EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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EMA/650464/2012
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Picato

International non-proprietary name: **ingenol mebutate**

Procedure No. **EMEA/H/C/002275**

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Product information

Name of the medicinal product:	Picato
Applicant:	LEO Pharma A/S Industriparken 55 DK-2750 Ballerup Denmark
Active substance:	ingenol mebutate
International Non-proprietary Name/Common Name:	ingenol mebutate
Pharmaco-therapeutic group (ATC Code):	Antibiotics and chemotherapeutics for dermatological use, other chemotherapeutics (D06BX02)
Therapeutic indication:	Picato is indicated for the cutaneous treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis in adults.
Pharmaceutical form:	Gel
Strengths:	150 micrograms/g, 500 micrograms/g
Route of administration:	Cutaneous use
Packaging:	Tube (HDPE/alu)
Package sizes:	3 tubes, 2 tubes,

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LIST OF ABBREVIATIONS

5-FU	5-Fluoro-uracil
AE	Adverse events
AET	Antimicrobial Effectiveness Testing
AK	Actinic Keratosis
AUC	Area under the curve
BCC	Basal Cell Carcinoma
CHMP	Commission on Human Medicinal Products
CI	Confidence Interval
C_{max}	Maximum blood concentration
CMH	Cochran-Mantel-Haenszel Test
CNS	Central nervous system
CSR	Clinical study report
CYP	Cytochrome P-450
DLT	Dose limiting toxicities
DMBA	7,12-Dimethylbenz(a)anthracene
ECG	Electrocardiograph
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EPAR	European Public Assessment Report
E. Peplus	Euphorbia peplus
FDA	Food and Drug administration
FTIR	Fourier Transform InfraRed spectrophotometry
GACP	Good Agricultural and Collection Practice
GC-MS	Gas Chromatographic-Mass Spectrometry
GSR	Global severity rating
HEC	Hydroxyethylcellulose
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonisation
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectroscopy
I_{kr}	Rapidly activating delayed rectifier K^+ channel
INN	International Nonproprietary Name

ITT	Intent to treat
IV	Intravenous
IVR/IWR	Interactive voice response/Interactive Web response
LLOQ	Lower limit of Quantification
LSR	Local skin reactions/responses
MTD	maximum tolerated dose
NMSC	non-melanotic skin cancer
NMT	No More Than
NOAEL	No observable adverse effect level
PEP005 Topical Gel	Picato (Ingenol mebutate) Gel
PD	Pharmacodynamics
PDCO	Paediatric Committee
Ph. Eur.	European Pharmacopeia
PK	Pharmacokinetics
PKC	Protein kinase C
PP	Per protocol
RCM	Reflectance confocal microscopy
RCT	Randomised Control Trial
RH	Relative Humidity
SAE	Serious Adverse events
SCC	Squamous cell carcinoma
SmPC	Summary of Product Characteristics
SOC	System Organ Class
T_{max}	Time to maximum concentration
TPA	12-O-tetradecanoylphorbol-13-acetate; phorbol 12-myristate 13-acetate
TSE	Transmissible Spongiform Encephalopathy
TSQM	Treatment Satisfaction Questionnaire for Medication
UI	International Unit
UK	United Kingdom
USA	United States of America
UV	ultraviolet

1. Background information on the procedure

1.1. Submission of the dossier

The applicant LEO Pharma A/S submitted on 27 July 2011 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Picato, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility for the centralised procedure was agreed upon by the EMA/CHMP on 26 January 2010.

The applicant applied for the following indication: topical treatment of actinic keratosis in adults.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or study.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/206/2010 on the granting of a (product-specific) waiver.

New active Substance status

The applicant requested the active substance ingenol mebutate contained in the above medicinal product to be considered as a new active substance in itself.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 22 July 2010. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

Picato has been given a Marketing Authorisation in the USA on 23 January 2012.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: **Robert James Hemmings** Co-Rapporteur: **Pierre Demolis**

- The application was received by the EMA on 27 July 2011.
- The procedure started on 17 August 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 04 November 2011. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 10 November 2011.
- During the meeting on 12-15 December 2011, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 15 December 2011.

- The applicant submitted the responses to the CHMP consolidated List of Questions on 15 May 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 2 July 2012.
- During the CHMP meeting on 16-19 July, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 17 August 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding issues to all CHMP members on 3 September 2012.
- During the meeting on 17-20 September 2012, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Picato on 20 September 2012.

2. Scientific discussion

2.1. Introduction

Problem statement

Actinic keratosis (AK) is a common skin condition visible as thickened, cornified, scaly lesions and characterised histologically by atypical epithelial proliferation. Actinic keratosis usually develops on areas that are frequently exposed to the sun (e.g., face, lips, ears, scalp, neck, forearms and back of the hands). Actinic keratosis is a carcinoma in situ with progression to invasive squamous cell carcinoma (SCC). Histological evidence shows that contiguous AK is present in 97% of SCC lesions on sun-damaged skin.

Actinic keratosis lesions are currently the most common epithelial premalignant lesions seen by dermatologists (Yu, et al., 2003). It is estimated that AK occurs in 11–50% of the population aged 40 and older in the United States (US) and Australia. In Europe the prevalence rate is 11-25% for people aged 40 or older. Patients with AK tend to have Fitzpatrick type I or II skin (fair skin) which burns and does not tan. In the US and Australia, the majority of people who develop AK lesions have fair skin with Fitzpatrick skin types I and II. The same is true for Northern European populations, such as the English, Irish, Scottish, and Scandinavians.

Currently available treatments for AK belong to two broad categories: surgical (e. g. cryosurgery or curettage) and conservative therapy. Medicinal products approved in the EU include 5-fluorouracil cream, imiquimod cream, diclofenac gel and photodynamic therapy (PDT) with amino-levulinic acid (ALA).

About the product

Picato is a topical gel for cutaneous application that contains the new active substance ingenol mebutate, which is a pure ingenol angelate obtained from the aerial parts of the plant species Euphorbia peplus L. by extraction and purification.

The mechanism of action of ingenol mebutate 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 by infiltration of immunocompetent cells. Results from two clinical trials on biological effects of ingenol mebutate have shown that topical administration induced epidermal necrosis and a profound inflammatory response in

both epidermis and the upper dermis of the treated skin, dominated by infiltrating T cells, neutrophils and macrophages. The proposed mechanism of action is stated to distinguish ingenol mebutate from current therapeutic options and provides a rationale for substantially shorter durations of treatment (two to three days) compared to authorised topical AK products.

The applicant applied for the indication: Picato is indicated for the topical treatment of actinic keratosis in adults. The finally approved indication was: Picato is indicated for the cutaneous treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis in adults.

For AK on face and scalp in adults, Picato 150 mcg/g gel should be applied to the affected area once daily for 3 consecutive days. For AK on trunk and extremities in adults, Picato 500 mcg/g gel should be applied to the affected area once daily for 2 consecutive days.

The content of one tube covers a treatment area of 25 cm^2 (e.g. 5 cm x 5 cm). The content of the tube should be applied to one treatment area of 25 cm^2. The tube is for single-use only and the tube should be discarded after use (see SmPC section 6.6). The gel from the tube should be squeezed onto a fingertip and spread evenly over the entire treatment area, allowing it to dry for 15 minutes. The content of one tube should be used for one treatment area of 25 cm^2. Picato is intended for single-use only.

Optimal therapeutic effect can be assessed approximately 8 weeks after treatment. If the treated area shows an incomplete response at the follow-up examination the treatment should be carefully re-evaluated.

Patients should be instructed to wash their hands with soap and water, immediately after applying Picato. If treating the hands, only the fingertip which is used for applying the gel should be washed. Washing and touching the treated area should be avoided for a period of 6 hours after application of Picato. Picato should not be applied immediately after taking a shower or less than 2 hours before bedtime. The treated area should not be covered with occlusive bandages after Picato is applied.

2.2. Quality aspects

2.2.1. Introduction

Picato is presented as an aqueous gel containing the active substance ingenol mebutate. Ingenol mebutate is obtained from the aerial parts of the plant species Euphorbia peplus L. by extraction and purification. *Euphorbia peplus* L. (E. peplus) is a member of the Euphorbiaceae family (Spurge family). The gel is presented in two strengths and is intended for the cutaneous treatment of actinic keratosis in adults, on the face and scalp (150 micrograms/gram gel) and on the trunk and extremities (500 micrograms/gram gel). The gel is presented in single use tubes; to be applied to the affected areas once a day for 3 consecutive days (face and scalp) or for 2 consecutive days (trunk and extremities). The finished product is a hydrophilic non-sterile gel. Other ingredients are defined in the SmPC, section 6.1. The appearance of the gel is the same for both strengths: a clear and colourless gel. The gel is presented in a laminate tube with a polyethylene screw cap.

2.2.2. Active Substance

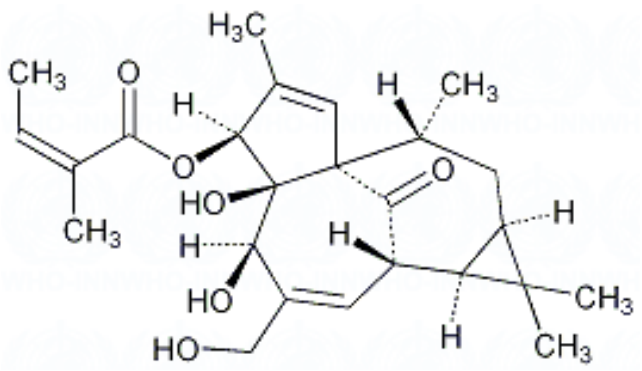
This medicinal product contains as active substance ingenol mebutate. Ingenol mebutate, a macrocyclic diterpene ester, is a purified ingenol angelate extracted from the aerial parts of *Euphorbia peplus* L. (Euphorbiaceae) plant.

The International Nonproprietary Name (INN) is ingenol mebutate and the chemical name is: (1aR,2S,5R,5aS,6S,8aS,9R,10aR)-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-

1a,2,5,5a,6,9,10,10aoctahydro-1H-2,8a-methanocyclopenta[a]cyclpropa[e][10]annulen-6-yl (2Z)-2-methylbut-2-enoate. The molecular formula and molecular weight are: $C_{25}H_{34}O_6$ and 430.53 g/mol.

Ingenol mebutate is a white to pale yellow crystalline powder and is freely soluble in isopropyl alcohol and benzyl alcohol. It is soluble in ethanol, methanol, acetone and acetonitrile and practically insoluble in n-heptane and water. Ingenol mebutate is unstable in water, is a very weak lipophilic acid with a stability pH optimum about 3.2. Ingenol mebutate is not hygroscopic and does not show polymorphism; it exists in only one crystalline form.

Figure 1- Ingenol mebutate structural formula



The molecule has eight chiral centres and one "non-restricted" double bond thus there is a theoretical possibility of up to 512 stereoisomers.

Manufacture

The ingenol mebutate is obtained from the dried, milled aerial parts of the plant by extraction followed by a series of purification steps. The final step of the process involves crystallisation. No chemical synthesis is involved. The applicant has confirmed that none of the materials used in the synthesis of the active substance present a risk of TSE contamination. Critical steps in the manufacturing process have been identified and relevant in process controls and intermediate specifications are in place.

Characterisation

The applicant has elucidated the structure of ingenol mebutate by elemental analysis, FTIR-, UV-, NMR- and mass spectroscopy as well as X-ray crystallography. All these investigations support the molecular formula. The applicant has adequately described the potential impurities in ingenol mebutate. Potential impurities have been presented with details of their origin, detection and level found, and discussed in relation to their potential carry-over into the final active substance. These include: i) organic impurities originating from the plant biosynthesis, ii) organic impurities formed during the manufacturing process, iii) degradation products, iv) residual solvents, v) heavy metals from the soil due to the origin from a plant material and vi) fungicide used during growth.

Specification

Ingenol mebutate is not described in the European Pharmacopoeia, hence the active substance is tested as per in-house specifications. The specifications include tests for: appearance, identification (by FTIR (Ph.Eur.) and HPLC), assay and organic impurities (by HPLC), residual solvents (by GC-MS), heavy metals (by ICP-OES), sulphated ash (Ph.Eur.) and water (micro determination per Ph.Eur.). Microbiological quality testing of the active substance is not performed. This is in line with ICH Q6A as

the manufacturing process includes the use of organic solvents that are toxic to microorganisms and the active substance itself does not support microbial growth.

The specifications and tests proposed by the active substance manufacturer are compliant with the relevant ICH guidelines and general requirements of Ph.Eur. The specifications are adequate to control the quality of the active substance. Limits of specified impurities are toxicologically acceptable. The analytical methods are appropriate and the validation data confirm the suitability of the proposed analytical procedures for their intended use. Batch analysis data (n=3 production scale batches) show compliance with the predefined active substance specifications and confirm consistency and uniformity of the active substance manufacture.

Stability

Ingenol mebutate powder is stored in glass vial with a rubber stopper and an aluminium crimp seal. The packaging material used for stability testing is the same as that used for storage and distribution.

The suitability of the container closure system has been demonstrated by the stability studies, showing that the active substance is effectively protected at long term conditions The stability studies have been conducted in accordance with ICH guidelines. A Long term stability study was conducted on five production scale batches, which have been stored up to 24 months at -20°C. Stability was tested according to the active substance specification and test methods, which showed to be stability indicating: appearance, assay and organic impurities.

Additional stability studies have been performed under the following conditions:

• Storage at 5°C for 24 months

• Storage at 25°C/60% RH for 6 months

The stability data presented support the proposed retest period and storage conditions in the proposed container closure system.

2.2.3. Finished Medicinal Product

Pharmaceutical Development

The applicant's objective was to develop a topical formulation, in which the active substance is chemically stable. In order to treat actinic keratosis on the face and scalp as well as the trunk and extremities, two strengths of the gel (0.015% w/w and 0.05% w/w) have been developed.

During development studies it was found that a gel formulation has superior properties to cream and ointment formulations. The proposed gel is formulated with conventional excipients such as hydroxyethylcellulose (HEC) as gelling agent and benzyl alcohol as preservative. The formulation development was based on an Quality By Design approach. A Quality Target Product Profile has been established and the Critical Quality Attributes of the finished product have been identified. The composition of the gel has remained the same during the development, apart from the grade of HEC. The recommended pH of a topical medicinal product is close to the pH of the skin. This is however not possible due to instability of the active substance with increasing pH. The active substance pH optimum is 3.2. The pH of the citrate buffer ensures that the pH requirement of 3.2 - 4.0 in the medicinal product is fulfilled.

The viscosity of the gel is dependent on the temperature. At 2-8 °C the viscosity is relatively high. After applying the finished product to the skin the gel becomes less viscous because of the combination

of increased temperature and the physical stress induced to the finished product by the application. This shear-thinning characteristic results in a gel, which is easily distributed on the skin.

The manufacturing method has been developed to avoid any heating steps taking account of the characteristics of the active substance. The development of the manufacturing process has been adequately performed.

The primary packaging is a single-dose laminate tube. The material complies with Ph. Eur. requirements and it is adequate to support the stability and use of the product.

Adventitious agents

No excipients of animal or human origin and no novel excipients are used in the manufacture of the finished product.

Manufacture of the product

The manufacturing of the gel comprises of the following main steps; dissolution, mixing and filling. No intermediates are isolated during the manufacturing process. The manufacturing process and equipment are adequately described. Critical process parameters have been defined and are controlled by relevant process controls. Suitable manufacturing validations have been performed. The applicant commits to perform process validation for each proposed batch size before these batch sizes are introduced for commercialisation.

Product Specification

The finished product release and shelf-life specifications include tests for appearance by visual inspection, identification (RT, UV spectrum) by UPLC, ingenol mebutate assay by UPLC, organic impurities by UPLC, benzyl alcohol assay by HPLC, pH (Ph. Eur.), viscosity (rheometer) and microbial quality (Ph. Eur.). Control of the finished product is satisfactory. The proposed test procedures and acceptance criteria comply with the requirements of the Ph. Eur. and ICH guidelines. All tests included in the specification have been satisfactorily described and validated. The UPLC method used for assay and related substances has been shown to be stability indicating. The finished product specification is acceptable and the specification limits are supported by batch data and stability results. Batch analyses are provided for all finished product batches used for toxicological studies and clinical trials. Batch analyses are also presented for production scale batches.

Stability of the product

The proposed container/closure is acceptable and sufficient to ensure the quality of the product throughout the proposed shelf-life. Stability studies have been conducted in accordance with ICH guidelines on both strengths of finished product, in the proposed commercial packaging. Production scale batches have been included in the stability studies.

Stability data for storage at long term (5°C) for up to 24 months and at accelerated storage condition (25°C /60%RH) for 6 months are available. Additional stability studies have been performed as needed. The stability data support the proposed shelf-life and storage conditions for the commercially packaged product under the conditions specified in the SmPC.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The quality of the active substance is adequately controlled and all excipients comply with the Ph.Eur. The pharmaceutical development demonstrates that a medicinal product of satisfactory quality has been developed. The manufacturing process is shown to consistently deliver a gel with the intended performance. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the proposed specifications and manufacturing controls. Appropriate packaging is used to ensure the product remains stable within the agreed shelf-life.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this medicinal product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

At the time of the CHMP opinion, there were no unresolved quality issues which could have an impact on the benefit/risk ratio of the medicinal product.

2.3. Non-clinical aspects

2.3.1. Introduction

The pharmacology and pharmacodynamics studies submitted included *in vitro* investigations of the effects on tumour cell lines such as human melanoma cells and *in vivo* investigations of the effects upon implanted tumours or ultra-violet B (UVB)-induced p53+ mutant patches in SKH-1 hairless mice.

The pharmacokinetics of ingenol mebutate was investigated mostly in the toxicology studies in rats and minipigs. Pharmacokinetics, metabolism and excretion were studied in rats, dogs and mini-pigs *in vivo* and / or *in vitro*. Absorption and metabolism in humans were studied *in vitro*.

The toxicology programme included single- and repeat-dose toxicity, genotoxicity, reproductive toxicity, antigenicity, toxicological qualification of impurities, haemocompatibility and platelet aggregation studies.

The Applicant received Scientific Advice from the CHMP. The non-clinical advice related particularly to the need of additional carcinogenicity studies and reproduction toxicology studies.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The summary of primary pharmacodynamic studies performed *in vitro* with ingenol mebutate and with isomers and metabolites are presented in Tables 1 and 2 respectively.

Table 1: Summary of primary pharmacodynamic studies performed *in vitro* with Ingenol Mebutate

Study type / Study Number	Test system	Noteworthy findings
Inhibition of Cell Growth/Survival: Effects of Ingenol Mebutate 4.1	D04, MM96L, SK Mel-28, DU 145, and MCF7 cells	Ingenol mebutate had an additive, rather than a synergistic, inhibition of cell growth.
Inhibition of Cell Growth: Time of Exposure 4.4c	D04, SK-Mel-28, MM96L, MCF7, and DU 145 cells	Varying treatment times had little effect on the resistant cell lines (SK-Mel-28, MM96L, and DU 145), but some improvement in cell survival was seen with early removal of mid-range drug concentrations within the sensitive D04 and MCF7 cell lines. The D04 and MCF7 cell lines showed some inhibition at very low doses (2 nM), whereas the other cell lines (MM96L, SK-Mel-28 and DU 145) were only inhibited at the highest dose (200 µM). Treatment times of 1 hour were effective for growth inhibition in the DU 145, MM96L and SK-Mel-28 cell lines.
Inhibition of Cell Growth: NCI Data P093	57 human tumour cell lines (IVCLSP Developmental Therapeutics Program, NCI/NIH)	The GI_{50} (nM) for cell lines MDA-MB-435 (breast), HCC-2998 (colon), COLO 205 (colon), TK-10 (kidney), K-562 (leukaemia), CCRF-CEM (leukaemia), HOP-62 (lung), NCI-H460 (lung) was 10 nM. The GI_{50} for A498 (kidney), SK-Mel-5 (melanoma), and MOLT-4 (leukaemia) were 13, 64, and 282 nM, respectively.
Inhibition of Cell Growth: Monolayer Assay P115A	Solid human tumour cell lines	100 µM ingenol mebutate displayed unspecific cytotoxicity in all tested cell lines. At concentrations ≤10 µM, it inhibited 8 of 10 leukaemia/lymphoma cell lines and 7 of 37 solid tumour cell lines.
Inhibition of Cell Growth: Continuous vs. Repeat Dosing Regimens 5 002	HOP62, A549, COLO 205, HT29, K-562, U-937, SK-Mel-28, and D04 cells; nontumour (normal) cells: NFF and PBMC	Ingenol mebutate was potent on several cell lines.
Clonogenic Assay P115B	54 tumour xenograft cell lines (leukaemias, lymphomas, and solid tumour models) and hematopoietic stem cells	Ingenol mebutate inhibited colony growth of leukaemias, lymphomas, and solid human tumour xenografts in a concentration- dependent manner. Antitumour potency (based on the mean IC_{70} value) was determined with 6.519 µM.
Cell Survival P025	MM127 cells	Ingenol mebutate (AN-1) was toxic at 10^{-5} dilution.
Cell Survival 4d.5	D04, DU 145, MM96L, MCF7, and NFF cells	Ingenol mebutate showed no synergism with SBHA, tamoxifen, gemcitabine, curcumin, N-acetylcarnitine, or EPA. Ingenol mebutate showed strongly synergised DHA and Ca ionophore in DU145 and NFF, possibly synergised retinal in D04. Ingenol mebutate moderately protected MCF7 and NFF from retinal.

5-FU = 5-Fluorouracil; AML = Acute myeloid leukaemia; *Bcl2* = B cell lymphoma 2; Ca = Calcium; CCCP = Carbonyl cyanide *m*-chlorophenylhydrazone; CTL = Cytotoxic T lymphocytes; DHA = Docosahexaenoic acid; DME = Ethylene glycol dimethyl ether; DMSO = Dimethyl sulfoxide; DNA = Deoxyribonucleic acid; DTT = Dithiothreitol; EDTA = Ethylenediaminetetra acetic acid; EPA = Eicosapentaenoic acid; ERK = Extracellular signal-regulated kinases; Flt3 = Fms-like tyrosinekinase receptor - 3; HAS2 = Hyaluron synthase - 2; IFN = Interferon; MAP = Mitogen-activated protein; MAPK = Mitogen-activated protein kinase; MEK = Mitogen-activated protein kinase kinase 1; MITF = Microphthalamia-associated transcription factor; MMP = Matrix metalloproteinase; NCI = National Cancer Institute; NFF = Neonatal foreskin fibroblast; NK = Natural killer; OCI = Ontario Cancer Institute; ODC = Ornithine decarboxylase; PBMC = Peripheral blood mononuclear cells; PKC = Protein kinase C; QIMR = Queensland Institute of Medical Research; SBHA = Suberic bis-hydroxamic acid; TPA = 12-O-tetradecanoylphorbol-13-acetate.

Table 2: Summary of primary pharmacodynamic studies performed *in vitro* with isomers and metabolites

Study type / Study Number	Test system	Test article	Noteworthy findings
Stability of Ingenol Mebutate and Isomers Isomerisation in Tissue Culture Media ± Cells 4e.4		Ingenol mebutate, PEP015, and PEP025	Ingenol mebutate rapidly isomerised in culture medium (57% after 3 hours) to form PEP015 (40% after 3 hours) and slowly isomerised to form PEP025 (3% after 3 hours). PEP015 rapidly isomerised in culture medium (50% after 3 hours) to form ingenol mebutate (40% after 3 hours). Ingenol mebutate then isomerised to form PEP025 (10% after 3 hours).
Inhibition of Cell Growth 4i.2	Nontumour (normal) cells: NFF(fibroblasts); tumour cell lines: K-562, COLO 205, HT29, A549 HOP-62, CI80-13S, PC-3, DU 145, SK-Mel-5, SK-Mel-28, T24, and T47D	Ingenol mebutate, PEP006, PEP008, and PEP010	The pattern of activity against the various cell lines was also similar for the Peplin compounds; high sensitivity was observed for SkMel-5, K-562, COLO 205, A549 and HOP-62. The stimulated lymphocytes (essentially T cells) were moderately sensitive.
Inhibition of Cell Growth/Survival: Effects of Crude/Partially Purified E. peplus Preparations P028	D04 cells	PEP006, PEP008, and ingenol mebutate	D04 E is a sensitive cell line. PEP006 (Ingenane 8) was toxic at 10^{-5} dilution; PEP008 (Ingenane 9) was toxic at 10^{-7} dilution; ingenol mebutate (AN-1) was toxic at 10^{-6} dilution; and TPA was toxic at 10^{-4} dilution.
Cell Survival P030	Mel-FH, Me4405, Mel-Rmu, Mel-RM, Mel0538, MM96L, D04, and NFF cells	PEP006, PEP008, and ingenol mebutate	D04L, Me4405, and Mel-RM are very sensitive cell lines. Mel-FH, Mel-Rmu, and MM96L are mildly sensitive cell lines (not very sensitive, but not as resistant as NFFs). NFF and Mel0538 are resistant cell line
Cell Survival P032	MDA-MB-435, 4T1.2, SW620, and COLO 16 cells	PEP006, PEP008, and ingenol mebutate	The only toxicity seen was with PEP006 (Ingenane 8) at 690 µM and PEP008 (Ingenane 9) at 33.5 µM. The only toxicity seen was with Ingenane 8 at 690 µM and Ingenane 9 at 33.5 µM. The only toxicity seen was with Ingenane 8 at 690 µM. TPA showed the strongest toxic effect to less than 16 nM. Ingenane 9 and ingenol mebutate (AN-1) were toxic at 335 nM and toxic at 69 µM, respectively.
Cell Survival P049	D04 cells	Crude sap (PEP001) contains: ingenol mebutate, PEP006, and PEP008	0.1 and 1 µg/mL of the PKC inhibitor bisindolylmaleimide- 1 inhibited killing of D04 melanoma cells by crude sap (MPA).
Inhibition of Cell Growth/Survival: Synergistic Inhibition of Cell Survival with Ingenol Mebutate P051	DU 145 cells	Ingenol mebutate, PEP006, and PEP008	Synergism of ingenol mebutate, PEP006, and PEP008 with cisplatin or bisindolymaleimide 1, radiotherapy, Taxol, and suberic bis-hydroxamic acid in inhibition of cell survival was tested. All drug combinations seemed to have an additive effect, except bisindolylmaleimide, which only differed slightly from the control.
Inhibition of Cell Growth/Survival: Effects of PEP015 and PEP025	D04, DU 145, MM96L, MCF7, SK-Mel-28, and	Ingenol mebutate, PEP015, and PEP025	Ingenol mebutate, PEP015, and PEP025 appear to have equivalent growth inhibition activity, requiring approximately 200 µM to achieve

Study type / Study Number	Test system	Test article	Noteworthy findings
4d.3	B16 cells		complete inhibition of growth (presumably cell death).
Inhibition of Cell Growth 4d.4	D04, DU 145, MM96L, MCF7, B16, and SK-Mel-28 cells	Ingenol mebutate, PEP015, and PEP025	PEP015 and PEP025 appear to have equivalent cytotoxic activity, requiring approximately 200 µM to achieve cell death. Ingenol mebutate appears to be more toxic than PEP015 and PEP025, with complete cell death being achieved at approximately 100 µM. The sensitive D04 cell line was an exception, with evidence of cell growth inhibition by ingenol mebutate and PEP015 occurring at approximately 200 nM and at 2 µM for PEP025. Complete cell death of D04 cells may not occur until the "high" concentrations of 100 or 200 µM.

Table 3 presents the summary of studies performed *in vitro* to investigate the mechanism of action of ingenol mebutate.

Table 3: Summary of other *in vitro* Primary Pharmacodynamic studies

Study type / Study Number	Test system	Test article	Noteworthy findings
Mechanism of Action: Induction of Necrosis Timing of Cell Death at High Dose (Pilot Study) 4b.4	DU 145, MM96L, B16, MCF7, and SK-Mel-28 cells	Ingenol mebutate	Several other methods were tested in MM96L cells (loss of neutral red, uptake of trypan blue, and loss of Erythrosin B) but were found to be unsuitable due to lack of sensitivity.
Apoptosis or Necrosis 4.4b	B16, D04, and MM96L cells	Ingenol mebutate	At 230 µM approximately half the MM96L cells were undergoing apoptosis, while the other half were in late stage apoptosis/necrosis. At 230 µM, the D04 cells appeared to be at late stage apoptosis/necrosis.
Apoptosis or Necrosis 4.11	B16 and Jurkat cells	Ingenol mebutate	Ingenol mebutate and PEP006 killed cells rapidly (3 to 5 hours) *in vitro* at around 100 µg/mL for ingenol mebutate and 50 µg/mL for PEP006. Ingenol mebutate and PEP006 clearly induced primary necrosis in B16 cells, and death was associated with mitochondrial swelling. LK2 cells behaved similarly after treatment with ingenol mebutate. Jurkat cells showed morphological changes characteristic of apoptosis following ingenol mebutate treatment, although chromatin margination and DNA laddering was not detected.
Mitochondrial Disruption 4b.5	MM127, Me10538, B16, Jurkat and normal rat hepatocytes	Ingenol mebutate and PEP006	Ingenol mebutate and PEP006 (100 µg/mL) caused a rapid (0.5 to 1 hour) loss in mitochondrial membrane potential (ΔΨm) in dividing cells. In over confluent cells treated with ingenol mebutate, PEP006, and TPA, loss of ΔΨm was slowed and was preceded by an increased mean red fluorescent intensity.
Cytotoxicity ± Glycine 4g.12	B16, LK2, D04, and NFF cells	Ingenol mebutate	The rapid lysis of cells by ingenol mebutate was not inhibited by glycine, EDTA, DTT, or KCl.
Mitochondrial Membrane	SK23, B16, and	Ingenol mebutate	Ingenol mebutate induced cell death by

Study type / Study Number	Test system	Test article	Noteworthy findings
Integrity, ADP:ATP Ratio, and Intracellular Calcium BHAMUK-001	HaCat cells		apoptosis in two skin cancer cell lines. In one of these (SK23) cells progressed rapidly to secondary necrosis.
Cytotoxicity: ADP:ATP Ratio and Caspase, Calpain, and Cathepsin Inhibitors PC042	MCF7 cells	Ingenol mebutate	Incubation of MCF7 cells with ingenol mebutate at 100 µg/mL induced rapid apoptosis, which led to secondary necrosis. The induction of death was caspase dependent, involving activation of executioner caspase 7 caspase is not present in these cells) but did not involve activation of calpains or cathepsins.
Apoptosis: PKC and MAPK Inhibitors PC043	Primary AML, CD34 positive, HL-60, and U837 cells	Ingenol mebutate	Ingenol mebutate induced p42/44 MAP kinase activation in leukaemic cells. This activation was downstream of PKC activation and both are required for induction of apoptosis by ingenol mebutate. Ingenol mebutate induced apoptosis in most AML subtypes, but M1 appeared to be most resistant. Sensitivity did not correlate with known mutations, such as Flt3 mutations.
Analysis of Protein Kinase Screen 4d.8	D04 cells	Ingenol mebutate	Treatment of D04 cells with ingenol mebutate (1 µg/mL; 1 hour) mediated an increase in the phosphorylation of, among other kinases, ERK 1, ERK 2, MKK 1, MKK2, and MKK6. Treatment of D04 cells with ingenol mebutate (1 µg/mL; 1 hour) mediated a decrease in phosphorylation of, among other kinases, PKCε, PKCα, and PKCδ. Phosphorylation of kinases is indicative of their activation, and conversely, dephosphorylation is indicative of deactivation.
Signal Transduction Proteins P115E	MEXF514L, GXF251L, and LECL HL-60 cells	Ingenol mebutate	No clear mechanism of action could be identified for ingenol mebutate.
Bipolar Assay: PKC and MAPK Activation, Cell Cycle, Gene Expression, and Dendriteinducing Factor 4i.5	NFF, MM96L, SK-Mel-28, D04, and COLO 205 cells	Ingenol mebutate	The present study report suggests the following sequence for induction of bipolar dendrites in MM96L melanoma cells: PKC activator (ingenol mebutate) > PKC activity > MEK activation of ERK > Brn-2 > MITF > Rho GTPases > bipolarity. Further elucidation of its origin at the molecular level may in due course lead to a more quantitative and mechanistically informative approach to characterising the action of PKC activators in melanoma cells.
MAPK Signalling: Summary of Data P094	D04 cells	Ingenol mebutate	Two groups of melanoma cells have been identified: one group that is exceptionally sensitive to ingenol mebutate-induced growth inhibition (1 to 10 ng/mL), and the other is insensitive (>1,000 ng/mL). Treatment of sensitive melanoma cell lines for 24 hours with ingenol mebutate (100 to 1,000 ng/mL) led to growth arrest, cell cycle blocking (G1 and G2/M), and permanent senescence. This

Study type / Study Number	Test system	Test article	Noteworthy findings
			activity of ingenol mebutate was inhibited using BIS (5 µM) and the MEK inhibitor, PD98059 (20 µM). Growth inhibition of sensitive melanoma cells was observed using lower doses of ingenol mebutate (e.g., 1 to 10 ng/mL).
Mechanism of Action: Modulation of Gene Expression Gene Arrays 4d.7	B16 and LK2 cells; C57BL/6 mouse	Ingenol mebutate	In the *in vitro* LK2 study on the OCI mouse chip, the expression of serum/glucocorticoid-regulated kinase (a member of the serine/threonine kinase family), fos oncogene, and MAP kinase kinase 3 was increased by ingenol mebutate. This finding may indicate possible signal transduction pathways following environmental stress. This is consistent with in vitro activation of the MAP kinase pathway found in other studies of ingenol mebutate.
Gene Expression Modulation 4d.9	THP-1	Ingenol mebutate	The concentrations of ingenol mebutate, TPA, and prostratin that were used for the microarray study (2.3 nM, 2.3 nM, and 2.3 µM, respectively) were selected as they induced differentiation of the THP-1 cell line to similar degrees. The observation of the induction of TXK tyrosine kinase may have identified the pathway for the induction of IFN-γ by ingenol mebutate in the *in vivo* treatment of B16 tumours.
Mechanism of Action: Induction of Proinflammatory Cytokines Immunostimulatory Effects (PBMCs) P115D	Human PBMCs	Ingenol mebutate	Ingenol mebutate has immunostimulatory properties, as apparent from the strong induction of IL-1β, TNF-α, IL-2, IL-6, and IL-8 in PBMCs. The induction of cytokines by ingenol mebutate was more effective at 1 nM than at 10 or 100 nM. Cytotoxic effects were observed at 100 nM ingenol mebutate but not at 10 or 1 nM.
Immunostimulatory Effects (PBMCs) P164C	Human PBMCs	Ingenol mebutate	Ingenol mebutate has the capacity for the induction of IL-1β, IL-6, IL-8, and TNF-α in PBMCs, even at very low concentrations like 0.01 nM. The induction of cytokines by ingenol mebutate was more effective at 24 hours than at 2 or 6 hours following PBMC exposure.
Mechanism of Action: Role of Neutrophils Respiratory Burst and Phagocytosis P091	Human PBMCs	Ingenol mebutate, PEP006, PEP008, Ingenane Mix (PEP003) contains: ingenol mebutate, PEP006, and PEP008	TPA and the Peplin compounds, but not bryostatin 1, induced respiratory burst and phagocytosis in human leucocytes, particularly monocytes and granulocytes (neutrophils). PKC was confirmed as the target for Peplin compounds by the ability of the PKC inhibitor to block the respiratory burst.
Mechanism of Action: Role of NK, CTL, and B cells Cell Lysis by CTL and Stimulation of CTL PQ8017-019	Human melanoma cells and PBMCs	Ingenane Mix (PEP003) contains: ingenol mebutate, PEP006, and PEP008	Pretreatment of melanoma cells with PEP003 increased the level of NK activity in cultures compared to both TPA and the control treatment at the E:T ratio of 45:1.
Stimulation of NK Activity	Human melanoma cells and	Ingenane Mix (PEP003) contains:	Pretreatment of melanoma cells with PEP003 increased the lysis of K-562

Study type / Study Number	Test system	Test article	Noteworthy findings
PQ8017-016	PBMCs	ingenol mebutate, PEP006, and PEP008	cells compared to both TPA and the control treatment at the E:T ratio of 45:1, suggesting that PEP003 increased NK activity in PBMCs from Patient A02.
Cell Lysis by CTL and Stimulation of CTL PQ8017-005	Human melanoma cells and PBMCs	Ingenane Mix (PEP003) contains: ingenol mebutate, PEP006, and PEP008	PEP003 reduced the ability of melanoma cells to restimulate a specific CTL immune response in vitro. It did not alter the ability of melanoma cells to be killed by CTL.
CD8+ T Cells: Anti-apoptosis and Cytotoxicity PC044	CD8+ T cell lines from human PBMCs	Ingenol mebutate	The data so far suggest that ingenol mebutate extends the lifespan of both CD4+ (previous work) and CD8+ activated T cells. The anti-apoptotic effect works on proliferating and non-proliferating cells to an equal extent. Neither an increase nor a decrease of cytotoxic activity was detected. Therefore the conclusion from the *in vitro* work so far is that the increase in vivo antitumour activity is due to the anti-apoptotic effect of ingenol mebutate rather than an increase in cytotoxicity.

Table 4 presents the summary of studies performed *in vivo* to investigate the mechanism of action of ingenol mebutate.

Table 4: Summary of primary pharmacodynamic studies (mechanism of action) performed *in vivo* with ingenol mebutate

Study type / Study Number	Test system	Noteworthy findings
Mechanism of Action: Role of Neutrophils Efficacy and Granulocytes 4d.6	LK2 mouse SCC/athymic nu/nu nude mouse	Cotreatment with granulocyte antiserum, PCA, or DTT did not inhibit the efficacy of ingenol mebutate against LK2 tumours in nude mice. Mice treated with ingenol mebutate and granulocyte antiserum took slightly longer to reach the level of inflammation formed in the ingenol mebutate control group. Eventually, however, an equivalent degree of inflammation was seen to the ingenol mebutate control group.
Topical Efficacy in the ypomorphic Mouse 4g.3	Hypomorphic (Itgb2) mouse	Ingenol mebutate failed to cure subcutaneous B16 tumours in the hypomorphic mouse. Although the area of erythema, which was observed after treatment with ingenol mebutate in the hypomorphic mice, was similar to that observed in C57BL/6 mice, the intensity (red colouration) was markedly lower.
The Role of the Innate Immune Response (Macrophages) in the Treatment of Murine Squamous Cell Carcinomas 4h.1iv	B16 mouse melanoma/op/op hypomorphic mouse and LK2 mouse SCC/nude mouse control and macrophage ablated	The high topical efficacy of ingenol mebutate against B16 melanomas in the op/op mouse suggests that macrophages are not required for ingenol mebutate antitumour action. The second mouse model, where macrophages were depleted by clodronate prior to ingenol mebutate treatment, gave a more equivocal result with a substantial delay in growth. However, most tumour sites eventually grew. The tumour growth curves from ingenol mebutate plus clodronate-treated mice are remarkably similar to those from neutrophildepleted mice obtained previously for ingenol mebutate. The rapid growth of B16 in untreated, macrophage-

		deficient mice was unexpected.
The Role of the Innate Immune Response (NK and B Cells) in the Treatment of Murine Squamous Cell Carcinomas 4h.1ii	LK2 mouse SCC/nude (control and NK depleted), and SCID mouse	NK cells do not play a major role in the topical efficacy of ingenol mebutate.
The Role of the Innate Immune Response (Granulocytes) in the Treatment of Murine Squamous Cell Carcinomas 4f.10vi	B16 mouse melanoma/ B6.129S7- Itgb2tm1Bay/J mouse and LK2 mouse/Foxn1nu (Balb/c nu/nu) mouse	Ingenol mebutate caused minimal erythema in the granulocyte-ablated mice, and the tumours were not cured. Cure, marked erythema, and excellent skin regeneration were seen in control mice. Ingenol mebutate was ineffective in curing B16 mouse melanomas in β2-integrin knockout C57BL/6 mice, where granulocytes cannot be mobilised. B16 tumours in normal C57BL/6 mice are normally cured by ingenol mebutate.
Mechanism of Action: Cognate Immune Response Anticancer Cytotoxic T Lymphocyte Induction 4.10	B16-OVA mouse melanoma and Lewis OVA lung tumour/C57BL/6 mouse	Intralesional treatment of primary tumours clearly induced a systemic effect that resulted in inhibition of the growth of secondary tumours. Evidence for the role for CD8 T cells in mediating this activity is provided by (1) no systemic effect in nu/nu mice, which have no functional CD8 T cells (2) induction of anticancer CD8 T cells by ingenol mebutate treatment, and (3) from the correlation of higher CD8 T cell activity in mice showing the best anti-Lewis lung secondary activity. The controls illustrated that (1) tumours must be present and (2) must be cured in order to generate the systemic anticancer activity.
Codelivery with Immunomodulators On Secondary Tumours 4d.10 and 4b.6	B16-OVA mouse melanoma and Lewis OVA lung tumour/C57BL/6 mouse	CpG, LPS, polyIC, and IL-2 were not able to synergise with ingenol mebutate-mediated curing of primary tumours to produce a better cure of secondary tumours. Immunomodulatory compounds that synergise with ingenol mebutate were not identified.

Table 5 presents the summary of other primary pharmacodynamic studies performed *in vivo* with ingenol mebutate.

Table 5: Summary of primary pharmacodynamic studies (<u>other studies</u>) performed *in vivo* with ingenol mebutate

Study type / Study Number	Test system	Noteworthy findings
AK/SCC Model development: SKH1 Model Mouse SCC Tumourigenicity: SKH1 and C3H/HeN Mice QIMR-07-030	Murine SCC cell lines T7 and UV-13-1 and Human TE354.T BCC cells/inbred C3H/HeN, inbred and outbred SKH1 mouse	The immune competent UV-13-1/C3H/HeN model of SCC was established. At a dose of 40 µg the percentage of cured tumours was 60%.
UV-induced p53+ Mutant Patches: SKH1 Mouse QIMR-07-085	SKH1/hr mouse	At 14 days following cessation of UV irradiation, reduced size of p53+ patches was noted; however, the number of patches remaining was sufficient for future experiments.
Topical Efficacy in Mouse Xenografts: Ingenol Mebutate in Initial Model B16 Melanoma	B16 (mouse melanoma)/ C57BL/6 mouse	Topical application for 3 days of the pure Peplin compounds, equivalent to ingenol mebutate and PEP008 alone or mixed, caused inhibition of growth of subcutaneous B16 melanoma tumours in C57BL/6 mice. The growth curves in the mouse model, however, may severely underestimate the efficacy of these

Study type / Study Number	Test system	Noteworthy findings
P092		drugs due to local tumour invasion into the muscle. Tumour site outcome was expressed quantitatively: five of six (80%) sites were cured in each group. This rises to 100% if the failures are attributed to muscle invasion before treatment.
Topical Efficacy in Mouse Xenografts: Optimisation of Ingenol Mebutate Dosage and Regimen Dose Response 4.3a	B16 mouse melanoma/ nude (Balb/c athymic), C57BL/6 mouse and LK2 mouse SCC/nude (Balb/c athymic)	In the nude (Balb/c athymic) mouse models, all doses were effective at curing B16 implanted tumours, and two doses (6 and 18 µg) were effective at curing LK2 implanted tumours. There was an average of only one breakthrough out of 16 sites in both nude (Balb/c athymic) models. In the LK2/nude (Balb/c athymic) model, treatment with 54 µg was above the MID; all animals were dead by Day 5. The 6-µg dose was the best tolerated dose with the least side effects on erythema and weight loss. In the B16/C57BL/6 model there was a poor response to treatment due to tumour breakthroughs occurring in all the doses.
Best Regimen 4.3b	B16 mouse melanoma/ C57BL/6 mouse, and LK2 mouse SCC cells/nude (Balb/c athymic)	In the B16/C57BL/6 model, 15 µg ingenol mebutate for 3 consecutive days was the most effective treatment regimen; however, the mice in this group still suffered delayed breakthrough tumours with only one of seven mice tumour free at Day 21. All the mice in the other treatment groups suffered breakthrough tumours. In the LK2/nude model, 10 µg ingenol mebutate for 3 consecutive days was the most effective treatment regimen. The 1 × 25 µg was also effective, but two mice were found deceased on Day 2 and one was found deceased on Day 30, leaving only one mouse alive. The 7 × 3 µg regimen had four breakthroughs out of 16 sites. The low average weight in the 3 × 10 µg group was mainly due to one mouse in the group with a rectal prolapse losing a lot of weight rather than a side effect of topical treatment.
Topical Efficacy in Mouse Xenografts: PK After Local Ingenol Mebutate Application Time Course of Recovery From Skin and Tumour 4.5b	B16 mouse melanoma/nude (Balb/c athymic) mouse	This study suggests that most of the topically applied ingenol mebutate is absorbed into the skin and lost (metabolised or systemically) in less than 1 hour following application.
Blood Levels 4.6	Nude (Balb/c athymic)	In this small scale pilot study, the results clearly show that there is very little detectable ingenol mebutate in the blood of mice after topical or intralesional treatment with ingenol mebutate.
Topical Efficacy in Mouse Xenografts: Optimisation of Ingenol Mebutate Formulation Ingenol MebutateFormulations 4e.3	B16 mouse melanoma/nude (Balb/c athymic) mouse	Isopropanol gel remained the most effective ingenol mebutate treatment vector; however, in this study, Formulation C appears to be potentially the next best candidate for clinical treatment. Formulation C did not have any "failed" treatments Breakthrough tumours arise from nests of B16 cells that have lodged in the muscle. These tumours can be identified.
Ingenol Mebutate Formulation 4f.9	B16 mouse melanoma/ C57BL/6 mouse and LK2 mouse SCC/Foxn1nu (nude Balb/c) mouse	Ingenol mebutate Formulation D was effective in the nude mouse LK2 model. Ingenol mebutate Formulation D inhibited the growth of the tumour but was not curative in the C57BL/6J mouse B16 model.
Mouse SCC: Cream vs. Gel 5_005	LK2 mouse SCC/nude mouse	The decoded results indicate that all ingenol mebutate gel or cream preparations gave an early inflammatory response and ablation of tumours, compared with the corresponding placebos. The LK2 tumours grew slightly slower than usual.

Study type / Study Number	Test system	Noteworthy findings
2005		In addition, a high proportion of tumours were found at time of treatment to be growing on the underlying muscle. The number of muscle-associated tumours increased with time, suggesting that tumour cells gradually escape from the subcutaneous site and invade locally. The results indicate that all ingenol mebutate preparations were effective in blocking the growth of the LK2 tumours.
Topical Efficacy in Mouse Xenografts: Comparison with Approved Topical Therapies 5-FU and Imiquimod 4.3c	B16 mouse melanoma/ C57BL/6 mouse	In this B16/C57BL/6 model, ingenol mebutate treatment induced a reduction in tumour size with either cure or delayed tumour recurrence. Ingenol mebutate cured or delayed recurrence of subcutaneously implanted B16 mouse melanoma tumours in C57BL/6 mice, whereas imiquimod and 5-FU did not.

Secondary pharmacodynamic studies

The potential for off-target activity of ingenol mebutate was investigated in various in vitro receptor-binding and enzyme assays (Study 13753). Ingenol mebutate (1.0 µM) was shown to exhibit minimal (<20%) inhibition or stimulation against the 112 individual receptors and 41 enzymes tested.

Following topical exposure to 2 µg/kg/day in humans (Study Report PA001), there is negligible risk of potential off-target activity and consequent adverse systemic effects in humans since this test concentration is much higher than the estimated human blood C_{max} of 2.54 x 10^{-7} µM.

Safety pharmacology programme

The safety pharmacology studies submitted are summarised in Table 6.

Table 6: Summary of Safety Pharmacology studies

Study type / Study Number / GLP status	Species / Strain	Route of administration	Doses (mg/kg)	Gender and number per group	Noteworthy findings
Cardiovascular System					
hERG 700517-1 GLP	Human embryonic kidney cells	*In vitro*	0, 0.5, 1.0, 2.5, and 5.0 µg/mL E-4031 (positive control) 500 nM	7 cells/ replicate 3 cells/ replicate	Current inhibition was equivalent to the decrease in net normalised current density. Ingenol mebutate did not inhibit hERG current up to the highest concentration tested. The IC_{50} was not calculable.
Cardiovascular/ Respiratory, Anaesthetised 2174-010 GLP	Beagle dog	IV, escalating dose, 0.5 mL/kg, 0.27 mL/min; minimum 25 min continuous observation period	0 µg/kg (×4) 0.3, 1, 3, and 10 µg/kg 0, 0.3, 1, 3, and 10 µg/kg	2M/2F 2M/2F 4M/4F	≥3 µg/kg: Transient decrease in femoral blood flow occurred at 3 µg/kg and sustained at 10 µg/kg. 10 µg/kg: Increases in diastolic pressure and heart rate, tachycardia, and increases in peak inspiratory flow (PIF) were noted. At ingenol mebutate doses of 0.3, 1, 3, or 10 µg/kg, Cmax values in males were less than the LLOQ of 0.1 ng/mL, 0.341, 1.10, and 3.32 ng/mL, respectively, and in females 0.106, 0.434, 1.48, and 4.29 ng/mL, respectively.
Cardiovascular/ Respiratory, Telemetry	Beagle dog	IV (slow bolus, target 3 minutes)	0, 1.5, 7.5, and 15 µg/kg	4M/4F	≥1.5 µg/kg: Non-dose-related increases in diastolic and mean blood pressures were observed in both genders.

Study type / Study Number / GLP status	Species / Strain	Route of administration	Doses (mg/kg)	Gender and number per group	Noteworthy findings
N106161 GLP		Once daily on study Days 1, 4, 8, and 11 (at least 48 hours between dosing) as 4 × 4 balanced Latin- Square crossover design replicated twice (i.e., once for each sex)			≥7.5 µg/kg: Body temperature decreased, salivation, emesis, alterations in faecal consistency, and panting were observed. Dose and duration related increases in heart rate occurred in both genders; statistically significant increases in respiratory rates in males and minute volumes in females were observed. 15 µg/kg: Systolic pressures decreased and statistically significant increases in respiratory rates in females and minute volumes in males were observed. ≤15 µg/kg: No changes in ECG rhythm, morphology, or interval measurements in either gender compared to baseline were observed.
Central Nervous System (CNS)					
Effects on General Activity And Behaviour 2174-008	Wistar rat	IV, 5 mL/kg, 1 mL/min with observations at 5, 15, 30, 60 and 120 mins	0, 1, 3, and 10 µg/kg	4M/4F	No animals died or were prematurely sacrificed. No significant behavioural or physiological changes postdosing.

Pharmacodynamic drug interactions

No relevant studies were submitted (see discussion on non-clinical aspects).

2.3.3. Pharmacokinetics

The rat and mini-pig were used to investigate the percutaneous absorption of ingenol mebutate both *in vivo* and *in vitro*. Pharmacokinetics, distribution, metabolism and excretion were evaluated in the rat, dog and mini-pig *in vivo* and/or *in vitro*. Human tissues were included for *in vitro* studies of absorption and metabolism. Systemic drug exposure in topical and intravenous toxicology studies of the rat, rabbit, dog and mini-pig was quantified via blood concentration monitoring. The potential for clinical drug interactions was assessed by evaluating *in vitro* the inhibition or induction of the major human isoforms of cytochrome P450 by ingenol mebutate.

After *in vitro* applications of 0.01%, 0.1% or 0.05% PEP005 Gels to rat, mini-pig and human skin preparations, the percutaneous absorption was generally low, with a range of 0.04% (mini-pig) to 8.68% (rat) across animal species and 0.16% to 1.93% in humans. The absorbed doses of [^{3}H]-ingenol mebutate were in the order of WI rat > SD rat > human > mini-pig. After topical administration of PEP005 Gel to mini-pigs, blood levels of ingenol mebutate were generally not detected, and when detected, ranged up to 0.1 ng/mL. After topical administration of ingenol mebutate to rats, blood levels were consistently quantifiable only at doses of 300 µg/kg or greater, in which case the absolute bioavailability was 2% to 4%.

After IV administration, a high to very high blood clearance, moderate to high volume of distribution at steady-state and short half-life were observed in rats, rabbits, dogs and minipigs. Following IV administration of [^{3}H]-ingenol mebutate to rats, drug-related radioactivity was well distributed to the tissues and there were no gender differences in the organs exposed but elimination was faster in females. In vitro, ingenol mebutate and its isomers were shown to have high plasma protein binding in rats, dogs, minipigs and humans (>99%). In rats, the majority of an intravenous dose of [^{3}H]-ingenol mebutate was excreted via the biliary route, with urinary excretion as a minor pathway.

Plasma clearance and volume of distribution (steady-state) in humans were estimated using a simple allometric correlation based on body weight. Using a one-compartment model with first-order absorption and elimination kinetics, it was estimated that dermal administration of the maximum intended clinical dose of 2 µg/kg/day would produce levels of ingenol mebutate in the blood below the LLOQ of 0.1 ng/mL. Blood clearance and volume of distribution at steady-state were predicted to range from approximately 0.22 to 1.01 L/h/kg and approximately 0.61 L/kg, respectively. The absorption rate constant and topical bioavailability was projected to be 0.0277 hours^{-1} and 0.21%, respectively. A human blood T_{max} of 2 hours and C_{max} of 0.107 pg/mL were predicted for a 2 µg/kg/day topical dose. A minimum topical dose of 2000 µg/kg/day to humans would be required to produce detectable blood levels.

The *in vitro* metabolism of ingenol mebutate was qualitatively similar in blood, skin homogenates and hepatocytes of rats, dogs, minipigs and humans. Ingenol mebutate was found to be relatively stable in blood and skin homogenates, and to undergo extensive metabolism in cryopreserved hepatocytes. The major pathway in rat, dog and minipig hepatocytes was hydrolysis to ingenol, whereas the major pathway in humans was hydroxylation of ingenol mebutate. In the skin of rats, dogs, minipigs and humans, rearrangement of ingenol mebutate was predominantly to PEP015 (~26% to ~31%) and, to a much lesser extent, PEP025 (~1% to ~2%); hydrolysis to ingenol was minimal (0% to 0.81%). However, after topical or IV administration of ingenol mebutate to rats and minipigs, PEP025 was not detected and PEP015 was less than 10% of the corresponding ingenol mebutate concentration in the blood.

No inhibition or induction of cytochrome P450 isoforms was found at concentrations much greater than the estimated human blood levels after dosing at the maximal clinical dose of 2 µg/kg/day.

2.3.4. Toxicology

Single-dose toxicity

The single-dose toxicity of ingenol mebutate was examined in rats, rabbits and minipigs.

The studies are summarised in Table 7.

Table 7: Summary of single-dose toxicity studies

Species Study ID GLP	Method of Administration Doses (mg/kg)	Major findings
Rat/Wistar Han 2174-004	IV (20% PEG 400 in 0.9% saline v:v) 0, 10, 20, 30, 40b µg/kg	≥0 µg/kg: Tachypnea ≥10 µg/kg: Limp ≥20 µg/kg: Mortality, ataxia, decreased activity, prone, bradypnea, hyperpnoea, piloerection, lethargy, dark tail ≥30 µg/kg: Hypothermia 40 µg/kg: Animals died immediately (preliminary study) Observed Maximum Nonlethal Dose: (10 µg/kg). Minimum Lethal Dose (MLD) was 20 µg/kg, and the IV no observable effect level (NOEL) was <10 µg/kg.
Rat/Crl:SD 665399	Instillation into urinary bladder Three vehicles: 1) 0.9% NaCl; 2) 20% PEG 400/0.9% NaCl; and 3) 8.5% PEG 400/0.9% NaCl 0, 0, 0, 1, 3, 10, 30, 50µg/kg	0 µg/kg: Vehicle Formulation 3 was associated with reddening of bladder >1 µg/kg: Lacrimation during dosing, dose-related bladder leakage; dose-related urinary bladder oedema and haemorrhage were reversible following a 7-day recovery period >30 µg/kg: Reddening in the urethra or urinary bladder, cystitis 50 µg/kg: Transient irregular/ laboured breathing, diffuse transitional hyperplasia in bladder Observed Maximum Nonlethal Dose: >50 µg/kg. No mortality at doses up to 50 µg/kg, and a no observable adverse effect level (NOAEL) of <1 µg/kg was identified.

Species Study ID GLP	Method of Administration Doses (mg/kg)	Major findings
Rabbit/Crl:NZW/Kbl.BR 2174-009	IV (20% PEG 400 in 0.9% saline v:v) 0, 1, 5, 10, 20, 30 µg/kg	≥1 µg/kg: Bruise erythema and oedema at the injection site ≥10 µg/kg: Tachypnea, miosis ≥20 µg/kg: Unresponsive pupil, ataxia (F), prone, salivation, lacrimation, lethargy, rales (F) 30 µg/kg: Decreased body weight gain during postdose observation period (M) Observed Maximum Nonlethal Dose: >30 µg/kg. No mortality was observed following IV ingenol mebutate dosing at ≤30 µg/kg, and a NOEL of 5 µg/kg was identified.
Mini-pig/ Göttingen ApS 2174-025	Topical, Semiocclusiveh (Aqueous Gel/ (w/w 30% isopropyl alcohol, 1.5% hydroxyethyl cellulose, 0.9 % benzyl alcohol)	≥0.083 µg/mm²: Erythema/eschar, oedema, skin sore, reddening, flaking, acanthosis, scab formation, acute necrotic dermatitis. 0.83 µg/mm²: Skin cracking, weeping, bruising. Observed Maximum Nonlethal Dose: >73 µg/kg.

Repeat-dose toxicity

The design and results of the repeat-dose topical dermal toxicity studies are summarised in Table 8.

Table 8: Summary of Pivotal Repeat-Dose Topical Dermal Toxicity studies

Species / Duration Study ID GLP	Method of Administration Doses (µg/kg/day)	NOAEL (mg/kg/d)	Major findings
Rats/Sprague Dawley (3days) Duration of Dosing: 3 days Duration of Postdose:14 days 2174-026	Topical 60, 300, 600	Systemic >600 µg/kg/day; Local <60 µg/kg/day	The administration of PEP005 to the rat dorsum at concentrations of 0.01and 0.1% was found to be irritating. Dose-related erythema and oedema were observed with the rapidity of onset, numbers of animals affected and the degree of response generally more severe in females than males. Microscopically, dose-related necrotising dermatitis, fasciitis/fibrosis, myositis, acanthosis, ulceration/erosion were observed. No systemic or target organ toxicity was noted at 600 µg/kg/day. Local dermal irritation was observed at 0.25 µg/mm2/day; a NOAEL for the irritant response could not be established.
Rats/Crl[CD]SD (13 weeks) Duration of Dosing: 13 weeks Duration of Postdose: 15 days 457405	Topical 0, 75, 150, 375, 750	<75 µg/kg/day	Repeat dose cycling with PEP005 in the range 0.0125-0.25 µg/mm2 for a period of up to 13 weeks resulted in dermal responses (epidermal hyperplasia and ulcerative dermatitis) which culminated in a number of treatment groups being unable to complete dosing (only Group 3 completed as planned) and will therefore, most likely preclude repeat dosing for longer periods. Dermal responses were so intense as to require the suspension of dosing at 0.125 and 0.25 µg/mm2 after Day 2 of the study. Moreover, animals dosed at 0.025 µg/mm2 alternate daily for only 6 days with a 15 day recovery period was the only repeat dose/recovery cycle regimen to complete the 13 week dosing cycle. Given the inability to define a repeat dose/recovery cycle regimen which could be used for possible future carcinogenicity studies it is proposed that an alternative repeat dose/recovery cycle route be selected for such studies.
Rat/Crl[CD]SD (6 months) Duration of Dosing: Once/day for 3	Topical 0, 25, 50, 100	Systemic >100 µg/kg/day; Local <25 µg/kg/day	There were no signs of systemic toxicity following repeat treatment/repeat cycle topical administration of 0.005%, 0.01%, and 0.02% PEP005 gel to rats for 6-months. Blood levels of PEP005 were not quantifiable in a majority of samples at an LLOQ of 0.1 ng/mL.

Species / Duration Study ID GLP	Method of Administration Doses (µg/kg/day)	NOAEL (mg/kg/d)	Major findings
days followed by a 4-week non-dosing period (Treatment Cycle) and repeated monthly for 6 months (7 cycles) Duration of Postdose: 4-week recovery period N106168A			Dose-related dermal irritation and microscopic changes consistent with active-chronic dermal inflammation at the dosing site were observed, and in rats treated with 0.02% PEP005 gel, an increase in circulating neutrophils consistent with dermal inflammation was noted. There was no evidence of alterations in dermal tolerance to PEP005 administration over the course of treatment (7 cycles). Following a 4-week recovery period, microscopic dermal observations in all rats were resolved (placebo and 0.005% PEP005) or nearly resolved (≥ 0.01% PEP005).
Mini-pig/Göttingen ApS (3 days) Duration of Dosing: 3 days Duration of Postdose: 1-14 days 2174-029	Topical 6, 30, 60	> 60 µg/kg/day; Local <6 µg/kg/day	Dermal doses of up to 0.1% PEP005 produced a spectrum of dermal responses that increased in severity with increasing dose. The intensity of these dermal responses was greater in males than females. In Groups 2 and 3 there was a dose response which limited the number of doses certain animals could receive, due to the intensity of the irritant response. In the toxicokinetic analysis minimal systemic exposure to PEP005b was observed. Histopathological changes included dermatitis, acanthosis, scab formation and ulceration/erosion. At Day 14, the overall level of these changes at sites receiving PEP005 was reduced when compared with Day 4 and was generally consistent with healing.
Göttingen ApS Mini-pig (3 days) Duration of Dosing: 3 days Duration of Postdose:1-14 days 507450	Topical 0.4, 1, 2, or 4	0.4 µg/kg/day (males) and 1 µg/kg/day (females)	The dermal administration of PEP005 for 3 days at concentrations of 0.001% or more produced, scab formation, dermatitis, acanothosis and parakeratosis at the test sites of increased incidence and severity with increasing concentrations seen at both 24 h or 14 day post treatment. Minimal to marked erosion/ulceration formation was observed at the test sites treated with PEP005 at all concentrations in the males and females at 0.005% and 0.01% 24 h post treatment. This change was not present in females at 0.0025% or lower and of minimal severity at one test site in one male at 0.001%. The intensity of these dermal responses was greater in males than females. Consequently, based on the dermal responses seen after 24 h post dose treatment and the clear evidence of ongoing recovery during the 14 day observation period, a concentration of 0.001% for males and 0.0025% for females was considered the NOAEL in this study.
Mini-pig/Göttingen ApS (13 weeks) Duration of Dosing: 3- or 5-day cycle (total of 4 dosing cycles) Duration of Postdose: 23- to 25-day inter-cycle, followed by an 8-week recovery period	Topical 0, 0.0125, 0.025, 0.05 µg/mm²/day (5 days), and 0.025, 0.05, 0.075 µg/mm²/day (3 days)	0.5 µg/kg/day (0.0125 µg/mm², males) for 5 consecutive days; 1.0 µg/kg/day (0.025 µg/mm², females) for 3 or 5 consecutive days	Following 13 weeks of monthly dermal administration of PEP005 at doses up to 0.05 µg/mm2 for 5 days and 0.075 µg/mm2 for 3 days (concentrations of 0.01% and 0.05%) produced acanthosis at all sites and erosion/ulceration at some sites with the highest incidence and severity at Site 3 (0.05 µg/mm2 for 5 days) was observed. The observed severity of the dermal reactions and histopathological changes, was greater in males than females and was increased at doses of > 0.05 ug/mm2. The dermal observations decreased in severity with repeated dosing over the 4 dosing cycles, indicating tolerance with repeat cycling. At 8 weeks post treatment no residual PEP005 treatment-related histopathological changes were noted at any dose or duration of treatment (3 or 5

Species / Duration Study ID GLP	Method of Administration Doses (µg/kg/day)	NOAEL (mg/kg/d)	Major findings
509987			days). Consequently, based on the dermal responses seen in the first two cycles and the clear evidence of tolerance with repeat cycling as well as ongoing recovery in the 8 week observation period, a dose at 0.0125 µg/mm2 for 5 days in males and 0.025 µg/mm2 for 3 or 5 days in females were considered the NOAEL in this study.
Mini-pig/Göttingen ApS (41 weeks) Duration of Dosing: 3 consecutive day cycle (total of 11 dosing cycles) Duration of Postdose: 25 day inter-cycle, followed by an 8-week recovery period 509992	Topical 0, 4, 8, 12	4.0 µg/kg/day (0.025 µg/mm²/day, males); 8.0 µg/kg/day (0.05 µg/mm²/day, females)	The dermal administration of PEP005 at doses up to 0.075 µg/mm2/day for 3 consecutive days (concentrations of 0.01% to 0.03%) to mini pigs produced ulcerative dermatitis at sites at all doses. The intensity and severity of the dermal reactions, which was confirmed histopathologically, were greater in males treated at >0.025 µg/mm2/day PEP005 gel than in females treated at >0.05 µg/mm2/day. The dermal observations generally decreased in severity with repeated dosing over the 11 dosing cycles, and dosing was completed at an increased total number of sites indicating increased tolerance with repeat cycling. At 8 weeks post treatment, no residual PEP005 gel treatment-related histopathological changes were noted at any dose and normal skin architecture was observed histopathologically.

Intravenous toxicity

The toxicity of repeat IV (bolus) administration of ingenol mebutate was evaluated for 7 days in mice, for up to 6 months in rats and for 28 days in mini-pigs. Four infusion studies (two in rats and two in dogs) were conducted to support other indications.

In mice dosed for 7 consecutive days, one animal receiving 60 µg/kg/day and all animals receiving ≥80 µg/kg/day were killed prematurely after ≤4 days of dosing because of the severity of physical signs, which were dose-related. Treatment-related effects observed at ≥30 µg/kg/day were piloerection, subdued behaviour, hunched appearance, irregular respiration; at ≥60 µg/kg/day, unkempt appearance, prostration, skin cold to the touch, slow respiration and partially closed eyes; at ≥80 µg/kg/day, body tremors; and at 100 µg/kg/day, dark extremities, discoloured skin and bulging eyes. There were no treatment-related effects on body weight, food consumption, or macroscopic or microscopic findings. The NOAEL for IV repeat dose ingenol mebutate was 30 µg/kg/day in mice.

In rats, no deaths, or macroscopic or microscopic findings of systemic toxicity were observed following 28 days repeat IV dosing at ≤15 µg/kg/day. Treatment-related effects included transient tachypnoea, which was not dose-related, lethargy and/or subdued behaviour and decreased food consumption at doses ≥5 µg/kg/day. A NOAEL of 7.5 µg/kg/day was identified.

IV administration of ingenol mebutate for six-months at doses ≥1.5 µg/kg/day twice weekly or at 15 µg/kg/day once weekly was associated with laboured/rapid respiration, lethargy, and premature death related to pulmonary vessel thrombosis. Reversible minor effects on body weight gain and food consumption were noted in rats given ≥7.5 µg/kg/day ingenol mebutate. Renal tubular adenoma (n = 2), and pituitary adenoma (n = 1) were observed in rats given 15 µg/kg/day ingenol mebutate twice weekly. Hyperplasia of the renal tubules was also seen in the animals with renal adenomas, and hyperplasia of the pituitary was seen in one male at 7.5 µg/kg/day and in 1, 5, 2, 0 and 1 female at 0, 1.5, 7.5, 15 twice per week and 15 µg/kg/day once per week. However, pituitary tumours are common in laboratory animals and the incidences were not dose-related. At recovery necropsy, a

thyroid carcinoma (n = 1) was observed in one male at 15 µg/kg/day twice per week. The NOAEL for bolus IV administration of ingenol mebutate was <1.5 µg/kg/day in rats.

In mini-pigs, no deaths occurred at 5 µg/kg/day ingenol mebutate for 4 consecutive days or ≤3 µg/kg/day for 28 days. Treatment-related effects were limited to sporadic and transient subdued behaviour, emesis and slightly reduced body weight gain post-dose at ≥2.5 µg/kg/day. There were no toxicologically significant macroscopic or microscopic findings. The NOAEL was 3 µg/kg/day.

Genotoxicity

Ingenol mebutate was evaluated for potential genotoxicity *in vitro* and *in vivo* studies. The design and results of the genotoxicity studies are summarized in Table 9.

Table 9. Overview of Genotoxicity studies performed with Ingenol Mebutate

Type of test / study ID / GLP status	Test system	Concentrations / Metabolising system	Results Positive / Negative / Equivocal
In Vitro			
Reverse mutation in bacterial cells 2174-001/GLP	Salmonella typhimurium	0.32, 1.6, 8, 40, 200, 1000 (µg/plate)	Negative
Mutation at the tk locus of mouse lymphoma cells 2174-002 / GLP	L5178Y	Experiment 1 S9- : 5, 10, 20, 30, 35, 40, 45,50 µg/mL S9+ : 10, 20, 30, 40, 50, 60, 70, 80 µg/mL Experiment 2 S9- : 25, 32.5, 40, 45, 50, 55, 60 µg/mL S9+ : 30, 40, 50, 60, 70, 80, 90 µg/mL	Negative
Morphological transformation of SHE cells AB34NU.310.BTL/ GLP	Syrian Hamster Embryo Cells	0.05, 0.1, 0.25, 0.50, 1.0, 2.5, 5.0 µg/mL	Positive
In Vivo			
Bone marrow micronuclei 2174-007/GLP	Rats/Wistar Han	5, 10, 20 mg/kg	Negative

Carcinogenicity

No studies were submitted (see discussion on non-clinical aspects).

Reproduction Toxicity

The reproductive toxicology studies performed with ingenol mebutate are presented in Table 10.

Table 10. Summary of Main Reproductive Toxicology Studies

Study type/ Study ID / GLP	Species; Number Female/ group	Route & dose (µg/kg)	Dosing period	Major findings	NOAEL (µg/kg & AUC)
Embryo-foetal development / Report 494300 / GLP	Rat / 20	Intravenous 0, 1.5, 3 or 5 at ~3 ml/min.	Days 6-16	One female died at 5 µg/kg. No other findings.	F0: 3 F1: 5 No toxicokinetics

Embryo-foetal development / Report 494321 / GLP	Rabbit / 20	Intravenous 0, 1, 2 or 4 at ~2 ml/min	Days 6 - 18	Increased breathing rate in females. Slight (non-significant) increase in foetal death at 4 µg/kg. Slightly reduced ossification at 2 and 4 µg/kg. Possible effect on blood vessels at 4 µg/kg. Increased incidence of fusion of the jugal to the zygomatic process of the maxilla at 4 µg/kg Increased incidence of 7th costal cartilage not articulating with sternum at all doses.	F0: <1 F1: 1. AUC not calculable; Cmax 0.233 ng/ml Toxicokinetics from preliminary study

Toxicokinetic data

Local Tolerance

No studies were submitted (see discussion on non-clinical aspects).

Other toxicity studies

Four main studies and one pilot study have been conducted specifically to qualify the impurities found in the drug product. A summary of these studies is presented in Table 11.

Table 11: Summary of studies about impurities

Species/Strain **Study Number**	**Method of Administration** **Duration of Dosing** **Doses**	**Noteworthy Findings**
Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* 8224378	*In vitro* Exp 1: 0, 1.6-5000 µg/plate Exp 2: 0, 15.81-5000 µg/plate	Cytotoxic Effects: Experiment 1: ≥1000 µg/plate, with or without S9 in strains TA1537 and TA102 only. Experiment 2: ≥1581 µg/plate, with or without S9 in all strains. Genotoxic Effects: None.
Mouse Lymphoma Study 8224381	*In vitro* Exp 1: 0, 10-75 µg/mL (w/o S9); 0, 10-100 µg/mL (with S9) Exp 2: 0, 30-100 µg/mL (w/o S9); 20-150 µg/mL (with S9)	Cytotoxic Effects: Experiment 1: Relative total growth reduced to 12% at 75 µg/mL for 3 hour treatments without S 9 or 24% at 100 µg/mL for 3 hour treatments with S-9. Experiment 2: Relative total growth reduced to 31% at 100 µg/mL for 24 hour treatments without S-9 or 2% at 150 µg/mL for 3 hour treatments with S 9. Genotoxic Effects: None.
7-Day Pilot IV Rat Study 8224379	IV 15 µg/kg/day	Isolated incidents of convulsions. Post dosing observations included decreased activity, piloerection, dyspnoea and blue colouration to the tail.
28-Day IV Rat Study 8224382	IV 0, 1.5, 7.5, 15 µg/kg/day	Isolated incidents of convulsions at the mid and high dose group. Post dosing observations included decreased activity, piloerection, dyspnoea and blue colouration to the tail. Changes to urine parameters were evident in a dose related manner along with a decrease in prostate weight. Microscopically there were changes at the tail vein injection site suggestive of minor irritation. Based on the clinical observations, the NOAEL is considered to be 1.5 µg/kg/day

3-Day Dermal Mini-pig Study 516774	Topical 0 and 0.05 µg/mm²; 0 and 500 µg/day	Ulcerative dermatitis, epidermal hyperplasia and scabbing at the dosed sites. At 28 days post treatment, scab formation or crusting was observed at a few PEP005 treated sites indicative of an ongoing recovery of the skin.

Ingenol mebutate was evaluated in a local lymph node assay in order to assess its potential for induction of an immunologically-mediated, delayed-onset hypersensitivity following topical application to CBA/CaOla (CBA/Ca) Hsd mice. The summary of the antigenicity study is presented in Table 12.

Table 12: Summary of study on antigenicity

Species/Strain **Study Number**	**Method of Administration** **Duration of Dosing** **Doses**	**Noteworthy Findings**
Mouse/ CBA/CaOlaHsd 2174-031	**Topical** 3 days **Preliminary Local Tolerance Phase:** 0.01%, 0.05%, 0.1% (v/v) in aqueous gel (w/w 30% isopropyl alcohol, 1.5% hydroxyethyl cellulose, 0.9% benzyl alcohol) **Main Study Phase:** Vehicle (0), 0.0025% (1.25 µg), 0.005% (2.5 µg), 0.01% (5 µg)a	**0.01%:** Slight oedema and discolouration after 3 applications **0.05% and 0.1%:** Severe irritation after 2 applications **Vehicle:** Mean DPM 59 **0.0025%:** Mean DPM 1846, SI 31 **0.005%:** Mean DPM 2497, SI 42 **0.01%:** Mean DPM 3526, SI 60 **α-Hexylcinnamaldehyde 25%:** Mean DPM 3957, SI 67

2.3.5. Ecotoxicity/environmental risk assessment

Results of submitted studies to evaluate the environmental risk from ingenol mebutate are summarised in table 13.

Table 13. Summary of main study results

Substance (INN/Invented Name):Picato/Ingenol mebutate			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	**OECD122**	**4.1**	**Not > 4.5** ; not PBT
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}		
	BCF		
Persistence	DT50 or ready biodegradability		
Toxicity	NOEC or CMR		
PBT-statement :	The compound is not considered as PBT nor vPvB The compound is considered as vPvB The compound is considered as PBT		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.0006 µg/l with maximum daily dose of PEP005 Gel equal to 250 mg	µg/l	**0.0006 µg/l < 0.01 µg/l.**
Other concerns (e.g. chemical class)			

2.3.6. Discussion and conclusion on the non-clinical aspects

Ingenol mebutate has multiple effects and inhibits tumour growth or induces tumour-cell death depending on the concentration and the type of cells. At high concentrations *in vitro* (e.g., 100 µg/mL), it appears to exert its cytotoxic effects via protein kinase C (PKC)-independent necrosis; at low concentrations (e.g., 10 ng/mL; 10,000-fold lower concentration), it stimulates PKC-dependent apoptosis or senescence. *In vivo*, dose-dependent inhibition of tumour growth was observed after topical dosing of 6 to 54 µg once daily for 3 days. Topical ingenol mebutate rapidly induces tumour cell death and an innate immune response, causing shrinkage of locally affected tumours, then inducing a tumour cell-specific immune response, characterised by infiltration of neutrophils and other immunocompetent cells. Ingenol mebutate administered intratumourally has also been shown to stimulate an acquired immune response through tumour-specific CD8+ T cell induction, resulting in further anti-tumour activity. Topical field treatment with 0.05% PEP005 of SKH1/hr mice with photo-damaged skin resulted in significantly fewer p53+ patches/cm^2, reduction of the UV induced-epidermal thickening and a reduction in cutaneous mast cells.

Ingenol mebutate has been shown to activate PKC. Activation of the various types of PKC can be either pro-tumourigenic or anti-tumourigenic depending on the isoform. The pattern of activation induced by ingenol mebutate is different from that of the known phorbol ester tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) and this has been used as an argument in support of the lower potential for tumour induction on the part of ingenol mebutate. Literature data were submitted in support of the view that ingenol mebutate is unlikely to be a tumour promoter (Kedei *et al*, 2004, Adolf W *et al*, 1983). *In vivo*, it was shown that ingenol mebutate did not promote tumours induced by 7,12-dimethylbenz (a) anthracene (DMBA) unlike TPA. The property of promotion was also shown to be linked to the length of the carbon chain. Esters with six carbon atoms or fewer were concluded to be very weak to weak promoters. Ingenol mebutate has four carbon atoms and hence falls into the category of very weak to weak promoters.

The applicant has conducted a large number of pharmacology studies in a wide range of tumour cell lines and tumour types. The studies are considered to support the clinical indication. The mechanism of action of ingenol mebutate is still being investigated however the data indicate that it has a novel dual mechanism of action (Ogbourne et al, 2004, Challacombe et al, 2006) that results in the rapid induction of cellular cytotoxicity, followed by immune cell-mediated cell death. It has been proposed that with a short course of therapy, this dual mechanism of action results in both a rapid and durable cure of tumours (Ogbourne et al, 2007). Several experimental techniques, including electron microscopy, flow cytometry and chromium (Cr) release, were used to determine the precise nature of cell death induced *in vitro*. Further studies on the mechanism of action are in progress and the interim analyses of these studies are discussed under primary and secondary clinical pharmacology.

Ingenol mebutate did not inhibit activity of the I_{Kr} ion channel in vitro or adversely affect QT intervals or electrocardiogram waveform morphology in vivo in dogs at doses of 5 µg/mL and 15 µg/kg, respectively. Mild haemodynamic effects (≥5 µg/kg) and increased respiration and minute volume (≥7.5 µg/kg) were observed in dogs following a single IV administration. No significant effects on central nervous system observations were noted in rats following IV administration of ≤10 µg/kg.

Since the intended use of ingenol mebutate is topical and systemic drug absorption after topical administration was not quantifiable except at high doses, no pharmacodynamic drug interaction studies were performed with ingenol mebutate. In addition, the intended short duration of administration (2 to 3 days) would be expected to minimise the potential for drug interactions.

The toxicity seen with dermal and intravenous dosing differed. The findings in the cutaneously dosed toxicity studies do not raise any concerns for clinical dosing. No target organs were identified other

than the skin and there was no evidence of squamous metaplasia. The dermally dosed studies obviated the need for any specific local tolerance studies.

Toxicity was found in studies conducted via the intravenous route to support other indications. Given the low cutaneous absorption, it is not clear how relevant the findings from the intravenous studies might be, notably the occurrence of tumours, particularly a thyroid carcinoma in one animal in the 6-month toxicity study in rats. However it is unlikely that the occurrence of tumours is relevant for clinical dosing for the following reasons: the intravenous route is not directly relevant to dermal dosing/ the dose at which the tumours occurred was in the lethal range/ the proposed clinical regimen is short/ the human exposure at clinical doses is minimal/the margin over human exposure at which the thyroid carcinoma occurred is of the order of 28,000 – 55,000; (the ICH guidance notes that findings occurring at >25 times the human exposure are unlikely to be relevant)/the no-effect dose for thyroid carcinoma was 7.5 µg/kg, suggesting that there is a threshold for the carcinogenicity/Ingenol mebutate was not genotoxic/there were no neoplastic or preneoplastic lesions in the 41-week dermal study in mini-pigs.

A proposal to include surveillance for AK to progress to SCC has been included in the Risk Management Plan, obviating the need for non-clinical carcinogenicity studies.

Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, repeated-dose toxicity and genotoxicity.

The non-clinical safety studies demonstrate that cutaneous administration of ingenol mebutate gel is well tolerated with any skin irritation being reversible and a negligible risk of systemic toxicity under the recommended conditions of use.

There are no data from the use of ingenol mebutate in pregnant women. Animal studies showed slight embryo-foetal toxicity. Risks to humans receiving cutaneous treatment with ingenol mebutate are considered unlikely as Picato is not absorbed systemically. As a precautionary measure, it is preferable to avoid the use of Picato during pregnancy.

No effects on the breastfed newborn/infant are anticipated as Picato is not absorbed systemically. The nursing mother should be instructed that the newborn/infant avoid physical contact with the treated area for a period of 6 hours after application of Picato.

No fertility studies have been performed with ingenol mebutate. The absence of fertility and pre-and post-natal developmental toxicity studies is acceptable.

The Applicant has submitted a phase I assessment. The $PEC_{surfacewater}$ was estimated at 0.0006 µg/l, considerably below the threshold to require further investigation. The data indicate that there is no potential for bioaccumulation of the acyl isomers. The medicinal product is unlikely to represent a risk for the environment following its prescribed usage in patients".

2.4. Clinical aspects

2.4.1. Introduction

The clinical documentation submitted in support of this application comprises data from 25 clinical studies. Of these, 18 were conducted in patients with AK. The remaining 7 studies contributed data to the safety profile of PEP005 Gel.

The applicant received scientific advice from the CHMP. The clinical advice related to the clinical development plans regarding long-term follow up and comparator studies.

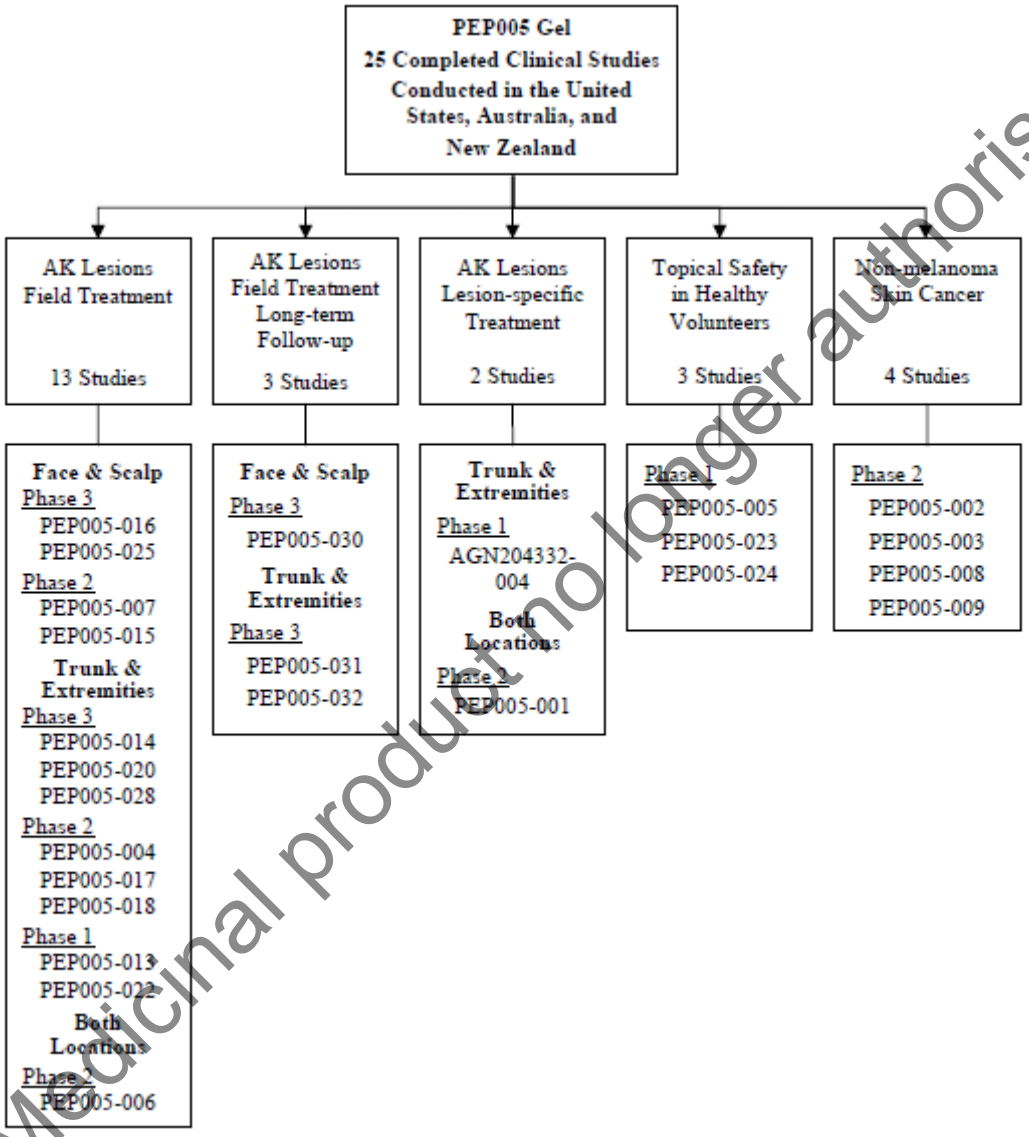
GCP

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

An overview of the clinical studies is provided below

- Tabular overview of clinical studies

Table 14: Clinical studies with ingenol mebutate



2.4.2. Pharmacokinetics

Pharmacokinetic samples were collected from a total of 32 patients enrolled in four different clinical studies: AGN 204332-004, PEP005-004, PEP005-013 and PEP005-017 (Table 15).

Table 15. 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.

The results of the PK analysis in all the above studies showed no detectable systemic absorption. Levels of ingenol mebutate and its acyl isomers PEP015 and PEP025 were below the LLOQ (0.1 ng/mL) in all samples collected.

Absorption

No measurable levels of ingenol or its isomers PEP015 and PEP025 were seen in any of the PK plasma samples in the clinical studies. This shows that any systemic absorption is below measurable limits of 0.1ng/ml.

Distribution

No clinical distribution/metabolism studies were submitted

Elimination

No relevant studies were submitted

Dose proportionality and time dependencies

No relevant studies were submitted

Special populations

No relevant studies were submitted

Pharmacokinetic interaction studies

No relevant studies were submitted

Pharmacokinetics using human biomaterials

No relevant studies were submitted

2.4.3. Pharmacodynamics

Mechanism of action

The mode of action of PEP005 has been established based on pre-clinical models using cell lines and animal models.

Primary and Secondary pharmacology

The applicant has submitted a number of pre-clinical pharmacology studies as described in table 16.

Table 16. Number of Pharmacology Studies by Study Type

		Route of Administration (In Vivo Studies Only)a		
Type of Study	**Number of Studies**	**Topical**	**IV/IP**	**Intralesional/SC**
Primary Pharmacodynamics				
In Vitro	60b	NAP	NAP	NAP
In Vivo	31b	25	0	8
Secondary Pharmacodynamics				
In Vitro	35b	NAP	NAP	NAP
In Vivo	14b	9	4	3
Safety Pharmacology				
In Vitro	1	NAP	NAP	NAP
In Vivo	3	0	3	0
Pharmacodynamic Drug Interactions	NAP	NAP	NAP	NAP

IP = Intraperitoneal; IV = Intravenous; NAP = Not applicable; SC = Subcutaneous.
a A study could have evaluated more than one route of administration.
b One study in primary pharmacodynamics and one study in secondary pharmacodynamics reported both in vitro and in vivo data.
c Two additional studies used other routes of administration (bladder instillation: one study; systemic: one study).

The key pharmacological and pharmacodynamic actions of ingenol mebutate as gathered from the above studies can be summarised as follows:

- inhibition the growth of various tumour cell lines in vitro, including lines derived from human melanomas
- shrinkage and removal of implanted tumours in in vivo mouse models
- induction of necrosis of tumour cells
- activation of classical Protein Kinase C isoforms, but not atypical isoforms, unlike TPA

- weak tumour promotion
- induction of pro-inflammatory cytokines
- extravasation of neutrophils
- induction of antibody-dependent cellular cytotoxicity
- production of reactive oxygen species
- production of tumour-specific CD8+ T cells

The applicant has provided the interim results of two clinical studies designed to assess the biological effects of PEP005 Gel, 0.05% in diseased and normal skin.

Study LP0041-02 focused on biological effects (necrosis and inflammation) in AK following treatment with PEP005 Gel, 0.05% administered for 2 consecutive days assessed by histology. The histological assessments were made at baseline (Day 1) and at Days 2 and3. The interim report included data from the first 8 patients out of the planned total of 27 patients.

The pharmacodynamics and safety conclusions from the interim report were:

• The degree of skin infiltration by leukocytes increased in epidermis and dermis of both normal skin and AK lesions after treatment and tended to be more severe in AK lesions than in normal skin.

• The degree of necrosis increased in epidermis of normal skin and AK lesions after treatment and tended to be more severe in AK lesions than in normal skin. Necrosis was not observed in the dermis.

• Haemorrhage (described as discrete extravasation of erythrocytes) was present in the epidermis of few and in the dermis of most normal skin and all AK lesion biopsies after treatment.

• At baseline, the dermis of the AK lesions was infiltrated by leukocytes and immune cells, notably T lymphocytes but also CD68 positive macrophages and CD1a positive antigen presenting cells and to a less extent B lymphocytes were evident.

• Treatment with ingenol mebutate induced immune response in both normal skin and AK lesions. The reaction in AK lesions tended to be stronger especially for infiltration by T and B lymphocytes.

• In the epidermis the immune response was dominated by neutrophils and in the dermis by CD4 positive T lymphocytes and CD68 positive macrophages and to a less extent CD8+ T lymphocytes and neutrophils. A slight increase in CD20 positive B lymphocytes and mast cells was also observed in the dermis.

• Normal skin showed activation of the vascular endothelium after treatment with ingenol mebutate, whereas vascular endothelium was already activated at baseline in AK skin and did not become further activated after ingenol mebutate treatment.

• The mRNA transcriptomics data indicated that the main responses to ingenol mebutate treatment in both normal skin and AK lesions were inflammatory response and response to wounding.

• The 8 subjects enrolled in the trial so far, reported 5 AEs, all non-serious. None led to withdrawal of treatment.

• Local skin responses were dominated by erythema and to a lesser extent swelling and vesiculation/pustulation. The skin reactions peaked at Day 3 and were almost gone at Day 29.

Study LP0041-03 focused on biological effects in AK following treatment with PEP005 Gel, 0.05% administered for 2 consecutive days assessed by reflectance confocal microscopy (RCM). The interim report included data from the first 8 patients out of the planned total of 24 patients.

The pharmacodynamics and safety conclusions from the interim report were:

• In the epidermis, inflammatory response and necrosis were observed in both normal skin treatment area and in the AK skin treatment area peaking at Day 3. Compared to clinical AK lesions the infiltration and necrosis in normal skin were milder and had a quicker recovery and the subclinical lesions showed an intermediate response.

• Pigmentation and fibrosis were unchanged at the end of the trial as compared to baseline. Solar elastosis remained unchanged for normal skin but decreased for both the clinical and subclinical AK lesions.

• Clinical assessment showed that 6 of 16 AK lesions were cleared at the end of the trial. The assessment of clearance was similar whether it was done clinically or by RCM; only one of 16 lesions was assessed differently by the two methods. At the end of the trial, 5 clinical and 11 sub clinical lesions showed typical honeycomb pattern and were hence completely cleared.

• Microvesicles, dyskeratotic cells and cells with dendritic shape reverted towards baseline levels at Day 57. Spongiosis was still present in the majority of AK spots.

• RCM and histological assessments of necrosis and the severity of inflammation were either identical or assessed as being more severe by RCM.

2.4.4. Discussion and conclusions on clinical pharmacology

Overall blood samples from 32 patients from 4 clinical studies in AK patients were analysed for levels of ingenol mebutate or its two isomers PEP015 and PEP025. Of these 25 were dosed with PEP005 gel (concentrations ranging from 0.01% for 1 day to 0.05% for 2 days) and 7 were dosed with vehicle. The treatment areas in some of the patients (n=16) were 100 cm^2 and a dose of 0.05% gel was applied for 2 days. 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 of 0.1 ng/ml).

The areas of application of the PEP005 gel in all these studies have been in the extremities. There is no information on levels of systemic absorption when the gel is applied to the facial skin and near mucous membranes (around lips), which is proposed in the intended clinical indication. The proposed dose on face and extremities are different, suggesting different tolerability and dermal absorption probably based on keratinisation. The applicant stated that as the calculated difference between actual systemic exposure and the required systemic exposure for measuring systemic levels is so large that minor increase in systemic absorption due to occlusion or due to application on face is unlikely to produce an increase in levels for which clinical studies would be warranted. This position is accepted and the CHMP concluded that further PK studies would not provide any useful information as long as the assay sensitivity is around 0.1ng/ml.

There is no knowledge of systemic absorption when the applied area is occluded or when the skin is damaged or has open-wounds. In normal clinical use, it is foreseeable that the gel should not be applied in such circumstances. The SmPC has been updated to reflect this.

No clinical distribution/metabolism studies have been performed to date because ingenol mebutate shows no systemic absorption when administered topically.

No clinical data on Dose proportionality and time dependencies is available and further clinical studies are not required because of negligible systemic exposure.

No population subgroup analyses have been performed for the purpose of PK or product metabolism analyses in humans. Such studies would not be relevant as no systemic levels of ingenol mebutate or its two isomers PEP015 and PEP025 have been quantifiable in the clinical studies evaluating PK till date.

No interaction studies have been performed. Interactions with systemically absorbed medicinal products are considered minimal as Picato is not absorbed systemically.

The need for human data on systemic secondary pharmacology of ingenol mebuate is considered limited as negligible systemic exposure has been demonstrated on topical application at the proposed doses.

The mechanism of action of ingenol mebutate in AK is not completely known. The two main mechanisms that have been proposed based on pre-clinical studies include 1) induction of local lesion cell death and 2) promoting an inflammatory response characterised by infiltration of neutrophils and other immunocompetent cells.

The two effects have been shown to be dependent on the topical concentrations of ingenol mebutate. A higher concentration of ingenol mebutate is achieved in the epidermis where the predominant action is induction of lesional cell death. In the dermis however, the concentrations of ingenol mebutate is lower and there is no cell death and only infiltration by inflammatory cells have been shown both in histological assessments and RCM assessment. Further these studies showed that the pathological skin is affected by these two mechanisms more than the normal skin, although normal skin is also involved. These findings explain the observations in the phase III studies where it is noted that there is a high degree of local skin reactions, which however resolve completely without any scarring. These observations further provide reassurance that the effects seen in clinical assessment of clearance of AK lesions are due to clearance of underlying pathology and not only due to a cosmetic superficial clearance. The interim results from the two clinical studies evaluating the biological effects of ingenol mebutate in AK patients are in line with a significant number of pre-clinical studies that have been conducted evaluating the dermal effects of ingenol mebutate.

2.5. Clinical efficacy

There are 6 clinical studies in AK patients which evaluate dose-response and 4 phase III studies which are the main pivotal studies.

2.5.1. Dose response studies

PEP005-001, PEP005-004, PEP005-007, PEP005-022, PEP005-006 and PEP005-015 are studies that evaluated different aspects of dose-response. Of these studies, PEP005-007 and PEP005-015 were in patients with AK lesions of head (face and scalp), while PEP005-022 and PEP005-004 were in patients with AK lesions of non-head locations (trunk and extremities). PEP005-001 and PEP005-006 were in patients with AK lesions of both head and non-head locations.

The main study for choosing the dose for phase III studies for treatment of AK lesions on head (face and scalp) location was PEP005-015 and on non-head (trunk and extremities) was PEP005-006.

The dose regimen at which **Study PEP005-007 (head)** was started, 0.025% for 2 days, was determined as the MTD for treatment of AK lesions on the face, as the next higher dose regimen of 0.025% for 3 days had two DLTs of severe application site oedema/vesiculo oedematous reaction and pustulation and swelling of the treatment area . Other dose regimens tested in this study included 0.025%, 0.0175% and 0.0125% applied for 2 consecutive days and 0.025%, 0.0175%, 0.0125%, 0.0075%, 0.0050%, and 0.0025% applied for 3 consecutive days.

Study PEP005-015 (head) used only one dose, 0.005% from the Study PEP005-007 and selected two intermediate doses 0.010% and 0.015% for the remaining two doses. Both the 2 days and 3 days frequency of application was evaluated with all three doses. Incidence of treatment related adverse events and compliance in the 3 days dosing regimen was dose-dependent in that higher doses were less compliant and had more treatment related adverse events. Complete clearance was dose-dependent in the 2 days dosing regimen. However the complete clearance in the 3 days dosing regimen for the 0.01% group was 18% compared to 33% for the 0.005% group (lower dose) and 50% for the 0.015% (higher dose) group. Therefore the dose of 0.015% for 3 days has been selected for further phase III development based on the best efficacy response obtained for any dose regimen in this study.

The doses investigated in **Study PEP005-004 (non-head)** were PEP005 Gel 0.01%, 0.025%, 0.05% or 0.075% once daily for 2 days. The treatment application in this study was lesion-specific and applied to an area of 9 cm^2 around the lesion. The dose of 0.05% PEP005 gel for 2 days was declared the MTD in treatment of AK on non-head locations. The next higher dose of 0.075% was the dose that had DLTs and therefore by default the next lower dose of 0.05% for 2 days was considered MTD.

Study PEP005-022 (non-head) used the proposed dose for non-head areas (0.05%). This study demonstrated that increasing the area of application of the PEP005 gel did not have an increase in the incidence of adverse events or on drop-out.

The doses investigated in **Study PEP005-001 (both head and non-head)** were 0.0025%, 0.01% and 0.05% administered on days 1 and 2 or days 1 and 8. Patients were randomised to one of two treatment schedules, Day 1 and Day 2 (Arm A) or Day 1 and Day 8 (Arm B) and within Arm A or B to one of three PEP005 Gel concentrations (0.0025%, 0.01%, and 0.05%). All patients were scheduled to receive two doses of study medication, on Days 1 and 2 in Arm A and on Days 1 and 8 in Arm B. All patients received both doses. A statistically significant difference in the percentage of patients who had complete clearance of ≥ 80% of AK lesions was observed when all treatment groups were compared ($p = 0.0082$). A statistically significant difference was also observed for the percentage of patients in the 0.05% PEP005 Gel group who had complete clearance of ≥ 80% of AK lesions when compared to vehicle gel ($p = 0.0185$).

Three dosing regimens (0.025% for 3 days, 0.05% for 2 days and 0.05% for 3 days) were evaluated in **study PEP005-006 (both head and non-head).** This study provided the primary basis for dosage selection for the non-head pivotal phase III studies.

The efficacy results, adverse events and dose-compliance were all seen to be dose-dependent. From lowest to highest PEP005 Gel concentration, complete clearance rates were 32%, 45.2 %, and 46.2 %, respectively, compared with 14% for vehicle gel ($p = 0.002$ for the two PEP005 Gel, 0.05% groups vs. vehicle gel). There was no significant difference between the 2 days and 3 days regimens. Partial clearance rates were 54%, 64%, and 74%, respectively, compared with 21% for vehicle gel ($p < 0.001$ for the two PEP005 Gel, 0.05% groups vs. vehicle gel); and the median reductions in the number of AK lesions were 75%, 83%, and 86%, respectively, compared with 0% for vehicle gel. The incidence of treatment related AEs was higher in the PEP005 Gel treatment groups compared with the vehicle gel, with the highest incidence in patients receiving PEP005 Gel, 0.05% for 3 days. Local skin responses showed a similar dose-response relationship across PEP005 Gel dose groups. Patient compliance showed a tendency to be better in the PEP005 Gel, 0.05% 2 days regimen (93%) compared with the PEP005 Gel, 0.05% 3 days regimen (85%).

Based on this study results, the applicant has chosen the dose of 0.05% for 2 days for further development in treatment of AK lesions in non-head locations.

2.5.2. Main studies

There are four main, phase III pivotal studies that are submitted in support of this application. They are PEP005-016, PEP005-025, PEP005-014 and PEP005-028. PEP005-016 and PEP005-025 studied patients with AK lesions in head (face and scalp) locations and the other two studies PEP005-014 and PEP005-028 in patients with AK lesions in non-head (trunks and extremities) locations. All these studies were identical in study-design which was the gold-standard, double-blind, randomised, controlled (vehicle-controlled) trials in support of this application.

PEP005-016 and PEP005-025 were two similar phase III studies that evaluated the efficacy and safety of PEP005 Gel 0.015% in AK patients when administered once daily for three consecutive days to a contiguous 25 sq cm area of the skin on the head (face and scalp).

PEP005-014 and PEP005-028 were two similar phase III studies that evaluated the efficacy and safety of PEP005 Gel 0.05% in AK patients when administered once daily for two consecutive days to a contiguous 25 sq cm area of the skin on non-head (trunk and extremities) locations.

Studies PEP005-016, PEP005-025, PEP005-014 and PEP005-028

PEP005-016 and PEP005-025 were two similar multi-center, randomised, parallel group, double-blind, vehicle-controlled phase III studies designed to evaluate the efficacy and safety of PEP005 Gel 0.015% in AK patients when administered once daily for 3 consecutive days to a contiguous 25 cm^2 area of the skin on the head (face and scalp).

PEP005-014 and PEP005-028 were two similar multi-center, randomised, parallel group, double-blind, vehicle-controlled phase III studies designed to evaluate the efficacy and safety of PEP005 Gel 0.05% in actinic keratosis patients when administered once daily for 2 consecutive days to a contiguous 25 cm^2 area of the skin on non-head locations (trunk and extremities).

Methods

Study Participants

- *Inclusion criteria*

PEP005-016 and PEP005-025

Male or female patients at least 18 years of age with 4 to 8 clinically typical, visible and discrete AK lesions within a contiguous 25 cm^2 treatment area on the head (face or scalp). Female patients had to be of non-child bearing potential or using effective contraception.

PEP005-014 and PEP005-028

Male or female patients at least 18 years of age with 4 to 8 clinically typical, visible, and discrete AK lesions within a contiguous 25 cm^2 treatment area on the trunk and extremities (i.e., non-head locations). Female patients had to be of non-child bearing potential or using effective contraception.

- *Exclusion criteria*

PEP005-016, PEP005-025, PEP005-014 and PEP005-028

Exclusion criteria included atypical AK lesions in the treatment area (e.g. hypertrophic, hyperkeratotic), AK lesions within 5 cm of an incompletely healed wound or within 10 cm of a suspected basal cell carcinoma or squamous cell carcinoma, cutaneous horns, recalcitrant disease – two previous cryosurgeries, previous treatment with PEP005 Gel in the selected treatment area, cryotherapy within 2 cm of the selected treatment area and within 2 weeks prior to screening, systemic cytotoxic agents

or immunosuppressants within 4 weeks prior to screening and 5-FU, imiquimod, diclofenac or photodynamic therapy within 2 cm of the selected treatment area and within 8 weeks prior to screening.

Treatments

PEP005-016 and PEP005-025

PEP005 Gel 0.015% administered once daily for three consecutive days to a contiguous 25 cm^2 area of the skin containing 4 to 8 AK lesions on the head (face and scalp). Study medication was packaged individually for each patient in a study medication kit containing 3 unit-dose tubes. Each unit-dose tube contained PEP005 Gel 0.015% or vehicle gel. Unit-dose tubes had a target fill weight of 0.49gms gel and were designed to deliver 230mg (250µl) gel, ensuring a consistent dose of 10µl/100mm^2. Study medication was applied by the patient at home on days 1, 2 and 3.

Safety assessments were performed were conducted on Days 4, 8, 15, 29 and 57.

PEP005-014 and PEP005-028

PEP005 Gel 0.05% administered once daily for two consecutive days to a contiguous 25 cm^2 area of the skin containing 4 to 8 AK lesions on non-head (trunk and extremities) locations. Study medication (PEP005 Gel, 0.05% or vehicle gel) was packaged individually for each patient in a study medication kit containing 2 unit-dose tubes. Study medication was applied by the patient at home on days 1 and 2.

Safety assessments were performed during study visits on Days 3, 8, 15, 29, and 57 following treatment.

In all studies, efficacy assessments were performed at baseline (Day 1 predose) and Day 57 (end of study). Patients completed the study on Day 57.

Post-study follow-up visits were required every 7 to 28 days for all patients who had unresolved treatment related adverse events (AEs) or local skin responses (LSRs) at Day 57. Patients were to be followed until either resolution or assessed as clinically stable. Patients with unresolved hypopigmentation or hyperpigmentation and/or scarring greater than baseline were required to undergo further post study follow-up every 28 days until resolution or for a period of six months post baseline (an additional four visits) unless deemed clinically insignificant.

Objectives

PEP005-016 and PEP005-025

The objectives of the studies were to evaluate the efficacy and safety of PEP005 Gel, 0.015%, compared to vehicle gel when administered once daily for 3 consecutive days to a contiguous 25 cm^2 area of skin on the head (face or scalp).

PEP005-014 and PEP005-028

The objectives of the studies were to evaluate the efficacy and safety of PEP005 Gel, 0.05% compared to vehicle gel when administered once daily for 2 consecutive days (Days 1 and 2) to a 25 cm^2 contiguous AK treatment area on non-head locations.

Outcomes/endpoints

PEP005-016, PEP005-025, PEP005-014 and PEP005-028

The primary endpoint was the complete clearance rate of AK lesions at the Day 57 visit. A patient with no clinically visible AK lesions in the selected treatment area was defined to have complete clearance.

The secondary endpoint was the partial clearance rate of AK lesions at the Day 57 visit. A patient with a 75% or greater reduction in the number of clinically visible AK lesions identified at baseline, in the selected treatment area was defined to have partial clearance.

The percent change from baseline to Day 57 in the total number of AK lesions was summarised as an additional efficacy endpoint.

Exploratory endpoints were patient-reported outcomes, including the Treatment Satisfaction Questionnaire for Medication (TSQM) at Day 57 and the Skindex-16 Dermatological Survey at baseline and Days 8, 29, and 57.

Safety endpoints were the incidence rate of adverse events (AEs) of serious adverse events (SAEs) and AEs leading to discontinuation of study medication as recorded throughout the study, the incidence rate and grade of local skin responses (LSRs), pigmentation and scarring following study treatment and the results of clinical laboratory tests, vital signs, physical examinations, and electrocardiogram (ECG) findings.

Sample size

PEP005-016 and PEP005-025

The phase IIb study, PEP005-015, demonstrated a complete clearance rate of 50% for PEP005 Gel, 0.015%, compared to a 9% clearance rate for vehicle gel, 57 days following treatment for 3 consecutive days in a study design similar to PEP005-016 and PEP005-025. Thus, statistical power greater than 95% could be achieved in a sample as small as 25 patients per treatment group. The choice of a sample size of 125 patients per group, in these Phase III studies was therefore motivated by the need to treat a sufficient number of individuals in pre-marketing studies to be able to estimate the incidence of common adverse drug events. Enrolment of 125 patients per group in each of two Phase 3 pivotal studies (PEP005-016 and PEP005-025) is consistent with this goal.

PEP005-014

A study population of approximately 250 patients (125 patients per treatment group) was considered sufficient to detect a difference in the complete clearance rate of AK lesions, allowing for at least a 20% difference between the treatment group and vehicle group(40% versus 20%) at Day 57. These rates correspond to an active-to-vehicle odds ratio of clearance rates of 2.67. Based on previous results of clinical studies, this sample size would provide at least 90% power with a two-sided $\alpha = 0.05$ using Fisher's exact test, assuming a 5% drop-out rate.

PEP005-028

Approximately 200 patients (100 per treatment arm) were planned for enrolment. Approximately 200 patients (100 per group) were to be randomised in this study. This sample size was based on comparing the treatment groups in terms of complete clearance rate of AK lesions. It was assumed that a 20% difference between the treatment group and vehicle group (30% vs. 10%) at Day 57 was of clinical interest. This sample size provided at least 90% power with a two-sided significance level of 0.05 (α=0.05) using the Chi-square test for homogeneity of proportions.

Randomisation

PEP005-016 and PEP005-025

Patients were randomised centrally to treatment in a 1:1 ratio through an interactive voice / web response (IVR/IWR) system. Randomisation was stratified by investigational site and by anatomical location of the treatment area (face or scalp). Enrolment was controlled so that approximately 20% of patients were treated on the scalp and approximately 80% of patients were treated on the face.

PEP005-014 and PEP005-028

Patients were randomised in a 1:1 ratio to study medication (PEP005 Gel, 0.05% or vehicle gel) using a central IVR/IWR system. Randomisation was stratified by investigational site and also by anatomical location of the selected treatment area (arm, back of hand, chest and other: back, shoulder, leg). A dynamic randomisation scheme was used in study PEP005-014 to obtain an approximate 1:1 ratio between treatment groups.

Blinding (masking)

All studies were double-blind.

Statistical methods

PEP005-016 and PEP005-025, PEP005-014 and PEP005-028

For the head locations and the non-head locations, the primary efficacy analysis in the Phase III studies compared complete clearance rates across treatment groups (active vs. vehicle) using the Cochran-Mantel-Haenszel (CMH) test statistic. Prior to performing the primary analysis of complete clearance rate, the Breslow-Day test was calculated using a significance level of 0.10 in order to investigate heterogeneity of the odds ratios across analysis sites.

The following populations were defined for analysis:

Intent-to-Treat (ITT) Population: All randomized patients. This is the primary population for efficacy analysis.

Safety Population: All randomized patients who have received at least one dose of study medication and who have had at least one post-baseline safety evaluation.

Per-Protocol (PP) Population: Patients in the ITT population who complete the study without any major protocol deviations.

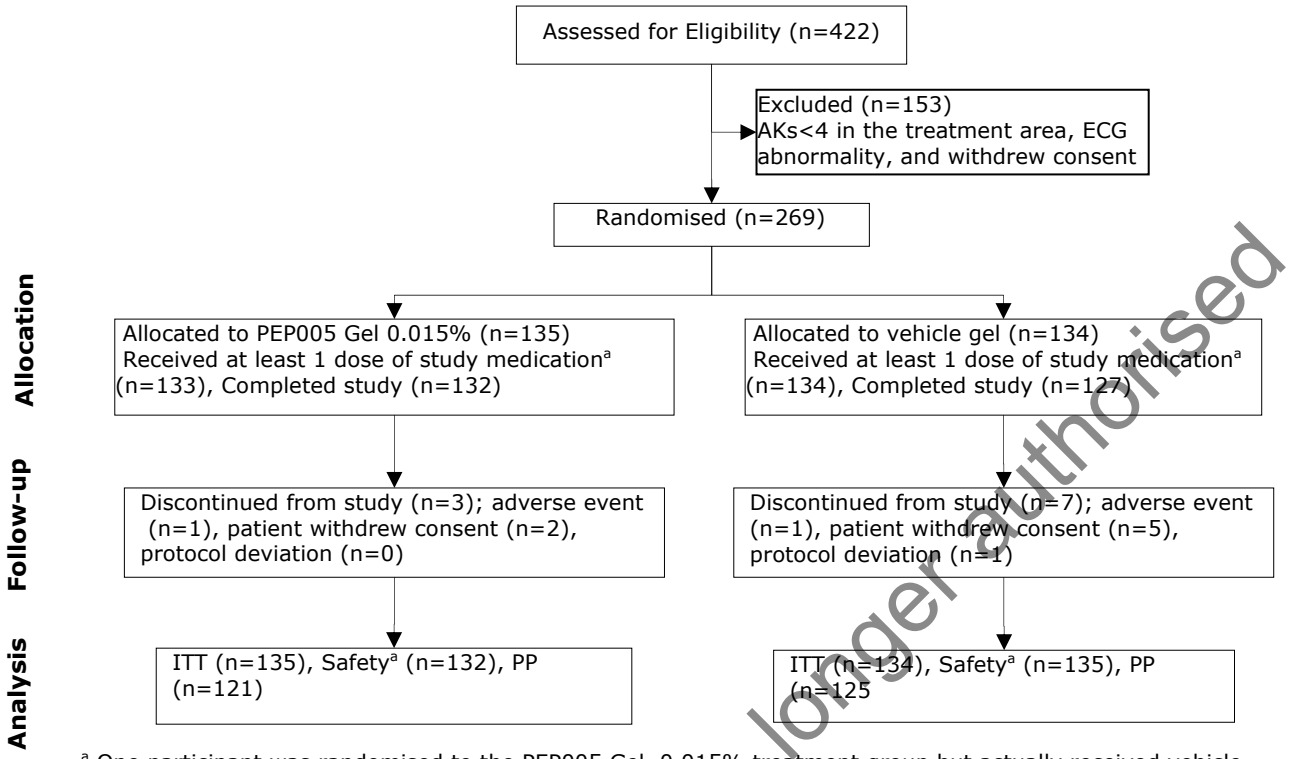
For study PEP005-014 one additional population was defined for analysis:

Evaluable: A subset of the ITT population that included all randomized patients who had AK lesion counts at both baseline (Day 1 predose) and Day 57.

Results

- **PEP005-016**

Participant flow



[a] One participant was randomised to the PEP005 Gel, 0.015% treatment group but actually received vehicle treatment because the site dispensed an incorrect kit and therefore, was included in the vehicle treatment group for all safety analyses.

Recruitment

First patient was randomised the 5th of June 2009 and the last patient completed Day 57 the 10th September 2009.

Conduct of the study

The following protocol deviations were noted as major protocol deviations and could lead to the exclusion of a patient from the PP population:

• Failure to meet all Inclusion/Exclusion criteria

• Usage of restricted medications/treatments, as defined in Section 9.4.7, Prohibited Treatments and Procedures During the Study;

• Failure to present an evaluable endpoint (AK lesion count) within a prespecified visit window of Day 57 (i.e., 50 ≤ study day ≤ 85);

• Noncompliance with the study treatment regimen (i.e., less than three applications of study medication);

• Failure to receive the study medication to which the patient was randomly assigned; or

• Unblinding of patient's treatment assignment at any time during the study.

Baseline data

Baseline demographic characteristics of the patients in PEP005-016 study are presented in Table 17.

Table 17. Demographic and baseline characteristics (study PEP005-016): ITT population

Parameter	PEP005 Gel 0.015% (N = 135)	Vehicle Gel (N = 134)	Total (N = 269)
Age (years)			
N	135	134	269
Mean (SD)	63.5 (10.5)	63.0 (10.0)	63.3 (10.2)
Minimum, Maximum	37, 88	40, 85	37, 88
Age Group [n (%)]			
< 65	71 (52.6)	77 (57.5)	148 (55.0)
≥ 65	64 (47.4)	57 (42.5)	121 (45.0)
Sex [n (%)]			
Male	116 (85.9)	120 (89.6)	236 (87.7)
Female	19 (14.1)	14 (10.4)	33 (12.3)
Race [n (%)]			
White	135 (100)	134 (100)	269 (100)
Fitzpatrick Skin Type [n (%)]			
Burns easily, rarely tans (I)	24 (17.8)	16 (11.9)	40 (14.9)
Burns easily, tans minimally (II)	58 (43.0)	53 (39.6)	111 (41.3)
Burns moderately, tans gradually (III)	44 (32.6)	59 (44.0)	103 (38.3)
Burns minimally, tans well (IV)	9 (6.7)	6 (4.5)	15 (5.6)
Rarely burns, tans profusely (V)	0	0	0
Never burns, deeply pigmented (VI)	0	0	0
Body Mass Index (kg/m^2)[a]			
N	135	134	269
Mean (SD)	28.5 (5.1)	28.0 (4.7)	28.2 (4.9)
Minimum, Maximum	19, 46	19, 45	19, 46

Note: Percentages are based on the total number of patients in each treatment group.

[a] Body Mass Index (BMI) is calculated as weight (kg) / [height (m)]^2.

The disease characteristics at baseline were:

Table 18. Baseline Disease Characteristics (study PEP005-016): ITT population

Parameter	PEP005 Gel 0.015% (N = 135) n (%)	Vehicle Gel (N = 134) n (%)	Total (N = 269) n (%)
Location of Treatment Area			
Face	109 (80.7)	109 (81.3)	218 (81.0)
Scalp	26 (19.3)	25 (18.7)	51 (19.0)
Baseline Lesion Count			
4	27 (20.0)	32 (23.9)	59 (21.9)
5	36 (26.7)	44 (32.8)	80 (29.7)
6	28 (20.7)	35 (26.1)	63 (23.4)
7	27 (20.0)	15 (11.2)	42 (15.6)
8	17 (12.6)	8 (6.0)	25 (9.3)

Numbers analysed

The number and percentage of patients included in each of the analysis populations by treatment group are presented in Table 19.

Table 19. Analysis populations

Population	PEP005 Gel 0.015%	Vehicle Gel	Total
ITT Population	135	134	269
Per Protocol Population[a, b]	121 (89.6%)	125 (93.3%)	246 (91.4%)
Safety Population[c]	132	135	267

Outcomes and estimation

Primary endpoint

The efficacy results in terms of the primary endpoint of complete clearance rate of AK lesions at Day 57 overall and by anatomic location are summarised in table 20.

Table 20. Complete clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-016): ITT population

	PEP005 Gel 0.015% (N = 135)	Vehicle Gel (N = 134)	*P* value
Overall			
Complete Clearance Rate [n/N (%)]	50/135 (37.0)	3/134 (2.2)	<0.001[a]
95% Confidence Interval [b]	28.9, 45.8	0.5, 6.4	
Breslow Day *P* value [c]			0.574
Face			
Complete Clearance Rate [n/N (%)]	46/109 (42.2)	3/109 (2.8)	<0.001[d]
95% Confidence Interval [b]	32.8, 52.0	0.6, 7.8	
Scalp			
Complete Clearance Rate [n/N (%)]	4/26 (15.4)	0/25	0.110[d]
95% Confidence Interval [b]	4.4, 34.9	0.0, 13.7	

[a] *P* values are from Cochran-Mantel-Haenszel test, stratified by analysis site. The *P* values ≤ 0.05 are considered statistically significant.

[b] Confidence intervals are calculated using the exact binomial distribution (Clopper-Pearson).

[c] *P* values ≤ 0.10 are considered statistically significant.

[d] *P* values are from Fisher's Exact test treatment group comparison. The *P* values ≤ 0.05 are considered statistically significant.

Secondary endpoint

The results of the partial clearance at Day 57 are presented in Table 21.

Table 21. Partial clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-016): ITT population

	PEP005 Gel 0.015% (N = 135)	Vehicle Gel (N = 134)	*P* value
Overall			
Partial Clearance Rate [n/N (%)]	81/135 (60.0)	9/134 (6.7)	<0.001[a]
95% Confidence Interval [b]	51.2, 68.3	3.1, 12.4	
Face			
Partial Clearance Rate [n/N (%)]	75/109 (68.8)	8/109 (7.3)	<0.001[c]
95% Confidence Interval [b]	59.2, 77.3	3.2, 14.0	
Scalp			
Partial Clearance Rate [n/N (%)]	6/26 (23.1)	1/25 (4.0)	0.099[c]
95% Confidence Interval [b]	9.0, 43.6	0.1, 20.4	

[a] *P* values are from Cochran-Mantel-Haenszel test, stratified by analysis site. The *P* values ≤ 0.05 are considered statistically significant.

[b] Confidence intervals are calculated using the exact binomial distribution (Clopper-Pearson).

[c] *P* values are from Fisher's Exact test treatment group comparison. The *P* values ≤ 0.05 are considered statistically significant.

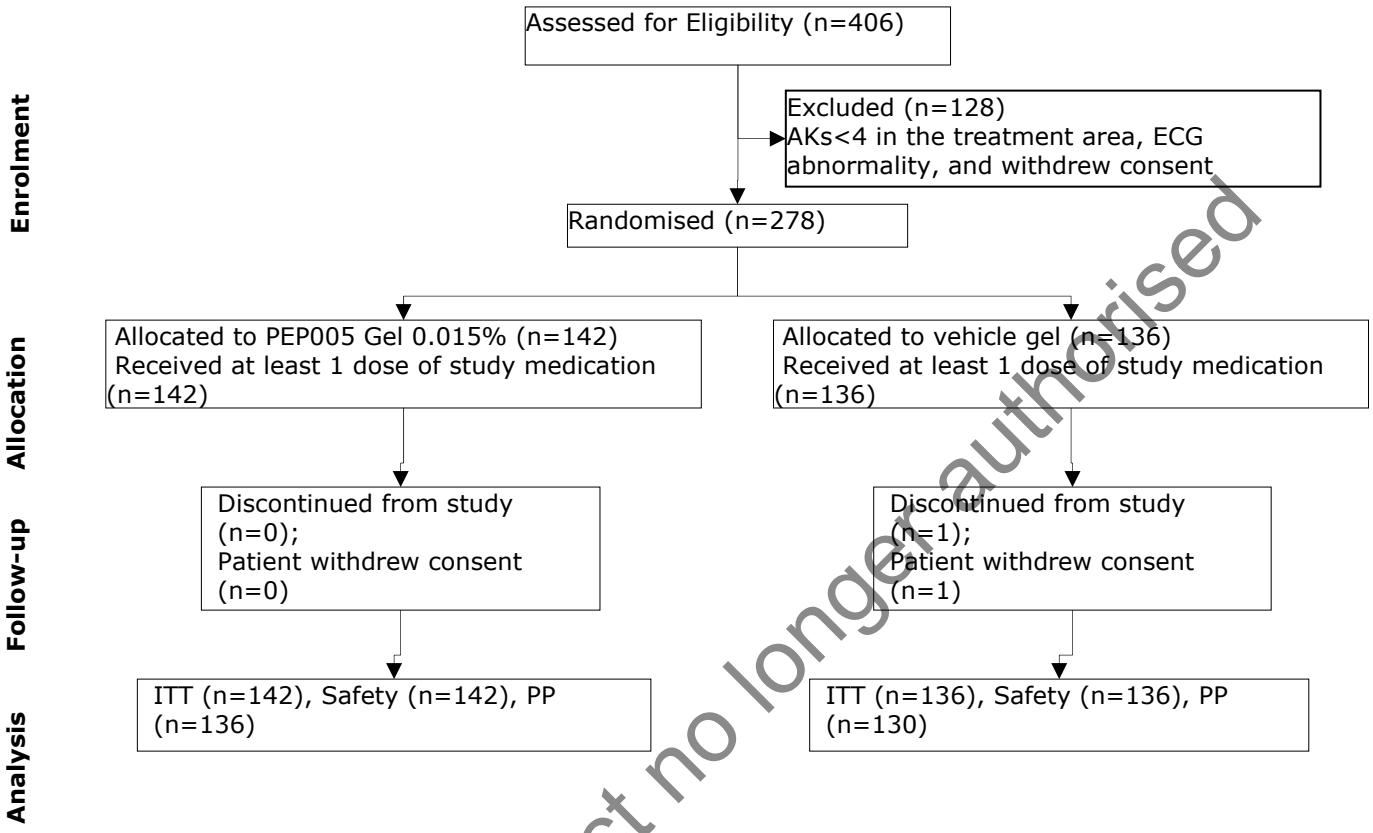
In the subgroup analyses, the complete clearance rate for each treatment group was analyzed by geographic region (US or AUS), gender, age group (<65 or ≥65 years), baseline AK lesion count (4, 5, 6 or 7, 8), skin type (Fitzpatrick I/II or III/IV/V/VI), and analysis site. Findings showed a higher complete clearance rate for PEP005 Gel-treated patients compared to vehicle patients in each category; the only category that did not show statistical significance was the geographic region of Australia (data not shown).

Patient reported outcomes included the TSQM and the Skindex-16 Dermatology Survey. Statistically significant, higher mean patient global satisfaction scores, measured by the TSQM, were seen in the PEP005 Gel group compared to the vehicle gel group ($p < 0.001$). For the Skindex-16 Dermatology Survey, a statistically significant difference was seen with PEP005-treated patients less bothered by each of the three domains (symptoms, emotions, and functioning) compared to vehicle gel; the positive effect was seen at Day 29 ($p < 0.001$, each domain) and continued at Day 57 ($p < 0.001$, each domain) (data not shown).

Ancillary analyses

- **PEP005-025**

Participant flow



Recruitment

First patient was randomised the 5th of June 2009 and the last patient completed Day 57 the 2nd September 2009.

Conduct of the study

The following protocol deviations were considered major:

- Failure to meet all Inclusion/Exclusion criteria;
- Usage of restricted medications/treatments, as defined in Section 9.4.8, Treatment Compliance;
- Failure to present an evaluable endpoint (AK lesion count) within a prespecified visit window of Day 57 (i.e., 50 ≤ study day ≤ 85);
- Noncompliance with the study treatment regimen (i.e., less than 3 applications of study medication);
- Failure to receive the study medication to which the patient was randomly assigned; or
- Unblinding of patient's treatment assignment at any time during the study.

Baseline data

Baseline demographic characteristics of the patients in PEP005-025 study are presented in Table 22.

Table 22. Demographic and baseline characteristics (study PEP005-025): ITT population

Parameter	PEP005 Gel 0.015% (N = 142)	Vehicle Gel (N = 136)	Total (N = 278)
Age (years)			
N	142	136	278
Mean (SD)	64.8 (11.2)	65.0 (10.1)	64.9 (10.7)
Minimum, Maximum	34, 88	46, 89	34, 89
Age Group [n (%)]			
< 65	73 (51.4)	63 (46.3)	136 (48.9)
≥ 65	69 (48.6)	73 (53.7)	142 (51.1)
Sex [n (%)]			
Male	117 (82.4)	112 (82.4)	229 (82.4)
Female	25 (17.6)	24 (17.6)	49 (17.6)
Race [n (%)]			
White	142 (100.0)	136 (100.0)	278 (100.0)
Fitzpatrick Skin Type [n (%)]			
Burns easily, rarely tans (I)	27 (19.0)	18 (13.2)	45 (16.2)
Burns easily, tans minimally (II)	65 (45.8)	59 (43.4)	124 (44.6)
Burns moderately, tans gradually (III)	40 (28.2)	52 (38.2)	92 (33.1)
Burns minimally, tans well (IV)	10 (7.0)	7 (5.1)	17 (6.1)
Rarely burns, tans profusely (V)	0	0	0
Never burns, deeply pigmented (VI)	0	0	0
Body Mass Index (kg/m^2)[a]			
N	142	136	278
Mean (SD)	28.3 (4.4)	28.1 (4.5)	28.2 (4.5)
Minimum, Maximum	20, 49	20, 48	20, 49

Note: Percentages are based on the total number of patients in each treatment group.

[a] Body Mass Index (BMI) is calculated as weight (kg) / [height (m)]^2.

The disease characteristics at baseline were:

Table 23. Baseline Disease Characteristics (study PEP005-025): ITT population

Parameter	PEP005 Gel 0.015% (N = 142) n (%)	Vehicle Gel (N = 136) n (%)	Total (N = 278) n (%)
Location of Treatment Area			
Face	111 (78.2)	111 (81.6)	222 (79.9)
Scalp	31 (21.8)	25 (18.4)	56 (20.1)
Baseline Lesion Count			
4	21 (14.8)	25 (18.4)	46 (16.5)
5	39 (27.5)	35 (25.7)	74 (26.6)
6	28 (19.7)	29 (21.3)	57 (20.5)
7	27 (19.0)	21 (15.4)	48 (17.3)
8	27 (19.0)	26 (19.1)	53 (19.1)

Numbers analysed

The number and percentage of patients included in each of the analysis populations by treatment group are presented in Table 24.

Table 24. Analysis populations

Population	PEP005 Gel 0.015%	Vehicle Gel	Total
ITT Population	142 (100.0)	136 (100.0)	278 (100.0)
Per Protocol Population[a, b]	136 (95.8)	130 (95.6)	266 (95.7)
Safety Population	142 (100.0)	136 (100.0)	278 (100.0)

[a] Percents are based on the total number of patients in the ITT population.

[b] Patients may have more than one reason for exclusion.

Outcomes and estimation

Primary endpoint

The efficacy results in terms of the primary endpoint of complete clearance rate of AK lesions at Day 57 overall and by anatomic location are summarised in table 25.

Table 25. Complete clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-025): ITT population

	PEP005 Gel 0.015% (N = 142)	Vehicle Gel (N = 136)	*P* value
Overall			
Complete Clearance Rate [n/N (%)]	67/142 (47.2)	7/136 (5.1)	<0.001[a]
95% Confidence Interval [b]	38.8, 55.7	2.1, 10.3	
Breslow Day *P* value [c]			0.306
Face			
Complete Clearance Rate [n/N (%)]	58/111 (52.3)	6/111 (5.4)	<0.001[d]
95% Confidence Interval [b]	42.6, 61.8	2.0, 11.4	
Scalp			
Complete Clearance Rate [n/N (%)]	9/31 (29.0)	1/25 (4.0)	0.031[d]
95% Confidence Interval [b]	14.2, 48.0	0.1, 20.4	

[a] P values are from Cochran-Mantel-Haenszel test, stratified by analysis site. The P values ≤ 0.05 are considered statistically significant.

[b] Confidence intervals are calculated using the exact binomial distribution (Clopper-Pearson).

[c] P values ≤ 0.10 are considered statistically significant.

[d] P values are from Fisher's Exact test treatment group comparison. The P values ≤ 0.05 are considered statistically significant.

Secondary endpoint

The results of the partial clearance at Day 57 are presented in Table 26.

Table 26. Partial clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-025): ITT population

	PEP005 Gel 0.015% (N = 142)	Vehicle Gel (N = 136)	*P* value
Overall			
Partial Clearance Rate [n/N (%)]	96/142 (67.6)	11/136 (8.1)	<0.001[a]
95% Confidence Interval [b]	59.2, 75.2	4.1, 14.0	
Face			
Partial Clearance Rate [n/N (%)]	82/111 (73.9)	10/111 (9.0)	<0.001[c]
95% Confidence Interval [b]	64.7, 81.8	4.4, 15.9	
Scalp			
Partial Clearance Rate [n/N (%)]	14/31 (45.2)	1/25 (4.0)	<0.001[c]
95% Confidence Interval [b]	27.3, 64.0	0.1, 20.4	

[a] *P* values are from Cochran-Mantel-Haenszel test, stratified by analysis site. The *P* values ≤ 0.05 are considered statistically significant.

[b] Confidence intervals are calculated using the exact binomial distribution (Clopper-Pearson).

[c] *P* values are from Fisher's Exact test treatment group comparison. The *P* values ≤ 0.05 are considered statistically significant.

In the subgroup analyses, the complete clearance rate for each treatment group was analyzed by geographic region (US or AUS), gender, age group (<65 or ≥65 years), baseline AK lesion count (4, 5, 6 or 7, 8), skin type (Fitzpatrick I/II or III/IV/V/VI), and analysis site. Findings showed a higher complete clearance rate for PEP005 Gel-treated patients compared to vehicle patients in each category; the only category that did not show statistical significance was the geographic region of Australia (data not shown).

Patient reported outcomes included the TSQM and the Skindex-16 Dermatology Survey. Statistically significant, higher mean patient global satisfaction scores, measured by the TSQM, were seen in the PEP005 Gel group compared to the vehicle gel group ($p < 0.001$). For the Skindex-16 Dermatology Survey, a statistically significant difference was seen with PEP005 Gel-treated patients less bothered by each of the three domains (symptoms, emotions, and functioning) compared to vehicle gel; the positive effect was seen at Day 29 ($p < 0.001$, each domain) and continued at Day 57 ($p < 0.001$, each domain) (data not shown).

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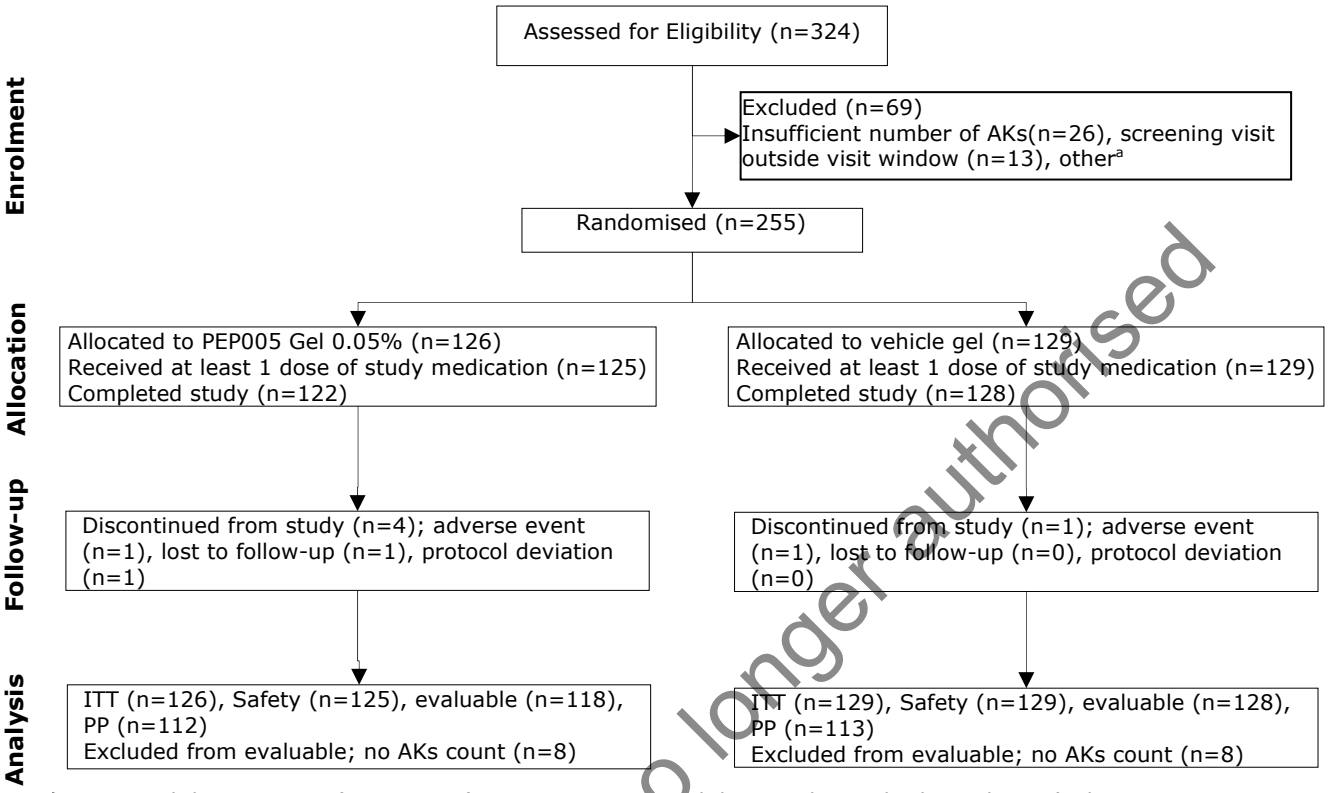
- **PEP005-014**

Participant flow



[a]Patient withdrew consent (12 patients); 6 patients were withdraw at the medical coordinator's discretion, 4 patients had exclusionary laboratory tests, 2 patients were taking prohibitive medications, and 6 patients were withdrawn for other reasons

Recruitment

First patient was randomised the 5th of September 2008 and the last patient completed Day 57 the 23rd February 2009.

Conduct of the study

The summary of major protocol deviations for the ITT population is presented in Table 27.

Table 27. Summary of major protocol deviations (study PEP005-014): ITT population

	PEP005, 0.05% (N = 126) n (%)	Vehicle (N = 129) n (%)	Total (N = 255) n (%)
Patients with major protocol deviations	12 (9.5)	16 (12.4)	28 (11.0)
Deviation Type			
Other protocol noncompliance	8 (6.3)	11 (8.5)	19 (7.5)
Ineligible patient/study criteria not satisfied	8 (6.3)	9 (7.0)	17 (6.7)
Prohibited medication/procedure	1 (0.8)	4 (3.1)	5 (2.0)
Procedures/visit not done	0	1 (0.8)	1 (0.4)
Randomization error/kit assignment error for any reason	0	1 (0.8)	1 (0.4)
Noncompliance with study medication	0	0	0

Baseline data

Baseline demographic characteristics of the patients in PEP005-014 study are presented in Table 28.

Table 28. Demographic and baseline characteristics (study PEP005-014): ITT population

Parameter	PEP005, 0.05% (N = 126)	Vehicle (N = 129)	Total (N = 255)
Age (years)			
n	126	129	255
Mean ± SD	67.3 ±10.59	66.9 ± 9.89	67.1 ± 10.22
Range	43 – 88	36 – 87	36 – 88
Sex [n (%)]			
Male	86 (68.3)	73 (56.6)	159 (62.4)
Female	40 (31.7)	56 (43.4)	96 (37.6)
Childbearing potential	4 (3.2)	2 (1.6)	6 (2.4)
Race [n (%)]			
White	126 (100)	129 (100)	255 (100)
BMI (kg/m^2)[a]			
n	126	128	254
Mean ± SD	28.045 ± 4.5852	28.467 ± 5.3782	28.258 ± 4.9952
Range	13.94 –40.81	18.22 – 47.82	13.94 – 47.82
Fitzpatrick Skin Type			
I = Burns easily, rarely tans	26 (20.6)	31 (24.0)	57 (22.4)
II = Burns easily, tans minimally	69 (54.8)	73 (56.6)	142 (55.7)
III = Burns moderately, tans gradually	21 (16.7)	21 (16.3)	42 (16.5)
IV = Burns minimally, tans well	10 (7.9)	4 (3.1)	14 (5.5)

BMI = body mass index; SD = standard deviation

Percentages based on number of non-missing values in each group.

[a] BMI = weight (kg) / [height (cm)]^2.

The disease characteristics at baseline were:

Table 29. Baseline Disease Characteristics (study PEP005-014): ITT population

Parameter	PEP005, 0.05% (N = 126)	Vehicle (N = 129)	Total (N = 255)
Duration of AK (months)[a]			
n	125	128	253
Median	68.0	82.5	70.0
Range	0 – 586	0 – 586	0 – 586
No. lesions in the treatment area			
n	126	129	255
Mean ± SD	5.6 ± 1.31	5.5 ± 1.29	5.5 ± 1.30
Range	4 – 9	4 – 8	4 – 9
Location of treatment area [n (%)][b]			
Arm	84 (66.7)	82 (63.6)	166 (65.1)
Left	49 (58.3)	47 (57.3)	96 (57.8)
Right	35 (41.7)	35 (42.7)	70 (42.2)
Shoulder	0	2 (1.6)	2 (0.8)
Left	0	1 (50.0)	1 (50.0)
Right	0	1 (50.0)	1 (50.0)
Chest	9 (7.1)	8 (6.2)	17 (6.7)
Back	2 (1.6)	3 (2.3)	5 (2.0)
Back of hand	25 (19.8)	29 (22.5)	54 (21.2)
Left	16 (64.0)	16 (55.2)	32 (59.3)
Right	9 (36.0)	13 (44.8)	22 (40.7)
Leg	6 (4.8)	5 (3.9)	11 (4.3)
Left	4 (66.7)	3 (60.0)	7 (63.6)
Right	2 (33.3)	2 (40.0)	4 (36.4)

AK = actinic keratosis; SD = standard deviation

Percentages based on number of non-missing values in each group unless otherwise specified by footnote.

[a] Calculated as integer of [(date of informed consent – date of first diagnosis) / 365.25] × 12.

[b] Left and right percentages based on number of patients with the specified treatment location in each group (left/right percentages add to 100%).

Numbers analysed

The number and percentage of patients included in each of the analysis populations by treatment group are presented in Table 30.

Table 30. Analysis populations

	PEP005, 0.05%	Vehicle	Total
Randomized/ITT population[a]	126 (100%)	129 (100%)	255 (100%)
Safety population[b]	125 (99.2%)	129 (100%)	254 (99.6%)
Evaluable population[c]	118 (93.7%)	128 (99.2%)	246 (96.5%)
PP population[d]	112 (88.9%)	113 (87.6%)	225 (88.2%)
Excluded from PP population	14	16	30
Reasons for exclusion from PP population			
Major protocol deviation (see Table 6)	12	16	28
Did not apply study medication on Day 1 and Day 2	2	0	2

AK = actinic keratosis; ITT = intent-to-treat; PP = per-protocol

Percentages based on number of randomized patients in each treatment group.

[a] All patients randomized to study treatment. Patients were counted in the treatment group to which they were randomized, regardless of the study medication they received.

[b] Patients in the ITT population who received at least one dose of study medication and had at least one postbaseline safety evaluation. Patients were counted in the group in which they were actually treated.

[c] A subset of the ITT population that included all randomized patients who had AK lesion counts at both baseline (Day 1 predose) and Day 57.

[d] A subset of the ITT population that included all randomized patients considered to be sufficiently compliant with the protocol.

Outcomes and estimation

Primary endpoint

The efficacy results in terms of the primary endpoint of complete clearance rate of AK lesions at Day 57 overall and by anatomic location are summarised in table 31.

Table 31. Complete clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-014): ITT population

Clinical Assessment	PEP005, 0.05% (N = 126)	Vehicle (N = 129)	p-value
All Anatomical Locations			
Observed complete clearance, n (%) [95% CI][a]	35 (27.8%) [20.2%, 36.46%]	6 (4.7%) [1.7%, 9.9%]	<0.0001[c]
Difference between treatment groups [95% CI][b]	23.13% [14.5%, 31.8%]		
CMH weighted complete clearance rate [95% CI][d]	27.3% [20.2%, 34.5%]	4.9% [1.2%, 8.7%]	
Difference between treatment groups [95% CI][e]	22.4% [14.3%, 30.5%]		<0.0001[e]
Anatomical Location			
Arm			
Observed complete clearance, n/N (%) [95% CI][a]	22/84 (26.2%) [17.2%, 36.9%]	4/82 (4.9%) [1.3%, 12.0%]	
Difference between treatment groups [95% CI][b]	21.3% [10.8%, 31.8%]		
Back of hand			
Observed complete clearance, n/N (%) [95% CI][a]	4/25 (16.0%) [4.5%, 36.1%]	0/29 [0, 11.9%]	
Difference between treatment groups [95% CI][b]	16.0% [01.6%, 30.4%]		
Chest			
Observed complete clearance, n/N (%) [95% CI][a]	8/9 (88.9%) [51.8%, 99.7%]	1/8 (12.5%) [0.3%, 52.7%]	
Difference between treatment groups [95% CI][b]	76.4% [45.6%, 100.0%]		
Other[f]			
Observed complete clearance, n/N (%) [95% CI][a]	1/8 (12.5%) [0.3%, 52.7%]	1/10 (10.0%) [0.2%, 44.5%]	
Difference between treatment groups [95% CI][b]	2.5% [-27.0%, 32.0%]		

CI = confidence interval

Complete clearance defined as no clinically visible AK lesions in the treatment area at Day 57. Missing values and values outside analysis windows were imputed using the last observation carried forward method.

[a] Exact CI for complete clearance rate

[b] Asymptotic CI for the difference in complete clearance rate between treatment groups (active – vehicle).

[c] p-value for the difference from Cochran-Mantel-Haenszel (CMH) test controlling for anatomical location.

[d] Asymptotic CI. Complete clearance rate weighted over anatomical location.

[e] Asymptotic CI for the difference between treatment groups (active – vehicle); p-value from Cochran chi-square test.

[f] Other = shoulder, back, and leg.

Secondary endpoint

The results of the partial clearance at Day 57 are presented in Table 32.

Table 32. Partial clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-014): ITT population

Clinical Assessment	PEP005, 0.05% (N = 126)	Vehicle (N = 129)	p-value
All Anatomical Locations			
Observed partial clearance, n (%) [95% CI][a]	56 (44.4%) [35.6%, 53.6%]	9 (7.0%) [3.2%, 12.8%]	<0.0001[c]
Difference between treatment groups [95% CI][b]	37.5% [27.73%, 47.19%]		
CMH weighted partial clearance rate [95% CI][d]	44.1% [35.9%, 52.3%]	7.3% [2.8%, 11.9%]	
Difference between treatment groups [95% CI][e]	36.8% [27.4%, 46.1%]		<0.0001[c]
Anatomical Location			
Arm			
Observed partial clearance, n/N (%) [95% CI][a]	40/84 (47.6%) [36.6%, 58.8%]	7/82 (8.5%) [3.50%, 16.8%]	
Difference between treatment groups [95% CI][b]	39.1% [26.8%, 51.4%]		
Back of hand			
Observed partial clearance, n/N (%) [95% CI][a]	6/25 (24.0%) [9.4%, 45.1%]	0/29 [0, 11.9%]	
Difference between treatment groups [95% CI][b]	24.0% [7.3%, 40.7%]		
Chest			
Observed partial clearance, n/N (%) [95% CI][a]	8/9 (88.9%) [51.8%, 99.7%]	1/8 (12.5%) [0.3%, 52.6%]	
Difference between treatment groups [95% CI][b]	76.4% [45.6%, 100%]		
Other[f]			
Observed partial clearance, n/N (%) [95% CI][a]	2/8 (25.0%) [3.2%, 65.1%]	1/10 (10.0%) [0.3%, 44.5%]	
Difference between treatment groups [95% CI][b]	15.0% [-20.3%, 50.3%]		

CI = confidence interval

Partial clearance defined as ≥75% reduction in the number of AK lesions identified at baseline in the treatment area at Day 57. Missing values and values outside analysis windows were imputed using the last observation carried forward method.

[a] Exact CI for complete clearance rate

[b] Asymptotic CI for the difference in partial clearance rate between treatment groups (active – vehicle).

[c] p-value for the difference from Cochran-Mantel-Haenszel (CMH) test controlling for anatomical location.

[d] Asymptotic CI. Partial clearance rate weighted over anatomical location.

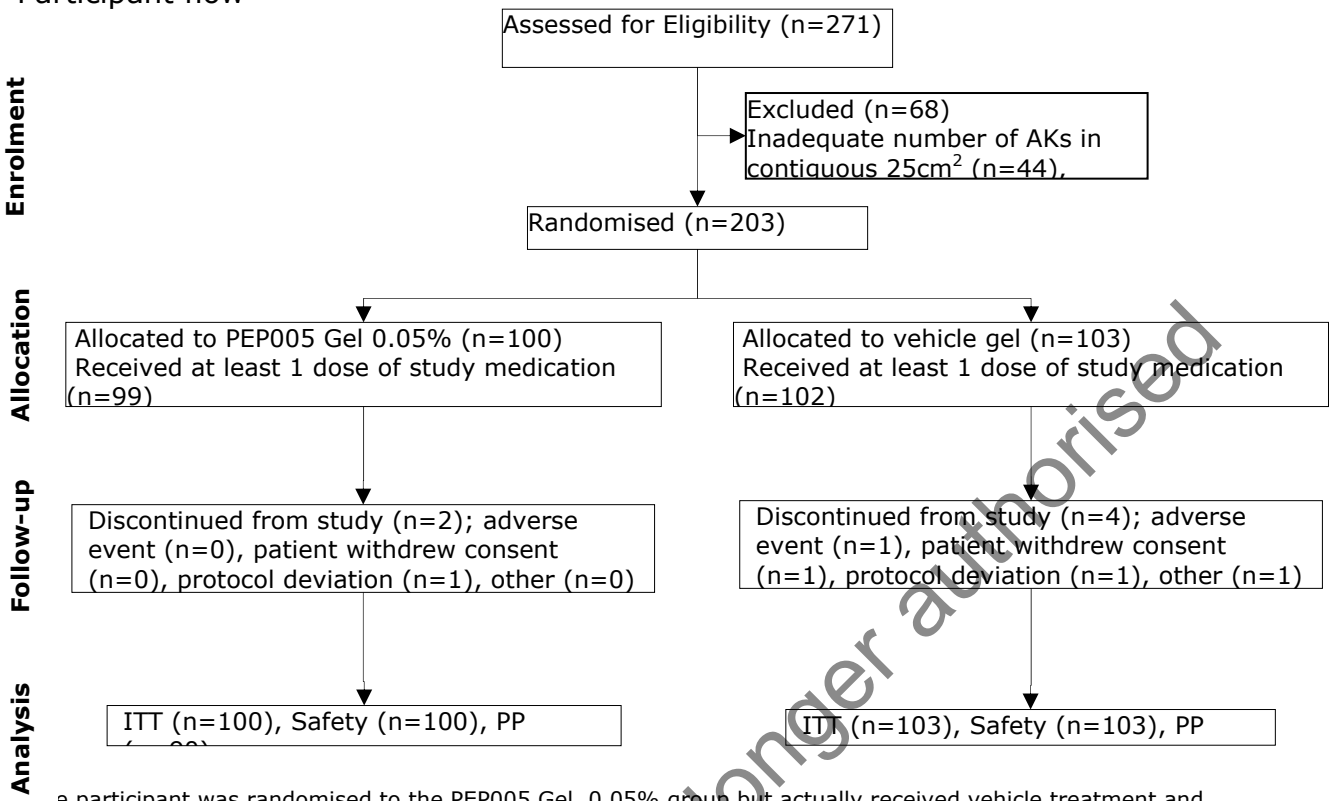
[e] Asymptotic CI for the difference between treatment groups (active – vehicle); p-value from Cochran chi-square test.

[f] Other = shoulder, back, and leg.

Patient-reported Global Satisfaction mean score at Day 57, as measured by the TSQM, was statistically significantly higher in the PEP005 Gel, 0.05% group (71.3) relative to the vehicle group (47.8) ($p < 0.0001$), indicating a significantly higher level of overall satisfaction with PEP005 Gel, 0.05% relative to vehicle. For the Skindex-16 Dermatology Survey, mean scores for all three domain scores (Symptoms, Emotions, and Functioning) at Day 57 were decreased from baseline both in the PEP005 Gel, 0.05% group and the vehicle group, indicating improvement in patient concern regarding their skin condition in both treatment groups. There were no statistically significant differences between treatment groups in any of the three domains at Day 57 (data not shown).

PEP005-028

Participant flow



e participant was randomised to the PEP005 Gel, 0.05% group but actually received vehicle treatment and, therefore, was included in the vehicle group for all safety analyses. Patient 64/005 was randomised to the vehicle group but actually received PEP005 Gel, 0.05% treatment and, therefore, was included in the PEP005 Gel, 0.05% group for all safety analyses.

[b]Patient withdrew consent (n=12); withdrawn at the medical coordinator's discretion (n=6), exclusionary laboratory tests (n=4), taking prohibitive medications (n=2), and withdrawn for other reasons (n=2)

Recruitment

First patient was randomised the 20th of July 2009 and the last follow-up date for any patient was 27 October 2009.

Conduct of the study

The summary of major protocol deviations for the ITT population is presented in Table 33.

Table 33. Summary of major protocol deviations (study PEP005-028): ITT population

	PEP005, 0.05% (N = 100) n (%)	Vehicle (N = 103) n (%)	Total (N = 203) n (%)
Patients with major protocol deviations	10 (10.0)	8 (7.8)	18 (8.9)
Deviation Type			
Exclusionary medication	6 (6.0)	3 (2.9)	9 (4.4)
Early termination/Day 57 visit outside window	2 (2.0)	3 (2.9)	5 (2.5)
Did not receive randomized treatment	1 (1.0)	1 (1.0)	2 (1.0)
Less than 2 study medication applications	1 (1.0)	0	1 (0.5)
Medication applied to incorrect area	1 (1.0)	0	1 (0.5)
Concomitant procedure	0	1 (1.0)	1 (0.5)

Percentages based on number of patients in each treatment group.

Patient counted once within each deviation type. Patients could have more than one type of protocol deviation.

Baseline data

Baseline demographic characteristics of the patients in PEP005-028 study are presented in Table 34.

Table 34. Demographic and baseline characteristics (study PEP005-028): ITT population

Parameter	PEP005, 0.05% (N = 100)	Vehicle (N = 103)	Total (N = 203)
Age (years)			
n	100	103	203
Mean ± SD	65.3 ± 10.2	64.9 ± 10.7	65.1 ± 10.4
Range	43 – 87	34 – 89	34 – 89
Age group [n (%)]			
< 65 years	48 (48.0)	52 (50.5)	100 (49.3)
≥ 65 years	52 (52.0)	51 (49.5)	103 (50.7)
Sex [n (%)]			
Male	59 (59.0)	68 (66.0)	127 (62.6)
Female	41 (41.0)	35 (34.0)	76 (37.4)
Race [n (%)]			
White	100 (100)	103 (100)	203 (100)
BMI (kg/m^2)[a]			
n	100	103	203
Mean ± SD	28.5 ± 5.6	28.2 ± 5.5	28.3 ± 5.6
Range	19 – 45	18 – 47	18 – 47
Fitzpatrick Skin Type [n (%)]			
I = Burns easily, rarely tans	26 (26.0)	24 (23.3)	50 (24.6)
II = Burns easily, tans minimally	36 (36.0)	45 (43.7)	81 (39.9)
III = Burns moderately, tans gradually	31 (31.0)	27 (26.2)	58 (28.6)
IV = Burns minimally, tans well	5 (5.0)	7 (6.8)	12 (5.9)
V = Rarely burns, tans profusely	2 (2.0)	0	2 (1.0)

BMI = body mass index; SD = standard deviation

Percentages based on number of patients treated in each group.

[a] BMI = weight (kg) / [height (cm)]^2.

A summary of treatment area location and baseline lesion count by treatment group for the ITT population is presented in Table 35.

Table 35. Treatment area location and baseline lesion count - ITT population

Parameter	PEP005, 0.05% (N = 100) n (%)	Vehicle (N = 103) n (%)	Total (N = 203) n (%)
Location of treatment area			
Arm	59 (59.0)	67 (65.0)	126 (62.1)
Back of hand	28 (28.0)	27 (26.2)	55 (27.1)
Chest	5 (5.0)	3 (2.9)	8 (3.9)
Leg	3 (3.0)	5 (4.9)	8 (3.9)
Back	3 (3.0)	0	3 (1.5)
Shoulder	2 (2.0)	1 (1.0)	3 (1.5)
Baseline lesion count			
4	37 (37.0)	27 (26.2)	64 (31.5)
5	25 (25.0)	21 (20.4)	46 (22.7)
6	20 (20.0)	26 (25.2)	46 (22.7)
7	6 (6.0)	15 (14.6)	21 (10.3)
8	12 (12.0)	14 (13.6)	26 (12.8)

Numbers analysed

The number and percentage of patients included in each of the analysis populations by treatment group are presented in Table 36.

Table 36. Analysis populations

	PEP005, 0.05%	Vehicle	Total
Randomized/ITT population[a]	100 (100%)	103 (100%)	203 (100%)
Safety population[b]	100 (100%)	103 (100%)	203 (100%)
PP population[c]	90 (90.0%)	95 (92.2%)	185 (91.1%)
Excluded from PP population	10	8	18

Outcomes and estimation

Primary endpoint

The efficacy results in terms of the primary endpoint of complete clearance rate of AK lesions at Day 57 overall and by anatomic location are summarised in table 37.

Table 37. Complete clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-028): ITT population

Clinical Assessment	PEP005, 0.05% (N = 100)	Vehicle (N = 103)	p-value
All Anatomical Locations			
Complete clearance rate, n (%)	42 (42.0%)	5 (4.9%)	<0.001[a]
[95% CI][b]	[32.2%, 52.3%]	[1.6%, 11.0%]	
Breslow Day p-value[c]	0.579		
Anatomical Location			
Arm			
Complete clearance rate, n/N (%)	27/59 (45.8%)	3/67 (4.5%)	<0.001[d]
[95% CI][b]	[32.7%, 59.2%]	[0.9%, 12.5%]	
Back of hand			
Complete clearance rate, n/N (%)	6/28 (21.4%)	0/27	0.023[d]
[95% CI][b]	[8.3%, 41.0%]	[0, 12.8%]	
Chest			
Complete clearance rate, n/N (%)	3/5 (60.0%)	1/3 (33.3%)	1.000[d]
[95% CI][b]	[14.7%, 94.7%]	[0.8%, 90.6%]	
Other[e]			
Complete clearance rate, n/N (%)	6/8 (75.0%)	1/6 (16.7%)	0.103[d]
[95% CI][b]	[34.9%, 96.8%]	[0.4%, 64.1%]	

CI = confidence interval; CMH = Cochran-Mantel-Haenszel

Complete clearance defined as no clinically visible AK lesions in the treatment area at Day 57. Missing values and values outside analysis windows were imputed using the last observation carried forward method.

[a] p-value from CMH test, stratified by analysis site; p-values ≤0.05 are statistically significant.

[b] Confidence intervals calculated using the exact binomical distribution (Clopper-Pearson)

[c] p-values ≤0.10 are statistically significant.

[d] p-value from Fisher's Exact test; p-values ≤0.05 are statistically significant.

[e] Other = leg, back, and shoulder.

Secondary endpoint

The results of the partial clearance at Day 57 are presented in Table 38.

Table 38. Partial clearance rate of AK lesions at Day 57 overall and by anatomic location (study PEP005-028): ITT population

Clinical Assessment	PEP005, 0.05% (N = 100)	Vehicle (N = 103)	p-value
All Anatomical Locations			
Partial clearance rate, n (%)	55 (55.0%)	7 (6.8%)	<0.001[a]
[95% CI][b]	[44.7%, 65.0%]	[2.8%, 13.5%]	
Anatomical Location			
Arm			
Partial clearance rate, n/N (%)	36/59 (61.0%)	4/67 (6.0%)	<0.001[c]
[95% CI][b]	[47.4%, 73.5%]	[1.7%, 14.6%]	
Back of hand			
Partial clearance rate, n/N (%)	9/28 (32.1%)	1/27 (3.7%)	0.012[c]
[95% CI][b]	[15.9%, 52.4%]	[0.1%, 19.0%]	
Chest			
Partial clearance rate, n/N (%)	4/5 (80.0%)	1/3 (33.3%)	0.464[c]
[95% CI][b]	[28.4%, 99.5%]	[0.8%, 90.6%]	
Other[d]			
Partial clearance rate, n/N (%)	6/8 (75.0%)	1/6 (16.7%)	0.103[c]
[95% CI][b]	[34.9%, 96.8%]	[0.4%, 64.1%]	

CI = confidence interval; CMH = Cochran-Mantel-Haenszel

Partial clearance defined as ≥75% reduction in the number of AK lesions identified at baseline in the treatment area at Day 57. Missing values and values outside analysis windows were imputed using the last observation carried forward method.

[a] p-value from CMH test, stratified by analysis site; p-values ≤0.05 are statistically significant.

[b] Confidence intervals calculated using the exact binomical distribution (Clopper-Pearson)

[c] p-value from Fisher's Exact test; p-values ≤0.05 are statistically significant.

[d] Other = leg, back, and shoulder.

Patient-reported Global Satisfaction mean score at Day 57, as measured by the TSQM, was statistically significantly higher in the PEP005 Gel, 0.05% group (71.9) than in the vehicle group (34.3) (p <0.001), indicating a significantly higher level of overall satisfaction with PEP005 Gel, 0.05% relative to vehicle.

For the Skindex-16 Dermatology Survey, mean scores for all three Skindex-16 domains (Symptoms, Emotions, and Functioning) at Day 57 were decreased from baseline to a significantly greater degree in the PEP005 Gel, 0.05% group relative to the vehicle group (p ≤ 0.013), indicating a greater improvement in patient concern regarding their skin condition in the PEP005 Gel, 0.05% group relative to the vehicle group (data not shown).

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 39. Summary of Efficacy for trial PEP-005-016

Title: A multi-center, randomised, parallel group, double-blind, vehicle-controlled study to evaluate the efficacy and safety of ingenol mebutate gel 0.015% in patients with actinic keratosis on the head (face or scalp) (REGION-IIa)			
Study identifier	PEP-005-016		
Design	Randomised, multi-center, parallel group, double-blind, vehicle-controlled (1:1 ratio) phase III study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	Day 57 not applicable not applicable	
Hypothesis	Superiority of Picato over vehicle		
Treatments groups	Picato	Picato 135	
	Vehicle	Vehicle 134	
Endpoints and definitions	**Primary endpoint** complete clearance rate of AK lesions	complete clearance rate	Proportion of patients at the Day 57 visit with no clinically visible AK lesions in the selected treatment area for the ITT population.
Database lock	23 November 2009		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat Day 57			
Descriptive statistics and estimate variability	Treatment group	Picato	Vehicle	
	Number of subjects (ITT)	135	134	
	complete clearance rate (proportion responders)	37%	2.2 %	
	95% CI (Clopper Pearson)	(28.9, 45.8)	(0.5, 6.4)	

Effect estimate per comparison	Primary endpoint (complete clearance rate, ITT)	Comparison groups	Picato vs. vehicle
		P-value (Cochran-Mantel-Haenszel stratified by analysis site)	P<.0001

Table 40. Summary of Efficacy for trial PEP-005-025

Title A multi-center, randomised, parallel group, double-blind, vehicle-controlled study to evaluate the efficacy and safety of ingenol mebutate gel, 0.015% in patients with actinic keratosis on the head (face or scalp)(REGION-IIb)		
Study identifier	PEP-005-025	
Design	Randomised, multi-center, parallel group, double-blind, vehicle-controlled (1:1 ratio) phase III study	
	Duration of main phase: Duration of Run-in phase:	Day 57 not applicable

	Duration of Extension phase:		not applicable
Hypothesis	Superiority of Picato over vehicle		
Treatments groups	Picato		Picato 142
	Vehicle		Vehicle 136
Endpoints and definitions	**Primary endpoint** complete clearance rate of AK lesions	complete clearance rate	Proportion of patients at the Day 57 visit with no clinically visible AK lesions in the selected treatment area for the ITT population.
Database lock	23 November 2009		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat Day 57			
Descriptive statistics and estimate variability	Treatment group	Picato	Vehicle	
	Number of subjects (ITT)	142	136	
	complete clearance rate (proportion responders)	47.2 %	5.1 %	
	95% CI (Clopper Pearson)	(38.8, 55.7)	(2.1, 10.3)	

Effect estimate per comparison	Primary endpoint (complete clearance rate, ITT)	Comparison groups	Picato vs. vehicle
		P-value (Cochran-Mantel-Haenszel stratified by analysis site)	P<.0001

Table 41. Summary of Efficacy for trial PEP-005-014

Title :A multi-center, randomised, parallel group, double-blind, vehicle-controlled study to evaluate the efficacy and safety of ingenol mebutate gel, 0.05% in patients with actinic keratosis on non-head locations (REGION-1)			
Study identifier	PEP-005-014		
Design	Randomised, multi-center, parallel group, double-blind, vehicle-controlled (1:1 ratio) phase III study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Day 57 not applicable not applicable
Hypothesis	Superiority of Picato over vehicle		
Treatments groups	Picato		Picato 126
	Vehicle		Vehicle 129
Endpoints and definitions	**Primary endpoint** complete clearance rate of AK lesions	complete clearance rate	Proportion of patients at the Day 57 visit with no clinically visible AK lesions in the selected treatment area for the ITT population.

Database lock				
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat Day 57			
Descriptive statistics and estimate variability	Treatment group	Picato	Vehicle	
	Number of subjects (ITT)	126	129	
	complete clearance rate (proportion responders)	27.8%	4.7 %	
	95% CI (Exact)	(20.2, 36.46)	(1.7, 9.9)	

Effect estimate per comparison	Primary endpoint (complete clearance rate, ITT)	Comparison groups	Picato vs. vehicle
		Difference in proportions	23.13
		95% CI (asymptotic)	(14.5, 31.8)
		P-value (Cochran- Mantel-Haenszel test statistic stratifying on anatomical location)	P<.0001

Table 42. Summary of Efficacy for trial PEP-005-028

Title : A multi-center, randomised, parallel group, double-blind, vehicle-controlled study to evaluate the Efficacy and safety of ingenol mebutate gel, 0.05% In patients with actinic keratosis on non-head locations(REGION-Ib)			
Study identifier	PEP-005-028		
Design	Randomised, multi-center, parallel group, double-blind, vehicle-controlled (1:1 ratio) phase III study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Day 57 not applicable not applicable
Hypothesis	Superiority of Picato over vehicle		
Treatments groups	Picato		Picato 100
	Vehicle		Vehicle 103
Endpoints and definitions	**Primary endpoint** complete clearance rate of AK lesions	complete clearance rate	Proportion of patients at the Day 57 visit with no clinically visible AK lesions in the selected treatment area for the ITT population.
Database lock	9 December 2009		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat Day 57		

Descriptive statistics and estimate variability	Treatment group	Picato	Vehicle	
	Number of subjects (ITT)	100	103	
	complete clearance rate (proportion responders)	42.0%	4.9%	
	95% CI (Clopper Pearson)	(32.2, 52.3)	(1.6,11.0)	
Effect estimate per comparison	Primary endpoint (complete clearance rate, ITT)	Comparison groups	Picato vs. vehicle	
		P-value (Cochran-Mantel-Haenszel stratified by analysis site)	P<.001	

Analysis performed across trials (pooled analyses and meta-analysis)

The applicant has combined the population of selected studies and presented combined studies population analysis, for each anatomic location as described below. The applicant has also provided a justification for choosing the studies to combine and for excluding certain studies for these analyses.

- **Head (Face and scalp) Location:**

For the head location, five studies (PEP005-016, PEP005-025, PEP005-015, PEP005-006, and PEP005-007) provided efficacy data for field treatment of PEP005 Gel to a defined 25 cm^2 area of skin containing 4 to 8 AK lesions. Of these PEP005-006 and PEP005-007 did not use the proposed dose of 0.015% gel for 3 days and PEP005-007 was also an uncontrolled study. Hence these two studies were not included in the combined studies population.

The efficacy results of the combined studies are presented in Table 43.

Table 43. Efficacy results in combined studies population, head location: intent to treat population – Head (face and scalp) locations

	Controlled Phase 3 Studies[a]		Controlled Phase 2 and 3 Studies[b]	
Efficacy Parameter	PEP005, 0.015% (N=277)	Vehicle (N=270)	PEP005, 0.015% (N=309)	Vehicle (N=303)
Complete Clearance				
n (%)	117 (42.2)	10 (3.7)	133 (43.0)	13 (4.3)
95% CI	36.4, 48.3	1.8, 6.7	37.4, 48.8	2.3, 7.2
P value	<0.001			
Partial Clearance				
n (%)	177 (63.9)	20 (7.4)	200 (64.7)	24 (7.9)
95% CI	57.9, 69.6	4.6, 11.2	59.1, 70.1	5.1, 11.6
P value	<0.001			
Percent Reduction in AK Lesions				
n	273	269 3	05 3	02
Median	83 0		83 0	
Range	-50, 100	-100, 100	-50, 100	-100, 100
Total AK Lesion Count				
Baseline	1607 1	526	1784 1	707
End of study (Day 57)	444 1	310 4	81 1	454
Percent change	72 14		73 15	

CI = confidence interval

[a] Controlled Phase 3 studies (PEP005-016 and PEP005-025)

[b] Controlled Phase 2 and 3 studies (PEP005-015, PEP005-016, and PEP005-025); for study PEP005-015 only the active treatment group which evaluated the proposed

- **Non-Head (Trunk and Extremities) Location:**

For the non-head locations, six studies (PEP005-014, PEP005-028, PEP005-006, PEP005- 018, PEP005-020, and PEP005-017) provide efficacy data for the field treatment of PEP005 gel to an area of skin containing 4 to 8 AK lesions. Of these six studies, study PEP005-017 was a single centre study that involved application of the study drug to a 100 sq cm treatment area and hence was not included in the combined studies population. The remaining five studies have been used by the applicant in doing a combined studies analysis. For this analysis, only the sub-group of patients with AK lesions on non-head locations who received PEP005 Gel 0.05% for two consecutive days from study PEP005-006 was included.

The efficacy results of all five clinical studies are presented in Table 44.

Table 44. Efficacy results in combined studies population, head location: intent to treat population – Non-Head (trunk and extremities) locations

	Controlled Phase 3 Studies		Controlled Phase 2 and 3 Studies		Controlled and Uncontrolled Phase 2 and 3 Studies	
Efficacy Parameter	PEP005, 0.05% (N=226)	Vehicle (N=232)	PEP005, 0.05% (N=268)	Vehicle (N=275)	PEP005, 0.05% (N=381)	Vehicle (N=275)
Complete Clearance						
n (%)	77 (34.1)	11 (4.7)	96 (35.8)	17 (6.2)	139 (36.5)	17 (6.2)
95% CI	27.9, 40.6	2.4, 8.3	30.1, 41.9	3.6, 9.7	31.6, 41.5	3.6, 9.7
P value	<0.001					
Partial Clearance						
n (%)	111 (49.1)	16 (6.9)	138 (51.5)	25 (9.1)	199 (52.2)	25 (9.1)
95% CI	42.4, 55.8	4.0, 11.0	45.3, 57.6	6.0, 13.1	47.1, 57.3	6.0, 13.1
P value	<0.001					
Percent Reduction in AK Lesions						
N	220	229	261 2	71 3	74 2	71
Median	75 0	75		0	75	0
Range	-25 – 100	-33 – 100	-57 – 100	-33 – 100	-80 – 100	-33 – 100
Total AK Lesion Count						
Baseline	1204 1	274 1	426	1513	2073	1513
End of study (Day 57)	441	1078	508	1255	736	1256
Percent change	63 15	64		17	64	17

Clinical studies in special populations

No clinical study in special populations was submitted.

Supportive studies

- **Studies PEP005-018 and PEP005-020**

These are uncontrolled studies and are not very significant in drawing conclusions on the efficacy or safety of PEP005 gel. There were no findings in these studies that could raise concerns on the conclusions drawn from the other conducted studies (data not shown).

- **Long-term Follow-up Studies: PEP005-030, PEP005-031 and PEP005-032**

Three prospective, observational long term 1 year follow-up studies (PEP005-030, PEP005-031, and PEP005-032) were conducted to evaluate efficacy by recurrence of AK lesions in the treatment field, and safety in patients who had received treatment with Picato. Recurrence was defined as any newly identified AK lesion in the selected treatment area. One study included patients treated with Picato 150 mcg/g on the face or scalp for 3 days (PEP005-030) and two studies included patients treated with Picato 500 mcg/g on the trunk or extremities for 2 days (PEP005-031 and PEP005-032). Only those patients who achieved complete clearance in the treated area at the end (day 57) of four phase III

studies (PEP005-16, PEP005-25, PEP005-20 and PEP005-28) were eligible for long term follow-up. Patients were followed every 3 months for 12 months.

The results of the rate of recurrence of AK lesions on head and non-head locations are presented in Table 45.

Table 45. Rate of recurrence of actinic keratosis lesions

	Picato 150 mcg/g gel Face and scalp (n=108)	**Picato 500 mcg/g gel Trunk and extremities (n=76[c])**
Recurrence Rate 12 months KM estimate (95% CI)[a]	53.9% (44.6-63.7)	56.0% (45.1-67.6)
Lesion Based Recurrence Rate[b] 12 months Mean (SD)	12.8% (19.1)	13.2% (23.0)

[a] The recurrence rate is the Kaplan-Meier (KM) estimate at the target study date of the visit expressed as a percentage (95% CI). Recurrence was defined as any identified actinic keratosis lesion in the previously treated area for patients who achieved complete clearance at day 57 in the previous phase 3 studies.
[b] The lesion-based recurrence rate for each patient defined as the ratio of the number of actinic keratosis lesions at 12 months to the number of lesions at baseline in the previous phase 3 studies.
[c] Of these, 38 subjects were previously treated in a vehicle controlled phase 3 study and 38 subjects were previously treated in an uncontrolled phase 3 study.

Literature Review

The applicant has performed a systematic literature review. A comparison with the following treatments has been done: Imiquimod, Diclofenac, 5-Fluorouracil, Photodynamic Therapy, 5-aminolevulinic acid, aminolevulinic acid, methyl amino levulinate and cryotherapy.

The search was conducted in MEDLINE (1950 to 21 Nov 2011), EMBASE (1947 to 21 Nov 2011), International Pharmaceutical Abstracts (1970 to 21 Nov 2011) and PASCAL (1984 to 21 Nov 2011). A summary of results is described below:

Imiquimod: Imiquomod 5% in the 4week treatment – 4 week treatment off – 4 week treatment regimen showed higher efficacy (54% complete clearance) than ingenol mebutate (42%) as judged by absolute clearance rate, while based on relative clearance rate ingenol is better (11.3) than imiquimod (7.89). With regard to the 12-month sustained clearance rate, imiquimod may be better (61%) than ingenol mebutate (46%).

Diclofenac: Diclofenac 3% gel bid for up to 90 days showed complete clearance of 40% (95% CI : 32-47%). Long term complete clearance where reported was around 18% (95% CI: 10-27%) as compared to ingenol mebutate (sustained clearance 0.46x0.42=19%)

5-Fluorouracil: The studies with 5-FU are quite small, the treatment duration variable, and the safety reporting inadequate. Therefore a comparison with ingenol mebutate is difficult and no conclusion regarding efficacy and safety of 5-FU compared to ingenol mebutate can be made based on the published data for 5-FU.

Photodynamic Therapy: The efficacy of PDT and ingenol mebutate seems to be in the same range but since one treatment is lesion-specific and the other field based the comparison is difficult. This applies also to the comparison of the safety, although it is likely that the local skin responses are more severe with ingenol mebutate.

Cryotherapy: Short term efficacy of cryotherapy can be high if aggressive freeze regimens are used but even then long term efficacy is low with this regimen. With "normal" freeze regimens, the short-

term efficacy of ingenol mebutate is probably on par with cryotherapy and the long-term efficacy is higher. Although the comparison is difficult, there is little doubt that short term LSRs are worse with ingenol mebutate than with cryotherapy.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The study designs of the dose-finding studies, the phase III studies and the long-term follow up studies to assess recurrence are appropriate.

The results from different dose-response studies have been used to define the maximal tolerated dose and the dose limiting toxicities. Different dosing regimens like 2 and 3 days dosing regimens for both anatomic locations and consecutive day dosing (day 1 and 2) versus dosing on day 1 and 8 have been assessed and the study designs are appropriate for the objectives.

The choice of vehicle control in the phase III studies and the lack of an active control in the phase III studies are adequately justified. The lack of comparative data with other topical agents for AK is addressed by systematic literature review and retrospective comparison against published studies showing recurrence of lesions comparable to other topical treatments of AK. In addition the applicant committed to conduct an active comparator study (LP0041-63) to compare the cumulative incidence of SCC after treatment with ingenol mebutate and imiquimod cream, a 3-year follow up study.

The clinical studies typically involved treatment of 4-8 AK lesions up to 25 cm^2 area of skin involvement where the lesions were not hypertrophic or hyperkeratotic. In addition, people on immunosuppressants were excluded from participating in the clinical studies. The applicant has proposed the broad indication of topical treatment of AK without the above exclusions. However, the potential for different activity of Picato exists based on the thickness of the skin, different anatomic locations and the type of lesion. Moreover there is no clear justification or guidance for managing smaller areas of involvement, larger areas of involvement, non-contiguous areas of AK lesions, less well described areas like neck and smaller number of AK lesions. Based on these, the indication has been restricted to ''treatment for the cutaneous treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis in adults''. In addition, the SmPC has been revised to include advice on dose to be used in the treatment of AK lesions in the neck and a statement that Picato has not been evaluated in the treatment of AK on eyelids, the inside of nostrils or ears or the lip area. Finally it is highlighted in the SmPC that clinical data on treatment in immunocompromised patients is not available, but systemic risks are not expected since ingenol mebutate is not absorbed systemically.

The duration of observations following two doses and the timing of clinical assessments is supported by the observations in the phase II studies. The selection of doses for the phase III studies is also appropriately supported from phase II study results.

The number of protocol deviations is small in the four main clinical studies. Moreover the sample size in these studies was much larger than what would be required to demonstrate superiority of Picato over vehicle. In this background any minor deviations and imbalances in randomization that may have occurred is highly unlikely to impact on the conclusions drawn from these studies.

Efficacy data and additional analyses

The dose-response studies have shown that the dose of 0.025% for 2 days to be the MTD for Picato on the face and 0.025% for 3 days as the dose that caused DLTs. Similarly for the non-head locations the dose of 0.075% for 2 days was determined as causing DLT, and a dose of 0.05% for 2 days was defined as the MTD. The choice of 0.015% Picato gel for 3 days for treatment of AK lesions on face and

scalp and the choice of 0.05% Picato gel for 2 days for treatment of AK lesions on non-head locations is supported by the balance of efficacy and safety demonstrated by these doses in the phase II studies.

The four main phase III studies have shown that the primary efficacy endpoint was met in all four studies and the difference from vehicle control was statistically significant. The efficacy data from the four main clinical studies demonstrated that at the proposed doses, Picato has a statistically significant complete clearance of AK lesions as assessed by clinical examination as compared to the vehicle gel.

It is acknowledged that diagnosis and management of AK lesions is by clinical examination in routine practice and therefore this is a valid endpoint for phase III studies. However, histology is considered to be the confirmatory diagnosis and the clinical development program has not shown conclusive evidence of histological clearance of AK lesions to Picato. Based on the results of interim analyses of studies LP0041-02 and LP0041-03 (see primary and secondary pharmacology) histological data confirmed the biological effects of ingenol mebutate in the epidermis suggesting drug activity on the underlying pathology and interim RCM data confirmed biological effects on AK and confirming clearance of AK lesions. Therefore despite the lack of a "precise" treatment effect size through histological confirmation, there is conclusive evidence of efficacy and clearance of AK lesions with Picato. In addition, the CHMP recommended that the applicant conducts a study (LP0041-62) on histological confirmation of clinical clearance of AK following treatment with ingenol mebutate gel 0.05%. This analysis is not expected to significantly change the observed benefits but will provide concordance between clinical assessments and histological confirmation of clearance of AK lesion as all the phase III clinical studies used only clinical assessment to measure clearance of AK lesions. This data will help in more precisely defining the treatment effect size of ingenol mebutate. The MAH should submit the full protocol for review before the start of the study and provide an interim update for the clinical study at an appropriate time-point.

All the secondary endpoints were in line with the results of the primary efficacy endpoint and many of them were also statistically significant as compared to the vehicle treated control group. The patient reported outcomes were also in line with these observations.

The additional analysis done by the applicant in combining the results of all the phase III studies and relevant phase II study data based on anatomic location are also consistent with the above observations and support the conclusions of the phase III studies.

The three follow-up studies provided robust data on recurrence of AK lesions. It is noted that the estimated recurrence rates are high after treatment response with Picato, with more than 50% of patients who responded getting AK lesions within the treatment area within a year. Whilst this recurrence rate may arguably be comparable to other established treatments, this cannot be concluded without a proper, prospective comparator trial. In the post-approval commitment, the applicant is committed to generating controlled active comparator data. The dossier does not have data on re-treatment with Picato. The applicant has provided appropriate advice in the SmPC regarding these limitations as follows: ''Clinical data on treatment for more than one treatment course of 2 or 3 consecutive days is not available". Furthermore, the applicant made a commitment to conduct a repeat use study (LP0041-22) to obtain data on safety and efficacy of re-treatment.

Conclusions on clinical efficacy

Overall, the efficacy results are considered compelling enough to establish the clinical efficacy of the product.

2.6. Clinical safety

The data on safety profile of PEP005 gel were mainly obtained with 13 studies which are presented in Table 46.

Table 46. Summary of studies in AK patients who received field application of study medication

Protocol No. Study status and dates	Population	Locations (No. of Study Centers)	Design; Control Type	PEP005 Gel and Vehicle, Dose & Regimen	No. Patients by dose group; Entered[a]/ Completed	Gender M/F & Age range (years) for the Safety Population	Treatment Duration	Safety Assessments
Phase 3 Controlled Studies, Field Application, Actinic Keratosis								
PEP005-014 Completed Study dates: 5 Sep 2008 23 Feb 2009	AK lesions trunk and extremities	US (18) AUS (2)	Double-blind, parallel group, vehicle-controlled, field application (25 cm^2 treatment area)	0.05% qd Vehicle qd	126/122 129/128	158/96 36–88	2 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs, ECGs
PEP005-016 Completed Study dates: 5 Jun 2009 to 10 Sep 2009	AK lesions face and scalp	US (19) AUS (2)	Double-blind, parallel group, vehicle-controlled, field application (25 cm^2 treatment area)	0.015% qd Vehicle qd	135/132 134/127	235/32 37–88	3 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs, ECGs
PEP005-025 Completed Study dates: 1 Jun 2009 to 2 Sep 2009	AK lesions face and scalp	US (19) AUS (2)	Double-blind, parallel group, vehicle-controlled, field application (25 cm^2 treatment area)	0.015% qd Vehicle qd	142/142 136/135	229/49 34–89	3 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs, ECGs
PEP005-028 Completed Study dates: 22 Jul 2009 14 Oct 2009	AK lesions trunk and extremities	US (17)	Double-blind, parallel group, vehicle-controlled, field application (25 cm^2 treatment area)	0.05% qd Vehicle qd	100/98 103/99	127/76 34–89	2 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs, ECGs

Protocol No. Study status and dates	Population	Locations (No. of Study Centers)	Design; Control Type	PEP005 Gel and Vehicle, Dose & Regimen	No. Patients by dose group; Entered[a]/ Completed	Gender M/F & Age range (years) for the Safety Population	Treatment Duration	Safety Assessments
Other Controlled Studies, Field Application, Actinic Keratosis								
PEP005-006 Completed Study dates: 11 Sep 2006 19 Jun 2007	AK lesions face and scalp, trunk and extremities	US (22)	Double-blind, double dummy, parallel group, vehicle-controlled, dose ranging, field application (25 cm^2 treatment area) (Phase 2b study)	2 days 0.05% qd 3 days 0.025% qd 0.05% qd Vehicle qd	 55/54 50/50 57/57 60/59	178/44 43–85	2 D or 3 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs
PEP005-015 Completed Study dates: 24 Jun 2008 20 Oct 2008	AK lesions face and scalp	US (25) AUS (3)	Double-blind, parallel group, dose-ranging, vehicle-controlled, field application (25 cm^2 treatment area) (Phase 2 study)	2 days 0.005% qd 0.01% qd 0.015% qd Vehicle qd 3 days 0.005% qd 0.01% qd 0.015% qd Vehicle qd	 33/32 34/34 33/33 33/31 33/31 34/34 32/32 33/33	236/28 46–91	2 D or 3 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs
PEP005-017 Completed Study dates: 18 Mar 2009 27 May 2009	AK lesions trunk and extremities	US (1)	Double-blind, parallel group, vehicle-controlled, field application (100 cm^2 treatment area) (Phase 2 study)	0.05% qd Vehicle qd	13/13 3/3	6/10 48–79	2 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs

Protocol No. Study status and dates	Population	Locations (No. of Study Centers)	Design; Control Type	PEP005 Gel and Vehicle, Dose & Regimen	No. Patients by dose group; Entered[a]/ Completed	Gender M/F & Age range (years) for the Safety Population	Treatment Duration	Safety Assessments
Phase 3 Uncontrolled Studies, Field Application, Actinic Keratosis								
PEP005-020 Completed Study dates: 8 Jun 2009 2 Sep 2009	AK lesions trunk and extremities	US (8) AUS (3)	Open-label, uncontrolled, single arm, field application (25 cm^2 treatment area) (Phase 3b study)	0.05% qd	102/102	68/34 38–88	2 D	AEs, LSRs, pigmentation and scarring, abnormal proliferation, vital signs
Phase 2 Uncontrolled Studies, Field Application, Actinic Keratosis								
PEP005-004 Completed Study dates: 7 Sep 2005 14 Mar 2006	AK lesions trunk and extremities	US (1)	Open-label, uncontrolled, dose escalation, field application to a single lesion (9 cm^2 treatment area) (Phase 2b study)	0.01% qd 0.025%qd 0.05% qd 0.075% qd	3/3 3/3 10/10 6/6	16/6 64–87	2 D	AEs (including assessments of local skin reactions, scarring, and abnormal proliferation), clinical laboratory assessments, vital signs, determination of MTD
PEP005-007 Completed Study dates: 18 Jan 2007 13 Nov 2007	AK lesions face and scalp	AUS/NZ (9)	Open-label, dose escalation, field application (25 cm^2 treatment area) (Phase 2a study)	2 days 0.0125% qd 0.0175% qd 0.025% qd 3 days 0.0025% qd 0.005% qd 0.0075% qd 0.0125% qd 0.0175% qd 0.025% qd	 3/3 3/3 30/30 6/6 8/8 9/9 11/10 10/9 8/8	65/23 42–89	2 D or 3 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs, determination of MTD

Protocol No. Study status and dates	Population	Locations (No. of Study Centers)	Design; Control Type	PEP005 Gel and Vehicle, Dose & Regimen	No. Patients by dose group; Entered[a]/ Completed	Gender M/F & Age range (years) for the Safety Population	Treatment Duration	Safety Assessments
Phase 2 Uncontrolled Studies, Field Application, Actinic Keratosis (Cont'd)								
PEP005-018 Completed Study dates: 11 Oct 2007 18 Dec 2007	AK lesions trunk and extremities	US (4)	Open-label, single-arm, uncontrolled, field application, (25 cm^2 treatment area) (Phase 2 study)	0.05% qd	11/11	11/0 57–82	2 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs
Phase 1 Uncontrolled Studies, Field Application, Actinic Keratosis								
PEP005-013 Completed Study dates: 17 Oct 2007 23 Apr 2008	AK lesions trunk and extremities	AUS (1)	Open-label, PK, field application (100 cm^2 treatment area) (Phase 1 study)	0.05% qd	6/6	6/0 56–81	2 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs
PEP005-022 Completed Study dates: 3 Apr 2008 4 Sep 2008	AK lesions trunk and extremities	US (8) AUS (4)	Open-label, assessment of application to treatment areas ranging from 25 to 100 cm^2 (Phase 1 study)	0.05% qd	64/63	64/0 44–85	2 D	AEs, LSR scoring, pigmentation and scarring, abnormal proliferation, clinical laboratory assessments, vital signs

[a]The term 'entered' refers to the number of patients randomized (for randomized studies) or treated (for non-randomized studies)

Patient exposure

Across the 13 studies that evaluated field applications of study medication for treatment of AK lesions, 1165 patients received PEP005 gel and 632 patients received vehicle gel. The disposition of patients is presented in Table 47.

Table 47. Disposition of patients in the AK field treatment studies

	Controlled Phase 3 Studies						All AK Field Treatment Studies	
	Face and Scalp[a]		Trunk and Extremities[b]		Face/Scalp and Trunk/Extremities Combined[c]		All Locations[d]	
	0.015% PEP005 Gel	Vehicle	0.05% PEP005 Gel	Vehicle	PEP005 Gel	Vehicle	PEP005 Gel	Vehicle
ITT Population (Randomized)	276	271	226	232	502	503	1172	632
Safety Population (Treated)	274 (99.3%)	271 (100.0%)	225 (99.6%)	232 (100.0%)	499 (99.4%)	503 (100.0%)	1165 (99.4%)	632 (100.0%)
Patients Terminating the Study Early	3 (1.1%)	8 (3.0%)	6 (2.7%)	5 (2.2%)	9 (1.8%)	13 (2.6%)	19 (1.6%)	16 (2.5%)
Reason for Early Termination								
Abnormal Lab Test/Adverse Event	1 (0.4%)	1 (0.4%)	2 (0.9%)	2 (0.9%)	3 (0.6%)	3 (0.6%)	4 (0.3%)	3 (0.5%)
Consent Withdrawn / Subject Decision	2 (0.7%)	6 (2.2%)	0 (0.0%)	1 (0.4%)	2 (0.4%)	7 (1.4%)	5 (0.4%)	9 (1.4%)
Lost to Follow-up	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	3 (0.3%)	0 (0.0%)
No Actinic Keratosis Lesions	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Protocol Deviation/Violation	0 (0.0%)	1 (0.4%)	2 (0.9%)	1 (0.4%)	2 (0.4%)	2 (0.4%)	3 (0.3%)	2 (0.3%)
Screening or Inclusion / Exclusion Failure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Applicant/Investigator Decision	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Other	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)	1 (0.2%)	1 (0.2%)	2 (0.2%)	2 (0.3%)

The summary of treatment exposure (Table 48) reflects the actual number of days that patients applied study medication to a treatment location.

Table 48. Summary of treatment exposure

Days of Treatment	Controlled Phase 3 Studies						All AK Field Treatment Studies	
	Face and Scalp[a]		Trunk and Extremities[b]		Face/Scalp and Trunk/Extremities Combined[c]		All Locations[d]	
	0.015% PEP005 Gel (N=274)	Vehicle (N=271)	0.05% PEP005 Gel (N=225)	Vehicle (N=232)	PEP005 Gel (N=499)	Vehicle (N=503)	PEP005 Gel (N=1165)	Vehicle (N=632)
1	3 (1.1%)	0 (0.0%)	3 (1.3%)	0 (0.0%)	6 (1.2%)	0 (0.0%)	42 (3.6%)	1 (0.2%)
2	2 (0.7%)	0 (0.0%)	222 (98.7%)	232 (100.0%)	224 (44.9%)	232 (46.1%)	648 (55.6%)	268 (42.4%)
3	269 (98.2%)	271 (100.0%)	0 (0.0%)	0 (0.0%)	269 (53.9%)	271 (53.9%)	475 (40.8%)	363 (57.4%)

Adverse events

The data of adverse events were collected and reported without the inclusion of local skin responses/reactions (LSRs).

Across all AK field treatment studies, 42.5% of PEP005 Gel-treated patients had an AE compared with 24.2% of vehicle-treated patients. The higher incidence of AEs in the PEP005 Gel-treated group is attributed to a higher rate of application site reactions occurring in patients treated with active rather than vehicle gel. Table x gives an overview of adverse events in trials with PEP005 gel in AK patients.

Table 49. Overview of Adverse events

	Controlled Phase 3 Studies				All AK Studies	
	Face and Scalp		Trunk and Extremities		All Locations	
	0.015% PEP005 Gel (N=274)	Vehicle (N=271)	0.05% PEP005 Gel (N=225)	Vehicle (N=232)	PEP005 Gel (N=1165)	Vehicle (N=632)
Patients with one or	102	60	75	63	495	153 (24.2%

more AEs	(37.2%)	(22.1%)	(33.3%)	(27.2%)	(42.5%)	)
Patients with one or more treatment-related AEs	72 (26.3%)	11 (4.1%)	29 (12.9%)	2 (0.9%)	312 (26.8%)	22 (3.5%)
Patients with one or more severe AEs	8 (2.9%)	4 (1.5%)	5 (2.2%)	4 (1.7%)	37 (3.2%)	10 (1.6%)
Patients with one or more severe treatment-related AEs	4 (1.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	17 (1.5%)	0 (0.0%)
Patients with one or more AEs leading to discontinuation of study drug	3 (1.1%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	37 (3.2%)	0 (0.0%)
Patients with one or more AEs leading to discontinuation from the study	1 (0.4%)	1 (0.4%)	2 (0.9%)	2 (0.9%)	4 (0.3%)	3 (0.5%)
Patients with one or more SAEs	6 (2.2%)	5 (1.8%)	8 (3.6%)	12 (5.2%)	49 (4.2%)	23 (3.6%)

The SOC of general disorders and administration site conditions had the highest incidence of AEs for patients treated with PEP005 Gel (22.7% vs. 2.8% for patients treated with vehicle). Within this SOC, application site pruritus, application site pain, and application site irritation were the most frequently reported AEs and were predominantly reported for patients treated with PEP005 Gel rather than patients treated with vehicle. Table x presents a summary of all adverse events with an incidence of > 1% that were reported in the AK trials of PEP005 gel.

Table 50. Summary of adverse events with an incidence of 1% for the PEP005 gel-treated patients in the All AK population by PEP005 gel concentration

System Organ Class Preferred Term	< 0.015% PEP005 Gel (N=175)	0.015% PEP005 Gel (N=339)	> 0.015% & < 0.05% PEP005 Gel (N=102)	0.05% PEP005 Gel (N=543)	> 0.05% PEP005 Gel (N=6)
All Systems	79 (45.1%)	132 (38.9%)	48 (47.1%)	231 (42.5%)	5 (83.3%)
General Disorders and Administration Site Conditions	47 (26.9%)	71 (20.9%)	21 (20.6%)	120 (22.1%)	5 (83.3%)
Application Site Pruritus	21 (12.0%)	23 (6.8%)	5 (4.9%)	72 (13.3%)	5 (83.3%)
Application Site Pain	7 (4.0%)	43 (12.7%)	7 (6.9%)	33 (6.1%)	2 (33.3%)
Application Site Irritation	16 (9.1%)	16 (4.7%)	4 (3.9%)	43 (7.9%)	2 (33.3%)
Application Site Paraesthesia	8 (4.6%)	3 (0.9%)	2 (2.0%)	8 (1.5%)	0 (0.0%)
Application Site Discomfort	7 (4.0%)	3 (0.9%)	2 (2.0%)	3 (0.6%)	0 (0.0%)
Application Site Reaction	1 (0.6%)	0 (0.0%)	4 (3.9%)	9 (1.7%)	0 (0.0%)
Infections and Infestations	12 (6.9%)	26 (7.7%)	8 (7.8%)	40 (7.4%)	2 (33.3%)
Nasopharyngitis	3 (1.7%)	1 (0.3%)	0 (0.0%)	9 (1.7%)	0 (0.0%)
Skin and Subcutaneous Tissue Disorders	5 (2.9%)	16 (4.7%)	6 (5.9%)	28 (5.2%)	1 (16.7%)
Periorbital Oedema	1 (0.6%)	9 (2.7%)	2 (2.0%)	0 (0.0%)	0 (0.0%)
Injury, Poisoning and Procedural Complications	4 (2.3%)	12 (3.5%)	4 (3.9%)	25 (4.6%)	0 (0.0%)
Nervous System Disorders	7 (4.0%)	14 (4.1%)	7 (6.9%)	13 (2.4%)	0 (0.0%)
Headache	6 (3.4%)	9 (2.7%)	6 (5.9%)	3 (0.6%)	0 (0.0%)
Neoplasms Benign, Malignant & Unspecified (Incl Cysts & Polyps)	2 (1.1%)	5 (1.5%)	5 (4.9%)	23 (4.2%)	0 (0.0%)
Basal Cell Carcinoma	1 (0.6%)	4 (1.2%)	2 (2.0%)	10 (1.8%)	0 (0.0%)
Investigations	5 (2.9%)	5 (1.5%)	3 (2.9%)	18 (3.3%)	0 (0.0%)
Musculoskeletal and Connective Tissue Disorders	7 (4.0%)	6 (1.8%)	0 (0.0%)	19 (3.5%)	0 (0.0%)
Respiratory, Thoracic and Mediastinal Disorders	2 (1.1%)	7 (2.1%)	6 (5.9%)	13 (2.4%)	0 (0.0%)
Gastrointestinal Disorders	5 (2.9%)	8 (2.4%)	2 (2.0%)	14 (2.6%)	0 (0.0%)
Cardiac Disorders	2 (1.1%)	3 (0.9%)	2 (2.0%)	13 (2.4%)	0 (0.0%)
Eye Disorders	5 (2.9%)	16 (4.7%)	4 (3.9%)	2 (0.4%)	0 (0.0%)
Vascular Disorders	2 (1.1%)	2 (0.6%)	3 (2.9%)	7 (1.3%)	0 (0.0%)
Hypertension	2 (1.1%)	2 (0.6%)	2 (2.0%)	6 (1.1%)	0 (0.0%)

Treatment-emergent AEs with an incidence of ≥ 1% in any treatment group that were considered related in the field application are summarized in Table 51.

Table 51. Summary of Treatment-Emergent adverse events considered related to study medication with an incidence of ≥ 1% in any group

	Controlled Phase 3 Studies						All Field Application AK Studies	
	Face and Scalp[a]		Trunk and Extremities[b]		Face/Scalp and Trunk/Extremities Combined[c]		All Locations[d]	
System Organ Class Preferred Term	0.015% PEP005 Gel (N=274)	Vehicle (N=271)	0.05% PEP005 Gel (N=225)	Vehicle (N=232)	PEP005 Gel (N=499)	Vehicle (N=503)	PEP005 Gel (N=1165)	Vehicle (N=632)
Any AE All Systems	72 (26.3%)	11 (4.1%)	29 (12.9%)	2 (0.9%)	101 (20.2%)	13 (2.6%)	312 (26.8%)	22 (3.5%)
General Disorders and Administration Site Conditions	51 (18.6%)	4 (1.5%)	25 (11.1%)	1 (0.4%)	76 (15.2%)	5 (1.0%)	259 (22.2%)	8 (1.3%)
Application Site Pruritus	22 (8.0%)	3 (1.1%)	18 (8.0%)	0 (0.0%)	40 (8.0%)	3 (0.6%)	125 (10.7%)	4 (0.6%)
Application Site Pain	38 (13.9%)	1 (0.4%)	4 (1.8%)	0 (0.0%)	42 (8.4%)	1 (0.2%)	91 (7.8%)	2 (0.3%)
Application Site Irritation	5 (1.8%)	0 (0.0%)	8 (3.6%)	1 (0.4%)	13 (2.6%)	1 (0.2%)	81 (7.0%)	2 (0.3%)
Application Site Paraesthesia	2 (0.7%)	0 (0.0%)	2 (0.9%)	0 (0.0%)	4 (0.8%)	0 (0.0%)	21 (1.8%)	1 (0.2%)
Application Site Discomfort	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	15 (1.3%)	0 (0.0%)
Application Site Reaction	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (1.2%)	0 (0.0%)
Skin and Subcutaneous Tissue Disorders	7 (2.6%)	0 (0.0%)	5 (2.2%)	0 (0.0%)	12 (2.4%)	0 (0.0%)	23 (2.0%)	2 (0.3%)
Periorbital Oedema	7 (2.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (1.4%)	0 (0.0%)	12 (1.0%)	0 (0.0%)
Nervous System Disorders	5 (1.8%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	5 (1.0%)	1 (0.2%)	23 (2.0%)	1 (0.2%)
Headache	5 (1.8%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	5 (1.0%)	1 (0.2%)	18 (1.5%)	1 (0.2%)
Eye Disorders	7 (2.6%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	7 (1.4%)	1 (0.2%)	22 (1.9%)	1 (0.2%)
Eyelid Oedema	3 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.6%)	0 (0.0%)	9 (0.8%)	0 (0.0%)
Infections and Infestations	7 (2.6%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	7 (1.4%)	1 (0.2%)	18 (1.5%)	1 (0.2%)
Application Site Infection	7 (2.6%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	7 (1.4%)	1 (0.2%)	10 (0.9%)	1 (0.2%)
Investigations	3 (1.1%)	3 (1.1%)	0 (0.0%)	0 (0.0%)	3 (0.6%)	3 (0.6%)	5 (0.4%)	4 (0.6%)

Local Skin Responses (LSR)

Following application of study medication, most PEP005 Gel-treated patients showed an increase in LSR scores relative to baseline, whereas most patients treated with vehicle showed no change from baseline LSR score; approximately 95% of AK patients who received field applications of PEP005 Gel vs. 36% of vehicle-treated patients had a post-treatment increase in LSR score.

The local skin responses are transient and typically occur within 1 day of treatment initiation and peak in intensity up to 1 week following completion of treatment. These effects typically resolve within 2 weeks of treatment initiation for areas treated on the face and scalp and within 4 weeks of treatment initiation for areas treated on the trunk and extremities.

For both treatment locations (face/scalp and trunk/extremities), erythema and flaking/scaling were the most common LSRs, followed by crusting and swelling. Erythema and flaking/scaling were present to some extent prior to application of study medication (reflecting presence of localised irritation at the lesion site); and these persisted with vehicle treatment and worsened following PEP005 Gel treatment. Local reactions assigned a maximum score of 4 (i.e., extending beyond the treatment area) were observed more frequently for erythema than the other LSRs, independent of treatment location. Table 52 gives a summary of patients with a post-baseline LSR score of Grade 4 based on the types of local skin reaction.

Table 52. Summary of Patients with a Maximum Post-baseline Local Skin Response Score in the Controlled Phase 3 Studies by Treatment Location

Local Skin Response	Maximum Grade of 4 Post Baseline defined as follows	Controlled Phase 3 Studies			
		Face/Scalp		Trunk/Extremities	
		0.015% PEP005 Gel (N=274)	Vehicle (N=271)	0.05% PEP005 Gel (N=225)	Vehicle (N=232)
Erythema	Red extending outside treatment area	66(24.1%)	0 (0.0%)	34 (15.1%)	0 (0.0%)
Flaking / Scaling	Scaling extending outside treatment area	25 (9.1%)	0 (0.0%)	18 (8.0%)	0 (0.0%)
Crusting	Crusting extending outside treatment area	16 (5.8%)	0 (0.0%)	8 (3.6%)	0 (0.0%)
Swelling	Marked swelling extending outside treatment area	14 (5.1%)	0 (0.0%)	7 (3.1%)	0 (0.0%)
Vesiculation / Pustulation	Transudate or pustules, with or without vesicles extending outside treatment area	15 (5.5%)	0 (0.0%)	3 (1.3%)	0 (0.0%)
Erosion / Ulceration	Black eschar or ulceration	1 (0.4%)	0 (0.0%)	2 (0.9%)	0 (0.0%)

The applicant has performed a systematic literature review in order to compare the safety of Picato with other available treatment options. Based on the main results, treatment with Imiquomod 5% showed the same types of local skin responses as ingenol, however the percentage of patients experiencing a maximum grade erythema was higher in the imiquimod (31%) as compared to ingenol (24%). The majority of adverse events (96%) after treatment with diclofenac were mild to moderate, indicating a low incidence of severe skin reaction.

Long Term Safety

The long term safety was assessed in studies PEP005-030, PEP005-031, and PEP005-032. These studies followed up patients from earlier treatment studies.

During follow-up, no patient received PEP005 Gel, and 14 patients prematurely discontinued due to: withdrawal of consent (9 patients), protocol violation (2 patients), lost to follow-up (1 patient), investigator decision (1 patient), and inability to return to the study site for the 12-month visit (1 patient).

Over 12 months of follow-up, 3 of the 198 patients had an AE within the selected treatment area that consisted of a mild sun burn, a moderate haematoma, and a mild rash. All 3 AEs occurred approximately 8 to 9 months after the start of follow-up; all events resolved within 2 weeks of onset, and all were considered not related to the study drug received during the prior study.

Of the patients withdrawn from the follow-up studies due to the change in eligibility criteria, one patient had an AE of BCC within the treatment area, which was reported on Day 100 of study PEP005-031. The BCC was graded as moderate and considered by the investigator as not related to study

medication. No action was taken, and at the time of last contact, there was little or no change in the BCC. This event was reclassified as an SAE by the applicant following review of AEs in the clinical database.

Serious adverse event/deaths/other significant events

SAEs

Among the patients who received field applications of PEP005 Gel or vehicle for treatment of AK lesions, SAEs were identified by both the investigator and applicant for 4.2% of patients in the PEP005 Gel group and 3.6% of patients in the vehicle group. Of these SAEs, BCC (occurring in 1.5% of PEP005 Gel-treated patients and 1.1% of vehicle treated patients) and SCC (0.9% of PEP005 Gel-treated patients and 0.8% of vehicle-treated patients) were the most frequently reported for both treatment groups (reflecting both investigator- and applicant-identified events; all serious events of BCC and approximately half of the reported SCCs were reclassified as SAEs by the applicant). Three patients (all treated with PEP005 Gel, 0.05%) had an SAE that was assessed as treatment-related; 1 patient had Bowen's disease, graded as mild and 2 patients had SCC, 1 graded as mild and the other graded as moderate. For all 3 patients, the SAE resolved following excision.

Table 53. Summary of serious adverse events (including both investigator-determined and applicant-determined events)

	Controlled Phase 3 Studies						All Field Application AK Studies	
	Face and Scalp[a]		Trunk and Extremities[b]		Face/Scalp and Trunk/Extremities Combined[c]		All Locations[d]	
System Organ Class Preferred Term	0.015% PEP005 Gel (N=274)	Vehicle (N=271)	0.05% PEP005 Gel (N=225)	Vehicle (N=232)	PEP005 Gel (N=499)	Vehicle (N=503)	PEP005 Gel (N=1165)	Vehicle (N=632)
Serious AEs – All Systems	6 (2.2%)	5 (1.8%)	8 (3.6%)	12 (5.2%)	14 (2.8%)	17 (3.4%)	49 (4.2%)	23 (3.6%)
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	3 (1.1%)	3 (1.1%)	5 (2.2%)	9 (3.9%)	8 (1.6%)	12 (2.4%)	30 (2.6%)	16 (2.5%)
Basal Cell Carcinoma	3 (1.1%)	1 (0.4%)	3 (1.3%)	4 (1.7%)	6 (1.2%)	5 (1.0%)	17 (1.5%)	7 (1.1%)
Squamous Cell Carcinoma	0 (0.0%)	0 (0.0%)	1 (0.4%)	3 (1.3%)	1 (0.2%)	3 (0.6%)	11 (0.9%)	5 (0.8%)
Bowen's Disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Malignant Melanoma	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	1 (0.1%)	1 (0.2%)
Neoplasm Skin	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Basosquamous Carcinoma	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Breast Cancer	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Lymphoma	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Cardiac Disorders	1 (0.4%)	0 (0.0%)	1 (0.4%)	3 (1.3%)	2 (0.4%)	3 (0.6%)	6 (0.5%)	5 (0.8%)
Angina Pectoris	0 (0.0%)	0 (0.0%)	1 (0.4%)	2 (0.9%)	1 (0.2%)	2 (0.4%)	2 (0.2%)	2 (0.3%)
Atrial Fibrillation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	1 (0.2%)
Myocardial Infarction	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.2%)	1 (0.2%)	1 (0.1%)	1 (0.2%)
Aortic Valve Disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Coronary Artery Disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Acute Coronary Syndrome	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)
Injury, Poisoning & Procedural Complications	1 (0.4%)	2 (0.7%)	1 (0.4%)	0 (0.0%)	2 (0.4%)	2 (0.4%)	4 (0.3%)	2 (0.3%)
Cervical Vertebral Fracture	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Meniscus Lesion	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Muscle Strain	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Upper Limb Fracture	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Injury	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Vascular Pseudoaneurysm	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Gastrointestinal Disorders	1 (0.4%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	2 (0.4%)	0 (0.0%)	4 (0.3%)	0 (0.0%)
Abdominal Pain	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Gastrooesophageal Reflux Disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Pancreatitis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Small Intestinal Obstruction	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Musculoskeletal and Connective Tissue Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.3%)	0 (0.0%)
Back Pain	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Muscle Spasms	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Muscular Weakness	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Respiratory, Thoracic and Mediastinal Disorders	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	2 (0.2%)	1 (0.2%)
Chronic Obstructive Pulmonary Disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Hypoxia	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Pulmonary Embolism	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Infections and Infestations	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Campylobacter Infection	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Cellulitis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Vascular Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Aortic Aneurysm	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Hypertension	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
General Disorders & Administration Site Conditions	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Death	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Surgical and Medical Procedures	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Hip Arthroplasty	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	1 (0.1%)	0 (0.0%)

Deaths

Across all studies, there was one death, which occurred in a patient who received PEP005 Gel, 0.005% in study PEP005-015. The patient, a 58-year-old white male, died of coronary artery atherosclerosis and hypertension approximately 4 months after receiving study treatment. The investigator judged the death as not related to study medication.

Laboratory findings

The vast majority of clinical laboratory parameters (haematology and serum chemistry) were within normal limits at the time points measured in the studies. The proportion of patients with normal laboratory values at baseline followed by shifts away from this normal range at later time points was generally similar between treatment groups, with no apparent trend. There were no meaningful shifts or trends in any of the clinical laboratory parameters.

Safety in special populations

The study population had enough exposure to elderly in line with the prevalence of AK. Of the 1165 patients treated with Picato in the actinic keratosis clinical studies conducted with ingenol mebutate gel, 656 patients (56%) were 65 years and older, while 241 patients (21%) were 75 years and older. No overall differences in safety or efficacy were observed between younger and older patients.

Sub-group analyses of two AEs (application site pain and application site pruritus) were performed to assess relation between event and the following demographic and baseline characteristics. geographic location, age category, sex, race, Fitzpatrick skin type, treatment location, histories of cardiovascular disorders, endocrine disorders, allergy or immunologic disorders, gastrointestinal disorders, concentration, regimen (i.e., number of dosing days), prior cryotherapy, or prior therapy with either imiquimod or 5-fluorouracil. Based on these results, there was no association between any of these demographic and baseline characteristics and the occurrence of these two AEs.

Analysis of pooled data from all 4 phase 3 studies showed that patients treated on head with 0.015% PEP005 Gel for 3 days had a higher incidence of application site pain (13.9%, 95% CI: 10.0, 18.5) than patients treated on the trunk or extremities with 0.05% PEP005 Gel for 2 days (2.2%, 95% CI :0.7, 5.1); this effect was not observed for application site pruritus.

Safety related to drug-drug interactions and other interactions

No drug-drug interaction studies were submitted.

Discontinuation due to adverse events

The summary of adverse events that lead to discontinuation of treatment is provided in Table 54.

Table 54. Summary of adverse events leading to discontinuation of treatment

System Organ Class Preferred Term	Controlled Phase 3 Studies						All Field Application AK Studies	
	Face and Scalp[a]		Trunk and Extremities[b]		Face/Scalp and Trunk/Extremities Combined[c]		All Locations[d]	
	0.015% PEP005 Gel (N=274)	Vehicle (N=271)	0.05% PEP005 Gel (N=225)	Vehicle (N=232)	PEP005 Gel (N=499)	Vehicle (N=503)	PEP005 Gel (N=1165)	Vehicle (N=632)
All Systems	3 (1.1%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	4 (0.8%)	0 (0.0%)	37 (3.2%)	0 (0.0%)
General Disorders and Administration Site Conditions	3 (1.1%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	4 (0.8%)	0 (0.0%)	33 (2.8%)	0 (0.0%)
Application Site Irritation	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	16 (1.4%)	0 (0.0%)
Application Site Pain	2 (0.7%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	3 (0.6%)	0 (0.0%)	16 (1.4%)	0 (0.0%)
Application Site Vesicles	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.3%)	0 (0.0%)
Application Site Pruritus	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	3 (0.3%)	0 (0.0%)
Application Site Discomfort	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Application Site Swelling	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Application Site Discharge	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Erosion	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Erythema	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Oedema	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Paraesthesia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Warmth	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Scab	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Facial Pain	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Eye Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.3%)	0 (0.0%)
Eyelid Oedema	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Eye Oedema	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Eye Swelling	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Eyelid Ptosis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Infections and Infestations	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.3%)	0 (0.0%)
Application Site Infection	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Application Site Pustules	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Nasopharyngitis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Urinary Tract Infection	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Nervous System Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Headache	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Respiratory, Thoracic and Mediastinal Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Nasal Congestion	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)
Psychiatric Disorders	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
Insomnia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

The overall patient exposure is considered adequate and is typical of a control clinical trial population of AK. Certain populations like pregnant women, immunosuppressed patients or patients with serious systemic illness have been excluded and suitable precautions/warnings have been included in the SmPC.

Picato gel has a higher incidence of adverse events as compared to the vehicle gel. Most of this is due to occurrence of local skin reactions or application site adverse events, which is associated with the treatment effects of Picato. The most frequently reported adverse reactions are local skin responses including erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation and erosion/ulceration at the application site of Picato. Following the application of Picato, most patients (>95%) experienced one or more local skin responses. The local skin responses are transient and typically occur within 1 day of treatment initiation and peak in intensity up to 1 week following completion of treatment. These effects typically resolve within 2 weeks of treatment initiation for areas treated on the face and scalp and within 4 weeks of treatment initiation for areas treated on the trunk and extremities. It is seen that some form of local skin reaction was universally seen in patients treated with Picato. Based on the available data, these are mostly mild to moderate and recover without any sequel.

Administration of Picato is not recommended until the skin is healed from treatment with any previous medicinal product or surgical treatment. Picato should not be applied to open wounds or damaged skin where the skin barrier is compromised.

From the safety database all the adverse reactions reported in clinical trials have been included in the SmPC.

No interaction studies have been performed. Interactions with systemically absorbed medicinal products are considered minimal as Picato is not absorbed systemically.

Overdosing of Picato could result in an increased incidence of local skin responses. Management of overdose should consist of treatment of clinical symptoms.

A total of 198 patients with complete clearance at day 57 (184 treated with Picato and 14 treated with vehicle) were followed for additionally 12 months. The results did not change the safety profile of Picato.

The applicant has reviewed literature and provided active comparator data with other topical agents. The duration of local skin reactions is generally in line with treatment durations and Picato having the shortest duration (2 days) as compared to other treatments (treatment duration in weeks) has a comparatively more favourable duration of local skin reactions.

No data on re-treatment have been provided by the applicant. Given the universal occurrence of local skin responses, it is important to identify whether patients are willing to be re-treated with Picato gel after having an initial round of treatment. This would give a true reflection on the patient perception of the local skin reactions. Therefore, in order to get most optimal information about re-treatment, the applicant committed to provide data on safety and efficacy of re-treatment in case of recurrent lesions. Study LP0041-22 will evaluate the repeat use for multiple AK on face and scalp and the study report will be available in April 2014.

The main purpose of treatment of AK lesion is to prevent the conversion of the AK lesion to a SCC. An estimate of the risk of conversion to SCC can only be made in long term follow up studies. However, it is noted that the long-term follow up studies submitted by the applicant only followed up patients who successfully responded to treatment in the initial treatment studies. This excluded patients who did not respond and who may be considered to therefore be at a higher risk of conversion to SCC. However it is acknowledged that the treatment duration is for 2 days and the safety data of follow up till 57 days after treatment is complete and does not raise any significant safety concerns. The safety profile showed only local skin responses that resolve completely. The skewing of data is therefore only in relation to the long-term potential safety concern of increasing the risk of conversion to SCC. It is acknowledged that even if the long-term safety data was complete (and included the patients who had not responded) a conclusion on the long-term safety in terms of risk of conversion to SCC would not be possible given the low rates of occurrence of this risk and the confounding due to the back-ground rate of AK conversion to SCC without any application of treatments. In order to explore this low but significant safety concern the applicant committed to conduct a post-approval study (LP0041-63) to compare the cumulative incidence of SCC after treatment with ingenol mebutate and imiquimod cream (a 3-year follow up study). The report of the study will be available in October 2018.

2.6.2. Conclusions on the clinical safety

The overall tolerability and safety profile of Picato is acceptable. The adverse events rates are significantly higher in the Picato group as compared to the vehicle group, but most of these are mild to moderate local skin reactions that generally resolved spontaneously. From the retrospective comparative safety data provided by the applicant where they extensively reviewed literature for the safety profiles of other topical agents in the treatment of AK, it is seen that the incidence and severity

of local skin responses after treatment with Picato is comparable to at least one of the other topical agents.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The applicant submitted a risk management plan

Table 55. Summary of the risk management plan

Safety concerns	Proposed Pharmacovigilance activities	Proposed risk minimisation activities
Important identified risks		
Local Skin Responses	Routine pharmacovigilance activities	A warning is included in the label describing the occurrence and clinical nature of Local skin responses: EU SmPC Section 4.4: Local skin responses such as erythema, flaking/scaling, and crusting should be expected to occur after cutaneous application of Picato (see section 4.8). Localised skin responses are transient and typically occur within 1 day of treatment initiation and peak in intensity up to 1 week following completion of treatment. Localised skin responses typically resolve within 2 weeks of treatment initiation when treating areas on the face and scalp and within 4 weeks of treatment initiation when treating areas on the trunk and extremities. Treatment effect may not be adequately assessed until resolution of local skin responses. EU PIL section 2: You should expect to get local skin responses after treatment with this medicine (see section 4). Contact your doctor if these local skin responses get severe. Further, the label gives the instructions for proper use of the product in order to minimise additional risk. *EU SmPC Section 4.4: Administration of Picato is not recommended until the skin is healed from treatment with any previous medicinal product or surgical treatment. Picato should not be applied* to open wounds or damaged skin where the skin barrier is compromised. *EU PIL section 2:Make sure that your skin has healed from any other treatments or surgery before using this medicine. Do not apply Picato on open-wounds or damaged skin.* LSRs are presented in the ADR table in the label. *EU SmPC section 4.8:* *Application site erosion* *Application site vesicles* *Application site swelling* *Application site exfoliation* *Application site scab*

		Application site erythema *Application site pustules* Application site ulcer *EU PIL section 4:* *Some of the outer layer of your skin may wear away (erosion)* *Blisters (vesicles, pustules)* *Swelling* *Peeling (exfoliation)* *Scabs* Redness due to widening of the small blood vessels (erythema)
Eye Disorders	Routine pharmacovigilance activities	The label gives detailed instructions for proper use of the product in order to minimise any risks with the product. EU SmPC Section 4.4: Contact with the eyes should be avoided. If accidental exposure occurs, the eyes should be flushed immediately with large amounts of water, and the patient should seek medical care as soon as possible. Eye disorders such as eye pain, eyelid oedema and periorbital oedema should be expected to occur after accidental eye exposure of Picato (see section 4.8). *EU PIL section 2: Avoid contact with eyes. In the event of accidental contact, remove the gel by rinsing with plenty of water and contact your doctor as soon as possible.* LSRs are presented in the ADR table in the label. *EU SmPC Section 4.8:* *Eye pain* *Eyelid oedema* *Periorbital oedema* *EU PIL section 2:* *Swelling of the area around the eye (periorbital oedema)* *Swelling (oedema) of your eye lid* *Eye pain*
Important potential risks		
AK to SCC progression	Routine pharmacovigilance activities. The pharmacovigilance system in place ensures early detection of the potential risk. Additional pharmacovigilance activities include a planned clinical trial to investigate the impact of treatment with ingenol mebutate gel on progression of AK to SCC.	A description has been included in the EU SmPC describing the observed incidence of SCC following ingenol mebutate gel treatment after 12 month follow up: *EU SmPC section 5.1: Risk of progression to squamous cell carcinoma.* *At end of study (day 57), the rate of squamous cell carcinoma (SCC) reported in the treatment area was comparable in patients treated with ingenol mebutate gel (0.3%, 3 of 1165 patients) and in vehicle treated patients (0.3%, 2 of 632 patients) in the actinic keratosis clinical studies conducted with ingenol mebutate gel.* *SCC in the treatment area was reported in no patients (0 of 184 patients previously treated with ingenol mebutate gel) in the three prospective, observational long term 1 year follow-up studies.*
Overdose after treatment at multiple locations	Routine pharmacovigilance activities.	The label gives the instructions for proper use of the product in order to minimise additional risk: *EU SmPC section 4.2: Method of administration* The content of one tube covers a treatment area of 25 cm2 (e.g. 5 cm x 5 cm). The content of the tube should be applied to one treatment area of 25 cm2. *EU PIL section 3: Apply the content of one tube to one area of 25 cm2 (e.g. 5 cm x 5 cm).*

Important Missing Information		
Retreatment of patients	Routine pharmacovigilance activities. Additional pharmacovigilance activities include a trial to assess the safety profile of patients retreated with ingenol mebutate gel.	In EU the proposed label describes that each tube is a unit dose for one treatment area only and should be discarded after use: EU SmPC section 4.2: The tube is for single-use only and the tube should be discarded after use (see section 6.6). *EU PIL section 2: This medicine is intended to treat one area of 25 cm2 for three/two days. This should be strictly adhered to.* *EU PIL section 5: For single use only. Do not re-use the tubes once opened.*
Immunocompromised patients	Routine pharmacovigilance activities.	The label describes that that there is no clinical experience in immunocompromised patients.. *EU SmPC section 4.2: Clinical data on treatment in immunocompromised patients is not available, but systemic risks are not expected since ingenol mebutate is not absorbed systemically.*
Phototoxicity	Routine pharmacovigilance activities	Routine risk minimisation activities including instructions in label of proper use of the product. *EU SmPC section 4.4: Studies have been conducted to assess the effects of UV irradiation of the skin following single and multiple applications of ingenol mebutate gel, 100 mcg/g. Ingenol mebutate gel did not demonstrate any potential for photo irritation or photo allergic effects. However, due to the nature of the disease, excessive exposure to sunlight (including sunlamps and tanning beds) should be avoided or minimised.* *EU PIL section 2: Avoid sunlight as much as possible (including sunlamps and tanning beds).*
Hypersensitivity	Routine pharmacovigilance activities	Routine risk minimisation activities including instructions in label for proper use of the product. *EU SmPC section 4.3: Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.* *EU PIL section 2: If you are allergic to ingenol mebutate or any of the other ingredients of this medicine (listed in section 6).*
Hypopigmentation	Routine pharmacovigilance activities	The label gives the instructions for proper use of the product in order to minimise additional risk of Hypopigmentation: *EU SmPC section 4.4: Due to the nature of the disease, excessive exposure to sunlight (including sunlamps and tanning beds) should be avoided or minimised.* *EU PIL section 2: Avoid sunlight as much as possible (including sunlamps and tanning beds).*

The CHMP, having considered the data submitted, was of the opinion that the below pharmacovigilance activities in addition to the use of routine pharmacovigilance are needed to investigate further some of the safety concerns:

Table 56: Additional pharmacovigilance activities

Description	Due date
The MAH should conduct a study (LP0041-63) in order to investigate the Risk of squamous cell carcinoma on skin areas treated with ingenol mebutate gel 0.015% and imiquimod 5% cream. The MAH should submit the full protocol for review before the start of the study and provide an interim update for the clinical study at an appropriate time-point.	October 2018
The MAH should conduct a study (LP0041-22) with Ingenol mebutate gel 0.015% in order to investigate the repeat use for multiple actinic keratosis on face and scalp.	April 2014

No additional risk minimisation activities were required beyond those included in the product information.

2.8. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

Picato gel has been shown to lead to a clinically and statistically significant increase in complete clearance of AK lesions from the treated area as compared to vehicle. The secondary endpoints of partial clinical clearance of AK lesions and a median percent reduction in the number of AK lesions after treatment with Picato has been demonstrated in robust phase III studies. The patient reported outcomes were also in line with these observations.

Uncertainty in the knowledge about the beneficial effects

The lack of data to establish concordance between the clinical clearance and histological confirmation after treatment with Picato makes it difficult to confirm the treatment effect size. In this context however, the following points are noted: The treatment effect size as measured by clinical clearance is highly significant as compared to placebo and therefore this lack of confirmation by histology is not considered a major uncertainty. Both the short-term and long-term clearance rates of AK lesions after treatment with Picato are comparable with the clearance rates (from published literature) of at least one other licensed treatment for AK. The study on biological effects of Picato shows clearance of AK lesions as assessed by reflectance confocal microscopy. This data provides reassurance on the drug effects on the underlying pathology. Further there is histopathological evidence to show that Picato affects all the relevant layers of the skin (necrosis is seen in all layers of the epidermis). In published literature, there is reasonably good concordance between histological clearance of AKs and clinical diagnosis of clearance, for example in study by Szeimies et al 2004. Based on this concordance, in clinical practice, AK lesions are diagnosed and managed routinely by clinical diagnosis. Finally, the CHMP recommended that the applicant conducts a study (LP0041-62) on histological confirmation of

clinical clearance of AK following treatment with Picato gel in order to provide concordance between clinical assessments and histological confirmation of clearance of AK lesion.

The dossier also lacks prospective active comparator trial. However, robust and detailed comparisons of efficacy of Picato with other active treatments based on literature search have been provided. Although this data has limitations of any retrospective comparison to published studies it suggests that the rate of complete clearance of AK lesions and the rate of recurrence of AK lesions are comparable to at least one other topical agent approved for the treatment of AK. In the absence of prospective controlled trial data, an accurate perspective on the relative efficacy of Picato gel in relation to available treatment options cannot be concluded. However this is not considered a major uncertainty considering the novel mechanism of action, the acceptable topical safety profile and the comparable efficacy and safety profile of Picato with other topical agents available for AK. In addition, the applicant committed to conduct an active comparator study (LP0041-63, 3-year follow up study) to compare the cumulative incidence of SCC after treatment with ingenol mebutate and imiquimod cream.

Risks

Unfavourable effects

The most frequently reported adverse reactions assessed by investigators are local skin responses including erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation and erosion/ulceration at the application site of ingenol mebutate gel. Following the application of Picato, most patients (>95%) experienced one or more LSRs. The LSRs are transient and typically occur within 1 day of treatment initiation and peak in intensity up to 1 week following completion of treatment. These effects typically resolve within 2 weeks of treatment initiation for areas treated on the face and scalp and within 4 weeks of treatment initiation for areas treated on the trunk and extremities.

Retrospective comparison of the safety profile of Picato with other active topical treatments of AK has been done by extensive literature search. Such a comparison is fraught with difficulties in drawing any conclusions, however this does show that the incidence and severity of local skin reactions after treatment with Picato is comparable to at least one other approved topical agent in the treatment of AK lesions. The duration of skin responses is generally in line with the duration of treatment and therefore is lower with Picato as compared to some other topical agents.

Uncertainty in the knowledge about the unfavourable effects

Based on the long-term safety data, only patients who responded with complete clinical clearance after initial treatment were followed up. Patients who did not respond to Picato gel and who may potentially be at a higher risk of occurrence of SCC were not followed up. The available data does not allow a conclusion on the long-term safety of Picato gel. However it is acknowledged that even if the long-term safety data from patients who did not respond were available it would not have been possible to draw conclusions on the potential risk of increasing incidence due to the low rates of occurrence of this event. A long term study is required to make any meaningful conclusions on this long-term potential safety concern. The applicant committed to conduct a 3-year follow up active comparator study to compare the cumulative incidence of SCC after treatment with Picato and imiquimod cream.

No data on re-treatment have been provided by the applicant. Given the universal occurrence of local skin responses, it is important to identify whether patients are willing to be re-treated with Picato gel after having an initial round of treatment. This would give a true reflection on the patient perception of the local skin reactions. Therefore, in order to get most optimal information about re-treatment, the applicant committed to provide data on safety and efficacy of re-treatment in case of recurrent lesions.

Study LP0041-22 will evaluate the repeat use for multiple AK on face and scalp and the study report will be available in April 2014.

Balance

Importance of favourable and unfavourable effects

Complete AK clearance is a relevant clinical endpoint. The results are considered to be robust, consistent, and of clinical relevance. Results in key secondary endpoints supported the observed improvement in complete AK clearance and the patient reported outcomes were also in line with these observations.

Picato has an acceptable safety profile and the reported adverse events are largely local skin reactions that resolve completely. Though the incidence of local skin reactions is very high, in that almost all treated patients get these reactions, this is comparable to the safety profile of at least one other topical agent approved in the treatment of AK lesion.

Benefit-risk balance

Overall, the efficacy of ingenol mebutate has been demonstrated. The development of Picato, as a topical cutaneous gel for AK, to be applied for a short duration of 2 or 3 days and which can be self-applied by the patients is a major therapeutic advantage over other active treatments for AK.

The safety profile is acceptable and the reported adverse events are largely local skin reactions that generally resolve completely.

Discussion on the benefit-risk assessment

The benefit-risk balance for ingenol mebutate for the cutaneous treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis in adults is considered positive. The favourable effects outweigh the negative effects and moreover Picato has the major therapeutic advantages of short treatment duration and can be 'self-applied' by the patients as compared to other active treatments for AK currently available.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers that the risk-benefit balance of Picato in the cutaneous treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis in adults is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Conditions and requirements of the Marketing Authorisation

Risk Management System

The MAH must ensure that the system of pharmacovigilance, presented in Module 1.8.1 of the marketing authorisation, is in place and functioning before and whilst the product is on the market.

The MAH shall perform the pharmacovigilance activities detailed in the Pharmacovigilance Plan as agreed in version 3 of the Risk Management Plan presented in Module 1.8.2. of the Marketing Authorisation and any subsequent updates of the RMP agreed by the CHMP.

As per the CHMP Guideline on Risk Management Systems for medicinal products for human use, the updated RMP should be submitted at the same time as the next Periodic Safety Update Report (PSUR).

In addition, an updated RMP should be submitted:

- When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan or risk minimisation activities
- Within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached
- at the request of the EMA

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Not applicable

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that ingenol mebutate is qualified as a new active substance.

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