CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207695Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 207695
Submission date: 1/7/2016
Drug: crisaborole ointment (2%)
Applicant: Anacor Pharmaceuticals Inc.
Indication: Topical treatment of mild to moderate atopic dermatitis in patients 2 years of age and older

Reviewing Division: Division of Dermatology and Dental Products

Discussion:
The pharmacology/toxicology reviewer and supervisor recommended that this NDA could be approved for the indications listed above. The applicant provided a sufficient nonclinical package to support approval.

Two year carcinogenicity studies were conducted with crisaborole. One was by the oral route in rats and one was by the topical route in mice. The executive carcinogenicity assessment committee found that the studies were adequate. No drug-related neoplasms were noted in the mouse study. There was a drug-related increased incidence of granular cell tumors in the uterus with cervix or vagina (combined) in high dose female rats. The clinical relevance of this finding is not clear.

Reproductive and developmental toxicity studies were conducted in rats and rabbits and only showed some effects in the presence of maternal toxicity.

The appropriate Established Pharmacologic Class for crisaborole is “Phosphodiesterase 4 Inhibitor”, which is the same term used for other molecules in this class.

Conclusions:
I agree that this NDA can be approved from a pharmacology/toxicology perspective and that no additional nonclinical studies are needed. I have provided suggestions for labeling separately.

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/s/

PAUL C BROWN
11/21/2016

Memorandum
To: NDA 207695
From: Barbara Hill, Ph.D., Pharmacology/Toxicology Supervisor
Re:
Receipt date: 10/26/2016
SDN: 16
Submission type: Quality/Response to Information Request
Drug: Eucrisa (crisaborole) ointment, 2%
Indication: Atopic Dermatitis
Route: Topical
Sponsor: Anacor Pharmaceuticals Inc.

Background:

Crisaborole is a PDE-4 inhibitor. Crisaborole ointment is being developed for the topical treatment of atopic dermatitis. The current submission contains information concerning potential genotoxic impurities that may be contained in the crisaborole drug substance.

Review of submitted nonclinical information:

The sponsor states that based on the results of the comprehensive mutagenic impurity risk assessment (b) (4) for potential genotoxic impurities assessment, they have confirmed that there are two known mutagenic impurities (b) (4) . These two impurities are categorized as Class 2 impurities.

The Sponsor has also determined that two additional potential impurities (b) (4) are categorized as Class 5 impurities after negative results from exploratory Ames assays. The two exploratory Ames assays are reviewed below.

Study 1

Title: Exploratory bacterial mutagenicity assay of (b) (4) a potential impurity of crisaborole
Study number: 16GR313
Testing Facility: Pfizer

The potential mutagenic potential of (b) (4) was evaluated in an exploratory non-GLP Ames assay. In the definitive assay, *Salmonella* (TA 1535, TA 1537, TA 98 and TA 100) and *E. coli* (WP2 *uvrA pKM101*) stains were exposed in triplicate to (b) (4) concentrations ranging from (b) (4) mg/plate in the presence and absence of metabolic activation. In the absence of S9 metabolic activation, dose-related cytotoxicity was observed at concentrations (b) (4) mg/plate with TA 100 and at (b) (4)

(b) (4)

mg/plate with TA 1537. In the presence of S9 metabolic activation, dose-related cytotoxicity was observed at concentrations [redacted] mg/plate with TA 100 and at the [redacted] mg/plate with TA 98 and TA 1537. The positive and negative controls responded appropriately in this Ames assay. The mean number of revertants per plate was similar compared to the negative controls in all of the treated cultures.

[redacted] was negative for the induction of reverse mutations in all strains when tested up to the maximum applied concentration of [redacted] mg/plate both in the absence and presence of metabolic activation. [redacted], a potential impurity of crisaborole, was not mutagenic in this exploratory Ames assay.

Study 2

Title: Exploratory bacterial mutagenicity assay of [redacted], a potential impurity of crisaborole
Study number: 16GR318
Testing Facility: Pfizer

The potential mutagenic potential of [redacted] was evaluated in an exploratory non-GLP Ames assay. In the definitive assay, *Salmonella* (TA 1535, TA 1537, TA 98 and TA 100) and *E. coli* (WP2 *uvrA pKM101*) stains were exposed in triplicate to [redacted] concentrations ranging from [redacted] mg/plate in the presence and absence of metabolic activation. Dose related cytotoxicity was not observed in any of the tester stains in either the presence or absence of S9. The positive and negative controls responded appropriately in this Ames assay. The mean number of revertants per plate was similar compared to the negative controls in all of the treated cultures.

[redacted] was negative for the induction of reverse mutations in all strains when tested up to the maximum applied concentration of [redacted] mg/plate both in the absence and presence of metabolic activation. [redacted] a potential impurity of crisaborole, was not mutagenic in this Ames assay.

Specifications for the two potential mutagenic impurities

The sponsor has proposed a revision to the crisaborole drug substance specification to include controls for the two potential mutagenic impurities at levels aligned with the ICH M7 guidance document and the threshold of toxicological concern (TTC).

The TTC is based on the maximum application of crisaborole in the Phase 3 clinical studies of [redacted] g crisaborole/day. The maximum dose used for the TTC is very conservative and would not apply to the majority of patients that would use crisaborole ointment. The sponsor proposed TTC for crisaborole based on an acceptable intake of a mutagenic impurity of [redacted] µg/day for long-term chronic dosing, per the ICH M7 guidance, is [redacted] ppm. The sponsor calculation is provided below.

TTC = [redacted] µg/day
Maximum daily dose = [redacted] g/day

Concentration limit = [redacted] ppm

The sponsor has proposed a specification for [redacted] of "NMT [redacted] ppm" for each potential mutagenic impurity in the crisaborole drug substance. The sponsor's proposed specification for [redacted] is acceptable from a Pharmacology/Toxicology perspective.

The sponsor proposed specification for the [redacted] non-mutagenic impurity of "NMT [redacted]%", for the [redacted] non-mutagenic impurity of "NMT [redacted]%" and for total impurities of "NMT [redacted]%" has been previously determined to be supported by nonclinical data in the original Pharmacology/Toxicology review written by Dr. Kumar Mainigi.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

The sponsor has proposed a specification for [redacted] of "NMT [redacted] ppm" for each potential mutagenic impurity in the crisaborole drug substance. This specification is based on the appropriate TTC calculation per the ICH M7 guidance document. The sponsor's proposed specification for [redacted] is acceptable from a Pharmacology/Toxicology perspective.

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/s/

BARBARA A HILL
11/17/2016

Pharmacology/Toxicology Supervisory Memorandum

NDA number:	207695
Supporting document:	1
CDER Stamp Date:	January 7, 2016
Type of submission:	Original NDA; 505(b)(1)
Applicant:	Anacor Pharmaceuticals, Inc.
Supervisor name:	Barbara Hill, PhD
Review Division:	Dermatology and Dental Products
Date:	August 16, 2016
Product:	Eucrisa (crisaborole) ointment, 2%
Pharmacologic class:	Phosphodiesterase 4 inhibitor
Indication:	Topical treatment of mild to moderate atopic dermatitis in patients 2 years of age and older

General comments:

- I concur with the overall assessment and conclusions contained in Dr. Kumar Mainigi's Pharmacology/Toxicology review for this drug product.
- I concur that there are no nonclinical approval issues for this drug product and that this NDA is approvable from a Pharmacology/Toxicology perspective.
- I concur that there are no nonclinical Post-Marketing Requirements recommended for this NDA.
- I concur with the recommended nonclinical labeling changes proposed by Dr. Mainigi for Eucrisa contained in Section 1.3.3 of his review which include:
    - Pharmacologic Class designation of "phosphodiesterase 4 inhibitor"
    - The revisions proposed for Sections 8.1, 12.1 and 13.1 of the label

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/s/

BARBARA A HILL
08/16/2016

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	**207695**
Supporting document/s:	SD-1
Applicant's letter date:	01-07-2016
CDER stamp date:	01-07-2016
Product:	**EUCRISA** (crisaborole), Ointment , 2%
Indication:	Mild to moderate atopic dermatitis
Applicant:	ANACOR Pharmaceuticals, Palo Alto, CA
Review Division:	Dermatology and Dental Products
Reviewer:	Kumar D. Mainigi, MSc., M.P.H., Ph.D., DABT
Supervisor/Team Leader:	Barbara Hill, Ph.D
Division Director:	Kendal Marcus, MD
Project Manager:	Lydia Springs

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of [NDA 207695] are owned by [ANACOR Pharmaceuticals] or are data for which [ANACOR Pharmaceuticals] has obtained a written right of reference. Any information or data necessary for approval of [NDA 207695] that [ANACOR Pharmaceuticals] does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of [NDA 207695].

TABLE OF CONTENTS

Table of Tables

1 Executive Summary

1.1 Introduction

The first IND (102317) for New Molecular Entity Crisaborole (AN2728) aimed at the treatment of atopic dermatitis (AD) was received in 2008; a total of 33 *in vitro* assays and short-term animal studies were evaluated under this submission. The second IND (077537) directed to treat mild to moderate AD and psoriasis was received in December 2010, and was reviewed along with additional supplements in 2011; a total of 30 non-clinical studies were evaluated. In order to complete the non-clinical safety profile to support chronic use of EUCRISA (crisaborole) ointment, 2%, the sponsor had also conducted the following recommended studies.

1. A 6-month rat oral study
2. A 9-month dermal toxicity study in minipigs
3. A 2-year oral carcinogenicity in rats
4. A 2-year dermal carcinogenicity study in mice
5. A perinatal and postnatal oral developmental toxicity study in rats

1.2 Brief Discussion of Nonclinical Findings

In the recommended dosing regimen, AD patients will receive a total of 6 grams of crisaborol, 2% ointment in two daily topical applications. [Data provided by the medical reviewer, based on three identical Phase 3 studies, where each subject received a total of 170 grams of crisaborole 2% ointment in 28 days]. In conventional calculations, assuming 100% systemic absorption, a 60 kg subject will receive 74mg crisaborole/m^2/day, the maximum recommended human dose (MRHD). Most non-clinical studies were conducted at dose levels much greater than the MRHD, and two critical dermal studies were conducted using up to the maximum feasible topical dose level of 7% crisaborole, ointment.

Note: MRHD in terms of mg/m^2 is only employed in single-dose oral studies (e.g. Safety Pharmacology) where conventionally no pharmacokinetic analyses are conducted, and therefore, the systemic absorption is assumed to be100 percent.

In vitro assays for safety pharmacology revealed that crisaborole did not affect the common receptors, ion channels, and monoamine transporters; the drug also tested as a low-potency HERG-channel blocker.

In animal safety pharmacology studies, drug did not damage the functioning of cardiovascular system in rats and mice at an oral dose of 1000mg/kg (40 and 81 MRHD).

In single-dose (30,100,300mg/kg) male dog study, one high-dose animal died from hypertensive shock, however, at the same dose level, EKG, QTc intervals, and

locomotor activity were not affected. Absolutely, no adverse cardiovascular effects were observed at the mid-dose level (27 MRHD).

Cardiovascular functions in minipigs treated with crisaborole, 5% ointment for three months remained normal. In 9-month minipig dermal study, two daily applications of 7% crisaborole ointment did not cause any changes in ECGs during the entire treatment and 1-month recovery period.

In single dose dog oral study, the bioavailability at 300mg/kg (81 MRHD) was only 0.8%. In single dose rat intravenous study, the elimination half-life in the abdominal fat was 1.2 hours; no drug retention was observed in major organs. In 90-day minipig dermal study (0%, 0.5% 2%, 5% crisaborole ointment), with minimal absorption, the approximate drug accumulation did not exceed 2-4x; apparently a concentration below the threshold level for minimum systemic toxicity.

In a set of *in vitro* assays using freshly isolated human hepatocytes from three healthy volunteers, drug did not induce any critical drug metabolizing CYP enzymes, especially CYP3A4, the most prominent CYP in humans induced by a broad spectrum of drugs. These findings suggest minimal (if any) drug-drug interactions.

In assays with human hepatic microsomes, drug did not exhibit any significant inhibition of CYP450 isoenzymes of C subfamily involved in drug metabolism. Together, these isoforms are estimated to account for metabolism of 20% of the prescribed drugs.

In mouse local lymph node assay, crisaborole at 1, 5, and 10% (w/v) levels did not produce any skin sensitization. In primary ocular and skin irritation assays in rabbits, 2% AN2728 ointment was tested as a mild to moderate irritant.

During 6-month of oral treatment, crisaborole at the highest dose level of 450mg/kg/day (NOAEL) did not cause any local or systemic toxicity in rats. Pharmacokinetic data at the end of treatment period did not indicate any accumulation of parent drug or its two major toxicologically/pharmacologically inert metabolites, deboronated crisaborole (AN7602) and carboxy-AN7602.

In 3-month minipig dermal study (vehicle, 0.5%, 2%, and 5% crisaborole ointment B) with one-month recovery period, two daily applications did not produce any systemic toxicity at NOAEL of 5 percent.

On day-90, $AUC_{(0\text{-}24\ hr)}$ values for 2% crisaborole ointment (the recommended human dose) and 5% crisaborole ointment in males were 1,080 ng·hr/ml and 1,450 ng·hr/ml, respectively. The corresponding $AUC_{0\text{-}24\ hr}$ values in females were 851 ng·hr/ml and 1,330 ng·hr/ml, respectively. Taking into account, the pediatric mean $AUC_{0\text{-}24\ hr}$ of 1,320 ng·hr/ml, the safety margin at the NOAEL of 5% crisaborole ointment is approximately equivalent to the maximum recommended human dose (MRHD) based on AUC comparisons.

Following two daily applications of crisaborole ointment at the maximum feasible strength of 7% (w/v) for 9 months, absolutely no systemic toxicity was exhibited by minipigs. Sporadically distributed dermal lesions (e.g. hyperkeratosis/parakeratosis) among groups including controls, were due to slight traumatic dermal abrasion developed during handling of large animals.

A minimal systemic absorption of crisaborole did not provide enough meaningful toxicokinetic data to determine systemic bioavailability of drug. However, the available data definitely supported an absolute lack of systemic toxicity during nine months of drug treatment and one month of recovery periods.

The highest test concentration of 7% crisaborole (3.5 times the applied human concentration) was established as NOAEL for drug.

Irrespective of the concentration level and the gender, $AUC_{(0\text{-}24\ hr)}$ in males on day 168 and 252 ranged from lowest of 639 ng·hr/ml to highest of 1,130 ng·hr/ml, expressing a relatively low systemic margin of safety compared to the human pediatric mean $AUC_{0\text{-}24\ hr}$ of 1,320 ng·hr/ml (see table 2 in this review).

Crisaborole tested non-mutagenic in Ames assays conducted using four *Salmonella* and one *E. coli* strains in presence/absence of Aroclor- induced rat liver S9 fraction. Drug also did not induce any structural/numerical chromosomal aberrations in activated/non-activated (Aroclor-S9) human peripheral blood lymphocytes. In rat micronucleus assay, crisaborole at dose levels up to 2,000mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes.

In 104-week rat oral carcinogenicity study (0, 30, 100, and 300mg/kg/day), no treatment related non neoplastic lesions were found. A drug related increased incidence of benign granular cell tumors in uterus with cervix or vagina combined was noted in high-dose females (control 6/65; low-dose 7/65; mid-dose 4/65; high-dose 20/65). Relevance of this finding in humans is unknown.

No drug-related neoplastic findings were noted at topical doses up to 7% crisaborole ointment in a dermal carcinogenicity study conducted in CD-1 mice.

Oral rat and rabbit embryofetal development studies were conducted with crisaborole. Oral doses up to 300 mg/kg/day crisaborole were administered to pregnant rats during the period of organogenesis and oral doses up to 100 mg/kg/day crisaborole were administered to pregnant rabbits during the period of oganogeneis. No drug related fetal malformations were noted in the rat or rabbit embyrofetal development studies.

No drug related effects on fetal development were noted in the rat prenatal/postnatal development study conducted at oral doses up to 600 mg/kg/day crisaborole administered to pregnant rats during gestation and lactation. No drug related effects on fertility were noted in male or female rats administered oral doses up to 600 mg/kg/day crisaborole prior to and during early pregnancy.

In a nut shell, a diversified group of 68 non-clinical *in vitro* assays and whole animal studies in multiple species has projected a safety profile with a comfortable margin for human users.

1.3 Recommendations

1.3.1 Approvability

Yes

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

It is recommended that the underlined wording be inserted into the label and the ~~strikeout~~ wording be deleted from the label below. The PLLR subheadings in Sections 8.1 and 8.2 should be in underlined format which has been proposed by the sponsor. The PLLR Sections 8.1 and 8.2 of the label have been discussed with the DPMH reviewer, Dr. Jane Liedtka. Some recommended general edits for the clinical sections of the label have been incorporated into this label. More specific clinical portions of Sections 8.1 and 8.2 will be addressed by the clinical reviewer.

The multiple of human exposure calculations based on AUC comparisons for this label are provided in Appendix 1. A clean copy of the proposed nonclinical portions of this label is provided in Appendix 2.

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE

EURISA ~~TRADENAME~~ is a (b) (4) phosphodiesterase-4 (b) (4) inhibitor indicated for topical treatment of mild to moderate atopic dermatitis in patients 2 years of age and older. (1)

Reviewer's comments: The pharmacologic class for crisaborole should be "phosphodiesterase 4 inhibitor" not " (b) (4) ". (b) (4)

Therefore, it is recommended to just use "phosphodiesterase 4 inhibitor" as the pharmacologic class.

8 USES IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no available data (b) (4) with ~~TRADENAME~~ EUCRISA in pregnant women to inform the drug-associated risk for major birth defects and miscarriage. In animal reproduction studies, there were no adverse developmental (b) (4) effects observed with oral administration of crisaborole in pregnant rats and rabbits during organogenesis at doses up to 5 (b) (4) and 3 (b) (4) times, respectively, the maximum recommended human dose (MRHD) [*see Data*].

The background risk of major birth defects and miscarriage for the indicated population is unknown. (b) (4) the background risk of major birth defects in the U.S. general population is 2% to 4% and of miscarriage are 15% to 20% of clinically recognized pregnancies.

Data

Animal Data

(b) (4) Rat and rabbit embryo-fetal development was assessed after oral administration of crisaborole. Crisaborole did not cause adverse effects to the fetus (b) (4) at oral doses up to 300 mg/kg/day in pregnant ~~(~~rats~~)~~ during the period of organogenesis (5 times the MRHD on an AUC comparison basis). No treatment-related fetal malformations were noted after oral treatment with crisaborole in pregnant rats at doses up to 600 mg/kg/day (18 times the MRHD on an AUC comparison basis) during the period of organogenesis. Maternal toxicity was produced at the high dose of 600 mg/kg/day in pregnant rats and was associated with findings of decreased fetal body weight and delayed skeletal ossification. Crisaborole did not cause adverse effects to the fetus at oral doses up to 100 mg/kg/day in pregnant rabbits during the period of organogenesis (3 times the MRHD on an AUC comparison basis). (b) (4)

In a (b) (4) /postnatal development (b) (4) -study, pregnant (b) (4) rats were treated with crisaborole at doses of 150, 300 and 600 mg/kg/day by gavage during gestation and lactation (from gestation day 7 through day 2 (b) (4) of lactation). Crisaborole did not have any adverse effects on fetal development at doses up to 600 mg/kg/day (18 times the MRHD on an AUC comparison basis). Maternal toxicity was produced at the high dose of 600 mg/kg/day in pregnant rats and was associated with findings of stillbirths, pup mortality and reduced pup body weights. (b) (4)

(b) (4)

8.2 Lactation

Risk Summary

There is no information available on the presence of (b) (4) EUCRISA in human milk, the effects of the drug on the breastfed infant or the effects of the drug on the milk production. The development and health benefits of breastfeeding should be considered along with the mother's clinical need for (b) (4) EUCRISA and any potential adverse effects on breastfeeding on the breastfed infant from (b) (4) EUCRISA or from the underlying maternal condition.

Reviewer's comment: No animal data is available for Section 8.2 of the label. The clinical reviewer will determine appropriate edits for Section 8.2 of the label.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Crisaborole is a (b) (4) phosphodiesterase 4 (PDE-4) inhibitor (b) (4) PDE-4 inhibition results in increased intracellular cyclic adenosine monophosphate (cAMP) levels. (b) (4) ~~T~~The specific mechanism(s) by which crisaborole exerts its therapeutic action for the treatment of atopic dermatitis is not (b) (4) ~~well defined,~~ (b) (4)

13 NONCLINICAL

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4) In an oral carcinogenicity study in Sprague-Dawley rats, oral doses of 30, 100, and 300 mg/kg/day crisaborole were administered to rats once daily. A drug related increased incidence of granular cell tumors in the uterus with cervix or vagina (combined) was noted in 300 mg/kg/day crisaborole treated female rats (2 times the MRHD on an AUC comparison basis). The clinical r~~R~~elevance of this finding in humans is unknown.

In a dermal carcinogenicity study in CD-1 mice, topical doses of 2%, 5% and 7% crisaborole ointment were administered once daily. No drug related neoplastic findings were noted at topical doses up to 7% crisaborole ointment (2 times the MRHD on an AUC comparison basis).

Crisaborole revealed no evidence of mutagenic or clastogenic potential based on the results of two in vitro genotoxicity tests (Ames assay and human lymphocyte chromosomal aberration assay) and one in vivo genotoxicity test (rat micronucleus assay).

(b) (4) No effects on (b) (4) fertility were observed in male or female rats (b) (4) that were administered oral doses up to 600 mg/kg/day crisaborole (b) (4) (18 times the MRHD on an AUC comparison basis) prior to and during early pregnancy.

2 Drug Information

2.1 Drug

CAS Registry Number: 906673-24-3

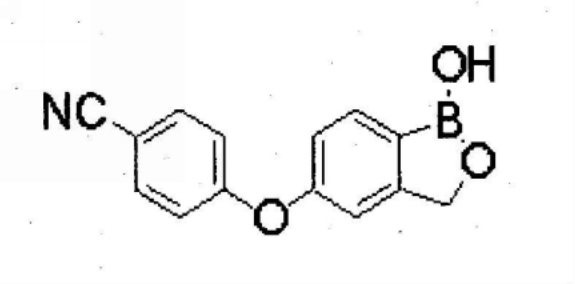
Generic Name: Crisaborole (USAN)

Code Name: AN2728; 5116-P54

Chemical Names: 5-(4-cyanophenoxy)-1, 3-dihydro-1-hydroxy-[2,1]-benzoxaborole

Molecular Formula/Molecular Weight: $C_{14}H_{10}BNO_3$ / 251.05

Structure or Biochemical Description:



Pharmacologic class: Phosphodiesterase 4 inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 077537
IND 102317

2.3 Drug Formulation

Composition of Crisaborole Ointment, 2%

Ingredient	%W/W	Function
Crisaborole	2.00	Active agent
Propylene Glycol, USP	(b) (4)	
Butylated Hydroxytoluene, NF		
Mono- and Di-glycerides, NF		
Paraffin Wax, NF		
White Petrolatum, USP		
Edetate Calcium Disodium, USP		

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

A number of synthetic and degradation impurities were found in drug lots used in early animal and some human studies. However, no specific impurity related systemic toxicity was observed in these studies. The total impurity (b) (4)%) was gradually reduced to (b) (4)% in later batches. Lots with low levels ((b) (4)%) of total impurity were tested in non-clinical and some Phase2 clinical studies. Absolutely, no systemic toxicity was observed in 9-month minipig dermal study conducted at the maximum feasible dose of crisaborole, ointment 7%, therefore, no further discussion about the qualification/ quantification of any impurity for any objective is warranted.

The sponsor has set a specification for (b) (4) of (b) (4)ppm in the drug product. This level is below the guidance for industry ICH Q3D *Elemental Impurities* permitted oral concentration in a drug product for (b) (4) of (b) (4)ppm. Therefore, the proposed specification for (b) (4) in this drug product is acceptable from a Pharmacology/Toxicology perspective.

2.6 Proposed Clinical Population and Dosing Regimen

Two daily topical applications on affected areas with mild to moderate atopic dermatitis will be applied in patients 2 years of age or older.

2.7 Regulatory Background

To date, crisaborole has not been approved by FDA.

End of Phase 2 meeting conducted on February 26, 2014
Pre-NDA meeting conducted on September 23, 2015

3 Studies Submitted

3.1 Studies Reviewed

1. A 6-month oral rat study with one-month recovery period

2. A 9-month dermal minipig study with one-month recovery period

3. Oral perinatal/postnatal development rat study including postnatal behavioral/functional evaluation

4. Dermal mouse carcinogenicity study (reviewed by Dr. Hill)

5. Oral rat carcinogenicity study (reviewed by Dr. Hill)

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

INDs 102317 and 077537

4 Pharmacology

4.1 Primary Pharmacology

It is suggested that the New Molecular Entity Crisaborole, an anti-inflammatory agent, acts by inhibiting the release of cytokines. A number of *in vitro* and *in vivo* studies were conducted to support the proposed primary mechanism of action involving inhibition of phosphodiesterases (e.g. PDE4) blocking the signal for release of pro-inflammatory cytokines.

Drug inhibited the release of several pro-inflammatory cytokines from the peripheral blood mononuclear cells (PBMCs) stimulated with lipopolysaccharide (LPS), phytochemagglutinin (PHA), or concanavalin A. Drug did not inhibit metaloproteinases, however, it caused significant (77%) inhibition of prostaglandin secretion; it also inhibited ICAM/VCAM (cell adhesion molecules) mediated cell adhesion.

Seven out of eight crisaborole formulations at 5% strength (b) (4) ointments, (b) (4) exhibited significant anti-inflammatory activity in mouse phorbol ester ear edema model; inhibition by the proposed clinical formulation (AN2728 ointment) was 74 percent.

4.2 Secondary Pharmacology

No category specific studies were reported.

4.3 Safety Pharmacology

In vitro safety pharmacology assays conducted at multiples of MRHD (mg/m^2), drug did not affect the common receptors, ion channels, and monoamine transporters; the drug also tested as a low-potency HERG-channel blocker.

In safety pharmacology studies, crisaborole did not damage the functioning of cardiovascular system in rats and mice at an oral dose of 1000mg/kg (125 and 62 MRHD as mg/m^2). In male dogs (30,100,300mg/kg), one high-dose animal died from hypertensive shock, however, at the same dose level, EKG, QTc intervals, and locomotor activity were not affected. Absolutely, no adverse cardiovascular effects were observed at the mid-dose level (42 MRHD). Cardiovascular functions in minipigs treated with crisaborole, ointment 5%, remained normal throughout 3-month treatment period. In 9-month minipig dermal study, absolutely no drug related changes in ECGs were observed throughout the treatment and one-month recovery period.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

In a set of *in vitro* assays using freshly isolated human hepatocytes from three healthy volunteers, crisaborole did not induce any drug metabolizing CYP enzymes, especially CYP3A4, the most prominent CYP in humans induced by a broad spectrum of prescribed drugs. These findings suggest minimal (if any) drug-drug interactions.

Six metabolites of parent drug were characterized in assays conducted with primary hepatocytes from mouse, rat, dog, minipig and human. The parent drug was mainly biotransformed to its primary metabolite AN7602 via oxidative deboronation/hydrolysis. Drug was also metabolized by Phase II enzymes via sulfation (metabolite M9), and glucuronidation (M3 and M14), and by Phase I enzymes via oxidation to metabolite AN8323. M9 was a common metabolite among the test species. No unique metabolite(s) was identified in human hepatocytes.

In assays with human hepatic microsomes, drug did not cause any significant inhibition of CYP450 isoenzymes of C subfamily involved in drug metabolism. Together, these isoforms are estimated to account for metabolism of 20% of the prescribed drugs in humans.

In vitro data suggested that anti-inflammatory action of crisaborole was due to the inhibition of phosphodiesterse 4, which in turn blocked the release of pro-inflammatory cytokines and chemokines.

5.2 Toxicokinetics

Included in toxicity studies

6 General Toxicology

6.1 Single-Dose Toxicity

Not required

6.2 Repeat-Dose Toxicity

Study title: **A 6-Month study of AN2728 by oral gavage in rats, including a 1-month recovery evaluation**

Study no.:	003-NCL TX-052-01
Study report location:	SD-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06-19-2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	#02110076/100%

Key Study Findings: During 6-month of oral treatment, crisaborole at the highest dose level of 450mg/kg/day (NOAEL) did not cause any local or systemic toxicity. Pharmacokinetic data at the end of treatment period did not indicate any accumulation of parent drug or its two major metabolites.

Methods

Doses:	0, 50, 150, and 450mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	10mL/kg
Formulation/Vehicle:	Drug in 1% (w/v) carboxymethylcellulose
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	10/sex/group for main study animals and 5/sex/group for one month recovery animals
Age:	Approximately seven weeks
Weight:	Males, 181-292g; females, 151-222g
Satellite groups:	Pharmacokinetic analyses: 3 rats/sex in control, and 6 rats/sex in drug groups
Unique study design:	None
Deviation from study protocol:	None
	Animals received daily dose of test substance or control for 182 days. On days 183 and 211, rats were sacrificed after completion of treatment and recovery period, respectively.

Observations and Results

Mortality/Clinical Signs

A total of 6 deaths (5M+F) occurred during the main study. Reportedly, none of these causalities were drug and or dose-related.

Sex/group	Day of death	Cause of death	Clinical signs
M/control	82	Faulty gavage	Consistent diarrhea
M/low-dose	37	Euthanized	Consistent diarrhea
M/low-dose	139	Faulty gavage	Consistent diarrhea
M/High-dose	84	Faulty gavage	Excessive salivation
M/high-dose	100	Euthanized	Palate ulceration/bleeding
F/control	187	Euthanized	Bleeding

A significant ($p< 0.05$) incidence of sparse hair coat of the limbs more pronounced in high-dose rats, and localized alopecia in high-dose females ($p<0.01$) were considered to be of common occurrence in the laboratory environment.

Body Weights/Feed Consumption

Body weights and food consumption were recorded weekly

Sporadically distributed but statistically significant reduction in body weights in both sexes did not exhibit any dose-related trend, and therefore, were not considered to be of any toxicological significance. At completion of recovery period, the average body weights in low-, mid-, and high-dose males were 98, 92, and 96% of controls. The corresponding values in females at the end of treatment period were 97, 93, and 92 percent.

A similar pattern was observed for food consumption. Relative food consumption values were unaffected during the recovery period.

Ophthalmoscopy

Examinations in all rats were conducted prior to the first dose and during week of sacrifice.

Retinal degeneration in one high-dose male and retinal scar in a control male were considered incidental (related to age and strain), and not drug or dose-related.

ECG

No electrocardiograms were recorded.

Hematology/Coagulation/Clinical Chemistry

Blood samples from all survivors collected on days 183 (end of treatment) and 211 (end of recovery) were analyzed for 16, 3, and 19 hematology, coagulation, and clinical chemistry parameters, respectively.

In high-dose males mean corpuscular hemoglobin concentration (MCHC) was significantly ($p<0.5$) reduced on day 183; on the same day, Hb, hematocrit, MCH and MCHC were significantly ($p<0.05$) reduced in high-dose females.

At recovery, statistically significant changes in hematologic parameters were low in magnitude and within the range of biological variation.
Significant ($p<0.05$) reductions in APTT in the high-dose males and prothrombin time at all dose levels in males at the end of treatment period were of very small magnitude (0.85 to 0.95x control), and therefore, were not considered to be of any toxicological concern.

A number of significant ($p<0.05$) randomly distributed changes (decrease/increase) in several clinical chemistry parameters, because of low magnitude, were not considered to be of any toxicological significance. Most of the values were within the range of biological variation.

Urinalysis

Overnight urine samples to determine 14 parameters were collected a day prior to sacrifice.

No significant inter-group differences in any urinary parameter were recorded.

Gross Pathology

Comprehensive necropsy examinations were conducted at the end of treatment and recovery periods.

No treatment-related inter-group differences in gross pathology lesions were observed.

Organ Weights

A total of 18 major internal and external organs were weighed to determine the absolute and relative weights to body weights.

No drug or dose-related absolute organ weights or organ to body weight ratios were recorded. A few sporadically distributed but statistically significant changes did not exhibit any trend, and therefore, were considered incidental or related to differences in sexual maturity. A similar pattern was also observed in organ weight/body weight ratio changes.

Histopathology

A total of 52 organs/tissues were subjected to microscopic evaluation.

Adequate Battery
Yes
Peer Review

Yes

Histological Findings: The cause of death of high-dose male (day 84) remained unexplained. Deaths in one control female and one low-dose male were due to esophageal perforation (faulty gavage). Both, a control female (day 187) and a high-dose male (day 100) exhibited inflammatory lesions consisted with bacterial infection.

Unique renal neoplasms in three rats (one male each from mid- and high-dose groups), and one low-dose female were considered spontaneous, and not drug-related. A CNS tumor in an olfactory bulb and spreading caudally in the neuropil was related to microbiological elements in the neuropil.

Special Evaluation

None

Toxicokinetics

Blood samples from pharmacokinetic groups (3/sex/group) were collected pre-dose, 0.5, 1, 2, 6, and 24 hours post-dose on days 1, 88, and 176. Plasma samples were analyzed for parent drug and two major metabolites, AN7602 and AN8323.

Following the first dose, the plasma drug levels were slightly higher in males at all dose levels. The amount of parent drug was 3-4 times greater than AN7602 and 1.5-2 times greater than AN8323. On day 176, all dose groups exhibited up to 2 times increase in AN7602 and up to 6 times in AN8323 (table1).

Table1. Mean plasma amounts of parent drug (Crisaborole, AN2728) and metabolites AN7602 and AN8323 in high-dose (NOAEL) groups on day 176.

Parameters:	AN2728	AN7602	AN8323
		Males	
C_{max} (µg/mL)	3.94	1.86	55.50
AUC_{0-t} (µg.h/mL)	13.30	17.40	930.00
		Females	
C_{max} (µg/mL)	3.15	2.64	91.80
AUC_{0-t} (µg.h/mL)	16.00	10.10	1170.00

The half-life of crisaborole ranged from 2.3 to 5.7 hours with Tmax of 0.5 to 1.0 hour. On day 176, Tmax of 8323 was 2-6 hours; however, terminal half-life was not calculated.

Dosing Solution Analysis: Crisaborole was not detected in any vehicle control samples. Drug concentration in the dosing solutions ranged from 88 to 106% of the targeted values. The calculated RSD of dosing solutions did not exceed 9 percent. Drug concentration and homogeneity of drug test solutions met the conventionally accepted

criteria of within or equal to±15% of the nominal concentrations and ≤10% RSD of individual concentration groups.

Study title: **AN2728: A 39-Week Dermal Toxicity Study with Twice Daily Application of AN2728 Ointment Followed By a 4-Week Recovery Period in Gottingen Minipigs**

Study no.: 003-NCL TX-051-01	
Study report location:	SD-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03-29-2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crisaborole, EAB/EHH (2%), EAC/EHK (5%), EAD/EHL (7%), and 97.9-104%

Key Study Findings: Following two daily applications of crisaborole ointment at the maximum feasible strength of 7%, absolutely no systemic toxicity was exhibited by minipigs. Sporadically distributed dermal lesions (e.g. hyperkeratosis/parakeratosis) among all groups including controls, were due to slight traumatic dermal abrasion developed during handling of large animals.

A minimal systemic absorption of crisaborole did not provide enough meaningful toxicokinetic data to determine realistic drug bioavailability. However, the available data definitely supported lack of systemic toxicity during nine months of drug treatment and one month of recovery periods.

The highest test concentration of 7% crisaborole was established as NOAEL for drug.

Methods

Doses:	0% (vehicle), 2%, 5%, and 7% crisaborole ointments
Frequency of dosing:	Twice daily
Route of administration:	Topical
Dose volume:	5mg/cm^2
Formulation/Vehicle:	Drug in vehicle
Species/Strain:	Minipig/Gottingen
Number/Sex/Group:	3/sex/group in control and drug groups, and 2/sex/group in recovery period
Age	4-5 months
Weight:	Males, 8.6-11.2kg; and females, 9.0-11.5kg
Satellite groups:	None
Unique study design:	Nothing significant
Deviation from study protocol:	Nothing significant

Observations and Results

Mortality/Clinical Signs

No drug-related deaths occurred during the study period.

Incidental dermal reactions such as red patches on the skin and scabs were observed during the pretreatment, treatment and recovery periods in all groups including controls. Clinical signs observed during the treatment period such as restricted use of limbs/paws, uncoordinated gait, vocalization, skin lump, reduced activity and crawling also occurred across the groups including controls.

A total of 5 drug treated animals (2 low-doses, one mid-dose, and 2 high-dose) exhibited crouching, partly closed eyes, warm to touch, coughing, shaking/shivering, or tremors. These adverse effects were not dose-related, and mostly resolved without any medical treatment.

Skin lesions (Modified Draize Scoring): In general, drug/vehicle related skin reactions on the application sites were observed among all the groups including controls. Incidences included mild to severe erythema with or without edema throughout the treatment and recovery periods. Lesions resolved with time, and therefore, were not considered to be treatment related.

Body Weights/Feed Consumption:

Body weights and food consumption were determined weekly and daily, respectively.

No statistically significant intergroup differences in any parameters were recorded**.**

Ophthalmoscopy

Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were conducted on all animals during weeks 13 and 39.

No drug treatment related changes in eye morphology were observed.

ECG

Electrocardiograms obtained from all animals prior to drug application, and during weeks 13 and 39, were used for qualitative and quantitative assessments of gross changes that may be indicative of cardiac electrical dysfunction and potential presence of involving heart rate (lead II), sinus and atrioventricular rhythm.

There were no drug related changes in ECGs during the entire treatment and recovery periods.

Hematology/Clinical Chemistry/Coagulation/Urinalysis

Overnight fasting samples (blood/urine) for clinical pathology were collected from all animals prior to initiation of treatment, and from overnight fasting animals during treatment weeks 13 and 39, and at the end of the recovery period.

No inter-group differences in hematology (14 parameters), clinical chemistry (18), coagulation (APTT and PT), and urinary (11) parameters were recorded.

Gross Pathology

Comprehensive necropsy examinations were conducted at the end of treatment and recovery periods.

Two out of six high-dose animals exhibited a few dark red areas in the lumbar portion of the dorsal dermal application site; Incidence was attributed to traumatic skin abrasion, not to drug application. Furthermore, no correlating microscopic changes were observed.

At recovery as well, no drug-treatment related macroscopic lesions were observed.

Organ Weights

Thirteen major organs were weighed to determine their absolute and relative to body weights.

Low testes weights in one male from each drug group, were correlated with the procedure-related microscopic changes in the organ, not to repeated drug applications.

Histopathology

Adequate Battery: Yes

Following processing, a total of 44 organs/tissues were subjected to microscopic examination.

Peer Review: Yes

Histological Findings: Infrequent appearance of microscopic lesions of low intensity on and around the application sites was observed in all groups including controls. These dermal reactions (e.g. hyperkeratosis/parakeratosis, epidermis crusts, perivascular edema/hemorrhage) attributed to slight traumatic dermal abrasion, were also correlated with the macroscopic findings of dark areas and wounds (gross pathology above).

Minimal to moderate hypoplasia/atrophy in testes of 2 males in each group including controls was correlated with lower organ weights (above). These changes were caused by restraining relatively large animals in sitting position in the final week of treatment to facilitate venipuncture, resulting in slight compression of the scrotum and testes. Same lesions of diminished intensity were observed during the recovery phase. Once again, none of these changes were caused by drug or vehicle treatment.

Special Evaluation

None

Toxicokinetics

Blood samples were collected from all animals on days 1,168 and 252 prior to test application and at 1, 2, 4, 8, and 24 hours post-dose.

Compared to single dose, repeated applications of crisaborole ointments for 168 and 252 days increased Cmax 3-6 times; a slight dose-related trend was only observed between days 1 and 168. Differences in exposure between the mid and high dose were insignificant, indicating that maximum feasible dose of 7% was almost a saturation level. Toxicokinetic profiles were similar in both sexes, although values were generally higher in males (table2).

Because of limited toxicokinetic data, it was not possible to determine the most critical safety parameter of bioavailability (F). Available data also strongly supported the absolute lack of expression of any systemic toxicity over nine months of twice daily applications of clinical formulations.

Two major metabolites of crisaborole (AN7602, the deboronated-crisaborole, AN8323 or carboxyl-AN7602) were detected in the plasma after the first application, and thereafter. On day 1, the parent drug was ~3-4xs greater than the AN7602 and ~1.5 to2x greater than AN8323. After repeated applications, all dose groups exhibited increase in AN7602 (2-6x) and AN8323 (5-10X) (table3)

Table 2: Pharmacokinetic profile of crisaborole on days 1, 168, and 252 following two daily topical applications of ointment.

Day	Parameter	Male			Female		
		2%	5%	7%	2%	5%	7%
1	Cmax, ng/mL	15.8	16.7	17.9	12.0	16.1	11.9
	Tmax, h	4.80	4.80	6.40	8.80	6.80	2.80
	$T_{1/2}$, h	NC[1]	25.6	NC[1]	NC[1]	14.5	37.2
	$AUC_{(0-24)}$, ng·h/mL	287	295	343	211	255	213
	$AUC_{(0-inf)}$, ng·h/mL	NC[2]	NC[2]	NC[2]	NC[2]	NC[2]	NC[2]
	Cmax Ratio (Male/Female)	1.32	1.04	1.50	-	-	-
	$AUC_{(0-24)}$ Ratio (Male/Female)	1.36	1.16	1.61	-	-	-
168	Cmax, ng/mL	56.9	63.4	66.6	61.6	66.6	67.3
	Tmax, h	2.20	1.60	1.00	1.00	2.20	2.80
	$T_{1/2}$, h	17.6	24.4	21.4	19.2	18.5	18.4
	$AUC_{(0-24)}$, ng·h/mL	802	916	774	809	1,020	988
	Cmax Ratio (Male/Female)	0.924	0.952	0.990	-	-	-
	$AUC_{(0-24)}$ Ratio (Male/Female)	0.991	0.898	0.783	-	-	-
	Cmax Ratio (Day 168/Day 1)	3.60	3.80	3.72	5.13	4.14	5.66
	$AUC_{(0-24)}$ Ratio (Day 168/Day 1)	2.79	3.11	2.26	3.83	4.00	4.64
252	Cmax, ng/mL	41.8	57.9	55.9	43.6	76.0	68.0
	Tmax, h	2.80	2.40	3.20	1.40	2.40	3.20
	$T_{1/2}$, h	16.8	21.8	31.3	19.6	18.4	27.2
	$AUC_{(0-24)}$, ng·h/mL	639	906	867	731	1,130	1,100
	Cmax Ratio (Male/Female)	0.959	0.762	0.822	-	-	-
	$AUC_{(0-24)}$ Ratio (Male/Female)	0.874	0.802	0.788	-	-	-
	Cmax Ratio (Day 252/Day 1)	2.65	3.47	3.12	3.63	4.72	5.71
	$AUC_{(0-24)}$ Ratio (Day 252/Day 1)	2.23	3.07	2.53	3.46	4.43	5.16

NC[1] - Not Calculated; due to not enough data points in the post distribution phase
NC[2] - Not Calculated; either greater than twenty percent extrapolation from $AUC_{(0-T)}$ or the $T_{1/2}$ was not calculated

Table3. Mean levels of crisaborole (AN2728) and its major metabolites AN7602 (deboronated crisaborole) and AN8323 (carboxyl-AN7602) at the NOAEL of 7% ointment on day 252.

	Gender	C_{max} (ng/mL)	$AUC_{(0-24)}$ (ng·h/mL)
AN2728	M	55.9	867
	F	68.0	1100
AN7602	M	16.5	248
	F	19.2	285
AN8323	M	82.2	1410
	F	126	2150

Dosing Solution Analysis: Samples of test formulations collected during the treatment period and at the end of study, were used to determine level of parent drug.

No crisaborole was found in the vehicle samples, and samples in drug formulations were within ±15% of the targeted parent drug concentration.

7 Genetic Toxicology

Summary: Crisaborole tested non-mutagenic in Ames assays conducted using four *Salmonella* and one *E. coli* strains in presence/absence of Aroclor- induced rat liver S9 fraction. Drug also did not induce any structural/numerical chromosomal aberrations in activated/non-activated (Aroclor-S9) human peripheral blood lymphocytes. In rat micronucleus assay, crisaborole at dose levels up to 2,000mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes.

8 Carcinogenicity

Summary: In 2-year carcinogenicity studies, no evidence of crisaborole-induced tumors was observed in mice at the maximal feasible dermal dose of 7% ointment or in rats at oral dose of 300 mg/kg/day in males and 100 mg/kg/day in females, respectively. Benign granular cell tumors of the female reproductive tract (uterus with cervix and vagina combined) were observed in the oral rat carcinogenicity study at a dose of 300 mg/kg/day. Relevance of this finding in humans is unknown.

For detailed reviews of carcinogenicity studies by Dr. Barbara Hill, see appendices 3 and 4.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Summary: An oral rat fertility study (0, 150, 300, and 600 mg/kg/day crisaborole) was conducted in male and female rats. The NOAEL for reproduction was established at the highest dose level. No effects on fertility were observed in male or female rats that

were administered oral doses up to 600 mg/kg/day crisaborole prior to and during early pregnancy.

9.2 Embryonic Fetal Development

Summary: Oral rat (0, 150, 300, and 600mg/kg/day crisaborole) and rabbit (0, 25, 50, and 100mg/kg/day crisaborole) embrofetal developmental studies were conducted. NOAELs for fetal malformations and maternal toxicity were set at 300 and 100 mg/kg/day crisaborole in rats and rabbits, respectively. Maternal toxicity with associated findings of decreased fetal body weight and delayed skeletal ossification was noted at 600 mg/kg/day crisaraborole in rats.

9.3 Prenatal and Postnatal Development

Study title: **A developmental and perinatal/postnatal reproduction study of AN2728 by oral gavage and rats, including a postnatal behavioral/functional evaluation**

Study no.:	003-NCL TX-056-01
Study report location:	SD-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06-30-2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crisaborole, batch#13-451 and 99.9%

Key Study Findings: Because of reduced gain in body weight and food consumption during gestation and lactation periods at the high-dose level of 600mg/kg/day, the NOAEL for maternal toxicity was established at the mid-dose level of 300mg/kg/day.

The reproductive NOAEL in females was 600mg/kg/day; and the NOAEL for viability and growth in the offspring was 300mg/kg/day.

Methods

Doses:	Maternal dose levels: 0,150, 300, and 600mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	5mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	1% (w/v) carboxymethylcellulose
Species/Strain:	Rat/Sprague-Dawley Crl:CD(SD) females
Number/Sex/Group:	F_0 Generation: 25 pregnant females F_1 Generation: 25 pups/sex/group
Satellite groups:	None
Study design:	F_0 Generation: Dams received daily dose of test material from gestation day 7 through lactation day 20 (rats that delivered a litter) or gestation day 24 (rats that did not deliver a litter). Doses were based on mostly recorded body weight. F1 Generation: Pups did not receive any test material directly, assuming they will be exposed via the maternal milk during the lactation period. Note from the study director: “This study was designed to evaluate ICH Harmonized Tripartite Guideline stages C to F to the reproductive process, but did not include the evaluation of caesarean- delivered fetuses (stages C and D), because the evaluation was performed in supplementary study”.
Deviation from study protocol:	Nothing significant

Observations and Results

F_0 Dams

Survival: All females survived to scheduled necropsy.

Clinical signs: Two high-dose dams exhibited dehydration and hunched posture during first lactation week; one of these mothers delivered all dead pups on lactation day 6.

Other clinical signs such as sparse hair coat, scab on the head, chromodacryorrhea, and localized alopecia were not considered to be drug or dose related; these lesions are commonly developed in the environment of animal facilities.

Body weight: In the high-dose group, gain in body weight during the entire gestation dose period (days 7 to 20) was significantly (<0.05) reduced (91% of control). The corresponding values at the low-and mid-dose levels were 99, and 94%, respectively.

A similar pattern of decreased gain in body weight in the high-dose dams was maintained during lactation days 1 to 4 (92% of control); however, the gain in this group was comparable to controls in remainder of the lactation period.

The absolute body weight and weight gain in the low- and mid-dose groups were not affected during the lactation period. The gain and average absolute body weights were significantly (p< 0.01-0.05) increased in three drug groups.

The absolute (g/day) and relative (g/kg/day) food consumption were significantly (p<0.05/<0.01) reduced in high-dose mothers throughout the entire gestation period. The absolute food consumption values for the entire lactation period in drug groups were 102, 102, and 92 percent of controls, respectively.

In nutshell, absolute and relative food consumption values during the gestation and lactation periods were not affected in the low- and mid-dose dams.

Uterine content:	The rate of pregnancy in controls (25/25=100%), low-(22/25=88%), mid-(24/25=96%, and high-dose (25/25=100%) females, did not indicate any drug or dose-related trend. Crisaborole also had no effect on the number of delivered litters, duration of gestation, and number of implantation sites. All drug-related adverse effects were restricted to high-dose (600mg/kg/day) females. Thus, the percentage of still born pups was increased and live pups was decreased (both at $p<0.01$). The number of pups that were found dead or missing and presumed cannibalized on lactation days 1-2, to 4-5 to 7 were significantly increased ($p<01$) at various points during the lactation period. A litter in high-dose group had all pups alive, but found dead on lactation day one. In the high-dose group, pups weights were significantly ($p<0.01$) reduced during the first week of lactation (14-16% lower than controls).
Necropsy observation:	Maternal necropsy examination did not reveal any treatment-related changes.
Toxicokinetics:	No analyses were conducted.
Dosing Solution Analysis	No parent drug was detected in vehicle control samples. Mean sample concentration level of crisaborole in individual dosing solutions was within and equal to 15% of the target concentration. For homogeneity, RSD of target concentration was <5% for each group.
Other:	None

F_1 Generation

Survival: The viability index (number of live pups on day 4 postpartum/number of live born pups on day 1 postpartum) was significantly ($p<0.01$) reduced by 16 percent; however, lactation index was only reduced by 3 percent ($p<0.01$).

Clinical signs: At high-dose level (5/6 litters), pups looked pale with mild to moderate dehydration. No other signs in F_1 generation were drug or dose-related. Clinical signs in control group (1-3 litters) included cold to touch, scabbing, purple body or lower midline, bradypnea, restlessness, sparse hair coat, thinness, bent tail, laceration of the head, and missing tip of tail.

Body weight: Changes in gain in body weights were mostly restricted to the high-dose group. Body weights in males from day 29 through 85 postpartum were significantly reduced (6-8% at $p<0.01$-0.05). However, gain in body weight in high-dose females was only reduced (6-8% at $p<0.01$) during the first four weeks of post weaning.
At mid-dose level of 300mg/kg/day, body weights in both sexes were not affected during the post-weaning, pre-cohabitation, and gestation periods.

Feed consumption: The absolute food consumption in high-dose males was significantly ($p<0.01$) reduced between days 22-29 (-16%) and 29-36 (-10%), respectively. Thereafter, no significant inter-group difference in food consumption was recorded.

The absolute food consumption in high-dose females was significantly ($p<0.01$) reduced between days 22-29 (-10%), 29-36 (-11%), and 36-43 (-8%).

Physical development: All pups that lived to scheduled sacrifice on lactation day 21 appeared normal at necropsy. Sexual maturation was not affected at the maternal high-dose level of 600mg/kg/day. The average day on which preputial separation or vaginal potency occurred was comparable among the four groups.

The drug at the high dose level had no statistically significant effect on learning, short-term retention, or response inhibition in rats of either sexes, as

confirmed in a passive avoidance paradigm. Also, no statistically significant inter-group differences in water maze performance were observed.

Reproduction: All reproduction functions such as number of days in cohabitation, mating and fertility indices, the number of rats that mated, and number of pregnancies per number of rats in cohabitation were similar among the four groups.

Other: Examination of ovarian and uterine content revealed a significant ($p<0.05$) reduction in number of corpora lutea (9%) and implantations (11%) in high-dose F_1 females. Other parameters such as pre-implantation loss, litter sizes, live and dead fetuses, and early and late resorptions were not significantly different among four test groups.

F_2 Generation

Survival: There were no significant inter-group differences in live fetuses (table4)

Body weight: There were no significant inter-group differences in body weights (table4)

External evaluation: The only gross external alteration was a short tail in a single fetus in the mid-dose group.

Male/Female ratio: Control=1.02, low-dose=0.9, mid-dose=1.0, and high-dose=1.2

Other: Cesarean-sectioning revealed no significant drug-related changes up to 600mg/kg/day in average parameters such as pre-implantation loss, litter sizes, live fetuses, early and late resorptions, post-implantation loss, fetal body weights, percent resorted concept uses, and live male fetuses, and there were no dead fetuses. (table4).

All placentae appeared normal, however, litter averages for corpora lutea and implantation was significantly reduced (11% at $p<0.05$) at the high-dose group (table4). Accordingly, this decrease was still within the range of historical control data of animal testing laboratory.

Table 4. A summary of perinatal/postnatal parameters in F_2 generation.

Dose groups (mg/kg/day): Parameter	0	150	300	600
Litter with 1 or more live fetuses	23	21	24	22
Implantation (Mean/SD)	16.3/2.0[1]	16.0/1.2	15.8/2.1	1.5/2.4*
Live fetuses	352	326	358	308
Live fetal body weight (g/litter)	5.6	5.6	5.3	5.6
% Resorption conspectuses/litter	5.6/8.7[1]	2.7/4.2	6.1/8.6	3.4/6.8

*$p<0.05$ [1] standard deviation

10 Special Toxicology Studies

In mouse local lymph node assay, crisaborole at 1, 5, and 10% (w/v) levels did not produce any skin sensitization. In primary ocular and skin irritation assays in rabbits, 2% AN2728 ointment was tested as a mild to moderate irritant.

11 Integrated Summary and Safety Evaluation

To support daily dosing regimen of 6 grams of crisaborole ointment, 2% (MRHD equivalent to 74mg/m^2/day based on 60kg subject) in atopic dermatitis patient the sponsor has presented a non-clinical safety profile consisting of 68 assays/studies conducted under two original INDs (102317 and 077537) and relevant supplements. Almost all studies were conducted at multiples of MRHD; critical dermal studies were conducted at the maximum feasible dose level of 7% crisaborole ointment. During early process of drug development, investigations were conducted in multiple species (mouse, rat, dog, minipig, ferret, and suncus) using multiple routes (oral, dermal, intravenous, and intramuscular) of drug administration. Eventually, rat and minipig were employed as the most sensitive and resistant species, respectively.

Note: MRHD in terms of mg/m^2 is only employed in single-dose oral studies (e.g. Safety Pharmacology) where conventionally no pharmacokinetic analyses are conducted, and therefore, the systemic absorption is assumed to be 100 percent.

In vitro assays for safety pharmacology revealed that crisaborole did not affect the common receptors, ion channels, and monoamine transporters; the drug also tested as a low-potency HERG-channel blocker.

In animal safety pharmacology studies, drug did not damage the functioning of cardiovascular system in rats and mice at an oral dose of 1000mg/kg (40 and 81 MRHD).

In single-dose (30,100,300mg/kg) male dog study, one high-dose animal died from hypertensive shock, however, at the same dose level, EKG, QTc intervals, and locomotor activity were not affected. Absolutely, no adverse cardiovascular effects were observed at the mid-dose level (27 MRHD).

Cardiovascular functions in minipigs treated with crisaborole, 5% ointment for three months remained normal. In 9-month minipig dermal study, two daily applications of 7% crisaborole ointment did not cause any changes in ECGs during the entire treatment and 1-month recovery period.

In single dose dog oral study, the bioavailability at 300mg/kg (81 MRHD) was only 0.8%. In single dose rat intravenous study, the elimination half-life in the abdominal fat was 1.2 hours; no drug retention was observed in major organs. In 90-day minipig

dermal study (0%, 0.5%, 2%, 5% crisaborole ointment), with minimal absorption, the approximate drug accumulation did not exceed 2-4x; apparently a concentration below the threshold level for minimum systemic toxicity.

During 6-month of oral treatment, crisaborole at the highest dose level of 450mg/kg/day (NOAEL) did not cause any local or systemic toxicity in rats. Pharmacokinetic data at the end of treatment period did not indicate any accumulation of parent drug or its two major toxicologically/pharmacologically inert metabolites, deboronated crisaborole (AN7602) and carboxy-AN7602.

In 3-month minipig dermal study (vehicle, 0.5%, 2%, and 5% crisaborole ointment B) with one-month recovery period, two daily applications did not produce any systemic toxicity at NOAEL of 5 percent.

On day-90, $AUC_{(0\text{-}24\ hr)}$ values for 2% crisaborole ointment (the recommended human dose) and 5% crisaborole ointment in males were 1,080 ng·hr/ml and 1,450 ng·hr/ml, respectively. The corresponding $AUC_{0\text{-}24\ hr}$ values in females were 851 ng·hr/ml and 1,330 ng·hr/ml, respectively. Taking into account, the pediatric mean $AUC_{0\text{-}24\ hr}$ of 1,320 ng·hr/ml, the safety margin at the NOAEL of 5% crisaborole ointment is approximately equivalent to the MRHD based on AUC comparisons.

Following two daily applications of crisaborole ointment at the maximum feasible strength of 7% (w/v) for 9 months, absolutely no systemic toxicity was exhibited by minipigs. Sporadically distributed dermal lesions (e.g. hyperkeratosis/parakeratosis) among groups including controls, were due to slight traumatic dermal abrasion developed during handling of large animals.

A minimal systemic absorption of crisaborole did not provide enough meaningful toxicokinetic data to determine systemic bioavailability of drug. However, the available data definitely supported an absolute lack of systemic toxicity during nine months of drug treatment and one month of recovery periods.

The highest test concentration of 7% crisaborole (3.5 times the applied human concentration) was established as NOAEL for drug.

Irrespective of the concentration level and gender, $AUC_{(0\text{-}24\ hr)}$ in males on day 168 and 252 ranged from lowest of 639 ng·hr/ml to highest of 1,130 ng·hr/ml, expressing a relatively low systemic margin of safety compared to the human pediatric mean $AUC_{0\text{-}24\ hr}$ 1,320 ng·hr/ml (see table 2 in this review).

Crisaborole tested non-mutagenic in Ames assays conducted using four *Salmonella* and one *E. coli* strains in presence/absence of Aroclor- induced rat liver S9 fraction. Drug also did not induce any structural/numerical chromosomal aberrations in activated/non-activated (Aroclor-S9) human peripheral blood lymphocytes. In rat

micronucleus assay, crisaborole at dose levels up to 2,000mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes.

In 104-week rat oral carcinogenicity study (0, 30, 100, and 300mg/kg/day), no treatment related non neoplastic lesions were found. A drug related increased incidence of benign granular cell tumors in uterus with cervix or vagina (combined) was noted in high-dose females (control 6/65; low-dose 7/65; mid-dose 4/65; high-dose 20/65). Relevance of this finding in humans is unknown.

In 104-week mouse dermal carcinogenicity study, daily topical applications of vehicle, 2, 5, or 7% crisaborole ointment did not produce any treatment related neoplastic lesions.

In rat fertility and early development oral study (0, 150, 300, and 600mg/kg/day), because of pronounced incidences of urine-stained abdominal fur, significant decrease in gain in body weight, and food consumption, the NOAEL for maternal toxicity was set at the mid-dose level (300mg/kg/day). However, since none of the fertility and litter parameters were affected at any dose level, the NOAEL for reproduction for both sexes was considered to be 600mg/kg/day, the highest dose tested**.**

In rat oral embryonic fetal development study (0, 150, 300, and 600mg/kg/day) study, reduced maternal body weights (-26%) in the high-dose group during the most critical period of skeletal development (gestation days 7-10) resulted in significant fetal body weight reduction and incomplete ossification of bones. Such effects at the low- and mid-dose levels were of insignificant magnitude. NOAEL for maternal and fetotoxicity (delayed skeletal development) was considered to be 300mg/kg/day.

In rabbit oral embryonic fetal development study (0, 25, 50, and 100mg/kg/day), with rapid absorption, Cmax at all dose levels was achieved within ½ hour. After 13 daily doses, there was no indication of any significant drug accumulation. Drug at the highest dose level did not cause any malformations or variations in fetuses, therefore, the NOAEL for maternal and fetal toxicity was considered to be 100mg/kg/day, the highest dose tested.

A comprehensive nonclinical safety profile for EUCRISA (crisaborole), Ointment, 2% supports the safety of the proposed clinical dosing regimen for the topical treatment of mild to moderate atopic dermatitis. This NDA is approvable from a Pharmacology/Toxicology perspective.

12 Appendix/Attachments

Appendix 1: Multiple of human exposure calculations

Human PK data: mean $AUC_{0\text{-}24\ hr}$ = 1320 ng·h/mL on Day 8 (per the clinical pharmacology reviewer, Dr. Chinmay Shukla)

The pharmacokinetics of crisaborole ointment, 2% were investigated in 34 pediatric subjects aged 2 to 17 years of age with mild to moderate atopic dermatitis in a clinical pharmacology maximal use systemic exposure study. Following twice daily topical application of crisaborole ointment, 2% to 27% to 92% BSA, the mean $AUC_{0\text{-}24\ hr}$ on day 8 was 1320 ng·h/mL.

A) Carcinogenicity studies

1) Oral rat carcinogenicity study

Week 26 $AUC_{0-24\ hr}$ values for crisaborole at 300 mg/kg/day dose:
Males – 5910 ng·h/mL; Females – 2860 ng·h/mL

Multiple of human exposure for 300 mg/kg/day in females is 2X (2860 ng·h/mL ÷ 1320 ng·h/mL = 2.1)

2) Dermal mouse carcinogenicity study

Day 181 $AUC_{0-24\ hr}$ values for 7% crisaborole ointment dose:
Males – 1920 ng·h/mL; Females – 2030 ng·h/mL; Average – 1975 ng·h/mL

Multiple of human exposure for 7% crisaborole ointment is 2X (1975 ng·h/mL ÷ 1320 ng·h/mL = 1.5)

B) Reproductive toxicity studies

1) Oral rat embryofetal development study

GD 17 $AUC_{0\text{-}24\ hr}$ for 600 mg/kg crisaborole dose: 24000 ng·h/mL
GD 17 $AUC_{0\text{-}24\ hr}$ for 300 mg/kg crisaborole dose: 6270 ng·h/mL

Multiple of human exposure for 600 mg/kg/day pregnant rats is 18X (24000 ng·h/mL ÷ 1320 ng·h/mL = 18.1)

Multiple of human exposure for 300 mg/kg/day pregnant rats is 5X (6270 ng·h/mL ÷ 1320 ng·h/mL = 4.8)

2) *Oral rabbit embryofetal development study*

GD 19 $AUC_{0\text{-}24\ hr}$ for 100 mg/kg crisaborole dose: 4070 ng·h/mL

Multiple of human exposure for 100 mg/kg/day pregnant rats is 3X (4070 ng·h/mL ÷ 1320 ng·h/mL = 3.1)

3) Oral rat peri- and post-natal development study

GD 17 $AUC_{0\text{-}24\ hr}$ for 600 mg/kg crisaborole dose: 24000 ng·h/mL

Multiple of human exposure for 600 mg/kg/day pregnant rats is 18X (24000 ng·h/mL ÷ 1320 ng·h/mL = 18.1)

4) Oral rat fertility study

GD 17 $AUC_{0\text{-}24\ hr}$ for 600 mg/kg crisaborole dose: 24000 ng·h/mL
Multiple of human exposure for 600 mg/kg/day pregnant rats is 18X (24000 ng·h/mL ÷ 1320 ng·h/mL = 18.1)

Appendix 2: Clean version of proposed nonclinical portions of the label

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE

EUCRISA is a phosphodiesterase 4 inhibitor indicated for topical treatment of mild to moderate atopic dermatitis in patients 2 years of age and older. (1)

8 USES IN SPECIFIC POPULATIONS
8.1 Pregnancy
Risk Summary
There are no available data with EUCRISA in pregnant women to inform the drug-associated risk for major birth defects and miscarriage. In animal reproduction studies, there were no adverse developmental effects observed with oral administration of crisaborole in pregnant rats and rabbits during organogenesis at doses up to 5 and 3 times, respectively, the maximum recommended human dose (MRHD) [see Data].

The background risk of major birth defects and miscarriage for the indicated population is unknown. However, the background risk of major birth defects in the U.S. general population is 2% to 4% and of miscarriage is 15% to 20% of clinically recognized pregnancies.

Data
Animal Data
Rat and rabbit embryo-fetal development was assessed after oral administration of crisaborole. Crisaborole did not cause adverse effects to the fetus at oral doses up to 300 mg/kg/day in pregnant rats during the period of organogenesis (5 times the MRHD on an AUC comparison basis). No treatment-related fetal malformations were noted after oral treatment with crisaborole in pregnant rats at doses up to 600 mg/kg/day (18 times the MRHD on an AUC comparison basis) during the period of organogenesis. Maternal toxicity was produced at the high dose of 600 mg/kg/day in pregnant rats and was associated with findings of decreased fetal body weight and delayed skeletal ossification. Crisaborole did not cause adverse effects to the fetus at oral doses up to the highest dose tested of 100 mg/kg/day in pregnant rabbits during the period of organogenesis (3 times the MRHD on an AUC comparison basis).

In a prenatal/postnatal development study, pregnant rats were treated with crisaborole at doses of 150, 300 and 600 mg/kg/day by oral gavage during gestation and lactation (from gestation day 7 through day 20 of lactation). Crisaborole did not have any adverse effects on fetal development at doses up to 600 mg/kg/day (18 times the MRHD on an AUC comparison basis). Maternal toxicity was produced at the high dose of 600 mg/kg/day in pregnant rats and was associated with findings of stillbirths, pup mortality and reduced pup weights.

8.2 Lactation

Risk Summary

There is no information available on the presence of EUCRISA in human milk, the effects of the drug on the breastfed infant or the effects of the drug on milk production. The development and health benefits of breastfeeding should be considered along with the mother's clinical need for EUCRISA and any potential adverse effects on the breastfed infant from EUCRISA or from the underlying maternal condition.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Crisaborole is a phosphodiesterase 4 (PDE-4) inhibitor. PDE-4 inhibition results in increased intracellular cyclic adenosine monophosphate (cAMP) levels. The specific mechanism(s) by which crisaborole exerts its therapeutic action for the treatment of atopic dermatits is not (b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In an oral carcinogenicity study in Sprague-Dawley rats, oral doses of 30, 100 and 300 mg/kg/day crisaborole were administered to rats once daily. A drug related increased incidence of granular cell tumors in the uterus with cervix or vagina (combined) was noted in 300 mg/kg/day crisaborole treated female rats (2 times the MRHD on an AUC comparison basis). The clinical relevance of this finding (b) (4) is unknown.

In a dermal carcinogenicity study in CD-1 mice, topical doses of 2%, 5% and 7% crisaborole ointment were administered (b) (4) once daily. No drug related neoplastic findings were noted at topical doses up to 7% crisaborole ointment (2 times the MRHD on an AUC comparison basis).

Crisaborole revealed no evidence of mutagenic or clastogenic potential based on the results of two in vitro genotoxicity tests (Ames assay and human lymphocyte chromosomal aberration assay) and one in vivo genotoxicity test (rat micronucleus assay).

No effects on fertility were observed in male or female rats that were administered oral doses up to 600 mg/kg/day crisaborole (18 times the MRHD on an AUC comparison basis) prior to and during early pregnancy.

Appendix 3: Dermal mouse carcinogenicity study review (reviewed by Barbara Hill, PhD)

Study title AN2728 ointment: A 104 week dermal oncogenicity study in mice

Study no.:	003-NCL TX-050-01
Study report location:	SDN 1, electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 17, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AN2728 ointment vehicle, lot # EAA-1/EHG-1, N/A AN2728 ointment, 2%, lot # EAB/EHH, 96.1% AN2728 ointment, 5%, lot # EAC/EHK, 95.8% AN2728 ointment, 7%, lot # EAD/EHL, 95.0%
CAC concurrence:	Yes, 7-5-11

Key Study Findings

No treatment related neoplastic or non-neoplastic findings were noted in this study.

Adequacy of Carcinogenicity Study

The two year dermal mouse carcinogenicity study appears to be adequate.

Appropriateness of Test Models

Conduct of a dermal mouse carcinogenicity study is appropriate for this drug product to evaluate possible tumors after topical administration which is the clinical route of administration.

Evaluation of Tumor Findings

No treatment related tumors were noted in this study.

Methods

Doses:	0 (untreated), 0 (vehicle), 2%, 5% and 7% AN2728 ointment
Frequency of dosing:	Once daily
Dose volume:	1.1 µl/g
Route of administration:	Topical to a clipped unoccluded treatment site equal to 10% BSA
Formulation/Vehicle:	Propylene glycol, (b) (4) %, butylated hydroxytoluene, (b) (4) %, (b) (4) %, (b) (4) %, (b) (4) %, (b) (4) %, white petrolatum, (b) (4) %
Basis of dose selection:	High dose based on maximum feasible dose which was well tolerated in a 13 week dermal mouse toxicity study
Species/Strain:	Mice/CD-1
Number/Sex/Group:	65/sex/group
Age:	6 weeks; Males: 26.1 – 35.3 grams; Females: 21.4 – 28.2 grams
Animal housing:	Single animal housing
Paradigm for dietary restriction:	N/A
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetic animals: untreated control: 9/sex vehicle control and test article groups: 40/sex/group
Deviation from study protocol:	None of significance

Observations and Results

Mortality

Mortality was assessed twice daily. No treatment related effects on mortality were noted in this study.

During the course of the main study, the following animals died or were euthanized *in extremis*: 40 males and 45 females in the untreated control group, 31 males and 44 females in the vehicle control group, 44 males and 44 females dosed in the low dose group, 39 males and 45 females in the mid dose group, and 36 males and 38 females dosed in the high dose group.

During Week 99, the untreated female control group survival had declined to a total of 19 animals. Based on the criteria recommended by the Exec CAC, all female groups were terminated during week 99. All male groups completed 104 weeks of treatment.

The causes of the moribundity/death of these animals varied across groups with no apparent dose relationship and were consistent with common causes of death of aging mice of this strain. The most frequent causes of moribundity/death included urogenital inflammation/obstruction/calculi in males and chronic progressive nephropathy/uremia and lymphoid tumors in females.

Clinical Signs

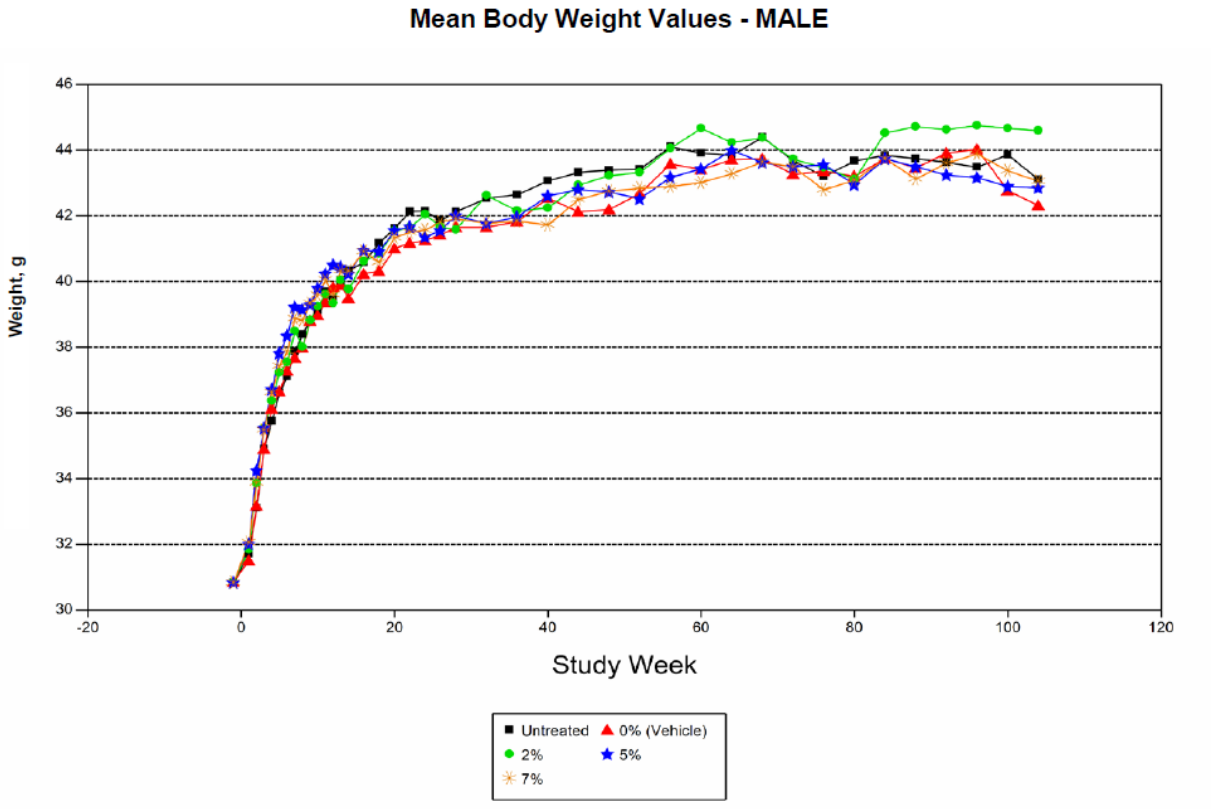
Clinical signs were assessed once weekly. No treatment related effects on clinical signs were noted in this study.

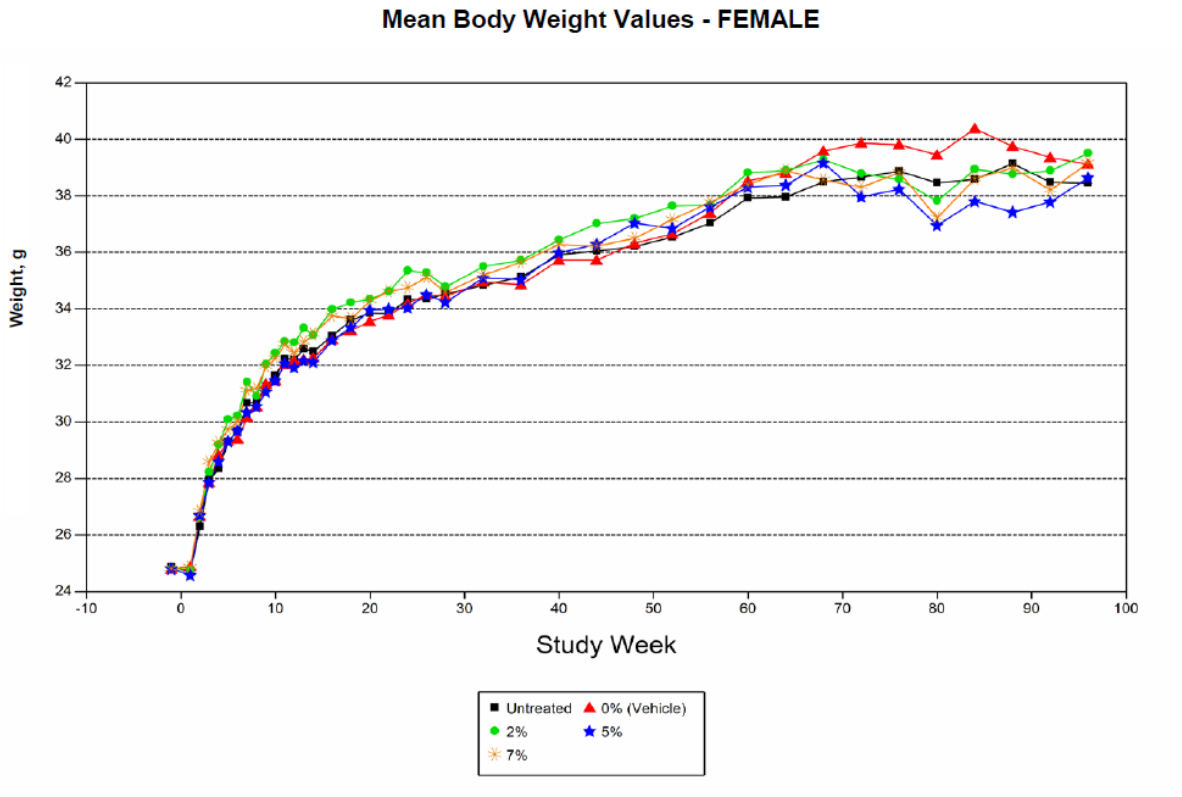
Dermal Irritation

Dermal irritation was assessed once weekly. No treatment related effects on dermal irritation were noted in this study.

Body Weights

Body weights were assessed weekly for the first 14 weeks, every two weeks until week 28 and every four weeks thereafter. No treatment related effects on body weight were noted in this study. Male and female body weight curves are provided below (copied from NDA submission).





Feed Consumption

Food consumption was assessed weekly for the first 14 weeks, every two weeks until week 28 and every four weeks thereafter. No treatment related effects on food consumption was noted in this study.

Ophthalmology

Ophthalmologic examinations were conducted on all animals at pretest and 6, 12 and 18 months and prior to scheduled necropsy. No treatment related effects on ophthalmologic parameters was noted in this study.

Gross Pathology

A complete gross necropsy with collection of tissues was performed for each animal at the end of treatment. No treatment related effects on macroscopic parameters were noted in this study.

Histopathology

The following tissues were collected for histopathological evaluation.

- Adrenal (2)
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]
- Clitoral gland (2)
- Coagulating gland (2)
- Epididymis (2)
- Eye including optic nerve (2)
- Gallbladder
- GALT [Gut Associated Lymphoid Tissue]
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2) with oviduct (2)
  - testis (2)
- Gross lesions
- Harderian gland (2)
- Heart
- Joint, tibiofemoral
- Kidney (2)
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [collected whole; 2 examined]
- Lung [collected whole; all lobes examined]
- Lymph nodes: mandibular [2 collected; 1 examined], mesenteric, and regional where applicable
- Mammary gland [only process females]
- Nose [levels a, b, c, and d]
- Pancreas
- Pharynx
- Pituitary
- Preputial gland (2)
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin, inguinal
- Skin [treated and untreated]
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen
- Thymus
- Thyroid/parathyroid (2)
- Tissue masses
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina
- Zymbal's gland (2)

---

[a]Bone marrow smears were collected at necropsies and held.

(2) Paired organ

Histopathological evaluation of all collected tissues was performed for all animals in all dose groups in this study.

Peer Review
Yes

Neoplastic

The sponsor states that there are no treatment related neoplastic findings in this study.

The CDER statistical reviewer, Dr. Steven Thomson, generated the following table in his review that lists the potentially statistically significant results for organ-tumor combinations in mice.

Table 6. Organ-Tumor Combinations in Mice with Results Close to Statistical Significance

Overall Results organ/tumor	Tumor Incidence Notrt	Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh plow vsVeh	pmed vsVeh pntrt vsVeh
Male Mice								
testes								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	48.4	55.7	45.0	43.1	46.3			
ADENOMA, INTERSTITIAL CELL	4	1	2	3	5	.0297	.0669 .4162	.2145 .9804
Female Mice								
bone marrow, femur								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	44.7	46.1	44.8	39.2	46.4			
LYMPHOMA	1	0	2	2	6	.0045	.4543 1	.5000 1
lung								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	45.2	47.2	44.2	38.3	46.0			
ADENOMA, BRONCHIOLAR ALVEOLAR	1	4	4	2	9	.0126	.4361 .1944	.1733 .1944
nose, level c								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	44.6	46.8	44.6	39.0	46.0			
LYMPHOMA	0	2	3	2	6	.0132	.2117 .2584	.1207 .2584
skin, inguinal								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	44.6	46.4	44.5	39.5	45.6			
LYMPHOMA	0	1	2	3	4	.0157	.0995 .5111	.2471 .5111
tongue								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	44.7	46.3	44.4	39.5	46.2			
LYMPHOMA	1	1	3	3	6	.0125	.2636 .7638	.3081 .7638
uterus with cervix								
# Evaluated	65	65	65	65	65			
Adj. # at Risk	44.6	46.1	44.0	38.3	44.6			
GRANULAR CELL TUMOR	0	0	0	1	2	.0297	.4634 .	. .
Adj. # at Risk	44.6	46.4	44.7	39.4	44.2			
HEMANGIOMA	0	4	3	2	2	.4356	.2177 .0639	.1207 .0639

The tumors identified by Dr. Thomson in the previous table are considered common tumors. The CDER eCAC criteria for considering a common tumor as treatment related are that both the trend and pairwise comparison for high dose versus vehicle have a p-value less than 0.01. None of the tumors listed in the previous table meet this criteria. Therefore, no treatment related tumors were noted in this study.

Non Neoplastic

No treatment related non neoplastic lesions were noted in this study.

Toxicokinetics

Blood samples for toxicokinetic assessment were collected from 3 animals/sex/group/timepoint on day 1 and week 26 at 0 (pre-dose), 0.5, 1, 3, 8 and 24 hours post dose. Plasma concentrations of the parent (AN2728) and the two major metabolites (AN7602 and AN8323) were determined from the collected blood samples.

The toxicokinetic parameters determined from this study are provided in the following table (copied from the NDA submission). The toxicokinetic parameters for the parent compound (AN2728) are provided in the following table.

Summary TK Parameters of AN2728 in Mice on Days 1 and 181 Following Once Daily Dermal Administration of 2, 5, and 7% AN2728 Topical Ointment

AN2728 Topical Ointment (%)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-T} (ng*hr/mL)	$t_{1/2}$ (hr)
Day 1					
2	Male	200	0.5	380	4.99
	Female	239	0.5	557	5.81
5	Male	1890	1.00	2840	7.53
	Female	418	0.5	985	4.19
7	Male	449	1.00	1090	4.52
	Female	1640	1.00	3030	5.39
Day 181					
2	Male	64.1	1.00	290	6.05
	Female	123	1.00	349	7.10
5	Male	240	0.5	815	10.2
	Female	407	1.00	2030	3.66
7	Male	350	3.00	1920	NC
	Female	1410	0.5	2030	3.83

AN2728 exposure was slightly higher in females compared to males across all dose groups, except for the Day 1 mid dose assessment. No significant accumulation of AN2728 was noted in this study. Systemic exposure increased with dose between the low and mid dose groups but appeared to plateau between the mid and high dose groups.

The metabolites, AN7602 and AN8323, were detected in plasma following dermal application of AN2728 ointment. No apparent accumulation of AN7602 was observed in male or female mice at all doses, except for mid dose females which showed mild

accumulation. Days 1 and 181 exposures of AN2728 were ~2-6 fold higher than that of the metabolite, AN7602, in both sexes.

The toxicokinetic parameters for the metabolite, AN7602, are provided in the following table.

Summary TK Parameters of AN7602 in Mice on Days 1 and 181 Following Once Daily Dermal Administration of 2, 5, and 7% AN2728 Topical Ointment

AN2728 Topical Ointment (%)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-T} (ng*hr/mL)	$t_{1/2}$ (hr)
Day 1					
2	Male	50.0	0.5	127	4.92
	Female	53.2	0.5	116	1.45
5	Male	243	1.00	443	7.93
	Female	93.0	0.5	251	3.94
7	Male	138	1.00	750	2.87
	Female	272	1.00	539	4.29
Day 181					
2	Male	25.6	1.00	91.8	NC
	Female	32.1	1.00	79.9	NC
5	Male	76.9	0.5	323	7.31
	Female	116	1.00	593	NC
7	Male	105	3.00	749	NC
	Female	264	0.5	511	4.21

NC - Not Calculated

AN8323 exhibited no accumulation in both male and female mice at all doses. The exposure of the metabolite, AN8323, was up to 10 fold higher than exposure of the parent compound, AN2728.

The toxicokinetic parameters for the metabolite, AN8323, are provided in the following table.

Summary TK Parameters of AN8323 in Mice on Days 1 and 181 Following Once Daily Dermal Administration of 2, 5, and 7% AN2728 Topical Ointment					
AN2728 Topical Ointment (%)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-T} (ng*hr/mL)	$t_{1/2}$ (hr)
Day 1					
2	Male	406	1.00	2700	4.18
	Female	699	3.00	5090	NC
5	Male	1310	1.00	7600	4.36
	Female	1520	3.00	8900	NC
7	Male	1340	3.00	10100	NC
	Female	1810	3.00	12700	NC
Day 181					
2	Male	223	3.00	1160	NC
	Female	365	3.00	3520	NC
5	Male	385	3.00	3350	NC
	Female	1550	3.00	8170	NC
7	Male	707	3.00	5030	NC
	Female	1410	3.00	7720	NC
NC - Not Calculated					

Dosing Solution Analysis

The dosing solution analysis demonstrated that the concentration of the dose groups ranged from 82.6% to 103.4% which is acceptable.

Appendix 4: Oral rat carcinogenicity study review (reviewed by Barbara Hill, PhD)

Study title: AN2728: A 104-week oral oncogenicity study in rats

Study no.:	003-NCL TX-049-01
Study report location:	SDN 1, electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 22, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AN2728; lot # SLBH3881V, 02110076, 02120059; 98.5%
CAC concurrence:	Yes, 7-5-11

Key Study Findings

A drug related increased incidence of granular cell tumors in the uterus with cervix or vagina, combined, was noted in high dose female rats (vehicle control, 6/65; low dose, 7/65; mid dose, 4/65; high dose, 20/65). No treatment related non-neoplastic findings were noted in this study.

Adequacy of Carcinogenicity Study

The two year oral rat carcinogenicity study appears to be adequate.

Appropriateness of Test Models

Conduct of an oral rat carcinogenicity study is appropriate for this drug product to evaluate possible systemic tumors if adequate systemic exposure is achieved after topical administration.

Evaluation of Tumor Findings

A drug related increased incidence of granular cell tumors in the uterus with cervix or vagina, combined, was noted in high dose female rats (vehicle control, 6/65; low dose, 7/65; mid dose, 4/65; high dose, 20/65).

Methods

Doses:	0 (vehicle), 30, 100 and 300 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg/day
Route of administration:	Oral (gavage)
Formulation/Vehicle:	1% carboxymethylcellulose
Basis of dose selection:	MTD based on mortality at 1000 mg/kg/day in males in a 4 week study and mortality, clinical signs and hematological changes at 1000 mg/kg/day in females in a 4 week study
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	65/sex/group
Age:	6 weeks; Males: 204 - 262 grams; Females: 152 - 206 grams
Animal housing:	Animals were pair-housed in solid bottom cages with nonaromatic bedding in an environmentally controlled room. One untreated animal/sex was added to each group to accommodate pair housing. Untreated animals were euthanized at study termination.
Paradigm for dietary restriction:	N/A
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetic animals: vehicle control: 5/sex Test article groups: 9/sex/group
Deviation from study protocol:	None of significance

Observations and Results

Mortality

Mortality was assessed twice daily. No treatment related effects on mortality were noted in this study.

During the course of the main study, the following animals died or were euthanized *in extremis*: 46 males and 46 females in the vehicle control group, 31 males and 42 females dosed in the low dose group, 30 males and 44 females in the mid dose group, and 38 males and 49 females dosed in the high dose group.

During Week 89 (males) and Week 94 (females), the vehicle control group survival had declined to a total of 20 animals or less. Based on the criteria recommended by the Exec CAC, all male groups were terminated during week 89 and all female groups were terminated during week 94.

The causes of the moribundity/death of these animals varied across groups with no apparent dose relationship and were consistent with common causes of death of aging rats of this strain. The most frequent causes of moribundity/death included pituitary gland tumors in both sexes and mammary gland tumors in females.

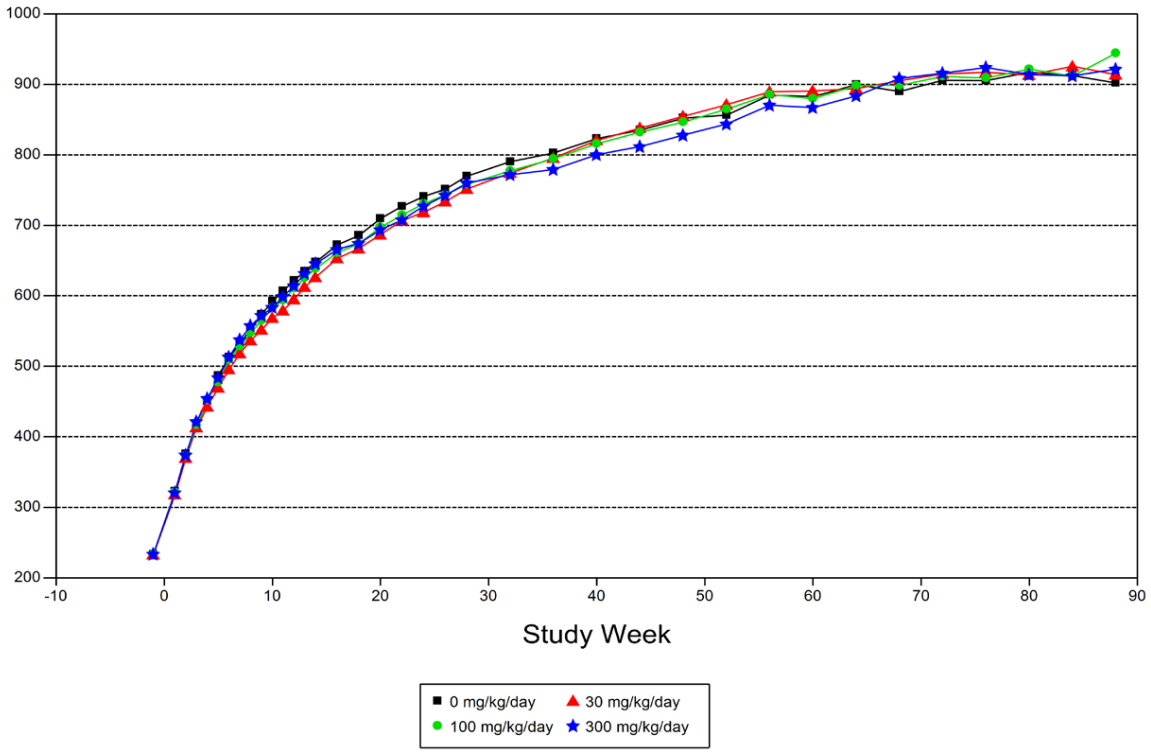
Clinical Signs

Clinical signs were assessed once daily. No treatment related effects on clinical signs were noted in this study.

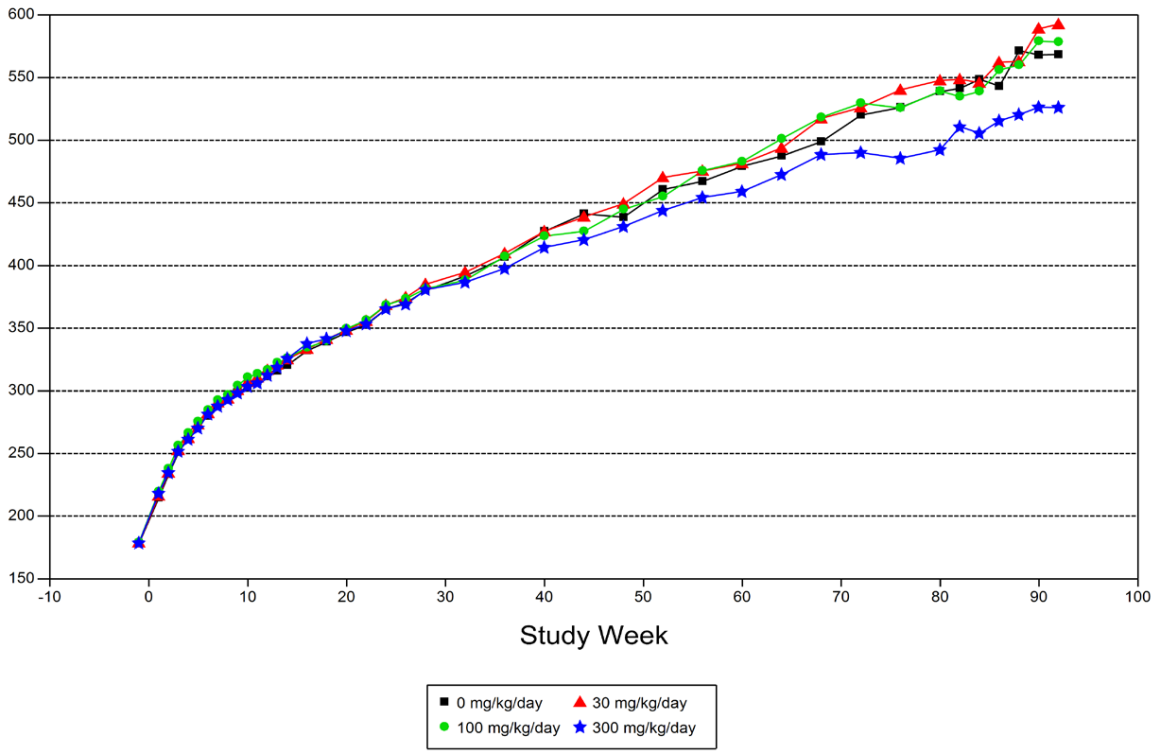
Body Weights

Body weights were assessed weekly for the first 14 weeks, every two weeks until week 28 and once every four weeks thereafter. No treatment related effects on body weight were noted in this study. Male and female body weight curves are provided below (copied from NDA submission).

Male mean body weights



Female mean body weights



Feed Consumption

Food consumption was assessed weekly for the first 14 weeks, every two weeks until week 28 and once every four weeks thereafter. No treatment related effects on food consumption were noted in this study.

Ophthalmology

Ophthalmologic examinations were conducted on all animals at pretest and 6, 12 and 18 months and prior to scheduled necropsy. No treatment related effects on ophthalmologic parameters were noted in this study.

Gross Pathology

A complete gross necropsy with collection of tissues was performed for each animal at the end of treatment. No treatment related effects on macroscopic parameters were noted in this study.

Histopathology

The following tissues were collected for histopathological evaluation.

- Adrenal (2)
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]
- Clitoral gland (2)
- Coagulating gland (2)
- Epididymis (2)
- Eye including optic nerve (2)
- GALT [Gut Associated Lymphoid Tissue]
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2) with oviduct (2)
  - testis (2)
- Gross lesions
- Harderian gland (2)
- Heart
- Joint, tibiofemoral
- Kidney (2)
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [3 sections collected; 2 examined]
- Lung [collected whole; 2 sections examined]
- Lymph nodes: mandibular [2 collected; 1 examined], mesenteric, and regional where applicable
- Mammary gland [only process females]
- Nose [levels a, b, c, and d]
- Pancreas
- Pharynx
- Pituitary
- Preputial gland (2)
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen
- Thymus
- Thyroid/parathyroid (2)
- Tissue masses
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina
- Zymbal's gland (2)

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[a]Bone marrow smears were collected at necropsies and held.

(2) Paired organ

Histopathological evaluation of all collected tissues was performed for all animals in all dose groups in this study.

Peer Review
Yes

Neoplastic

The sponsor states that an increased incidence of granular cell tumors in the distal reproductive tract (uterus with cervix and vagina) were noted in high dose females.

The sponsor states that the higher incidence of benign granular cell tumors in the distal reproductive tract of high dose females was considered AN2728-related given the significant results of statistical analyses of the incidence of these tumors (Fisher's Exact Test, Cochran-Armitage Trend Test, and Peto Test) and that the incidence of benign granular cell tumors in the distal reproductive tract of high dose females exceeded the historical control range at this facility.

(b) (4) historical control data from 26 control groups from 20 recent 2-year studies in Sprague-Dawley rats that occurred between 4/1/2009 through 5/1/2014

indicated an incidence rate of benign granular cell tumors in the distal female reproductive tract (uterus with cervix and vagina) ranging from 0 to 23.33% with an average incidence rate of 5.50%. The incidence of benign granular cell tumors in the distal reproductive tract of high dose females in the current study (29.23%) exceeded the historical control range. The incidence rates of benign granular cell tumors in the distal reproductive tract of low and mid dose females was 9.23% and 6.15%, respectively, which were lower than that observed in vehicle control females (9.38%) and were within the historical control range.

The CDER statistical reviewer, Dr. Steven Thomson, generated the following table in his review that lists the potentially statistically significant results for organ-tumor combinations in rats.

Table 5. Organ-Tumor Combinations in Rats with Results Close to Statistical Significance

	Tumor Incidence				Significance Levels			
Organ/Tumor	Veh	Low	Med	High	ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Rats								
Testes								
# Evaluated	65	65	65	65				
Adj. # at Risk	45.1	52.0	48.5	49.9				
ADENOMA, INTERSTITIAL CELL	0	0	1	4	.0057	.0695	.5161	.
Female Rats								
uterus with cervix								
# Evaluated	65	65	65	65				
Adj. # at Risk	46.6	47.8	48.9	48.6				
GRANULAR CELL TUMOR	1	0	4	8	.0008	.0179	.1943	1
vagina								
# Evaluated	65	65	65	65				
Adj. # at Risk	47.2	48.0	48.9	50.6				
GRANULAR CELL TUMOR	5	7	2	15	.0026	.0166	.9477	.3793

The tumors identified by Dr. Thomson in the previous table are considered common tumors. The CDER Executive Carcinogenicity Assessment Committee (eCAC) criteria for considering a common tumor as treatment related are that both the trend and pairwise comparison for high dose versus vehicle have a p-value less than 0.01.

The incidence of interstitial cell adenoma in the testis in males has a trend p-value of 0.0057 which is slightly less than 0.01 but has a pairwise p-value for vehicle versus high dose males of 0.0695 which is much greater than the 0.01 level. Therefore, it does not appear that this meets the CDER eCAC criteria for statistical significance.

The eCAC requested that a statistical analysis for the incidence of granular cell tumor of the uterus with cervix or vagina, combined, be conducted for this study. Dr. Feng Zhou performed the requested statistical analysis and the results from that statistical analysis are provided in the following table.

Organ Name	Tumor Name	0 mkd VC N=65	30 mkd LD N=65	100 mkd MD N=65	300 mkd HD N=65	P-Value			
						Dos Response	VC vs. LD	VC vs. MD	VC vs. HD
uterus with cer	GRANULAR CELL TUMOR	1	0	4	8	<0 001*	1.0000	0.1947	0 0162
vagina	GRANULAR CELL TUMOR	5	7	2	15	0.0024*	0.3950	0.9483	0 0162
uterus cervix vagina	**GRANULAR CELL TUMOR**	**6**	**7**	**4**	**20**	**<0.001***	**0.5177**	**0.8518**	**0.0032***

The incidence of granular cell tumor in the uterus with cervix in females has a trend p-value of <0.001 which is significantly less than 0.01 and a pairwise p-value for vehicle versus high dose females of 0.0162 which is slightly greater than the 0.01 level. The incidence of granular cell tumor in the vagina of female rats has trend p-value of 0.0024 which is less than 0.01 and a pairwise p-value for vehicle versus high dose females of 0.0162 which is slightly greater than the 0.01 level. The incidence of granular cell tumor in the uterus with cervix or vagina, combined, in females has a trend p-value of <0.001 which is significantly less than 0.01 and a pairwise p-value for vehicle versus high dose females of 0.0032 which is less than the 0.01 level. Therefore, a drug related increased incidence of granular cell tumors in the uterus with cervix or vagina, combined, was noted in high dose female rats.

Non Neoplastic

No treatment related non neoplastic lesions were noted in this study.

Toxicokinetics

Blood samples for toxicokinetic assessment were collected from 3 animals/sex/group/timepoint on day 1 and week 26 at 0 (pre-dose), 0.5, 1, 2, 5 and 24 hours post dose. Plasma concentrations of the parent (AN2728) and the two major metabolites (AN7602 and AN8323) were determined from the collected blood samples.

The toxicokinetic parameters determined from this study are provided in the following tables (copied from the NDA submission). The toxicokinetic parameters for the parent compound (AN2728) are provided in the following table.

Summary TK Parameters of AN2728 in Rats During Weeks 1 and 26 Following Once Daily Oral Dosing of AN2728					
Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-T} (ng*hr/mL)	$t_{1/2}$ (hr)
Week 1					
30	Male	83.1	0.5	178	120
	Female	78.9	0.5	88	NC
100	Male	575	0.5	1380	1.72
	Female	345	0.5	920	1.37
300	Male	4260	0.5	9190	0.868
	Female	1210	0.5	3350	1.14
Week 26					
30	Male	72.7	0.5	149	1.33
	Female	88.3	0.5	134	1.46
100	Male	247	1.00	633	NC
	Female	481	1.00	1110	5.05
300	Male	2000	1.00	5910	4.00
	Female	950	1.00	2860	3.62
NC - Not Calculated					

There was no apparent accumulation of AN2728 exposure upon multiple dosing for 26 weeks. Increases in AN2728 exposure with increasing dose were generally more than dose proportional. Following a single dose of AN2728, male rats had slightly higher exposure of AN2728 than female rats. There were no consistent gender-related differences in TK parameters of AN2728 at Week 26.

The metabolites, AN7602 and AN8323, were detected in plasma following oral dosing of AN2728. The increases in AN7602 and AN8323 exposures with increasing AN2728 dose were generally more than dose proportional following single or multiple doses. After 26 weeks of dosing, there was a slight increase in AN7602 exposure in males at the 300 mg/kg/day dose (up to 3 fold), but not at other doses. Week 1 exposure of the parent compound AN2728 was ~2-4 fold higher than that of the metabolite AN7602 in males and similar to AN7602 in females. The exposure of the parent compound AN2728 at Week 26 in females was similar to that of the metabolite AN7602 at all doses.

The toxicokinetic parameters for the metabolite, AN7602, are provided in the following table.

Summary TK Parameters of AN7602 in Rats During Weeks 1 and 26 Following Once Daily Oral Dosing of AN2728					
Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-T} (ng*hr/mL)	$t_{1/2}$ (hr)
Week 1					
30	Male	31.0	0.5	48.1	NC
	Female	79.8	0.5	82.8	NC
100	Male	185	1.00	582	NC
	Female	350	1.00	822	NC
300	Male	952	1.00	2500	NC
	Female	1260	1.00	3310	NC
Week 26					
30	Male	40.0	0.5	49.0	NC
	Female	73.9	0.5	77.1	NC
100	Male	158	1.00	538	6.13
	Female	532	1.00	919	NC
300	Male	2040	1.00	6350	NC
	Female	1460	1.00	3240	5.10
NC - Not Calculated					

AN8323 exposure at Week 26 was generally higher than at Week 1 for all dose groups (up to 5 fold). Week 1 exposure of the parent compound AN2728 was ~3 to 30 fold lower than exposure of AN8323. The exposure of the parent compound AN2728 at Week 26 was up to 65 fold lower than exposure of the metabolite AN8323 at Week 26 and Week 1.

The toxicokinetic parameters for the metabolite, AN8323, are provided in the following table.

Summary TK Parameters of AN8323 in Rats During Weeks 1 and 26 Following Once Daily Oral Dosing of AN2728					
Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-T} (ng*hr/mL)	$t_{1/2}$ (hr)
Week 1					
30	Male	133	1.00	542	NC
	Female	462	2.00	1970	NC
100	Male	914	2.00	9540	NC
	Female	2310	2.00	24600	NC
300	Male	5680	1.00	70400	2.86
	Female	10900	2.00	98100	NC
Week 26					
30	Male	346	1.00	2050	7.46
	Female	570	2.00	5040	NC
100	Male	3150	1.00	12400	5.77
	Female	4740	1.00	22900	4.83
300	Male	26600	6.00	383000	NC
	Female	14400	1.00	133000	6.33
NC - Not Calculated					

Dosing Solution Analysis

The dosing solution analysis demonstrated that the concentration of the dose groups ranged from 98.5 to 100.2%, which is acceptable.

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/s/

DAIVENDER K MAINIGI
08/15/2016

BARBARA A HILL
08/15/2016