SYNOPSIS

Name of Sponsor/Company: LEO Pharma A/S

Name of Finished Product: Ingenol mebutate 0.05%

Name of Active Ingredient: Ingenol mebutate

Title of trial: PEP005 Gel – Biological Effects in Actinic Keratosis assessed by Reflectance Confocal Microscopy. A phase 1, two-stage, single-centre, open label, within- and between-subject comparison trial to explore the biological effects of PEP005 (ingenol mebutate) Gel, 0.05%, applied once daily for 2 consecutive days in subjects with actinic keratosis on the upper extremity. A single-arm first stage followed by a two-arm, parallel group, randomized, placebo (vehicle) -controlled second stage.

Investigators: Prof. Dr. med. [Redacted], Germany.

Trial centre: The trial was conducted at one centre in [Redacted] Germany

Publication (reference): None

Trial period (years)
date of first enrolment: 27-Sep-2011
date of last completed: 22-May-2012

Objectives:
Primary: To compare the inflammation and necrosis in clinical AK lesions, subclinical AK lesions and normal skin following treatment with ingenol mebutate gel, 0.05% administered for two consecutive days as assessed by RCM.
Secondary:
- To compare inflammation and necrosis in clinical AK lesions, subclinical AK lesions and normal skin between subjects treated with ingenol mebutate gel (formerly known as PEP005 Gel), 0.05% and Placebo (vehicle gel) as assessed by RCM
- To compare inflammation in clinical AK lesions, subclinical AK lesions and normal skin following treatment with ingenol mebutate gel*, 0.05% and Placebo (vehicle gel) as assessed by visual inspection
- To describe the changes in pigmentation, solar elastosis and fibrosis in clinical AK lesions, subclinical AK lesions and normal skin in response to the treatment with ingenol mebutate gel, 0.05% as assessed by RCM
- To confirm the RCM diagnosis of subclinical AK lesions and RCM grading of inflammation and necrosis against histology
- To determine AK clearance following treatment with ingenol mebutate gel, 0.05% using RCM

Methodology:
This open label, two-stage, single centre, within- and between subject comparison trial comprised 24 subjects, 8 in Stage 1 and 16 in Stage 2. The two stages consisted of a single-arm first stage followed by a two-arm, parallel group, randomized, placebo (vehicle) -controlled second stage. All subjects in Stage 1 received ingenol mebutate gel. The subjects in Stage 2 received either ingenol mebutate gel or placebo (vehicle gel) randomised 1:1. Investigational drug was applied by the physician in the clinic daily for two consecutive days (study days 1 and 2) on two selected treatment areas suitable for
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### Individual Trial Table
Referring to Part of the Dossier

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### RCM
AK Treatment Area (A contiguous area of 25 cm² of skin on the upper extremity (incl. dorsum manus) and Normal Skin Treatment Area (A contiguous area of 25 cm² of normal skin that had no or only minimal sun-damage from the inner upper arm).

A biopsy was conducted on a clinical and subclinical lesion within the AK Treatment Area at Day 3 and RCM was done up to 5 times on the same spots of skin throughout the trial.

### Number of subjects (planned and analysed):
The trial population was planned to include 24 subjects. All 24 subjects were included in the full analysis set and the safety analysis set.

### Diagnosis and main criteria for inclusion:
1. Male or female subjects at least 18 years of age
2. Subjects with at least 3 clinically typical, visible and discrete AK lesions and 3 subclinical AK lesions within a contiguous 25 cm² area (AK Treatment Area) on the upper extremitiy suitable for RCM.
3. Subjects must have a 25 cm² area of normal skin on the inner upper arm suitable for RCM
4. Female subjects must be of either:
   - Non-childbearing potential, post-menopausal, or have a confirmed clinical history of sterility (e.g. the subject is without a uterus) or,
   - Childbearing potential, provided there is a confirmed negative urine pregnancy test prior to exposure, to rule out pregnancy.
5. Female subjects of childbearing potential must be willing to consent to using high effective methods of contraception (Pearl index < 1%) at study entry and for the duration of the trial participation.

### Test product, dose and mode of administration, batch number:
Ingenol mebutate gel, 0.05%, topical administration, batch number [redacted]

### Duration of treatment:
Each subject received two ingenol mebutate gel or vehicle gel applications; once daily for two consecutive days

### Reference therapy, dose and mode of administration, batch number:
Placebo (vehicle gel), topical administration, batch number [redacted]

### Criteria for evaluation:
**Pharmacodynamic evaluation:**
The primary outcome measures were:
1. Change from baseline in the degree of infiltration of the epidermis by inflammatory cells following treatment with ingenol mebutate gel, 0.05% as assessed by RCM
2. Change from baseline in degree of infiltration of the dermis by inflammatory cells following treatment with ingenol mebutate gel, 0.05% as assessed by RCM
3. Change from baseline in the degree of necrosis in the epidermis following treatment with ingenol mebutate gel, 0.05% as assessed by RCM
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4. Change from baseline in the degree of necrosis in the dermis following treatment with ingenol mebutate gel, 0.05% as assessed by RCM

The secondary outcome measures were:
1. Change from baseline in the degree of necrosis and infiltration of the epidermis by inflammatory cells as assessed by RCM for subjects in Stage 2
2. Change from baseline in the degree of necrosis and infiltration of the dermis by inflammatory cells as assessed by RCM for subjects in Stage 2
3. Change from baseline in RCM spot reaction as assessed by visual inspection on a 5-point scale for subjects in Stage 2
4. Description of change from baseline in pigmentation, solar elastosis and fibrosis as assessed by RCM following treatment with ingenol mebutate gel, 0.05%
5. Concordance of AK diagnosis, inflammation and necrosis grading as assessed by RCM and histology for all subjects
6. Complete clearance of clinical AK lesions and subclinical AK lesions as defined through RCM following treatment with ingenol mebutate gel, 0.05%

Safety:
1. Reported Adverse Events and their seriousness, intensity and causality
2. Evaluation of vital signs, reasons for withdrawal and withdrawal rate as well as local skin response

Statistical methods:
There was no formal statistical hypothesis to be evaluated. Trial results were summarised using descriptive statistics.

SUMMARY - CONCLUSIONS

PHARMACODYNAMIC RESULTS:

Preliminary analysis of the first 8 patients showed that the depth of resolution of RCM was decreased for some days after treatment with ingenol mebutate gel. It was not possible to extract meaningful data from the dermis by RCM on Day 2, 3 and 8. Therefore infiltration and necrosis in the dermis as evaluated by RCM was not analysed.

The clinical RCM spot reaction assessment was developed for the present study however its implementation was not successful. Therefore this endpoint is not presented.

- In the epidermis, after treatment with ingenol mebutate gel an inflammatory response was observed by RCM in both normal skin and in the clinical AKs and subclinical AKs peaking at Day 3. Hereafter it decreased and was back to baseline levels at the end of the trial (Day 57). The infiltration tended to be milder and had a quicker recovery in the normal skin compared to clinical and subclinical AKs.
- In the epidermis, after treatment with ingenol mebutate gel necrosis was observed by RCM in both normal skin and in the clinical and subclinical AKs peaking at Day 3. Hereafter the degree of necrosis diminished and baseline levels were rapidly reached at Day 8 for normal skin and by the end of trial for clinical and subclinical AKs. Compared to clinical AK lesions, the necrosis in normal skin was milder and the subclinical lesions showed an intermediate response.
- The effect of gel vehicle on the infiltration of epidermis by inflammatory cells as well as on necrosis was mini-
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- Pigmentation as assessed by RCM tended to decrease after treatment with ingenol mebutate gel while fibrosis remained unchanged. Solar elastosis did not change for normal skin but decreased for both the clinical and sub-clinical AK lesions.
- Clinical assessment showed that 14 of 32 ingenol mebutate gel treated AKs and 1 out of 16 vehicle treated AKs were cleared at the end of the trial.
- Assessment of the AK lesions by RCM after ingenol mebutate treatment showed 11 out of 32 lesions cleared (primary assessor) and 9 out of 32 lesions cleared (secondary assessor) at the end of the trial. 23 of 32 subclinical AKs (primary assessor) and 18 of 32 subclinical AKs (secondary assessor) showed typical honeycomb pattern and were hence completely cleared by treatment with ingenol mebutate gel.
- Histological and RCM assessments of necrosis were (except in one case) either identical or assessed as being more severe by RCM.

**SAFETY RESULTS:**
- 16 subjects treated with ingenol mebutate gel had 39 AEs of which 31 events were localised application site disorders (e.g. pain, burning sensation, pruritus) related to trial treatment.
- None of the subjects withdrew from trial or treatment due to AEs.
- One subject had two simultaneous events of severe pruritus (one inside AK treatment area and one inside normal skin treatment area). All other events were mild or moderately intense.
- One SAE (hypertensive crisis) unrelated to treatment was reported.
- Local skin responses were dominated by erythema, flaking/scaling and vesiculation. The skin reactions peaked at Day 3 or Day 8 and were resolved at Day 57. The AK treatment area tended to show a stronger response to treatment than the normal skin treatment area.

**CONCLUSION:**
In the present trial in subjects with AK on the upper extremity, treatment with ingenol mebutate gel induced inflammatory response and necrosis in the epidermis peaking the day after last treatment. Compared to clinical AK lesions the response in normal skin was milder and had a quicker recovery.
Ingenol mebutate gel was generally well tolerated with no unexpected signs of toxicity. The local skin responses were dominated by erythema, flaking/scaling and vesiculation peaking Day 3 or Day 8 and recovered by the end of the trial. In accordance with the more intense local inflammatory response and necrosis in the clinical and subclinical lesions, also the visible skin reactions tended to be more pronounced in the AK treatment area than in the normal skin treatment area.

**Date of the Report:** 26-Sep-2012