	2 (2.3%)
Bacterial skin infection	10 (5.5%)	9 (10.3%)
Elevated liver enzyme(s)	10 (5.5%)	2 (2.3%)
Urinary tract infection	10 (5.5%)	2 (2.3%)
Upset stomach, including flatulence and abdominal pain	10 (5.5%)	0
Leukopenia	9 (4.9%)	1 (1.1%)
Sneezing	8 (4.4%)	1 (1.1%)
Lipoma	7 (3.9%)	1 (1.1%)
Weight gain	7 (3.9%)	0
Increased water intake	4 (2.2%)	2 (2.3%)
Gingivitis (occurrence or worsening)	4 (2.2%)	0
Blood in stool	4 (2.2%)	0
Elevated total bilirubin	4 (2.2%)	0
Elevated triglyceride	4 (2.2%)	0
Histiocytoma	3 (1.7%)	0
Increased appetite	3 (1.7%)	0
Fungal skin infection	3 (1.7%)	2 (2.3%)
Weight loss	2 (1.1%)	1 (1.1%)
Metastatic neoplasia (i.e., hemangiosarcoma)	1 (0.6%)	0
Systemic fungal infection	1 (0.6%)	0
Mast cell tumor	1 (0.6%)	0

N=Number of dogs

Abnormal hematology results likely related to Zenrelia™ administration included thrombocytopenia, leukopenia, neutropenia, lymphopenia, eosinopenia, monocytopenia, and decreased red blood cell count.

Abnormal serum chemistry results potentially related to Zenrelia™ administration included increased hepatobiliary parameters (alanine transaminase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and total bilirubin), increased blood urea nitrogen (concurrently with an increase in creatinine in one dog), hypercholesterolemia, hypertriglyceridemia,

hypoalbuminemia (without a concurrent hyperglobulinemia), and hypoglobulinemia (with or without a decrease in total proteins).

Twelve Zenrelia™-treated dogs withdrew from the study early due to adverse reactions, nine of which were considered likely related to Zenrelia™ administration. These events included repeated episodes of vomiting, leukopenia, neutropenia, worsening of pre-existing lymphocytosis, enlargement of a non-resolving histiocytoma, eyelid mass with bacterial blepharitis, otitis interna with vestibular disease, urinary tract infection, and upper respiratory infection. Five of the placebo group dogs also withdrew from the study early due to an adverse reaction (i.e., lethargy, worsening of pre-existing lymphocytosis, occurrence of nystagmus, skin infection, and teat infection).

One Zenrelia™-treated dog was diagnosed with splenic and liver masses on Day 112. Histopathologic diagnosis after euthanasia one month later confirmed metastatic and splenic and hepatic hemangiosarcoma. Another Zenrelia™-treated dog experienced traumatic tendonitis and a puncture wound four days prior to study completion, progressing to a serious infection. The owner elected amputation after study completion. A third Zenrelia™-treated dog experienced a moderate neutropenia on Day 28 associated with a pre-existing subclinical urinary tract infection (UTI) that had progressed into a clinical UTI. The neutrophil count normalized seven days later while still receiving Zenrelia™, prior to exiting the study to receive antibiotics.

Conclusions: Once daily treatment with Zenrelia™ at the dose of 0.6-0.8 mg/kg was effective for the control of atopic dermatitis in client-owned dogs. Zenrelia™ may increase susceptibility to the development of neoplasia and infection. Zenrelia™ may also exacerbate progression of subclinical or uncomplicated infections into clinical or severe infections.

2. Field Study for Control of Pruritus Associated with Allergic Dermatitis

Title: Efficacy and Field Safety of Once Daily Oral Administration of LY3411067 for Control of Pruritus Associated with Allergic Dermatitis in Client-Owned Dogs. (Study No. ELA1900107)

Study Dates: July 16, 2020 to July 17, 2023

Study Locations: Investigators included board-certified diplomates of the American College of Veterinary Dermatology (DACVD) and general veterinary practitioners.

Battle Creek, MI	Manhattan, KS
Bradenton, FL	Nixa, MO
Columbia, SC	Plant City, FL
Dallas, TX	Springfield, MO
Decatur, IL	Starke, FL
Fort Collins, CO	Wheat Ridge, CO
Franklin, IN	Zachary, LA
Gilbert, AZ	

Study Design:

Objective: To evaluate the effectiveness and safety of once daily oral administration of Zenrelia™ for the control of pruritus associated with allergic dermatitis.

Study Animals: The study enrolled 306 client-owned dogs, 146 males and 160 female dogs, diagnosed with allergic dermatitis. Of the enrolled dogs, 152 were mixed-breed and 154 were pure-bred dogs, ranging in age from 1 to 15 years old and weighing between 3 to 83.3 kg. Commonly represented breeds enrolled were the Labrador Retriever (11%), Shih Tzu (11%), American Pit Bull Terrier (8.4%), Golden Retriever (6.5%), Bulldog (4.5%), and Dachshund (4.5%). Enrolled dogs were diagnosed with at least one of the following presumptive diagnoses for allergic dermatitis: atopic dermatitis, contact dermatitis, flea allergy dermatitis, food hypersensitivity, or other. See Table II.10 below for the presumptive diagnoses at enrollment for each treatment group. Enrollment required a minimum pruritus visual analog scale (PVAS) score of 6 (corresponding to moderate itching) as assessed by the owner. Dogs could be enrolled with concurrent health conditions if they were stable with their current treatment regimen and determined healthy enough to participate, as evaluated by the investigator. Dogs were required to be on a flea treatment or preventative throughout the study. Concomitant use of therapies which may interfere with the evaluation of effectiveness (e.g., glucocorticoids, cyclosporine, topical anesthetics, or other JAK inhibitors) were not allowed on study and required a wash-out period prior to enrollment.

Table II.10. Presumptive Diagnoses at Enrollment

Presumptive Diagnoses for Allergic Dermatitis*	**Zenrelia™ Group Percentage (%) of Dogs N=206**	**Placebo Group Percentage (%) of Dogs N=100**
Atopic Dermatitis†	92.2	92
Contact Dermatitis	23.3	26
Flea Allergy Dermatitis	15.5	19
Food Hypersensitivity	24.3	27
Other	2.9	5

* Dogs may have more than one diagnosis at enrollment.

† 45.1% Zenrelia™ group and 39% placebo group dogs had atopic dermatitis as the sole presumptive diagnosis.
N=Number of dogs

Experimental Design: This study was a masked, randomized, placebo-controlled, multi-site field study to assess the effectiveness and safety of Zenrelia™ for the control of pruritus in dogs diagnosed with allergic dermatitis. The study was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Effectiveness was determined on Day 7 by the reduction from baseline of pruritus as assessed by the owner on the PVAS. Dogs enrolled in the study were randomized to receive once daily Zenrelia™, at 0.6-0.8 mg/kg, or placebo for 28

days. An optional continuation phase was offered from Days 29 to 112, in which a dog could continue their assigned treatment.

Dogs were randomly assigned within each site, in a 2:1 ratio of Zenrelia™ to placebo, in order of enrollment. The treatment dispenser was the only unmasked study participant at each study site. The investigator, owner(s), and all other site personnel were masked to treatment assignments.

Table II.11. Treatment Groups

Treatment Group	Daily Dose Administered	Number of Dogs
Zenrelia™	0.6-0.8 mg/kg	206
Placebo*	0.0 mg/kg	100

* Placebo was the formulated tablet without the active ingredient, ilunocitinib

Drug Administration: Dogs were administered Zenrelia™ or placebo tablets once daily, at approximately the same time each day, beginning on Day 0. The appropriate combination of tablet strengths (or half tablets) or the equivalent number of placebo tablets was administered to each dog based on their body weight. Placebo tablets were identical in appearance to the Zenrelia™ tablets, containing all the same ingredients except for ilunocitinib. The assigned treatment could be given with or without food.

Measurements and Observations: Physical examination, hematology, serum chemistry, baseline assessments of pruritus on PVAS (by owner), and baseline assessment of dermatitis by the veterinarian using a dermatitis visual analog scale (DVAS) were recorded at enrollment on Day 0.

Owner assessments of pruritus on the PVAS were recorded daily at home on Days 1 to 7, 14, and 28. The PVAS is a vertical line scale with text descriptors of severity and behavior corresponding to degrees of pruritus, with 0 being a normal dog and 10 indicating extremely severe itching. Investigator assessments of dermatitis on the DVAS were recorded at clinic visits on Days 7 and 28. The DVAS is a scale from 0 to 10, with zero being no dermatitis present, and 10 being extremely severe dermatitis. Owner diaries recording treatment administration and owner observations were completed daily while the dog was in the study. Physical examinations, hematology, and serum chemistry were conducted at each clinic visit (Days 7 and 28).

For dogs enrolled in the optional continuation phase, owner assessment of pruritus was recorded on the PVAS at home on Days 56, 84, and 112. Physical examinations, hematology, serum chemistry, and investigator assessment of dermatitis (using the DVAS) were conducted on Days 56, 84, and 112. Urinalysis was performed prior to and at the end of the continuation phase (Days 28 and 112).

Treatment success for each dog was defined as at least a 50% reduction from baseline (Day 0) in owner-assessed PVAS score for pruritus on at least 5 out of the first 7 days of treatment. Dogs that did not achieve at least 50% reduction in pruritus on 5 out of the first 7 days were considered treatment failures. Dogs that were electively withdrawn from the study due to perceived lack of effectiveness,

or due to an adverse event on or before Day 7, were considered treatment failures.

Statistical Methods: Treatment success on Day 7 was assessed as a comparison of treatment success in each group, using a generalized linear mixed model (GLMM) with a logit link and binomial error. The statistical model included treatment as a fixed effect, and site and the site-by-treatment interaction as random effects. Estimated success proportions, their standard errors and corresponding 95% confidence intervals (CI) were obtained by back-transformations (ILINK) from the GLMM least square (LS) estimates.

Effectiveness was established if the proportion of dogs achieving treatment success in the Zenrelia™ group was statistically significantly different from ($p \leq 0.05$) and numerically greater than those in the placebo group.

Results:

Primary Effectiveness at Day 7:

The proportion of dogs that were treatment successes in the Zenrelia™ group was significantly different from ($p=0.006$) and greater than the placebo group. See Table II.12 below.

Table II.12. Estimated Proportion of Dogs Achieving Treatment Success on Day 7

Treatment Group	Number of Dogs with Treatment Success	Estimated Proportion of Success*	95% Confidence Interval
Zenrelia™ (N=193)	49	0.25	(0.21, 0.31)
Placebo (N=91)	7	0.08†	(0.03, 0.17)

* Based on back-transformed least squares means
† Placebo vs Zenrelia™ p=0.006
N=Number of dogs

In dogs with fleas present on Day 0, flea treatment alone did not result in a meaningful increase in treatment success in either treatment group.

Secondary Effectiveness Variables Assessed for PVAS Scores:

The Zenrelia™ group had a higher proportion of dogs with a ≥50% reduction from baseline in owner-assessed PVAS at every time point after Day 1, compared to the placebo group.

Table II.13. Proportion of Dogs with ≥50% Reduction from Baseline in Owner-Assessed PVAS Scores by Day

Day	Proportion of Zenrelia™ Group Dogs* (N)	Proportion of Placebo Group Dogs* (N)
1	0.09 (193)	0.09 (90)
2	0.17 (193)	0.07 (90)
3	0.33 (192)	0.13 (88)
4	0.40 (192)	0.15 (89)
5	0.46 (190)	0.16 (89)
6	0.50 (191)	0.20 (89)
7	0.53 (187)	0.25 (87)
14	0.69 (174)	0.23 (63)
28	0.81 (177)	0.37 (59)

* Based on back-transformed least squares means
N=Number of dogs with data for that time point

The estimated mean PVAS scores for the Zenrelia™ group were lower than the placebo group at all time points after Day 1.

Table II.14. Mean Owner-Assessed PVAS Scores (in cm) by Day

Day	Zenrelia™ Group Mean* (N)	Placebo Group Mean* (N)
0	7.9 (196)	7.5 (93)
1	6.7 (193)	6.7 (90)
2	5.8 (193)	6.4 (90)
3	5.0 (192)	6.2 (88)
4	4.5 (192)	5.9 (89)
5	4.1 (190)	5.7 (89)
6	3.8 (191)	5.7 (89)
7	3.6 (187)	5.6 (87)
14	2.9 (174)	5.4 (63)
28	2.2 (177)	4.7 (59)

* The arithmetic mean of owner-assessed PVAS scores
N=Number of dogs with data for that time point

Secondary Effectiveness Variables Assessed for Investigator DVAS Scores:

The mean DVAS scores for the Zenrelia™ group were lower than the placebo group at all time points.

Table II.15. Mean Investigator-Assessed DVAS Scores by Day

Day	Zenrelia™ Group Mean* (N)	Placebo Group Mean* (N)
0	5.1 (196)	4.7 (93)
7	2.7 (187)	3.6 (87)
28	1.5 (174)	2.9 (59)

* Arithmetic means
N=Number of dogs with data for that time point

Adverse Reactions: All 306 (206 Zenrelia™,100 placebo) enrolled dogs received at least one dose of their assigned treatment and were evaluated for safety. By Day 112, 84% of placebo-treated dogs and 49.5% of Zenrelia-treated dogs exited the study. Adverse reactions seen during the field study are summarized in Table II.16 below.

Table II.16. Adverse Reactions Through Day 112

Adverse Reaction	Zenrelia™ (N=206) Number of dogs (%)	Placebo (N=100) Number of dogs (%)
Vomiting or nausea	32 (15.5%)	11 (11%)
Diarrhea	26 (12.2%)	5 (5%)
Lethargy	25 (12.1%)	7 (7%)
Urinary tract infection	13 (6.3%)	2 (2%)
Anorexia	10 (4.9%)	3 (3%)
Coughing, wheezing, or difficulty breathing	9 (4.4%)	0
Elevated liver enzyme(s)	8 (3.9%)	0
Otitis externa	8 (3.9%)	5 (5%)
Increased water intake	7 (3.4%)	2 (2%)
Upset stomach, including flatulence, retching, and abdominal pain	5 (2.4%)	4 (4%)
Ocular discharge	5 (2.4%)	1 (1%)
Elevated triglyceride	5 (2.4%)	0
Dermal or subcutaneous growth (e.g., cyst, nodule)	3 (1.5%)	2 (2%)
Sneezing	3 (1.5%)	0
Blood in stool	3 (1.5%)	0
Increased urination	3 (1.5%)	0
Bacterial skin infection	2 (1%)	4 (4%)
Weight gain	2 (1%)	0
Neurological disorder (e.g., tremors, ataxia)	2 (1%)	0
Increased appetite	1 (0.5%)	0
Fungal skin infection	1 (0.5%)	0
Fever	1 (0.5%)	0
Hematuria (without urinary tract infection)	1 (0.5%)	0

N=Number of dogs

Abnormal hematology results likely related to Zenrelia™ administration included thrombocytosis, leukopenia, neutropenia, eosinopenia, and monocytopenia.

Abnormal serum chemistry results likely related to Zenrelia™ administration included increased hepatobiliary parameters (alanine transaminase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and total bilirubin), increased blood urea nitrogen, increased creatinine, hypertriglyceridemia, hypercholesterolemia, hypoproteinemia, and hypoglobulinemia (with or without a decrease in total protein).

Seven Zenrelia™-treated dogs withdrew from the study early due to adverse reactions, four of which were considered likely related to Zenrelia™ administration. These events included vomiting, lethargy, soft stool, neutropenia, increased liver enzymes, fever, abdominal discomfort, coughing, and wheezing. Four placebo group dogs also withdrew from the study early due to an adverse

reaction (i.e., splenic hemangiosarcoma, restlessness, abdominal pain, lethargy, and vomiting).

One Zenrelia™-treated dog experienced vomiting, dyspnea, depression, fever, abdominal discomfort, and slightly worsened azotemia on Day 4. The dog had a pre-existing elevated creatinine level. Another Zenrelia™-treated dog experienced vomiting, diarrhea, and lethargy starting on Day 1 and mild neutropenia on Day 3 that resolved 5 days later. A third Zenrelia™-treated dog experienced a neurological episode (ataxia, disorientation) on Day 78.

Conclusions: Once daily treatment with Zenrelia™ at the dose of 0.6-0.8 mg/kg was effective for the control of pruritus associated with allergic dermatitis in client-owned dogs. Gastrointestinal signs (e.g., vomiting, diarrhea, and anorexia), lethargy, urinary tract infections, and hematology and serum chemistry abnormalities, including leukopenia and increased hepatobiliary and renal parameters, are considered related to Zenrelia™ administration.

III. TARGET ANIMAL SAFETY

A. Margin of Safety Study

Title: LY3411067: A 6-Month Oral (Tablet) Safety Study in 11- to 12-month-old Beagle Dogs. (Study No. ELA210188)

Study Dates: September 13, 2021 to October 06, 2022

Study Location: Mattawan, Michigan

Study Design:

Objective: To evaluate the safety of Zenrelia™ when orally administered once daily (by tablet) at 0X, 1X, 2X, 3X, and 5X the maximum exposure dose (0.8 mg/kg/day), in the fed state to approximately 11- to 12-month-old beagle dogs for 182 consecutive days.

Study Animals: Forty beagle dogs (20 female and 20 male), approximately 11-12 months of age, weighing between 5.5 to 12.4 kg, and determined as healthy based on physical examination and clinical pathology were included in the study.

Experimental Design: The study was a masked, randomized, and controlled laboratory study. Dogs were randomized to treatment group following a randomized block design, stratified by sex with room as a blocking factor. Dogs were then assigned to cages within a room following a completely randomized design. This study was conducted in accordance with Good Laboratory Practices (GLP) regulations.

Table III.1. Treatment Groups

Group	Dose (mg/kg)	Number and Sex of Dogs
Control*	0	4 male, 4 female
1X	0.8	4 male, 4 female
2X	1.6	4 male, 4 female
3X	2.4	4 male, 4 female
5X	4	4 male, 4 female

* Control group was sham dosed

Drug Administration: Dogs in the Zenrelia™ treatment groups were administered the commercial formulation of Zenrelia™ once daily at 1, 2, 3, or 5X the maximum exposure dose of 0.8 mg/kg/day for 182 consecutive days. Dogs in the control group were sham dosed. Dogs were dosed in the fed state, the prandial state of maximum exposure.

Measurements and Observations: General health observations were conducted twice daily throughout the study. Detailed clinical observations (Day -14, -7, then once weekly), veterinary physical examinations (Day -6, then every 14 days), neurological examinations (Day -6, Day 183), and ophthalmoscopic examinations (Day -7, Day 182) were conducted. Body weight (Day -6, -1, then once weekly) and daily food consumption were measured. Samples for hematology, coagulation, serum chemistry, urinalysis, and C-reactive protein (CRP) assessment were collected on Days -16, -8, 29, 57, 85, 113, 141, 169, and 183. Samples for Zenrelia™ plasma concentrations were collected on Days 1, 85, and 182. Gross necropsy was performed on Day 183, and histopathologic examinations were performed on all dogs.

Statistical Methods: The experimental unit was the individual animal. All fixed model effects were tested at significance level of $\alpha=0.10$. Pairwise mean comparisons between each treatment group and the control group were performed using a significance level of $\alpha=0.10$. No adjustment was made for pair-wise comparisons. Endpoints which were measured once post-treatment were analyzed by Analysis of Variance (ANOVA) with treatment as a fixed effect. Endpoints which were measured multiple times post-treatment and included a pre-treatment measurement were analyzed by Mixed Model Repeated Measures Analysis of Covariance (RMANCOVA) with treatment, time, sex, the 2-way interactions, and the 3-way interaction as fixed effects. The last available pre-treatment value was used as a covariate and room was included as a random effect. All animal data were also summarized through descriptive statistics or frequency counts.

Results: All dogs completed the study and no serious adverse events occurred.

Clinical Observations and Physical Examinations: Zenrelia™-related clinical observations included a dose-dependent increase in the frequency and severity of interdigital furunculosis (cysts), with or without discharge on one or more paws, swollen and/or scabbing paws, and paw skin thickening and/or discoloration.

Clinical Pathology: Zenrelia™-related hemogram findings included a dose-dependent minimal to moderate decrease in hematocrit (HCT), hemoglobin (HGB), and red

blood cell count (RBC) without a corresponding increase in absolute reticulocytes. Other Zenrelia™-related findings included a minimal to mild decrease in mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and eosinophil counts. Abnormal clinical pathology observations considered secondary to the interdigital furunculosis, included minimal to moderate increases in fibrinogen concentrations, total protein, C-reactive protein, and globulin, and decreases in albumin, albumin/globulin ratio, and calcium levels. There were no Zenrelia™-related effects on lymphocytes, monocytes, and basophils. Two dogs in the 5X group had minimally lower myeloid:erythroid ratios consistent with a physiological bone marrow response to the lower red blood cell mass despite no apparent effect on absolute reticulocytes.

Pathology: Zenrelia™-related pathology changes included decreased prostate gland weights in the 5X group males. Six dogs had cutaneous lesions that correlated with microscopic findings. One of these dogs (3X group) had a papilloma on each paw with fragments of *Demodex canis*, and a follicular cyst within the markedly inflamed dermis. The other five dogs (5X group) had cutaneous lesions consistent with papillomas, and other changes secondary to inflammation, including dermatitis/furunculosis in three dogs and enlarged and mildly reactive draining lymph nodes in one dog.

Pharmacokinetics: Minimal accumulation was observed between Days 1 and 182 with geometric mean accumulation ratios for C_{max} and AUC_{last} between 1.1 and 1.6. After the first dose, C_{max} increased in a linear but less than proportional manner where a 5-fold increase in dose resulted in a 3.4-fold (90% confidence limit: 2.9-4) increase in C_{max}. There was a non-linear relationship between dose and AUC_{last} where a 5-fold increase in dose resulted in a 4.2-fold (90% confidence limit: 3.4-5.1) increase in AUC_{last}.

Table III.2. Mean of Ilunocitinib Plasma Pharmacokinetic Parameters (0.8 mg/kg group, Day 182)

Parameter	Estimate Geometric Mean (Coefficient of variation %)
C_{max} (ng/mL)	310 (20.6%)
T_{max}* (h)	2 (range: 1-2)
AUC_{last} (h*ng/mL)	1360 (25.1%)
AUC_{inf} (h*ng/mL)	1370 (25.5%)
t½ (h)	3.29 (11.9%)

* Median and range
h=hour
C_{max}=maximum plasma concentration
T_{max}=time to maximum plasma concentration
AUC_{last}=area under the curve from the time of dosing to the last quantifiable plasma concentration
AUC_{inf}=area under the curve from the time of dosing extrapolated to infinity
t½=half-life

Conclusion: This study supports the safe use of Zenrelia™ when used according to the label directions. Zenrelia™ demonstrated an adequate margin of safety when administered orally once daily to 11 to 12-month-old, fed beagle dogs for 6 months at

1, 2, 3, and 5X the maximum exposure dose of 0.8 mg/kg. Treatment with Zenrelia™ was associated with interdigital papillomas and/or dermatitis/furunculosis, localized demodicosis, and decreased prostate gland weights. Zenrelia™-related clinical pathology findings included decreases in HCT, HGB, and RBC counts without a corresponding increase in absolute reticulocyte count, and decreases in MCH, MCHC, and eosinophil counts.

B. Pilot Target Animal Safety Study

Title: 6-Month Pilot Study of LY3411067 by Oral Gavage in 9-Month-Old Beagle Dogs. (Study No. ELA1700407)

Study Design: The objective of the study was to evaluate the safety of a non-final formulation (oral suspension) of ilunocitinib when administered via gavage once daily for 6 months to dogs. Thirty-two, 9-month-old beagle dogs were randomized to one of four groups. Treated dogs were dosed at 0X, 1X, 3X, and 4.5X the maximum exposure dose of 0.8 mg/kg through Day 64. Due to serious adverse reactions in the 3X and 4.5X groups, the 4.5X group was decreased to 2X through Day 185. Control dogs were sham dosed. The study was not conducted in accordance with Good Laboratory Practices regulations.

Results: One dog in the 4.5X group and two dogs in the 3X group were prematurely euthanized (Days 52, 57, and 134, respectively) due to an acute onset of lethargy, labored breathing, fever, tremors, and pale gums, starting within four hours of dosing via oral gavage. Microscopic pathology findings in the three dogs included necrotizing hemorrhagic pneumonia. Two of these three dogs developed severe leukopenia and neutropenia and one dog had severe weight loss (~18% of body weight) in the two weeks prior to euthanasia.

Twelve dogs administered ilunocitinib, including the three dogs prematurely euthanized, had a decrease in at least one RBC parameter (HCT, HGB, or RBC count), without a corresponding increase in absolute reticulocyte count, at one or more timepoints during the study.

Additional microscopic findings included minimal to mild increased erythropoiesis and pigment in the spleen, and minimal to mild pigmented macrophages in the liver of male dogs in the 3X and 5X groups. Pigment seemed consistent with hemosiderin. The changes in the liver and spleen tended to occur in dogs that had a decrease in at least one RBC parameter at one or more timepoints during the study. Minimal adipocyte accumulation in the bone marrow was observed in the dogs administered ilunocitinib and tended to correlate with increased erythropoiesis in the spleen.

A higher incidence of fecal abnormalities (discolored, liquid, and mucoid feces) was observed in the 3X and 4.5X groups.

Conclusion: Non-final formulation (oral solution) ilunocitinib administration via gavage was associated with an acute onset of necrotizing hemorrhagic pneumonia in the 3X and 4.5X dose groups, which was considered secondary to ilunocitinib induced immunosuppression and gavage administration. Ilunocitinib-related clinical pathology findings included decreases in HCT, HGB, and RBC counts without a

corresponding increase in absolute reticulocyte count. Additional ilunocitinib-related microscopic pathology changes included minimal to mild increased erythropoiesis and pigment in the spleen, minimal to mild pigmented macrophages in the liver, and minimal adipocyte accumulation in the bone marrow.

C. Vaccine Response Study

Title: The Effect of Oral LY3411067 on the Response to Primary Vaccination in Dogs. (Study No. ELAVV200035)

Study Dates: April 20, 2021 to March 13, 2023

Study Location: Manhattan, Kansas

Study Design:

Objective: To evaluate the safety and the effect of Zenrelia™ on response to vaccination when orally administered once daily in vaccine naïve 10-month-old beagle dogs, prior to and following primary vaccination, at 3X the maximum exposure dose of 0.8 mg/kg for 89 days.

Study Animals: Sixteen vaccine-naïve beagle dogs (8 female and 8 male), approximately 10 months of age, weighing between 5.8 and 15.4 kg and determined as healthy based on physical examination, were included in the study. The dogs were raised in a separate biosecure facility and transported at approximately 10 months of age to the biosecure laboratory facility that conducted the study.

Experimental Design: The study was a masked, randomized, and controlled laboratory study. Dogs were randomized by sex to treatment group. Dogs were randomly assigned to pair housed cages by sex and treatment. This study was conducted in accordance with Good Laboratory Practices (GLP) regulations.

Table III.3. Treatment Groups

Group	Dose (mg/kg)	Number and Sex of Dogs
Control*	0	4 male, 4 female
3X	2.4	4 male, 4 female

* Control group was placebo dosed

Drug Administration: Dogs in the Zenrelia™ treatment group were administered the commercial formulation of Zenrelia™ once daily at 2.4 mg/kg (3X the maximum daily exposure dose) for 89 consecutive days. Dogs in the control group were placebo dosed. Dogs were dosed in the fed state, the prandial state of maximum exposure. The study included an 84-day recovery period in which no drug was administered.

Vaccines: A USDA-licensed multivalent modified live virus (MLV) vaccine containing canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus-2 (CAV-2), and canine parainfluenza virus (CPiV) was administered on Days 28 and 60. A single dose of a USDA-licensed killed rabies virus (RV) vaccine was administered on Day 60. The second administration of the multivalent MLV vaccine and single administration of the killed rabies vaccine was delayed from the originally planned

administration date by 4 days (from Day 56 to Day 60) due to suboptimal health of the dogs administered Zenrelia™; these dogs had clinical signs of *Cystoisospora canis* infection secondary to Zenrelia™-induced immunosuppression.

Measurements and Observations: General health observations were conducted daily until Day 88, then were conducted twice daily for the remainder of the study. Veterinary physical examinations (Days -10, 28, 60, then every 28 days), detailed clinical observations (weekly starting on Day -21 except on weeks when veterinary physical examinations were performed), and pre and post dosing clinical observations (Days 0 to 88) were conducted. Body weight (Day -21, -2, 2, 7, then once weekly) and daily dry food consumption were measured. Samples for hematology, serum chemistry, and urinalysis were collected on Days -21, -7, 28, 88, and 172. Serum antigen titer analysis was conducted on Day -21, -7, 28, 60, 88, 116 (rabies only), and 172.

Statistical Methods: The experimental unit was the cage. With the exception of vaccine titer data, all animal data were summarized through descriptive statistics or frequency counts. For variables measured at multiple timepoints, descriptive statistics of the cage averages and of individual animal data were presented by treatment group and day. Corresponding changes from baseline for each parameter were also summarized.

To conclude that Zenrelia™ did not interfere with the immune response to each vaccine, all treated dogs needed to achieve the following titer level on Day 88 for each vaccine antigen: ≥32 for canine distemper virus (CDV), ≥80 for canine parvovirus (CPV), ≥16 for canine parainfluenza virus (CPiV), ≥16 for canine adenovirus-2 (CAV-2), and >0.5 IU/mL for rabies.

Results: Two dogs in the Zenrelia™ group developed serious adverse reactions and were euthanized on Days 52 and 54, prior to evaluation of post-vaccine titers on Day 88. On Day 52, the first dog was euthanized due to lethargy, depression, poor body condition, and weakness. Necropsy revealed findings consistent with a colonic intussusception. The colonic intussusception was potentially related to a clinical *Cystoisospora canis* infection diagnosed on Day 35; this infection was secondary to Zenrelia™-induced immunosuppression. Clinical pathology findings on the day of euthanasia consisted of severe anemia and hypoalbuminemia. On Day 54, the second dog was euthanized due to lethargy, depression, poor body condition, and weakness. Necropsy revealed mesenteric lymphadenopathy, fat necrosis in the omentum and retroperitoneum, and suspected pancreatitis. Histopathology evaluation revealed marked necrotizing hepatitis and pancreatitis and evidence of systemic endotoxemia. Prominent intranuclear inclusion bodies were found in the liver and pancreas, consistent with adenoviral infection. Clinical pathology findings on the day of euthanasia consisted of severe anemia and leukopenia, severe hypoalbuminemia, and severely elevated liver enzymes (alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase) and bile acids. It was concluded that this dog acquired a vaccine-induced adenoviral hepatitis and pancreatitis secondary to Zenrelia™-induced immunosuppression.

Clinical Observations and Physical Examinations: Zenrelia™-related clinical observations included poor body condition, pale mucous membranes, lethargy,

diarrhea, emesis, weight loss, decreased appetite, and depression, potentially due to a clinical *C. canis* infection. A *C. canis* infection was diagnosed in seven of the eight dogs in the Zenrelia™ treatment group. No dogs in the control group were diagnosed with a *C. canis* infection. One dog in the control group had diarrhea on two days. Additional Zenrelia™-related clinical signs were interdigital cysts, lameness, and thickening and crusting of the ear margins.

Clinical Pathology: Zenrelia™-related hemogram findings included mild decrease in hematocrit (HCT), hemoglobin (HGB), and red blood cell (RBC) count on Day 28 with a corresponding increase in absolute reticulocyte count. In addition, the two dogs euthanized on Days 52 and 54 developed severe regenerative anemia. Zenrelia™-related clinical chemistry findings included mild to severe decreases in total serum protein, albumin, and globulins. The clinical pathology abnormalities are consistent with blood loss and gastrointestinal protein loss associated with clinical coccidiosis.

Antibody Titer Analyses: An adequate immune response to the CAV-2 and CPV vaccination was achieved in the six remaining dogs in the Zenrelia™ treatment group and all eight control dogs on Day 88. All eight dogs in the control group achieved an adequate immune response to the CDV and RV vaccination on Day 88.

Four of the remaining six dogs receiving Zenrelia™ failed to achieve an adequate immune response on Day 88 to the RV vaccination. One and three of these four dogs failed to achieve an adequate RV titer on Days 116 and 172 (27 and 83 days after discontinuing Zenrelia™), respectively. One control dog failed to achieve an adequate RV titer on Days 116 and 172 (Table III.4 below).

One of the remaining six dogs receiving Zenrelia™ failed to achieve an adequate immune response on Day 88 to the CDV vaccination. This dog also failed to achieve an adequate CDV titer on Day 172 and was the one dog that failed to achieve an adequate RV titer on Days 88, 116, and 172. In addition to Day 88, all control dogs achieved an adequate CDV titer response on Day 172 (Table III.5 below). All eight control dogs and remaining six Zenrelia-treated dogs achieved an adequate CAV-2 and CPV titer on Day 172. Due to failure of two control dogs to achieve a CPiV titer above the threshold on Day 88, the CPiV titer results were considered invalid.

Table III.4. Number of Dogs With an Inadequate Immune Response to RV Vaccine

Treatment Group	Day 88	Day 116*	Day 172†
Zenrelia™ (6 dogs)	4	1	3
Control (8 dogs)	0	1	1

* off Zenrelia™ for 27 days
† off Zenrelia™ for 83 days

Table III.5. Number of Dogs With an Inadequate Immune Response to CDV Vaccine

Treatment Group	Day 88	Day 172*
Zenrelia™ (6 dogs)	1	1
Control (8 dogs)	0	0

***** off Zenrelia™ for 83 days

Conclusions: This study demonstrates that it is not safe to administer vaccines in dogs concurrently receiving Zenrelia™. The administration of Zenrelia™ at 2.4 mg/kg/day (3X the maximum exposure dose) induced immunosuppression, which resulted in the emergence of fatal vaccine-induced adenoviral hepatitis and pancreatitis, infectious enteritis that potentially contributed to a fatal intussusception, and the failure to achieve an adequate serologic response to CDV and rabies vaccinations. In addition to the animal safety concerns identified in this study, the failure of 4 out of 6 dogs to mount an adequate serologic response to the killed rabies vaccine also raises a public health concern, given the serious zoonotic nature of rabies virus infections. These concerns can be mitigated by withholding Zenrelia™ administration before and after vaccination (Section III.D below).

Additionally, the Zenrelia™-induced immunosuppression resulted in secondary clinical *C. canis* infection, interdigital cysts, and thickening and crusting of the ear margins. Zenrelia™-related clinical pathology findings included decreases in HCT, HGB, and RBC counts with a corresponding increase in absolute reticulocyte count, and decreases in total serum protein, albumin, and globulins.

D. Time to Withhold Zenrelia™ Administration Before and After Vaccination

Due to the risks in immunocompromised animals of vaccine-induced disease associated with MLV vaccines and inadequate immune response to any vaccine, Zenrelia™ should be discontinued at least 28 days to 3 months prior to vaccination and should not be administered for at least 28 days after any vaccination.

The 28-day to 3-month time period to discontinue Zenrelia™ before vaccination is based on data from Study ELAVV200035. Although the study design did not include dogs that were vaccinated after discontinuing Zenrelia™, three of the four dogs in the Zenrelia™ group that failed to achieve an adequate immune response on Day 88 to RV vaccination did have an adequate immune response 27 days after discontinuing Zenrelia™. This data provides some evidence of recovery from drug-induced immunosuppression within 27 days. However, one of the four dogs in the Zenrelia™ group that failed to achieve an adequate immune response on Day 88 to RV vaccination continued to fail to achieve an adequate RV titer level after Zenrelia™ was discontinued (titers measured 27 and 83 days after discontinuing Zenrelia™). This dog also failed to achieve an adequate immune response on Day 88 to CDV vaccination and continued to fail to achieve an adequate CDV titer level 83 days after discontinuing Zenrelia™ (Tables III.4 and 5 above). A 3-month washout

period is supported by veterinary and human vaccination guidelines[1,2].

The 28-day time period to withhold Zenrelia™ after vaccination is based on published and unpublished data evaluating the duration of MLV vaccine virus shedding and published human vaccination guidelines[2 -8].

E. Foreign Experience

In a well-controlled field study conducted in Europe (Germany, Hungary, Ireland, and Portugal), one dog was diagnosed with a pyometra 46 days after starting Zenrelia™. Another dog was diagnosed with a seminoma 49 days after starting Zenrelia™.

IV. HUMAN FOOD SAFETY

This drug is intended for use in dogs. Because this new animal drug is not intended for use in food-producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

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[1] Squires, R., Crawford, C., Marcondes, M., & Whitley, N. (2024). 2024 guidelines for the vaccination of dogs and cats - compiled by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). *J Small Anim Pract*, 65(5): 277–316.
[2] Center for Disease Control and Prevention (CDC). (2023). *Altered Immunocompetence General Best Practice Guidelines for Immunization.* CDC. https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/immunocompetence.html
[3] Decaro, N., Crescenzo, G., Desario, C., Cavalli, A., Losurdo, M., Colaianni, M., Ventrella, G., Rizzi, S., Aulicino, S., Lucente, M., & Buonavoglia, C. (2014). Long-term viremia and fecal shedding in pups after modified-live canine parvovirus vaccination. *Vaccine*, 32(30): 3850–3853.
[4] Ruch-Gallie, R., Moroff, S., & Lappin, M. (2016). Adenovirus 2, *Bordetella bronchiseptica*, and parainfluenza molecular diagnostic assay results in puppies after vaccination with modified live vaccines. *J Vet Intern Med*, 30:164-166.
[5] Wilson, S., Illambas. J., Siedek, E., Thomas, A., King, V., Stirling, C., Plevová, E., Salt, J., & Sture, G. (2014). The administration of a single dose of a multivalent (DHPPiL4R) vaccine prevents clinical signs and mortality following virulent challenge with canine distemper virus, canine adenovirus or canine parvovirus. *Trials in Vaccinology*, 3:102-106.
[6] Bass, A., Chakravarty, E., Akl, E., Bingham, C., Calabrese, L., Cappelli, L., Johnson, S., Imundo, L., Winthrop, K., Arasaratnam, R., Baden, L., Berard, R., Bridges, S., Jr, Cheah, J., Curtis, J., Ferguson, P., Hakkarinen, I., Onel, K., Schultz, G., Sivaraman, V., … Reston, J. (2023). 2022 American College of Rheumatology guideline for vaccinations in patients with rheumatic and musculoskeletal diseases. *Arthritis care & research*, 75(3): 449–464.
[7] Papp, K., Haraoui, B., Kumar, D., Marshall, J., Bissonnette, R., Bitton, A., Bressler, B., Gooderham, M., Ho, V., Jamal, S., Pope, J., Steinhart, A., Vinh, D., & Wade, J. (2019). Vaccination guidelines for patients with immune-mediated disorders on immunosuppressive therapies. *J Cutan Med Surg*, 23(1):50-74.
[8] National Association of State Public Health Veterinarians, Compendium of Animal Rabies Prevention and Control Committee, Brown, C., Slavinski, S., Ettestad, P., Sidwa, T., & Sorhage, F. (2016). Compendium of Animal Rabies Prevention and Control, 2016. *J Am Vet Med Assoc*, 248(5): 505–517.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Zenrelia™:

Not for human use, Keep this drug out of reach of children. Wash hands immediately after handling tablets. In case of accidental ingestion, seek medical advice immediately.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that Zenrelia™, when used according to the label, is safe and effective for the conditions of use in the General Information Section above.

A. Marketing Status

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to rule out other diseases in the diagnosis of allergic and atopic dermatitis, and to monitor and ensure the safe use of the product, including treatment of any adverse reactions and determining the appropriate time to administer vaccines.

B. Exclusivity

Zenrelia™, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the FD&C Act because this is the first time we are approving this active moiety in a new animal drug application submitted under section 512(b)(1) of the FD&C Act.

C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.