### SYNOPSIS

**Name of Sponsor/Company:** LEO Pharma A/S  
**Name of Finished Product:** Ingenol mebutate gel, 0.05%  
**Name of Active Ingredient:** Ingenol mebutate

**Title of trial:** PEP005-Gel – Biological Effects in Actinic Keratosis assessed by histology. A Phase 1, single-centre, open-label, within-subject comparison trial to explore the biological effects of PEP005 (ingenol mebutate) Gel, 0.05%, applied once daily for two consecutive days in patients with actinic keratosis on the upper extremity.

**Investigators:**  
Prof. Dr. Germany

**Trial centre:** The trial was conducted at one centre in Germany

**Publication (reference):** None

**Trial period (years):**  
- **date of first enrolment:** 12-Sep-2011  
- **date of last completed:** 03-May-2012

**Phase of development:** Phase 1

**Objectives:**  
**Primary:** To explore the biological effects following treatment with ingenol mebutate gel, 0.05% administered for two consecutive days.  
**Secondary:** To compare the skin response in normal skin and AK lesions after treatment with ingenol mebutate gel 0.05% administered for two consecutive days

**Methodology:**  
This open label, single centre, within subject comparison trial comprised 27 subjects. Eligible subjects had ingenol mebutate gel applied by the physician in the clinic daily for two consecutive days (study days 1 and 2). The investigational product was applied on two selected treatment areas: AK Treatment Area (A contiguous area of 25 cm² of skin on the upper extremity (incl. dorsum manus) that contained 2 to 5 AK lesions. Additionally there was to be at least one AK lesion located within 1 to 5 cm outside of the selected AK Treatment Area) 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). Biopsies from the two selected treatment areas were conducted at Day 1, 2 and 3. Subsequent follow-up visits were conducted on Day 8 and Day 29.

### Table:

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**Number of subjects (planned and analysed):**  
The trial population was planned to include 27 subjects. One subject was excluded from the full analysis set due to an incorrect diagnosis (Stucco Keratosis, not Actinic Keratosis). The subject was included in the safety analysis set.

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**Diagnosis and main criteria for inclusion:**

1. Male or female patients at least 18 years of age
2. Patients with 2 to 5 clinically typical, visible and discrete AK lesions within a contiguous 25 cm² area (AK Treatment Area) on the upper extremity; with one additional AK lesion located 1 to 5 cm from the AK Treatment Area
3. Patients with a 25 cm² area of normal skin (Normal Skin Treatment Area) on the upper inner arm suitable for treatment and biopsy
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 CBC-C/Jun-2012.

**Duration of treatment:**
Each subject received two Ingenol mebutate gel applications; once daily for two consecutive days

**Reference therapy, dose and mode of administration, batch number:**
None

**Criteria for evaluation:**
Pharmacodynamic evaluation

The primary outcome measures were:
1. Degree of skin infiltration of leukocytes in haematoxylin & eosin-stained sections assessed on a scale from 0 to 3 in the AK biopsies from baseline, Day 2, and Day 3
2. Degree of necrosis of the epidermis and dermis in haematoxylin & eosin-stained sections assessed on a scale from 0 to 3 in the AK biopsies from baseline, Day 2, and Day 3

The secondary outcome measures were:
1. Degree of skin infiltration of leukocytes in haematoxylin & eosin-stained sections assessed on a scale from 0 to 3 in the normal skin biopsies from day 3 compared to base-line and to AK lesion biopsies
2. Degree of necrosis of the epidermis and dermis in haematoxylin & eosin-stained sections assessed on a scale from 0 to 3 in the normal skin biopsies from day 3 compared to base-line and to AK lesion biopsies
3. Degree of haemorrhage assessed by histology (binary outcome present/absent) in the Normal skin and AK lesion biopsies at all time points
4. Characterisation of immune cells such as T and B lymphocytes, polymorphonuclear cells, mast cells and antigen presenting cells in the Normal skin and AK lesion biopsies
5. Degree of apoptosis in the Normal skin and AK lesion biopsies
6. Degree of vascular endothelium activation in the Normal skin and AK lesion biopsies
7. Changes in mRNA and miRNA expression in the Normal skin and AK lesion biopsies
8. Expression of drug transporters in the Normal skin and AK lesion biopsies (P-gp)

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:
- The degree of skin infiltration by leukocytes increased in epidermis and dermis of both normal skin and AK lesions after treatment with ingenol mebutate gel, Figure 1
- The degree of necrosis was enhanced in epidermis of both normal skin and AK lesions after treatment. Necrosis was not observed in the dermis of either skin types after treatment except for a few of the 26 subjects, Figure 1
- Haemorrhage (described as discrete extravasation of erythrocytes) was present in the epidermis of 10 AK and 2 normal skin biopsies and in the dermis of around half of normal skin and AK lesion biopsies after treatment
- At baseline, the dermis of the AK lesions was infiltrated by leukocytes, notably T lymphocytes, CD68 positive macrophages/histiocytes and CD1a positive antigen-presenting cells (22 to 24 positive of 26 biopsies) and to a lesser extent by B lymphocytes (13 positive biopsies)
- In the epidermis, treatment with ingenol mebutate induced an immune response in both normal skin and AK lesions dominated by neutrophils, CD4 and CD8 positive T lymphocytes, and CD68 positive macrophages/histiocytes
- In the dermis, treatment with ingenol mebutate gel increased the scores of neutrophils, T lymphocytes, CD68 positive macrophages/histiocytes, mast cells and B lymphocytes in both normal skin and AK lesions
- An increased number of cells staining positive in the cc3 and TUNEL assays were seen in both epidermis and dermis at baseline in AK lesions. A further increase in cc3 and TUNEL was observed in both normal skin and AK lesions after treatment
- Normal skin showed activation of the vascular endothelium in dermis as evidenced by increased ICAM-1 expression after treatment with ingenol mebutate gel, whereas vascular endothelium was already activated at baseline in AK lesions and did not become further activated after ingenol mebutate treatment
- Gene expression profiles of skin biopsies from the treated areas were suggestive of changes in epidermal compartments, inflammatory responses and response to wounding, in agreement with the histology assessments.
SAFETY RESULTS:
- Overall, 67% of subjects had adverse events during the trial. Adverse events related to trial treatment were observed in 63% of subjects (17 of 27). These events include pruritus reported by 10 subjects (37%), burning sensation reported by 5 subjects (18.5%) and pain reported by 3 subjects (11.1%), all inside the treatment area. None of the reported events were severe or serious and none led to withdrawal from the trial.
- Local skin responses (LSRs) were dominated by erythema and to a lesser extent flaking/scaling, swelling and vesculation/pustulation. The mean LSR composite score peaked at Day 3 for normal skin (score 6.6) and Day 3 and 8 for AK skin (score 6.4) and was almost normalised at Day 29 (end of trial).

CONCLUSION:
In the present trial in subjects with AK on the upper extremity, treatment with ingenol mebutate gel induced epidermal necrosis in both AK lesions and normal skin as assessed by histology, whereas necrosis was infrequently observed in the dermis. An inflammatory response was observed in both normal skin and AK lesions. In normal skin a more pronounced
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change in expression profile was evident. The genes and miRNAs expressed in AK lesions and normal skin reflected changes in epidermal compartment, immune responses and wound healing. Ingenol mebutate gel was generally well tolerated with no unexpected signs of toxicity. The local skin responses were dominated by erythema and to a lesser extent flaking/scaling, swelling and vesiculation/pustulation. The skin reactions peaked at Day 3 for normal skin, at Day 3 and Day 8 for AK skin and was almost normalised by end of trial at Day 29.

23-Aug-2012