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PEP005 (ingenol mebutate) Gel

2.7.2 Summary of Clinical Pharmacology Studies

Module 2

LEO Pharma A/S
Clinical Development

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Module 2

Summary of Clinical Pharmacology Studies

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ABBREVIATIONS AND DEFINITION OF TERMS

ADCC	antibody dependent cellular cytotoxicity
AE	adverse event
AK	actinic keratosis
$AUC_{(0–24)}$	area under the concentration time curve from 0 to 24 hours
AUS	Australia
CD	cluster of differentiation
CFR	Code of Federal Regulations
CI	confidence interval
CL_b	blood clearance
C_{max}	maximum plasma concentration
CYP	cytochrome P450
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HIPAA	Health Information Portability and Accountability Act
HPLC-RAD	High performance liquid chromatography – radiometric detection
IC_{50}	50% inhibitory concentration
ICH	International Conference on Harmonisation
IV	Intravenous
ka	rate constant
K_i	partition coefficient
kp	permeation coefficient
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LLOQ	lower limit of quantitation
LSC	liquid solid chromatography
MTBE	methyl tert-butyl ether
MTD	maximum tolerated dose
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate
NDA	New Drug Application
NOAEL	no observable adverse effect level
NQ	not quantitated
PK	pharmacokinetic
$t_{1/2}$	half-life

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T_{max}	time to maximum plasma concentration
US	United States
V_{ss}	steady-state volume of distribution

1 BACKGROUND AND OVERVIEW

1.1 BACKGROUND

Ingenol mebutate drug substance is a pure ingenol angelate extracted from the Euphorbia peplus (*Euphorbiaceae*) plant. The angeloyl ingenol fraction has three isomers, nominally identified as ingenol mebutate, PEP015 (5-angeloyl ingenol isomer), and PEP025 (20-angeloyl ingenol isomer). The ingenol mebutate isomer is obtained during the extraction process. The pure drug substance, ingenol mebutate, is formulated as a topical gel referred to as PEP005 (ingenol mebutate) Gel or PEP005 Gel, which has remained unchanged throughout the clinical development program for topical treatment of actinic keratosis (AK). Ingenol mebutate is a pleiotropic effector that exerts a direct cytotoxic effect on tumour cells and modulates Protein Kinase C (PKC) isoforms. The anti-tumour activity of ingenol mebutate is associated with a direct cytotoxic effect on tumour cells and an enhanced innate and acquired immune response.[1,2,3,4,5,6] The mechanism of action in actinic keratosis is not fully understood. In vivo and in vitro models have shown a dual mechanism of action for the effects of ingenol mebutate: 1) induction of local lesion cell death and 2) promoting an inflammatory response characterised as infiltration of neutrophils and other immunocompetent cells.

Based on nonclinical studies, it is anticipated that topical PEP005 Gel will produce negligible systemic exposure in humans. Limited ingenol mebutate skin penetration into the systemic circulation was noted at topical doses of ≤ 600 and ≤ 60 µg/kg (≤ 0.250 µg/mm2) in rats and mini-pigs, respectively. Ingenol mebutate isomers were either undetectable (PEP025) or were ≤ 10% (PEP015) of the corresponding blood ingenol mebutate concentration in both species. Estimated ingenol mebutate topical bioavailability in rats was 2% to 4.%. Topical bioavailability in mini-pigs could not be estimated since ingenol mebutate was not detectable in blood. Based on the total applied topical dose, the maximum 3-day repeat PEP005 Gel doses were ~300-fold (600 µg/kg/day) and 30-fold (60 µg/kg/day) higher for rats and mini-pigs, respectively, than the intended clinical therapeutic dose (2 µg/kg/day for a 60-kg human) (Module 2.6.6, section 9).

As no systemic toxicity was noted in any of the dermal studies a safety margin of at least 49 in rats and 27 in minipigs has been established [Studies 2174/026, N1006168A, 2174/029, 509992]. The rat safety margin of 49 is considered very conservative as rat skin is much

thinner than human skin and the dermal bioavailability is known to be higher in rats. No mortality was observed following repeat intravenous (IV) administration of PEP005 at doses of ≤15 µg/kg/day for up to 28 days in rats [Study 2174-014]. A no observable adverse effect level (NOAEL) of 7.5 µg/kg/day was identified based on unspecific symptoms (subdued behavior, tachypnea, decreased body weight) observed at 15 µg/kg/day. At a dose level of 7.5 µg/kg/day PEP005 was detectable in rat plasma but due to the lack of systemic exposure in man, it is not possible to compare rat and human pharmacokinetic (PK) parameters and calculate traditional safety margins based on area under the concentration time curve (AUC) or maximum plasma concentration (C_{max}) values. The study, however, demonstrates that the safety margins derived from the dermal studies are extremely conservative and likely to be much higher (Module 2.6.6, section 9).

Following IV dosing in rats and mini-pigs, ingenol mebutate had high blood clearance (CL_b), a moderate steady-state volume of distribution (V_{ss}), and a short half-life ($t_{1/2}$) in both species. In rats, biliary and urinary excretion accounted for most of the IV administered ingenol mebutate.

Metabolic profiles of ingenol mebutate in blood and skin homogenates were similar across species, and chemical rearrangement of ingenol mebutate to yield PEP015 and PEP025 was evident. Ingenol mebutate was significantly metabolized in hepatocytes, yielding predominantly ingenol (rats, dogs, and mini-pigs) or hydroxyl-ingenol mebutate (humans).

In in vitro studies, ingenol mebutate did not inhibit or induce major human cytochrome P450 (CYP) isoforms and did not significantly inhibit or stimulate receptors or enzymes. Ingenol mebutate has no potential to cause clinically significant drug interactions.

1.2 OVERVIEW OF CLINICAL PHARMACOLOGY STUDIES

In total, there are 14 studies that provide information relevant to the clinical pharmacology of PEP005 Gel, 10 nonclinical studies and 4 clinical studies. A tabular overview of the 10 nonclinical studies is presented in Table 1. A more detailed description of the nonclinical studies with a tabular listing of findings is provided in Appendix Table 1 through Appendix Table 5. A tabular description of the 4 clinical studies is presented in Table 2.

Nonclinical Studies

Studies have been conducted using both non-radiolabeled and radiolabeled ingenol mebutate to evaluate penetration of PEP005 Gel through isolated human skin. For non-radiolabeled studies a highly sensitive and selective liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was developed and validated for measurement of ingenol mebutate and its two potential acyl migration products, PEP015 and PEP025, in receptor fluid from skin penetration assays [Study 2174/033]. The rate and extent of permeation of non radiolabeled ingenol mebutate through human dermatome membranes (containing stratum corneum, epidermis, and the upper layer of dermis) was examined using a flow-through membrane diffusion cell (Studies 2174/030 and 2174/032). In the skin flux study using radiolabeled PEP005 Gel ([^{3}H]-ingenol mebutate), the rate and extent of absorption were measured following topical application to human skin [Study 774782].

The metabolism of non-radiolabeled ingenol mebutate was initially qualitatively studied in human blood and isolated hepatocytes [Study 2174/028]. Subsequently, a pivotal study was conducted to investigate human tissue differences in rates and routes of metabolism of [^{3}H]-ingenol mebutate [Study 773824] in human whole blood, skin (skin homogenates), and liver (cryopreserved hepatocytes).

The potential for plasma protein binding of [^{3}H]-ingenol mebutate was evaluated in humans using equilibrium dialysis following incubation at 37°C for 2 hours [Study 182813]. Potential drug-drug interactions were evaluated in two types of in vitro studies. Ingenol mebutate was evaluated for potential inhibition and induction of major human CYP isoforms [Studies 779162 and 779157]. In a safety profile study, ingenol mebutate at a concentration of 1.0 x 10^{-06} M was evaluated for potential inhibition or stimulation against 112 individual receptors and 41 enzymes [Study 13753].

No clinical studies on human pharmacodynamics have been conducted to date. Consequently, human PD data are not available and no PK/PD correlation studies have been performed nor has any PK/PD relationship been established.

The mode of action of PEP005 has been established on preclinical models using cell lines and animal models. To date, human studies evaluating the mechanism of action have not been conducted. However, the Applicant plans to conduct two studies assessing the biological effects of PEP005 Gel, 0.05% in diseased and normal skin. These studies are planned to start

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4Q 2011. One study will assess the early (up to 2 days following application) biological effects (necrosis and inflammation), of PEP005 Gel, 0.05% in diseased and normal skin by histology. The second study will assess the early and late (up to 57 days after study medication application) biological effects of PEP005 Gel, 0.05% in diseased and normal skin, by histology and using confocal microscopy.

Clinical Studies

A highly sensitive and selective bioanalytical method was developed and validated for the simultaneous determination of ingenol mebutate and its potential acyl migration isomers, PEP015 and PEP025, in human whole blood containing EDTA as an anti-coagulant [Study 2174/16]. The method utilized liquid/liquid extraction with methyl tert-butyl ether (MTBE) for sample preparation and LC-MS/MS for detection. For ingenol mebutate, PEP015 and PEP025, the quantitation range was 0.01 to 20.0 ng/mL. Subsequent revalidation of the method was conducted over the range 0.1 to 20 ng/mL for ingenol mebutate, PEP015 and PEP025 [Study 2174/066]. Ingenol mebutate, PEP015, and PEP025 are unstable (liable to isomerism) in protic environments (e.g., water, methanol). In addition, exposure to raised temperatures exacerbates this instability. The isomerism is retarded at low pH . Isomerism under a variety of conditions was determined in a previous study [Study 2174/005]. It was shown during the initial validation that addition of orthophosphoric acid (0.1% final volume) prior to immediate freezing retards conversion between isomers when samples are stored at nominal -70ºC (4 months maximum time [Study 2174/034]). As study samples are acidified in this manner prior to freezing, calibration standards and quality control samples were prepared in the same manner. Storage stability has been demonstrated for PEP005, PEP015, and PEP025 in human whole blood for up to 10 weeks at nominal -20°C and 4 months stored at nominal -70°C [Study 2174/034]).

Blood samples were collected from patients in four of the clinical studies in the AK program [AGN204332-004, PEP005-004, PEP005-013 and PEP005-017] (Table 2).

AGN 204332-004 was a Phase 1, randomized, double-blind, vehicle-controlled, multicenter study designed primarily to evaluate the safety of a single application of PEP005 Gel, 0.01% compared to a vehicle gel in patients with AK lesions on the shoulder, chest back, and/or arm. Study drug was applied to individual AK lesions (lesion-specific therapy). Blood samples were collected from consenting patients predose and approximately 3 to 9 hours after dose

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application for analysis of ingenol mebutate, PEP015, and PEP025 levels. A total of 12 patients (8 on PEP005 Gel and 4 on vehicle) provided blood samples.

PEP005-004 was a Phase 2a, open-label, uncontrolled, dose-escalation study designed to determine the maximum tolerated dose (MTD) of PEP005 Gel, administered once daily for two consecutive days in patients with AK lesions on the shoulder, chest back, and/or arm. A single application of PEP005 Gel (escalating concentrations starting at 0.01%) was applied to a 9 cm^2 treatment area that included a single target AK lesion (field therapy). Blood samples were collected at baseline (before Day 1 treatment) and at 0.5, 1, 2 and 4 hours post treatment on study Day 2. Two patients treated with the 0.05% dose consented to blood samples.

PEP005-013 was a Phase 1, open-label, uncontrolled study in male patients with AK lesions on the dorsal aspect of the forearm. Patients received PEP005 Gel, 0.05% once daily for two consecutive days applied to a contiguous 100 cm^2 treatment area containing at least 5 AK lesions (field therapy). Blood samples were collected prior to and 24 hours following the Day 1 application, and at 30 minutes and 1, 2, 4, 8, 12, and 24 hours following the Day 2 application Three patients completed two consecutive days of treatment and provided a complete set of blood samples for PK analysis.

PEP005-017 was a phase 2, double-blind, vehicle-controlled study evaluating the pharmacokinetics of PEP005 Gel, 0.05% when used in a maximal-use setting. Patients received PEP005 Gel, 0.05% once daily for two consecutive days applied to a contiguous 100 cm^2 treatment area on the dorsal forearm containing multiple AK lesions. Blood samples were collected predose on Day 1, predose on Day 2 (+24 hour Day 1) and 30 minutes and 1, 2, 4, 8, 12, and 24 hours following the Day 2 dose application. Whole blood samples were to be quantified (C_{max}, T_{max} and $AUC_{(0–24)}$) for ingenol mebutate and its primary metabolites, PEP015 and PEP025 (lower limit of quantitation [LLOQ] = 0.1 ng/mL). Blood samples were collected from all 16 patients (13 on PEP005 Gel and 3 on vehicle) enrolled in the study.

Table 1 Nonclinical Studies and Human Pharmacokinetic Prediction Study with Information Relevant to the Clinical Pharmacology of PEP005 (ingenol mebutate) Gel

Study ID Locations (No. Study Sites)	Study Status Study Dates	Type of Study	Test Article	Study System (Human)	Parameters
2174/030 Covance Laboratories Limited, Harrogate, North Yorkshire, England, UK	Completed February, 2004	In vitro skin penetration	Ingenol mebutate	Skin	Absorption (% applied dose), penetration rate, lag time, permeability coefficient
2174/032 Covance Laboratories Limited, Harrogate, North Yorkshire, England, UK	Completed July, 2004	In vitro skin penetration	Ingenol mebutate	Skin	Absorption (% applied dose), penetration rate, lag time, permeability coefficient
774782 Charles River Laboratories, (formerly Inveresk) Tranent, Scotland, UK	Completed March, 2007	In vitro skin penetration	3H-Ingenol mebutate	Skin	Absorbed/ unabsorbed dose / dermal delivery (% applied dose), steady state flux
2174/028 Covance Laboratories Limited, Harrogate, North Yorkshire, England, UK	Completed November, 2004	In vitro metabolism	Ingenol mebutate	Fresh blood, hepatocytes	Metabolite detection
774824 Charles River Laboratories, (formerly Inveresk) Tranent, Scotland, UK	Completed March, 2007	In vitro metabolism	3H-Ingenol mebutate	Whole blood, skin homogenates, cryopreserved hepatocytes	Metabolites, percent of total radioactivity

182813 Charles River Laboratories, (formerly Inveresk) Tranent, Scotland, UK	Completed March, 2007	In vitro plasma protein binding	3H-Ingenol mebutate	Plasma	Percent bound
779162 Charles River Laboratories, (formerly Inveresk) Tranent, Scotland, UK	Completed July, 2008	In vitro drug-drug interaction	Ingenol mebutate	Liver microsomes	Inhibition of major CYP isoforms
779157 Charles River Laboratories, (formerly Inveresk) Tranent, Scotland, UK	Completed December, 2008	In vitro drug-drug interaction	Ingenol mebutate	Freshly isolated hepatocytes	Induction of major CYP isoforms
13753 Cerep, Le Bois l'Evêque, France	Completed January, 2008	In vitro drug-drug interaction	Ingenol mebutate	Receptor/ enzyme assay	Percent inhibition / stimulation of 112 receptors and 41 enzymes
Letter No. PA001 PharmAdvance Consulting, Inc. 97 Cottage Lane Aliso Viego, CA 92656, USA	Completed November, 2007	Human PK prediction after topical application	PEP005 (ingenol mebutate) Gel	NA	Projected estimates: blood clearance, volume of distribution at steady state, absorption rate, topical bioavailability, Cmax and Tmax at proposed topical clinical dose, minimum clinical topical dose to produce LLOQ in human blood

CYP = cytochrome P450; LLOQ = lower limit of quantitation; NA = not applicable PK = pharmacokinetic

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Table 2 Description of Clinical Studies with Information Relevant to the Clinical Pharmacology of PEP005 (Ingenol mebutate) Gel

Study ID Locations (No. Study Centers)	Study Status Study Dates[a] Enrollment[b]	Study Design and Control Type (Phase)	Study Objectives	Diagnosis Inclusion Criteria	PEP005 and Vehicle Dose, Regimen[c]	No. Patients[d,e]	Gender, M/F[e] Age Range (years)	No. Patients Providing Blood Samples	Sample Collection
AGN204332-004 US (4)	Completed 12-Aug-04 15-Oct-04 16 / 16	Double-blind, parallel group, vehicle-controlled (Phase 1)	Safety	≥5 AK lesions on shoulder, chest back, and/or arm	0.01% x 1d Vehicle x 1d	11/11 5/4	10/1 4/1 42 – 82	8 4	Predose and ~3 to 9 hrs postdose
PEP005-004 US (1)	Completed 7-Sep-05 14-Mar-06 22 / up to 34	Open-label, nonrandomized, uncontrolled, dose escalation (Phase 2a)	Safety (MTD), efficacy, PK	Target AK lesion diameter 3 mm to 15 mm on the shoulder, chest, back, or arm.	0.01% x 2d 0.025% x 2d 0.05% x 2d 0.075% x 2d	3/3 3/3 10/10 6/6	2/1 2/1 7/3 5/1 64 – 87	0 0 2 0	Predose and 0.5, 1, 2, and 4 hrs postdose
PEP005-013 AUS (1)	Completed 17 Oct 2007 23 Apr 2008 8 / 8	Open-label, nonrandomized, uncontrolled, maximal use (Phase 1)	PK, safety	Male, ≥5 AK lesions in a 100 cm^2 contiguous area on dorsal aspect of forearm	0.05% x 2d	8/6	8/0 56 – 81	3[f]	Day 1: predose and 24 hrs postdose Day 2: 30 min and 1, 2, 4, 8, 12, and 24 hrs postdose
PEP005-017 US (1)	Completed 18-Mar 09 27May09 16 / 15	Double-blind, parallel group, vehicle-controlled, maximal use (Phase 2)	PK, safety, efficacy	Multiple AK lesions in a 100 cm^2 contiguous area on dorsal aspect of one forearm	0.05% x 2d Vehicle x 2d	13/13 3/3	6/7 0/3 48 – 79	13 3	Day 1: predose Day 2: predose and 30 min and 1, 2, 4, 8, 12, and 24 hrs postdose

AK = actinic keratosis; AUS = Australia; d = day; F = female; hr = hour; M = male; min = minute; MTD = maximum tolerated dose; PK =pharmacokinetics; US = United States

[a] First patient randomized/treated to last patient/last followup
[b] Total enrolled / enrollment goal.
[c] All study and control drugs were applied topically.
[d] Number of patients entered / number of patients completed
[e] By dose group
[f] Patients who completed two consecutive days of treatment and provided a complete set of blood samples for PK analysis.

2 SUMMARY OF RESULTS OF INDIVIDUAL STUDIES

2.1 IN VITRO SKIN PENETRATION

(Appendix Table 1)

Studies 2174/030 (Module4.2.2.2\2174\030) and 2174/032 (Module4.2.2.2\2174\032)
A pilot, in vitro skin penetration study of PEP005 Gel was conducted at doses of 15 and 150 µg/cm^2 across a surface area of 64 mm^2 using a flow-through membrane diffusion cell model [Study 2174/030]. The percent of ingenol mebutate that penetrated through human dermatomal skin during the 6-hour study period was 0.67% at 15 µg/cm^2 and was 0.17% at 150 µg/cm^2. These findings indicate that minimal skin penetration of ingenol mebutate occurred at either dose in vitro during the 6-hour study period, although in this preliminary study, mass balance was not determined. The maximum rate of penetration through skin was 24.1 ng/cm^2/hr at 15 µg/cm^2 and was 60.9 ng/cm^2/hr at 150 µg/cm^2. These results indicate that the absorption of ingenol mebutate increased with increasing dose in human dermatomal skin in vitro.

Based on the results from the pilot study, an additional in vitro permeability study was conducted [Study 2174/032]. The second study used the same membrane diffusion cell model but included a longer exposure period (24 hours) and focused on the low dose application rate (15 µg/cm^2). The percent of ingenol mebutate that penetrated through the human dermatomal skin during the 24-hour study period was 2.1%, with a maximum rate of penetration of 18.4 ng/cm^2/hr. Consistent with the results from the 6-hour pilot study, the results from this 24-hour study indicated that there is minimal permeation of ingenol mebutate through skin.

Study 774782 (Module4.2.2.2\774782)
In a subsequent in vitro skin flux study using radiolabeled PEP005 Gel ([^{3}H]-ingenol mebutate) [Study 774782], the rate and extent of absorption were measured following topical application to human skin.

Split-thickness human skin membranes were mounted into flow through diffusion cells. Receptor fluid, saline (sodium chloride, 0.9%, w/v) containing sodium azide (ca 0.01%, w/v) and polyoxyethylene 20-cetyl ether (PEG, ca 6%, w/v) was pumped underneath the skin at a flow rate of 1.5 mL/h. The skin surface temperature was maintained at ca 32°C throughout the experiment. A tritiated water barrier integrity test was performed and any sample

exhibiting a permeation coefficient (kp) greater than 2.5 x 10^{-3} cm/h was excluded from subsequent absorption measurements.

[^{3}H]-ingenol mebutate was applied in a gel formulation (ca 0.05%, w/w) at a volume of 10 µL/cm^2 to human skin membranes mounted into flow through diffusion cells in vitro. Absorption was assessed by collecting receptor fluid in hourly fractions from 0 to 6 hours postdose and then 2-hourly fractions from 6 to 24 hours postdose. At 24 hours postdose, exposure was terminated by washing the skin surface with a 2% (v/v) commercial soap solution and drying with tissue paper (tissue swabs). The underside of the skin was rinsed with receptor fluid. The skin was then removed from the flow through cells, dried, and the stratum corneum was removed with 20 successive tape strips. During tape stripping it was noted that the epidermal/dermal junction had broken down and was very delicate. The remaining skin was divided into exposed and unexposed skin and solubilized with Soluene®. All liquid samples were analyzed by liquid scintillation counting and solid samples (tissue swabs) were analyzed by combustion/liquid scintillation counting.

For [^{3}H]-ingenol mebutate applied in a gel formulation at ca 0.05% (w/w) to human skin in vitro, the absorbed dose and dermal delivery were 0.21% (0.01 µg equiv./cm^2) and 0.91% (0.04 µg equiv./cm^2) of the applied dose, respectively. Most of the dose was removed by washing; the dislodgeable dose was 77.22% of the applied dose. The stratum corneum retained 23.70% of the applied dose. The total unabsorbed dose was 101.09% of the applied dose. The mass balance was essentially complete with 102.00% of the applied dose recovered. The steady state flux was 0.39 ng equiv./cm^2/hr over a period of 4 to 24 hours.

In conclusion, following topical application of [^{3}H]-ingenol mebutate in a gel formulation at ca 0.05% (w/w) to human skin in vitro, the absorbed dose of [^{3}H]-ingenol mebutate was 0.21% (0.01 µg equiv./cm^2) and dermal delivery of [^{3}H]-ingenol mebutate was 0.91%. The stratum corneum acted as a good barrier to absorption; total unabsorbed dose of [^{3}H]-ingenol mebutate recovered from the human skin was 101.09% of the applied dose.

2.2 IN VITRO METABOLISM

(Appendix Table 2)

Study 2174/028 (Module4.2.2.4\2174\028)

In vitro metabolism of ingenol mebutate was studied in hepatocytes isolated from rat, mini-pig, and human and in blood from rat, rabbit, mini-pig, and human [Study 2174/028]. The quantitation of ingenol mebutate and its metabolites and acyl migration products in blood or hepatocytes was not performed. PEP015, but not PEP025, was formed via acyl migration in blood of all species after one hour incubation of 10 µM ingenol mebutate at 37°C. Ingenol, the ester hydrolysis product of ingenol mebutate, was found in rabbit blood but not in rat, mini-pig, or human blood. Following 6 hours incubation of 10 µM ingenol mebutate with hepatocytes of rat, mini-pig, and human at 37°C, ingenol appeared to be the major metabolite in all species based on peak intensity. Other proposed metabolic pathways included hydroxylation of ingenol, sulfate conjugation of ingenol, and sulfate conjugation of ingenol mebutate. No apparent species differences in the metabolism of ingenol mebutate in blood or in hepatocytes were observed.

Study 774824 (Module4.2.2.4\774824)

A subsequent pivotal study was conducted to investigate human tissue differences in rates and routes of metabolism of [^{3}H]-ingenol mebutate [Study 774824]. The study was conducted in vitro and metabolite profiles were assessed via HPLC with on-line radiodetection and LC-MS/MS. The tissues investigated were whole blood, skin (skin homogenates), and liver (cryopreserved hepatocytes).

[^{3}H]-ingenol mebutate (1 and 10 µM) was found to be relatively metabolically stable in both whole blood and skin homogenates. In both tissues, there was some hydrolysis to yield ingenol, but this generally accounted for less than 1% of the radioactivity. Significant chemical rearrangement of [^{3}H]-ingenol mebutate to yield PEP015 and PEP025 was also evident in both tissues. PEP015 was the predominant product (up to 32% formation) in all samples. The presence of all components was confirmed by LC-MS/MS detection of the 10 µM samples.

In contrast to skin and blood, [^{3}H]-ingenol mebutate was found to undergo significant metabolism in cryopreserved hepatocytes. The metabolite profiles were similar across all samples; however, in humans the metabolite at Rt 38.1 min was the major metabolite in both

sexes accounting for ca 39.7% and 42.6% of the radioactivity at 180 min in males and females, respectively. In these samples ingenol accounted for ca 23.3% and 17.1%, respectively. A number of other peaks of radioactivity were evident in chromatograms from all samples, including the chemical rearrangement products PEP015 and PEP025.

The samples generated from incubations with 10 µM [^{3}H]-ingenol mebutate were analyzed by LC-MS/MS detection in order to provide tentative structural information for the metabolites formed. Following incubation with whole blood and skin homogenates the only metabolic product present was positively identified as ingenol (ammonium adduct observed at m/z 366 amu). In addition, the presence of the alternative isomeric forms, PEP015 and PEP025, was confirmed. Analysis of hepatocyte postincubation supernatants confirmed extensive metabolism of [^{3}H]-ingenol mebutate. In humans (both males and females), the major metabolic product was identified as a hydroxylated metabolite that was unnamed and referred to as PEP0XX (m/z 447 amu). By comparison with the molecular ions formed from ingenol mebutate, PEP015 and PEP025, it is considered likely that this component is hydroxy-ingenol mebutate, as both the molecular ion and an ammonium adduct (m/z 464 amu) were present (no molecular ion was observed following analysis of authentic PEP015 or PEP025. Two other hydroxylated metabolites of PEP0XX were also identified, neither of which formed an ammonium adduct. The presence of ingenol, hydroxyl-PEP0XX, and the isomeric forms of ingenol mebutate in hepatocyte postincubation supernatants was confirmed by both positive and negative ion electrospray MS.

In conclusion, of the tissues tested, extensive metabolism of [^{3}H]-ingenol mebutate was only evident in hepatocytes, and the principal routes of metabolism were identified as hydrolysis and hydroxylation.

2.3 IN VITRO PLASMA PROTEIN BINDING

(Appendix Table 3)

Study 182813 (Module4.2.2.3\182813)

The in vitro plasma protein binding of [^{3}H]-ingenol mebutate was investigated in plasma from human volunteers at concentrations of 0.5, 2, 5 and 20 ng/mL [Study 182813]. Plasma protein binding of total radioactivity was very high (> 99%) at concentrations investigated and no apparent differences were observed between sexes. Stability investigation of [^{3}H]-ingenol

mebutate in plasma and buffer showed that there was isomerization of [^{3}H]-ingenol mebutate to [^{3}H]-PEP015 and [^{3}H]-PEP025, which occurred to different degrees in plasma and in buffer. Therefore, it was not possible to determine the level of plasma protein binding for ingenol mebutate, PEP015, and PEP025 separately.

2.4 IN VITRO DRUG-DRUG INTERACTION

(Appendix Table 4)

Study 13753 (Module4.2.1.2\13753)

The potential for off-target activity of ingenol mebutate at a concentration of 1.0 µM was investigated in various in vitro receptor binding and enzyme assays [Study 13753]. Ingenol mebutate was shown to exhibit minimum (< 20%) inhibition or stimulation against the 112 individual receptors and 41 enzymes tested. Since this test concentration is much higher than the estimated human blood C_{max} of 2.54 x 10^{-7} µM following topical exposure to 2 µg/kg/day PEP005 Gel in humans, there is negligible risk of potential off-target activity and consequent adverse systemic effects in humans [Study Report PA001].

Studies 779162 (Module4.2.2.4\779162) and 779157 (Module4.2.2.4\779157)

The potential of ingenol mebutate to inhibit or induce the major human CYP isoforms that mediate the metabolism of therapeutic agents was assessed in vitro.

Human hepatic microsomal protein was incubated with selective CYP substrates in the presence of ingenol mebutate over a concentration range of 2 x 10^{-4} to 20 µM to evaluate potential inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 [Study 779162]. In addition, ingenol mebutate was preincubated with microsomal protein in the presence or absence of nicotinamide adenine dinucleotide phosphate (NADPH) cofactor to investigate the potential for mechanism-based inhibition of the CYP isoforms. No notable direct- or mechanism-based inhibition was observed following incubation or preincubation of ingenol mebutate at any test concentration. The 50% inhibitory concentration (IC_{50}) and partition coefficient (K_i) values were not calculated since less than 50% inhibition was observed for each of the CYP isoforms tested over a drug concentration range of 2 x 10^{-4} to 20 µM ingenol mebutate.

Fresh human hepatocytes were incubated in vitro with ingenol mebutate at concentrations of 2×10^{-4}, 0.02, and 2 µM to evaluate potential for induction of major inducible CYP isoforms of CYP1A, CYP2C, and CYP3A [Study 779157]. No induction of these isoforms was observed.

2.5 HUMAN PHARMACOKINETICS PREDICTION

(Appendix Table 5)

Report PA001 (Module4.2.2.7\PA001)

The human PK profile of topical application of PEP005 Gel was predicted using allometric scaling from in vivo animal PK and in vitro percutaneous absorption data. Pharmacokinetic projection was based on the following assumptions: (1) one compartment model with first-order absorption and elimination; (2) clearance and volume of distribution at steady-state do not change after dose escalation, i.e., linear pharmacokinetics; (3) human absorption rate constant is similar to that of rat; (4) 0.21% bioavailability (F%) for topical administration in humans based on percent of absorbed dose in vitro in human skin for the clinical gel formulation. The half-life after topical dosing at 0.05% ranged from 16.7 to 50.4 hours in rat [Study 2174/026]. Assuming flip-flop kinetics, the absorption rate constant (ka) in rat is calculated as $\ln 2/t_{1/2}$, which results in a mean value of 0.0277 $hour^{-1}$.

Based on the estimated ingenol mebutate blood clearance V_{ss}, assumed absorption rate constant, and topical bioavailability, the maximum intended clinical dose (2 µg/kg/day) would not produce measurable systemic blood levels of ingenol mebutate. Assuming linear PK, it is predicted that a minimal topical dose of 2000 µg/kg/day would be required to produce systemic C_{max} ingenol mebutate concentrations above the LLOQ (0.1 ng/mL) of established bioanalytical assay.

2.6 CLINICAL STUDIES: SYSTEMIC ABSORPTION FOLLOWING TOPICAL ADMINISTRATION IN PATIENTS WITH ACTINIC KERATOSIS

Pharmacokinetic samples were collected from a total of 32 patients (25 on PEP005 Gel and 7 on vehicle) enrolled in four independent PEP005 Gel clinical studies for the treatment of non-head AK lesions [AGN 204332-004, PEP005-004, PEP005-013, PEP005-017]. The highest concentration and treatment area evaluated was 0.05 µg/mm^2 of PEP005 Gel, 0.05% applied once daily to a 100 cm^2 area for two consecutive days [Protocols PEP005-013 and

PEP005-017]. Details of these studies including the complete safety and efficacy analyses can be found in the clinical study reports located in Module 5.

AGN204332-004 (Module5.3.5.1\AGN204332-004)

AGN204332-004 was a Phase 1, multicenter, randomized, double-blind, parallel group, vehicle-controlled study. The primary objective of this study was to determine the safety of 0.01% PEP005 Gel after a single application to AK lesions on the shoulders, chest, back and/or arms. Eligible patients were male or female, ≥ 18 years of age, with at least 5 AK lesions on one of the non-head locations listed above. Study drug (0.01% PEP005 Gel or vehicle) was applied directly to each of the selected lesions by a licensed physician or trained personnel at the study site using a positive displacement micropipette. A volume of 200 µL of study gel was distributed between the 5 lesions selected for treatment. Blood samples were collected from consenting patients prior to dose application and approximately 3 to 9 hours after dose application for analysis of ingenol mebutate, PEP015, and PEP025 levels. Safety and efficacy were assessed at follow-up visits on Days 7 and 14. If local skin reactions were present at Day 14, further followup visits were required until resolution of the event.

Sixteen patients were enrolled in the study and treated, 11 patients received 0.01% PEP005 Gel and 5 patients received vehicle gel. Blood samples were obtained from 12 patients (8 on 0.01% PEP005 Gel and 4 on vehicle). The results of the PK analysis showed no detectable systemic absorption. Levels of ingenol mebutate and its acyl isomers PEP015 and PEP025 were below the LLOQ (0.01 ng/mL) in all samples collected.

PEP005-004 (Module5.3.5.2\PEP005 004)

PEP005-004 was a Phase 2a, single center, open-label, nonrandomized, uncontrolled, dose-escalation study. The primary objective of this study was to determine the MTD of PEP005 Topical Gel when applied once daily for two consecutive days to a 9 cm^2 (3 cm x 3 cm) field surrounding a target AK lesion. Eligible patients were male or female, ≥ 18 years of age, with one AK lesion with a diameter between 3 mm and 15 mm on the shoulders, chest, back, or arms. A single application (90 µL) of PEP005 Topical Gel (0.01%, 0.025%, 0.05%, or 0.075%) was applied to the treatment area by a licensed medical practitioner at the study site. Blood samples from patients treated at the MTD were to be collected at baseline (before Day 1 treatment) and at 0.5, 1, 2 and 4 hours post treatment on study Day 2. Safety and

efficacy were assessed on Days 1, 2, 8, 15, and 29. If local skin AEs were present at Day 29, follow up visits were required until resolution of the event.

Twenty-two patients were enrolled in the study, 3 patients received 0.01% PEP005 Topical Gel, 3 patients received 0.025% PEP005 Topical Gel, 10 patients received 0.05% PEP005 Topical Gel, and 6 patients received 0.075% PEP005 Topical Gel. The MTD was determined to be 0.05%. Two patients from the 0.05% expansion group consented to provide blood samples for PK analysis. The results of the PK analysis showed no detectable systemic absorption. Levels of ingenol mebutate, PEP015, and PEP025 were below the LLOQ (0.01 ng/mL) in all samples collected.

PEP005-013 (Module5.3.3.2\PEP005 013)

PEP005-013 was a Phase 1, open label, PK, maximal use study. The primary objective of this study was to evaluate the extent of systemic absorption of ingenol mebutate when applied as 0.05% PEP005 Topical Gel on two consecutive days to a 100 cm^2 (5 cm x 20 cm) contiguous treatment area on the dorsal aspect of one forearm. Eligible patients were male, ≥ 18 years of age, with at least 5 AK lesions on either the right or left extensor (dorsal aspect) forearm. Study medication was applied in the clinic by site staff using a micropipette, as 1 mL in four aliquots of 250 µL. Blood samples were collected predose on Day 1, predose on Day 2, and at 30 minutes and 1, 2, 4, 8, 12 and 24 hours following the Day 2 application. Safety was assessed on Days 1, 2, 8, 29 and 57. For patients with unresolved safety concerns at Day 57, follow-up visits were required until the event resolved or was assessed as clinically stable. Eight adult male patients were enrolled in the study. Of the 8 patients enrolled, 6 completed the study. Three patients completed 2 days of study treatment and provided a complete set of blood samples for pharmacokinetic analysis. The results of the PK analysis revealed no detectable systemic exposure. Levels of ingenol mebutate, PEP015, and PEP025 were below the LLOQ (0.1 ng/mL) in all samples collected.

PEP005-017 (Module5.3.3.2\PEP005 017)

PEP005-017 was a Phase 2, single center, randomized, double-blind, vehicle-controlled study. The primary objective of this study was to evaluate the potential for systemic exposure of PEP005 Gel when applied in a maximal use setting in patients with AK. Eligible patients were male or female, ≥ 18 years of age, with 4 to 8 AK lesions within a 100 cm^2 contiguous treatment area on the dorsal aspect of one forearm. Patients were randomized to receive

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PEP005 Gel, 0.05% or vehicle gel in a 4:1 ratio, respectively. Study medication was applied on two consecutive days in the clinic by site staff. The total volume of study gel applied to treatment area was approximately 1 mL (4 individual unit dose tubes). Blood samples were collected predose on Day 1, predose on Day 2 (+24 hour Day 1), and at 30 minutes and 1, 2, 4, 8, 12 and 24 hours following the Day 2 dose application. Whole blood samples were to be quantified (C_{max}, T_{max} and $AUC_{(0–24)}$) for ingenol mebutate and its primary metabolites, PEP015 and PEP025. Safety and efficacy were assessed on Days 2, 3, 8, 15, 29 and 57. For patients with unresolved safety concerns at Day 57, follow-up visits were required until the event resolved or was assessed as clinically stable.

Sixteen patients were enrolled and randomized in the study, 13 patients to PEP005 Gel, 0.05% and 3 patients to vehicle gel. All 16 patients completed treatment as planned and provided a full set of blood samples for PK analysis. The results of the PK analysis revealed no detectable systemic exposure. Levels of ingenol mebutate, PEP015, and PEP025 were below the LLOQ (0.1 ng/mL) in all samples collected.

3 COMPARISON AND ANALYSES OF RESULTS ACROSS STUDIES

Results from both the in vitro and in vivo studies in humans demonstrated very little if any skin penetration of ingenol mebutate or its acyl isomers PEP015 or PEP025 into systemic circulation following topical dosing of PEP005 Gel.

In vitro skin penetration studies using non-radiolabeled PEP005 Gel [Studies 2174/030 and 2174/032] indicated minimal permeation of ingenol mebutate through human skin (Table 3).

Table 3 In Vitro Human Skin Penetration of Non-radiolabeled Ingenol Mebutate

Study PEP005 Dose	PEP005 absorption (% applied [ng])	Max. rate of Penetration ($ng/cm^2/h$)	Lag Time (hours)	Permeability Coefficient (cm/sec)
Pilot Study [Study 2174/030]				
15 $\mu g/cm^2$ PEP005 Gel	0.67 [64 ng]	24.1	1.75	6.69×10^{-7}
150 $\mu g/cm^2$ PEP005 Gel	0.17 [159 ng]	60.9	1.34	1.69×10^{-7}
Main Study [Study 2174/032]				
15 $\mu g/cm^2$ PEP005 Gel (±SD)	1.93 ± 1.10	17.5 ± 10.5	3.7 ± 1.6	$4.87 \times 10^{-8} \pm 2.92 \times 10^{-8}$

SD = standard deviation
Source: Module 4.2.2.2

Similar results were demonstrated when using radiolabeled PEP005 Gel ([3H]-ingenol mebutate) [Study 774782] (Table 4).

Table 4 In Vitro Human Skin Penetration of PEP005 Gel ([H^3]-Ingenol Mebutate)

Parameter	Study 774782 Results
Target Ingenol Mebutate Concentration (% w/w)	0.05
Ingenol Mebutate Concentration by Radioactivity (% w/w)	0.05
Application Level of Ingenol Mebutate by Radioactivity (µg equiv./cm^2)	4.34
Dislodgeable Dose (% Applied Dose)	77.22
Unabsorbed Dose (% Applied Dose)	101.09
Absorbed Dose (% Applied Dose)	0.21
Dermal Delivery (% Applied Dose)	0.91
Mass Balance (% Applied Dose)	102.00
Dislodgeable Dose (µg equiv./cm^2)	3.35
Unabsorbed Dose (µg equiv./cm^2)	4.39
Absorbed Dose (µg equiv./cm^2)	0.01
Dermal Delivery (µg equiv./cm^2)	0.04
Mass Balance (µg equiv./cm^2)	4.43
Lag Time (h)	1
Steady State Flux (ng equiv./cm^2/h)	0.39
Period of Steady State Flux (h)	4-24

Source: Module 4.2.2.2

In in vitro metabolic studies [Studies 2174/028 and 773824], ingenol mebutate was found to be relatively metabolically stable in both whole blood and skin homogenates. In both tissues there was some hydrolysis to yield ingenol, but this generally accounted for less than 1% of the radioactivity. Significant chemical rearrangement of ingenol mebutate to yield PEP015 and PEP025 was also evident in both tissues. PEP015 was the predominant product in all samples. In contrast to skin and blood, [3H]-ingenol mebutate was found to undergo significant metabolism in cryopreserved hepatocytes. The rate of metabolism was faster in females than in males, and the metabolite at Rt 38.1 min was the major metabolite in both males and females accounting for ca 39.7% and 42.6% of the radioactivity at 180 min in males and females, respectively. In these samples, ingenol accounted for ca 23.3% and 17.1%, respectively. Other peaks of radioactivity were evident in chromatograms from all samples, including the chemical rearrangement products PEP015 and PEP025.

Ingenol mebutate was highly plasma protein bound in vitro with > 99% binding of total radioactivity at the concentrations investigated and no apparent differences between sexes [Study 182813].

No notable direct- or mechanism-based inhibition or induction of the major hepatic drug metabolizing enzymes was observed in vitro in human liver microsomes or hepatocytes exposed to ingenol mebutate [Studies 779162 and 779157]. Additionally, ingenol mebutate did not produce any significant off-target activity in various in vitro receptor binding and enzyme assays [Study 13753]. Based on these findings, the risk of a clinically relevant PK drug interaction following topically application of PEP005 Gel at therapeutic concentrations is negligible.

The human PK profile of topical application of PEP005 Gel was predicted using allometric scaling from in vivo animal PK and in vitro percutaneous absorption data. Based on the estimated ingenol mebutate blood clearance Vss, assumed absorption rate constant, and topical bioavailability, the maximum intended clinical dose (2 µg/kg/day) would not produce measurable systemic blood levels of ingenol mebutate. Assuming linear PK, it is predicted that a minimal topical dose of 2000 µg/kg/day would be required to produce systemic C_{max} ingenol mebutate concentrations above the LLOQ (0.1 ng/mL) of established bioanalytical assay [Study Report PA001]. This quantity of drug is far in excess of possible clinical usage.

Pharmacokinetic samples were collected from a total of 32 patients (25 on PEP005 Gel and 7 on vehicle) enrolled in four independent PEP005 Gel clinical studies for the treatment of non-head AK lesions: AGN 204332-004, PEP005-004, PEP005-013, and PEP005-017. Of the various ingenol mebutate concentrations and treatment areas evaluated in these four studies, the greatest exposure was 0.05 µg/mm^2, reflecting application of the PEP005 Gel, 0.05% to a 100 cm^2 area, tested in studies PEP005-013 and PEP005-017, in which the PEP005 Gel formulation was applied to the dorsal forearm for two consecutive days. For these two studies, the selected treatment area was to include multiple AK lesions, encompassing a substantial area of skin that was chronically actinically damaged, such that there was dry, flaking, , , and/or otherwise irritated skin. Following application of the PEP005 Gel, skin damage or irritation is further compounded, not only through the mechanism of action of ingenol mebutate, but also through the potential skin irritant properties of isopropyl alcohol (a component of the PEP005 Gel formulation). Alcohols are hypothesized to extract intracellular lipids from the stratum corneum, thereby potentially producing further skin irritation (such as drying) and increasing percutaneous absorption. [7,8] In studies PEP005-013 and PEP005-017, blood sampling for determination of systemic ingenol mebutate and its isomers was peformed from 30 minutes through 24 hours following the second application of PEP005 Gel. In spite of the large treatment area, the extent of AK-

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damaged skin, and the effects of the PEP005 Gel, no systemic levels of ingenol mebutate or its two isomers, PEP015 and PEP025, were quantifiable in any of the blood samples collected for PK analysis (i.e., concentrations were below the LLOQ).

No clinical metabolism studies have been performed to date because ingenol mebutate shows no systemic absorption when administered topically. However, in vitro studies have demonstrated that ingenol mebutate undergoes significant metabolism in human hepatocytes, with the principal routes of metabolism identified as hydrolysis and hydroxylation. The major metabolic product was identified as a hydroxylated metabolite of PEP0XX (an unnamed metabolite of ingenol mebutate).

No population subgroup analyses have been performed for the purposes of PK or product metabolism analyses in humans. No clinical drug interaction studies have been conducted. Such studies would be impractical, as no systemic levels of ingenol mebutate or its two isomers, PEP015 and PEP025, have been quantifiable in any clinical studies evaluating PK to date (i.e., concentrations were below the LLOQ).

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4 SPECIAL STUDIES

Not applicable.

REFERENCES

1. Hampson P, Chahal H, Khanim F, et al. PEP005, a selective small-molecule activator of protein kinase C, has potent antileukemic activity mediated via the delta isoform of PKC. Blood 2005;106:1362-1368.
2. Kedei N, Lundberg DJ, Toth A, et al. Characterization of the interactions of ingenol 3-angelate with protein kinase C. Cancer Res 2004;64:3243-3255.
3. Cozzi SJ, Parsons PG, Ogbourne SM, et al. Induction of senescence in diterpene ester-treated melanoma cells via protein kinase C-dependent hyperactivation of the mitogen-activated protein kinase pathway. Cancer Res 2006;66:10083-10091.
4. Hampson P, Kavanagh D, Smith E, et al. The anti-tumor agent, ingenol-3-angelate (PEP005), promotes the recruitment of cytotoxic neutrophils by activation of vascular endothelial cells in the PKCδ dependent manner. Cancer Immunol Immunother 2008;57:1241-51.
5. Serova M, Ghoul A, Benhadji KA, et al. Effects of protein kinase C modulation by PEP005, a novel ingenol angelate, on mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling in cancer cells. Mol Cancer Ther 2008;7:915-922.
6. Olsnes AM, Ersvær E, Ryningen A, et al. The protein kinase C agonist PEP005 increases NF-kB expression, induces differentiation and increases constitutive chemokine release by primary acute myeloid leukaemia cells. Br J Haematol 2009;145:761-774.
7. Cohen DE. Irritant Contact Dermatitis. In: Bolognia, JL, Jorizzo JL, eds. *Dermatology*. 2nd ed. Oxford: Elsevier; p. 241-50.
8. Cohen DE, de Souza A. Contact dermititis in topical agents. In: Draelos ZD ed. Cosmetic Dermatology Products and Procedures. Oxford:Wiley-Blackwell; 2010: p. 55-61.

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2.7.2 Summary of Clinical Pharmacology Studies

APPENDIX

Appendix Table 1 Summary of In Vitro Skin Penetration Studies

Study Number: 2174/030		
Type of Study	In vitro human skin penetration study	
GLP Compliance	No	
Test Article	Ingenol mebutate	
Formulation/Vehicle	30% Isopropyl alcohol gel	
Method of Administration	In vitro gel application on a 0.64 cm^2 skin surface area	
Dose	150 mL/cm^2 of the 0.01% gel and 150 mL/cm^2 of the 0.1% gel	
Sample	Receptor fluid	
Sampling Period	0-0.5, 0.5-1, 1-1.5, 1.5-2, 2-3, 3-4, 4-5 and 5-6 hours postdose	
Analyte	Ingenol mebutate, PEP015, PEP025	
Assay	LC-MS/MS	
Test System	Human Skin	
Administered dose density	150 mg/cm^2	15 mg/cm^2
Absorption of ingenol mebutate, percent of applied dose[a]	0.17	0.67
Maximum rate of penetration, $ng/cm^2/hr$	60.9	24.1
Lag time, hrs	1.34	1.75
Permeability coefficient, cm/sec	$1.69x10^{-7}$	$6.69x10^{-7}$

LC-MS/MS = liquid chromatography with tandem mass spectrometry

[a] Absorption of ingenol mebutate was calculated from the sum of ingenol mebutate, PEP015 and PEP025 found in the receptor fluid over the 6 hour study period expressed as percentage of applied dose.

(Continued)

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 1 Summary of In Vitro Skin Penetration Studies (Cont'd.)

Study Number: 2174/032	
Type of Study	In vitro skin penetration study in human
GLP compliance	Yes
Test Article	Ingenol mebutate
Formulation/Vehicle	30% Isopropyl alcohol gel
Method of Administration	In vitro gel application on a 0.64 cm^2 skin surface area
Dose	150 mL/cm^2 of the 0.01% gel
Sample	Receptor fluid
Sampling period	0-0.5, 0.5-1, 1-1.5, 1.5-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-14, 14-16, 16-18, 18-20, 20-22 and 22-24 hours postdose
Analyte	Ingenol mebutate, PEP015 and PEP025
Assay	LC-MS/MS
Administered Dose Density	15 mg/cm^2
Test System	Human Skin
	Study Results
Absorption of ingenol mebutate , percent of applied dose (−SD)[a]	1.93 (− 1.10)
Maximum rate of penetration, ng/cm^2/hr (−SD)	17.5 (− 10.5)
Lag time, hours (−SD)	3.7 (− 1.6)
Permeability coefficient, cm/sec (−SD)	4.87 x 10^{-8} (− 2.92x10^{-8})

LC-MS/MS = liquid chromatography with tandem mass spectrometry; SD = standard deviation

[a] Absorption of ingenol mebutate was calculated from the sum of ingenol mebutate, PEP015 and PEP025 found in the receptor fluid over the 24 hour study period expressed as percentage of applied dose.

(Continued)

PEP005 (ingenol mebutate) Gel
2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 1 Summary of In Vitro Skin Penetration Studies (Cont'd.)

Study Number: 774782	
Type of Study	In vitro skin penetration study in human
GLP Compliance	Yes
Test Article	^{3}H-ingenol mebutate
Formulation/Vehicle	30% Isopropyl alcohol gel
Method of Administration	In vitro gel application on a 0.64 cm^2 skin surface area
Dose	10 mL/cm^2 of the 0.05% gel
Sample	Receptor fluid
Sampling period	0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, 18-20, 20-22 and 22-24 hours postdose
Analyte	Radioactivity
Assay	LSC
Administered Dose Density	5 mg/cm^2
Test System	Human Skin
	Study Results
Absorbed Dose, % of applied dose	0.21
Dermal Delivery, % of applied dose	0.91
Unabsorbed Dose, % of applied dose	101.09
Steady State Flux, ng equiv./ cm^2/hr	0.39
LSC = liquid solid chromatography Absorption of ingenol mebutate was calculated from the total radioactivity found in the receptor fluid over the 24 hour study period expressed as percentage of applied dose.	

Source: Module 4.2.2.2

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 2 Summary of In Vitro Metabolism Studies

Study Number: 2174/028						
Species	**Matrix**	**Test Article**	**Conc. of Substrate(s) (µM)**	**Sampling Period (hour)[a]**	**Analytical Method**	**Metabolite Detected**
Human	Fresh blood	Ingenol mebutate	10	1	LC-MS/MS	PEP015
Human	Hepatocytes	Ingenol mebutate	10	6	LC-MS/MS	Ingenol Sulphate conjugation of ingenol Sulphate conjugation of ingenol mebutate
[a] Incubation was conducted at 37 C for 1 hour in fresh blood or for 6 hours in hepatocytes						

Study Number: 774824						
GLP Compliance	Yes					
Test Article	^{3}H-ingenol mebutate					
Study System	In vitro whole blood		In vitro skin homogenates		In vitro cryopreserved hepatocytes	
Method	Incubation		Incubation		Incubation	
Sampling Period[b]	3 hours		3 hours		3 hours	
Concentration	1 µM		1 µM		1 µM	
Incubation Temperature	37 C		37 C		37 C	
Assay[c]	LC-MS/MS and LSC		LC-MS/MS and LSC		LC-MS/MS and LSC	
	Percent of Total Radioactivity		**Percent of Total Radioactivity**		**Percent of Total Radioactivity**	
Species – Human Compound	Male	Female	Male	Female	Male	Female
Parent	92.73	86.77	NA	65.14	0	0
M1	4.93	10.22	NA	30.51	0	0
M2	0.52	0.93	NA	2.36	0	0
M3	0.41	0.36	NA	0.47	23.34	17.07
M4	NQ	NQ	NA	NQ	13.04	12.04
M5	NQ	NQ	NA	NQ	8.16	12.43
M6	NQ	NQ	NA	NQ	39.70	42.64
Others	1.41	1.72	NA	1.52	15.76	15.82

LC-MS/MS = liquid chromatography with tandem mass spectrometry; LSC = liquid solid chromatography; NA = not applicable; NQ = not quantitated
[b] Incubation was conducted for 3 hours.
[c] Identity of each metabolite was characterized by LC-MS/MS and quantitation of parent drug and metabolites were determined by LSC.
Parent: ingenol mebutate, M1: PEP015, M2: PEP025, M3: ingenol, M4: hydroxyl-PEP015, M5: hydroxyl-PEP025, M6: hydroxyl-ingenol mebutate.

Source: Module 4.2.2.4

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 3 Summary of In Vitro Plasma Protein Binding Study: Test Article ^{3}H-Ingenol Mebutate

Study Number: 182813	
GLP Compliance	Yes
Study System	In vitro plasma
Method	Equilibrium dialysis
Sampling Period	2 hours
Incubation Temperature	37 C
Analyte	Total radioactivity
Assay	HPLC-RAD
Species	Human[a]
Concentration Tested (ng/mL)	0.5 – 20
% Bound[b]	> 99

HPLC-RAD = high performance liquid chromatography – radiometric detection

[a] Both male and female were tested.

[b] Total radioactivity instead of ingenol mebutate was used to calculated the plasma protein binding.

Source: Module 4.2.2.3

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate

Study Number: 779162												
Type of Study		An in vitro investigation to assess the potential inhibition of human cytochrome P450 enzymes by ingenol mebutate										
GLP Compliance		Yes										
Method		Human liver microsomes were used to assess the effect of ingenol mebutate on the metabolism of probe substrates for each of the major human drug metabolizing cytochrome P450's. Selective probe substrates were used to assess the following: CYP1A2 (phenacetin, 20 µM), CYP2A6 (coumarin, 2 µM), CYP2B6 (bupropion, 40µM), CYP2C8 (paclitaxel, 10 µM), CYP2C9 (tolbutamide, 100 µM), CYP2C19 (S-mephenytoin, 35 µM), CYP2D6 (bufuralol, 20 µM), CYP2E1 (chlorzoxazone, 100 µM) and CYP3A4 (testosterone, 100 µM; midazolam, 5 µM, and nifedipine, 25 µM).										
Description		Effect of ingenol mebutate on hepatic microsomal CYP activities (percent of control activity) – direct inhibition										
Test Item/ Inhibitor	**Concentration (µM)**	**Percent of Control Activity**										
		CYP1A2[a]	**CYP2A6[a]**	**CYP2B6**	**CYP2C8[a]**	**CYP2C9**	**CYP2C19**	**CYP2D6[a]**	**CYP2E1**	**CYP3A4 Testosterone**	**CYP3A4 Midazolam**	**CYP3A4 Nifedipine**
Ingenol mebutate	0	100.00 – 3.24	100.00 – 4.56	100.00[b]	100.00 – 12.83	100.00 – 13.60	100 – 7.97	100.00 – 1.06	100.00 – 8.55	100.00	100.00 – 5.41	100.00
	0.0002(0.002[a])	91.26 – 5.84	106.36 – 6.05	100.83[b]	96.23 – 15.35	126.81 – 8.43	94.59 – 7.18	93.98 – 0.80	85.03 – 2.88	110.01 – 26.21	97.76 – 1.59	112.59 – 4.37
	0.002 (0.02[a])	104.24 – 7.51	108.09 – 4.41	115.56 – 10.08	93.03 – 15.14	113.06 – 6.10	96.54 – 5.98	97.71[b]	94.60 – 9.72	88.82 – 19.95	107.50 – 3.96	117.73 – 7.44
	0.02 (0.2[a])	91.44 – 5.83	104.72 – 6.37	107.51 – 6.49	85.41 – 5.21	97.51 – 4.67	87.63 – 6.06	98.48 – 1.76	85.23 – 4.37	93.12 – 13.84	107.32 – 5.75	121.00[b]
	0.1 (1[a])	84.95 – 7.38	105.29 – 13.81	108.79 – 4.27	89.69 – 14.01	106.58 – 9.77	99.59 – 6.17	94.55[b]	87.22 – 6.18	101.35 – 6.31	117.92 – 3.57	107.06 – 3.32
	0.2 (2[a])	91.78 – 4.59	111.28 – 6.05	113.80 – 8.16	94.21 – 21.09	104.20 – 1.78	91.98 – 4.28	99.17 – 3.54	89.93[b]	92.35 – 15.42	111.09 – 6.24	106.70 – 1.29
	2 (20[a])	95.42 – 7.12	99.07 – 7.74	102.97 – 2.03	91.68 – 6.77	97.720 – 7.22	92.63 – 8.56	81.02 – 4.67	87.53 – 10.20	98.20[b]	96.31 – 3.45	109.19 – 10.04

(Continued)

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 779162 (Cont'd.)												
Description (Cont'd.)		Effect of ingenol mebutate on hepatic microsomal CYP activities (percent of control activity) – direct inhibition										
Test Item/ Inhibitor	**Concentration (µM)**	**Percent of Control Activity**										
		CYP1A2[a]	**CYP2A6[a]**	**CYP2B6**	**CYP2C8[a]**	**CYP2C9**	**CYP2C19**	**CYP2D6[a]**	**CYP2E1**	**CYP3A4 Testosterone**	**CYP3A4 Midazolam**	**CYP3A4 Nifedipine**
CYP Inhibitor	Control	100.00 – 9.75	100.00 – 8.08	100.00 – 11.75	100.00 – 11.59	100.00[b]	100.00 – 12.45	100.00 – 2.71	100.00 – 6.39	100.00 – 9.05	100.00 – 0.64	100.00 – 3.94
	Test	16.91 – 2.29	11.06 – 8.38	53.70 – 19.78	55.02 – 2.37	12.24 – 1.61	24.16 – 1.32	22.22 – 1.75	18.02 – 0.63	15.80 – 6.90	8.92 – 0.59	19.08 – 0.66
Description		Effect of ingenol mebutate on hepatic microsomal CYP activities (percent of control activity) – mechanism-based inhibition										
Test Item/ Inhibitor	Concentration (µM)	**Percent of Control Activity**										
		CYP1A2[a]	**CYP2A6[a]**	**CYP2B6**	**CYP2C8[a]**	**CYP2C9**	**CYP2C19**	**CYP2D6[a]**	**CYP2E1**	**CYP3A4 Testosterone**	**CYP3A4 Midazolam**	**CYP3A4 Nifedipine**
Ingenol mebutate	0 – 0 min pre-inc DMSO control	100.00 – 2.50	100.00 – 3.80	100.00	100.00 – 3.38	100.00 – 2.99	100 – 7.84	100.00 – 2.26	100.00	100.00 – 9.27	100.00	100.00
	2 (20[a]) – 0-min minus NADPH pre-inc	94.08 – 11.34	92.14 – 22.63	80.46 – 3.14	101.19 – 10.87	116.24 – 3.87	102.03 – 3.95	102.18 – 1.22	104.99 – 1.57	86.22[b]	91.95 – 1.33	98.87 – 1.18
	2 (20[a]) – 0 min plus NADPH pre-inc	100.00 – 2.17	101.50 – 4.76	95.41 –3.74	97.60 – 10.32	86.65 – 3.88	96.98 – 3.96	99.05 – 3.30	92.73[b]	95.06 – 22.65	92.92 – 7.46	93.75 – 2.65
	0 – 30 min pre-inc DMSO control	100.00 – 3.03	100.00 – 2.62	100.00 – 10.10	100.00 – 6.01	100.00 – 11.50	100.00 – 1.79	100.00 – 1.53	100.00	100.00 – 13.23	100.00	100.00
	2 (20[a]) – 30 min minus NADPH pre-inc	111.36 – 5.30	101.80 – 11.33	90.66 – 2.87	95.89 – 17.77	128.74 – 2.98	107.98 – 4.76	107.68 – 6.15	104.67 – 1.22	123.84 – 9.56	95.67 – 0.99	101.42 – 5.23
	2 (20[a]) – 30 min plus NADPH pre-inc	103.03 – 4.01	96.39 – 11.25	95.62 – 1.22	85.82 – 18.49	110.96 – 3.45	100.24 – 2.60	103.58 – 2.09	107.34 – 0.58	104.22 – 5.32	95.26 – 0.94	99.98 – 8.38

(Continued)

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 779162 (Cont'd.)												
Description		Effect of ingenol mebutate on hepatic microsomal CYP activities (reaction rates) – direct inhibition										
		Enzyme Activity (pmol/mg/min)										
Test Item/ Inhibitor	**Concentration (µM)**	**CYP1A2[a]**	**CYP2A6[a]**	**CYP2B6**	**CYP2C8[a]**	**CYP2C9**	**CYP2C19**	**CYP2D6[a]**	**CYP2E1**	**CYP3A4 Testosterone**	**CYP3A4 Midazolam**	**CYP3A4 Nifedipine**
Ingenol mebutate	0	128.07 – 4.16	193.77 – 8.83	122.04 – 1.66	22.53 – 2.89	67.78 – 9.22	10.22 – 0.81	30.99 – 0.33	181.02 – 15.48	1082.22 – 6.66	241.74 – 13.08	589.59 – 31.65
	0.0002(0.002[a])	116.88 – 7.48	206.10 – 11.72	123.05 – 5.93	21.68 – 3.46	85.95 – 5.72	9.67 – 0.73	29.12 – 0.25	153.93 – 5.22	1190.55 – 283.60	236.32 – 3.85	663.79 – 25.77
	0.002 (0.02[a])	133.50 – 9.61	209.46 – 8.55	141.03 – 12.30	20.96 – 3.41	76.62 – 4.13	9.87 – 0.61	30.28[b]	171.24 – 17.59	961.19 – 215.95	259.88 – 9.57	694.12 – 43.89
	0.02 (0.2[a])	117.10 – 7.47	202.91 – 12.35	131.21 – 7.92	19.24 – 1.17	66.08 – 3.16	8.96 – 0.62	30.52 – 0.55	154.28 – 7.90	1007.82 – 149.81	259.43 – 13.91	713.37 – 60.62
	0.1 (1[a])	108.79 – 9.46	204.02 – 26.75	132.78 – 5.21	20.21 – 3.16	72.23 – 6.62	10.18 – 0.63	29.30[b]	157.88 – 11.18	1096.83 – 68.32	285.05 – 8.63	631.23 – 19.56
	0.2 (2[a])	117.54 – 5.87	215.63 – 11.73	138.88 – 9.96	21.23 – 4.75	70.62 – 1.21	9.40 – 0.44	30.73 – 1.10	162.79[b]	999.41 – 166.90	268.56 – 15.08	629.11 – 7.6
	2 (20[a])	122.20 – 9.12	191.96 – 15.01	125.67 – 2.48	20.66 – 1.53	66.23 – 4.90	9.47 – 0.87	25.10 – 1.45	158.44 – 18.46	1062.73 – 36.30	232.82 – 8.35	643.75 – 47.39
CYP Inhibitor	Control	105.91 – 10.33	165.58 – 13.38	121.36 – 14.26	23.26 – 2.69	77.88[b]	30.36 – 3.78	48.07 – 1.30	787.43 – 50.29	2036.45 – 184.22	534.73 – 3.44	755.00 – 29.76
	Test	17.91 – 2.43	18.31 – 13.87	65.17 – 0.24	12.80 – 0.55	9.54 – 1.25	7.33 – 0.40	10.68 – 0.84	141.89 – 4.98	321.73 – 140.58	47.72 – 3.15	144.04 – 5.01

Data reported as mean values – standard deviation.
Control CYP inhibitors: CYP1A2-α-naphthoflavone (0.5 µm), CYP2A6-8-methoxypsoralen (6 µM), CYP2B6-ThioTEPA (20 µM), CYP2C8-Quercetin (10 µM), CYP2C9-sulphaphenazole (10 µM), CYP2C19-tranylcypromine (20 µM), CYP2D6-quinidine (5 µM), CYP2E1-diethyldithiocarbamate (10 µM), CYP3A4-ketoconazole (0.5 µM)
[a] Ingenol mebutate tested at 10 times the target concentrations due to a calculation error.
[b] Values calculated from two individual values only.

(Continued)

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 779157										
Type of Study		Evaluation of ingenol mebutate as an inducer of CYP1A2, CYP2C9, and CYP3A4 in fresh human hepatocytes								
GLP Compliance		Yes								
Method		Freshly isolated human hepatocytes from three donors were used to assess the potential for ingenol mebutate to induce major hepatic drug metabolizing enzymes. Ingenol mebutate (3 concentrations) were incubated with hepatocytes for up to 72 h before measurement of the metabolism of phenacetin (CYP1A), [^{14}C]-tolbutamide (CYP2C9), and [^{14}C]-testosterone (CYP3A).								
Description		Evaluation of ingenol mebutate as an inducer of CYP1A2, CYP2C9 and CYP3A4 in fresh human hepatocytes								
Control / Test Item/ Inducer	**Concentration (µM)**	**Mean Formation of Metabolite (pmol/10^6 hepatocytes/hour)**								
		Donor 1			**Donor 2**			**Donor 3**		
		CYP1A2	**CYP2C9**	**CYP3A4**	**CYP1A2**	**CYP2C9**	**CYP3A4**	**CYP1A2**	**CYP2C9**	**CYP3A4**
Metabolic Competence		628.6 ± 146.4	1324 ± 27	9543 ± 89	1581 ± 51	1060 ± 129	7896 ± 218	1443 ± 96	4199 ± 125.8	7761 ± 165
Media Control		54.48 ± 4.31	0.00	681.1 ± 73.7	81.98 ± 12.77	334.1 ± 28.1	537.1	105.10 ± 10.51	250.4 ± 23.5	495.8 ± 26.5
Vehicle Control (DMSO)		60.02 ± 5.51	270.6 ± 24.6	873.6 ± 182.3	84.29 ± 14.86	558.9 ± 113.6	596.8 ± 34.9	164.26 ± 5.22	412.0 ± 93.6	401.0 ± 32.6
Ingenol mebutate	0.0002 µM	68.64 ± 9.61	124.7 ± 113.4	817.3 ± 97.7	72.36 ± 4.63	451.0 ± 67.2	513.6 ± 48.0	175.01 ± 16.66	473.4 ± 15.5	440.1 ± 59.8
	0.02 µM	33.17 ± 6.00	161.6 ± 33.0	313.7 ± 16.4	54.86 ± 3.26	364.0 ± 98.5	374.7 ± 35.1	108.24 ± 13.43	301.2 ± 55.4	518.3 ± 108.7
	2 µM	25.79 ± 2.74	0.00	183.7 ± 35.8	56.67 ± 3.82	198.2 ± 32.6	239.1 ± 79.1	107.56 ± 9.77	128.9 ± 33.3	421.4 ± 137.8
CYP Inducer	Control	58.28 ± 10.08	207.6 ± 12.5	585.3 ± 81.4	77.62 ± 2.97	515.8 ± 114.6	642.3 ± 72.1	147.23 ± 10.76	395.6 ± 22.3	393.0 ± 54.3
	Test	1621 ± 283	538.5 ± 45.2	12263 ± 277	2026 ± 98.54	747.1 ± 156.0	10192.9 ± 1315.8	1465.55 ± 121.01	642.6 ± 46.5	5855.5 ± 166.2

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 779157 (Cont'd.)										
Description		Evaluation of ingenol mebutate as an inducer of CYP1A2, CYP2C9 and CYP3A4 in fresh human hepatocytes								
Control / Test Item/ Inducer	**Concentration (µM)**	**Induction Index[a]**								
		Donor 1			**Donor 2**			**Donor 3**		
		CYP1A2	**CYP2C9**	**CYP3A4**	**CYP1A2**	**CYP2C9**	**CYP3A4**	**CYP1A2**	**CYP2C9**	**CYP3A4**
Media Control		–	–	–	–	–	–	–	–	–
Vehicle Control (DMSO)		–	–	–	–	–	–	–	–	–
Ingenol mebutate	0.0002 µM	0.55	*	*	*	*	*	0.82	24.86	0.72
	0.02 µM	*	*	*	*	*	*	*	*	2.15
	2 µM	*	*	*	*	*	*	*	*	0.37
CYP Inducer	Control	–	–	–	–	–	–	–	–	–
	Test	27.8	2.6	21.0	26.10	1.45	15.9	9.95	1.62	14.9

CYP = cytochrome P450; DMSO = dimethyl sulfoxide; NADPH = nicotinamide adenine dinucleotide phosphate;

Data reported as mean values – standard deviation unless otherwise specified.

Metabolite formation: CYP1A2 paracetamol, CYP2C9 4-hydroxytolbutamide, CYP3A 6ß-hydroxytestosterone

Control CYP inducer s: CYP1A2 – omeprazole (30 µM), CYP2C9 – rifampicin (3µM), CYP3A – rifampicin (3µM)

[a] Values = fold induction relative to induction by positive control inducer, i.e., omeprazole or rifampicin. The positive control induction value was calculated relative to its corresponding control, i.e., ethanol (omeprazole) or DMSO (rifampicin).

* No fold induction

(Continued)

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
GLP Compliance	No
Test Article Concentration (M)	1.0E-06
Binding Site	**Mean Percent Inhibition (%)**
Adenosine, A_1 (Human cDNA)	-5
Adenosine, A_{2A} (Human cDNA)	-2
Adenosine, A_3 (Human cDNA)	-1
Adrenergic, $alpha_1$ (non-selective)	-3
Adrenergic, $alpha_2$ (non-selective)	-4
Adrenergic, $beta_1$ (Human cDNA)	-3
Adrenergic, $beta_2$ (Human cDNA)	7
Angiotensin-II, AT_1 (Human cDNA)	3
Angiotensin-II, AT_2 (Human cDNA)	-10
GABA, BZD (central) (Rat brain)	2
GABA, BZD (peripheral) (Rat brain)	1
Bombesin, BB (non-selective) (Rat brain)	-20
Bradykinin, B_1 (Human cDNA)	3
Bradykinin, B_2 (Human cDNA)	10
Calcitonin gene-related peptide, CGRP (Human cDNA)	-3
Cannabinoid, CB_1 (Human cDNA)	16
Cannabinoid, CB_2 (Human cDNA)	-2
Cholecystokinin, CCK_A(CCK_1) (Human cDNA)	-15
Cholecystokinin, CCK_B (CCK_2) (Human cDNA)	10
Corticotropin Releasing Factor, CRF1 (Human cDNA)	-8
Dopamine, D_1 (Human cDNA)	2

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753 (Cont'd.)	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
Binding Site	**Mean Percent Inhibition (%)**
Dopamine, D_1 (Human cDNA)	2
Dopamine, D_{2s} (Human cDNA)	-8
Dopamine, D_3 (Human cDNA)	-2
Dopamine, $D_{4.4}$ (Human cDNA)	5
Dopamine, D_5 (Human cDNA)	1
Endothelin, ET_A (Human cDNA)	0
Endothelin, ET_B (Human cDNA)	-16
GABA, GABA (non-selective)	12
Galanin, GAL_1 (Human cDNA)	-19
Galanin, GAL_2 (Human cDNA)	-5
Glutamate, AMPA (Rat brain)	4
Glutamate, kainate (Rat brain)	-10
Glutamate, NMDA (Rat brain)	-8
Glutamate, glycine (strychnine-insensitive)	18
Growth Factors, PDGF (mouse cell line)	0
Chemokines, CXCR2 (IL-8B) (Human cDNA)	-16
Cytokines, TNF-a (Human cDNA)	-17
Chemokines, CCR1 (Human cDNA)	-2
Chemokines, CCR2 (Human cDNA)	-8
Chemokines, CCR3 (Human cDNA)	-6
Histamine, H_1 (Human cDNA)	7
Histamine, H_2 (Human cDNA)	-5
Histamine, H_3 (Human cDNA)	14

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753 (Cont'd.)	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
Binding Site	**Mean Percent Inhibition (%)**
Imidazoline, I_1 (Bovine adrenals)	18
Imidazoline, I_2 (Rat brain)	-12
Leukotrienes, LTB_4 (BLT_1) (Human cDNA)	3
Leukotrienes, LTD_4 ($CysLT_1$) (Human cDNA)	-3
Melanocortin, MC_4 (Human cDNA)	-13
Melatonin, MT_1 (Human cDNA)	3
Muscarinic, M_1 (Human cDNA)	13
Muscarinic, M_2 (Human cDNA)	6
Muscarinic, M_3 (Human cDNA)	7
Muscarinic, M_4 (Human cDNA)	3
Muscarinic, M_5 (Human cDNA)	1
Neurokinin, NK_1 (Human cDNA)	0
Neurokinin, NK_2 (Human cDNA)	-2
Neurokinin, NK_3 (Human cDNA)	-7
Neuropeptide Y, Y_1 (Human cDNA)	3
Neuropeptide Y, Y_2 (Human cDNA)	2
Neurotensin, NT_1 (NTS_1) (Human cDNA)	-7
Neuromedin U, NmU2 (Human cDNA)	-9
Nicotinic, N(neuronal) (a-BGTX-insensitive) (a4b2) (Rat brain)	19
Opioid and opioid-like, $delta_2$ (DOP) (Human cDNA)	3
Opioid and opioid-like, kappa (KOP) (rat cDNA)	0
Opioid and opioid-like, mu (MOP) (agonist site) (Human cDNA)	1
Opioid and opioid-like, ORL1 (NOP) (Human cDNA)	8

(Continued)

PEP005 (ingenol mebutate) Gel
2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753 (Cont'd.)	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
Binding Site	**Mean Percent Inhibition (%)**
Vasoactive intestinal peptide, PACAP (PAC_1) (Human cDNA)	-10
PPAR, PPARg(Human cDNA)	-8
Platelet Activating Factor, PAF (Human cDNA)	6
Glutamate, PCP (Rat brain)	9
Prostanoid, EP_4 (Human cDNA)	9
Prostanoid, TP (TXA_2/PGH_2) (Human cDNA)	0
Prostanoid, IP (PGI2)-platelets (Human cDNA)	5
Purinergic, P2X (Rat urinary bladder)	-15
Purinergic, P2Y (Rat brain)	5
Serotonin, 5-HT_{1A} (Human cDNA)	-5
Serotonin, 5-HT_{1B} (Human cDNA)	-2
Serotonin, 5-HT_{1D} (Human cDNA)	-7
Serotonin, 5-HT_{2A} (Human cDNA)	11
Serotonin, 5-HT_{2B} (Human cDNA) (agonist site)	-7
Serotonin, 5-HT_{2C} (Human cDNA)	2
Serotonin, 5-HT_3 (Human cDNA)	-3
Serotonin, 5-HT_{4E} (Human cDNA)	1
Serotonin, 5-HT_{5A} (Human cDNA)	11
Serotonin, 5-HT_6 (Human cDNA)	0
Serotonin, 5-HT_7 (Human cDNA)	5
Sigma, sigma (non-selective) (Rat brain)	-8
Somatostatin, sst (non-selective) (Mouse cell line)	2
Glucocorticoid, GR (Human cell line)	-8

(Continued)

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753 (Cont'd.)	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
Binding Site	**Mean Percent Inhibition (%)**
Estrogen, ER (Human cell line)	-3
Progesterone, PR (Human cell line)	7
Androgen, AR (Human cell line)	-6
Thyrotropin Releasing Hormone, TRH_1 (Human cDNA)	-23
Urotensin-II, UT1 (Human cDNA)	17
Vasoactive intestinal peptide, VIP_1 ($VPAC_1$) (Human cDNA)	1
Vasopressin, V_{1a} (Human cDNA)	-1
Vasopressin, V_2 (Human cDNA)	6
Ca2+ channel (L, DHP site) (Rat brain)	-8
Ca2+ channel (L, diltiazem site) (benzodiazepines) (Rat brain)	-28
Ca2+ channel (L, verapamil site) (phenylalkylamines) (Rat brain)	-3
Ca2+ channel (N) (Rat brain)	-11
K+ channel, K_{ATP} (Rat brain)	-10
K+ channel, hERG (Human cDNA)	4
K+ channel, K_V (Rat brain)	-6
K+ channel, SK_{Ca} (Rat brain)	-9
Na+ channel (site 2) (Rat brain)	-2
GABA, Cl- channel (Rat brain)	6
Norepinephrine, NE transporter (Human cDNA)	0
Dopamine, DA transporter (Human cDNA)	-2
GABA, GABA transporter (Human cDNA)	-1
Choline, choline transporter (CHT1) (Human cDNA)	1

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753 (Cont'd.)	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
Binding Site	**Mean Percent Inhibition (%)**
Serotonin, 5HT transporter (Human cDNA)	-3
Phospholipase, PLA2 (Porcine pancreas)	-3
Cyclooxygenases, COX_1 (Human cDNA)	1
Cyclooxygenases, COX_2 (Human cDNA)	5
Lipoxygenase, 12-LO (Human cDNA)	1
No Synthases, constitutive NOS (endothelial) (Human cell line)	-11
Phosphodiesterases, PDE1 (Bovine brain)	-7
Phosphodiesterases, PDE2 (Human macrophages)	15
Phosphodiesterases, PDE3 (Human platelets)	-3
Phosphodiesterases, PDE4 (Human monocytes)	4
Phosphodiesterases, PDE5 (Human platelets)	-3
Phosphodiesterases, PDE6 (Bovine retina)	-2
Metalloproteases, ACE (Human cDNA)	5
Metalloproteases, ACE-2 (Human cDNA)	-1
Serine Proteases, elastase (Human leukocytes)	-4
Cysteine Proteases, caspase-3 (Human cDNA)	0
Cysteine Proteases, caspase-8 (Human cDNA)	6
Aspartic Proteases, cathepsin D (Human liver)	-4
Cysteine Proteases, cathepsin L (Human liver)	1
Metalloproteases, neutral endopeptidase (Human cell line)	0
Metalloproteases, MMP-1 (Human cDNA)	-5
Serine Proteases, tryptase (Human lung)	0
Phosphatases, phosphatase 1B (Human cDNA)	13

(Continued)

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 4 Summary of In Vitro Drug-Drug Interaction Studies: Test Article Ingenol Mebutate (Cont'd.)

Study Number: 13753 (Cont'd.)	
Type of Study	In vitro receptor binding and enzyme assays of ingenol mebutate
Binding Site	**Mean Percent Inhibition (%)**
CTK, ABl kinase (Human cDNA)	-2
AGC, Akt1/PKBa (Human cDNA)	-7
CAMK, CaMK2a (Human cDNA)	1
CAMK, CaMK4 (Human cDNA)	4
CMGC, CDC2/CDK1 (cycB) (Human cDNA)	1
RTK, FGFR2 kinase (Human cDNA)	-7
RTK, FLT-1 kinase (VEGER1) (Human cDNA)	0
RTK, IRK (InsR) (Human cDNA)	7
CTK, lyn A kinase (Human cDNA)	-9
STE, MEK1/MAP2K1 (Human cDNA)	-2
CMGC, p38 a kinase (Human cDNA)	2
AGC, PKA (Human cDNA)	0
AGC, PKCa (Human cDNA)	-2
TKL, RAF-1 kinase (Human cDNA)	3
CTK, ZAP70 kinase (Human cDNA)	-3
Monoamine & Neurotransmitter Synthesis & Metabolism, acetylcholinesterase (Human cDNA)	7
Monoamine & Neurotransmitter Synthesis & Metabolism, MAO-A (Human placenta)	-1
Monoamine & Neurotransmitter Synthesis & Metabolism, MAO-B (Human platelets)	-6
ATPASE, ATPase (Na+/K+) (Porcine brain)	-13

Source: Module 4.2.1.2; Module 4.2.2.4

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2.7.2 Summary of Clinical Pharmacology Studies

Appendix Table 5 Summary of Human Pharmacokinetics Prediction Study

Study Number: Letter No. PA001	
Type of Study	Human pharmacokinetics prediction
Test Article	PEP005 (ingenol mebutate) Gel
Method	Allometric scaling
Method of Administration	Topical
	Study Results
Blood clearance, L/hr/kg	0.22 to 1.01
Volume of distribution at steady state, L/kg	0.61
Absorption rate constant via topical dosing, hr^{-1}	0.0277
Topical Bioavailability, %	0.21
Cmax at the proposed topical clinical dose of 250 mL 0.05% Gel, ng/mL	0.000107
Tmax at the proposed topical clinical dose of 250 mL 0.05% Gel, hr	2
The minimum clinical topical dose to produce 0.1 ng/mL (lower limit of quantitation) in human blood, mg/kg/day	2000
Human pharmacokinetic projection after topical application of ingenol mebutate was based on the following assumptions: (1) one compartment model with first-order absorption and elimination; (2) clearance and volume of distribution at steady-state do not change after dose escalation, i.e., linear pharmacokinetics; (3) human absorption rate constant is similar to that of rat; (4) 0.21% bioavailability (F%) for topical administration in human based on percent of absorbed dose in vitro in human skin for the clinical gel formulation. The half-life after topical dosing at 0.05% ranged from 16.7 to 50.4 hours in rat (Nonclinical Study 2174/026). Assuming flip-flop kinetics, the absorption rate constant (ka) in rat is calculated as $\ln 2/t_{1/2}$, which results in a mean value of 0.0277 hr^{-1}	

Source: Module 4.2.2.7