Clinical Trial Report Synopsis

Biological effects of LEO 43204 in actinic keratosis assessed by histopathology

Design of trial:

Phase 1, multi-centre, open label, within-subject comparison trial to explore the biological effects of LEO 43204 Gel, 0.037%, applied once daily for 3 consecutive days in two groups of subjects with actinic keratosis on the arm including back of hand or scalp.

The clinical trial, including the archival of essential documents, was conducted in compliance with the clinical trial protocol, GCP, and the applicable regulatory requirement(s).
Clinical Trial Report Synopsis Statement

Approval Statement, LEO Pharma A/S

The following persons have approved this clinical trial report synopsis using electronic signatures as presented on the last page of this document:

PPD, M.Sc. Stat
Biostatistics Lead
Global Clinical Operations

PPD, MD, Ph.d
Medical Lead
Medical Science and Safety

Approval Statement, Coordinating Investigator

The coordinating investigator approves the clinical trial report synopsis by manually signing the International Coordinating Investigator Clinical Trial Report Approval Form, which is a separate document adjoined to the clinical trial report.

The following person has approved this clinical trial report synopsis:

Daniel Siegel, MD
Coordinating investigator
**Trial Registration Number**
NCT02600598

**Title of Trial**
Biological effects of LEO 43204 in actinic keratosis assessed by histopathology.

**Investigator**
Daniel Siegel M.D., Long Island Skin Cancer and Dermatology Surgery United States (US), was appointed as signatory investigator.

**Trial Centres**
This trial was conducted at 2 US centres and coordinated at the Long Island Skin Cancer and Dermatology Surgery.

**Publications**
None at the time of the final clinical trial report.

**Clinical Trial Period**
| Date of First Subject First Visit: 25-Jul-2016 | Development Phase |
| Date of Last Subject Last Visit: 16-Jan-2017 | Phase 1 |

**Objectives**
Primary objective:
To investigate the biological response to LEO 43204 Gel, 0.037% (investigational medicinal product [IMP]) in actinic keratosis (AK) lesions at the following, separate time points for subjects in Groups 1 and 2:
Group 1: shortly after the first dose (Day 1, 4 hours after application of IMP and after the skin had healed [Day 57]).
Group 2: during the peak of the local skin response (on Days 4 and 8).
Secondary objective:
To investigate the biological response to LEO 43204 Gel, 0.037% after the treated skin had healed (Day 57) compared with normal skin, for subjects in Group 1 only.

**Methodology**
This was a phase 1, multi-centre, open label, within-subject comparison trial.

**Number of Subjects Planned and Analysed**
It was planned to include 24 subjects; 25 subjects were enrolled including one screen failure. 24 subjects were allocated to 2 biopsy groups of 12 subjects each (Group 1 filled first and then Group 2); all were treated with IMP and analysed.

**Diagnosis and Main Criteria for Inclusion**
1. Informed consent form signed and dated prior to any trial related activities.
2. At least 5 non-keratotic, clinically typical, visible and discrete AK lesions within an affected 250 cm² area on the arm including back of hand or similar within 100-250 cm² on the scalp. At least 2 of the 5 AK lesions must measure ≥4 mm.
3. One additional 4mm AK lesion located in a non-treated area on the same arm or on the contralateral arm when using the arm including back of hand as treatment area or on the scalp when using scalp as treatment area.
4. An area of normal skin in close proximity to the treatment area or on the contralateral arm when using the arm including back of hand or on the scalp when using the scalp as treatment area.
5. Age ≥18 years.
6. Agreement from the subject to allow photographs of the selected treatment area to be taken and used as part of the trial data package.
7. Ability to follow trial instructions and likely to comply with all trial requirements.
8. Women of childbearing potential* had to be confirmed not pregnant by a negative pregnancy test prior to trial treatment.

*Women were considered of childbearing potential unless they had been post-menopausal for ≥1 year or had a confirmed clinical history of sterility (for example, hysterectomy or tubal litigation).

Women of childbearing potential had to be willing to use effective contraception from trial enrolment to completion. Effective contraception was defined as:
- Abstinence (when in line with the preferred and usual lifestyle of the subject).
- Vasectomised partner (given that the subject was monogamous).
- Intrauterine device.
- Double barrier method defined as 2 distinct barrier methods.
- Hormonal contraceptive (oral hormonal birth control, oestrogenic vaginal ring, percutaneous contraceptive patches, implants and injectable) for ≥1 menstrual cycle prior to enrolment.
**Test Product, Dose and Mode of Administration, Batch Number**

LEO 43204 Gel, 0.037%, administered topically by trial site staff and applied evenly as a thin and wet layer on the treatment area (250 cm² of skin on the arm including back of hand or 100-250 cm² of skin on the scalp); daily maximum 2 unit dose tubes.


**Duration of Treatment**

Once daily for 3 consecutive days approximately at the same time of the day.

**Reference Product, Dose and Mode of Administration, Batch Number**

No reference product was included.

**Criteria for Evaluation**

**Primary endpoint:**

Number of CD3+ T-lymphocytes assessed by immunohistochemical staining of biopsies from AK lesions collected at:

- Baseline and 56 days after first treatments (Group 1).
- Baseline, Day 4 and Day 8 (Group 2).

**Secondary endpoints:**

Number of infiltrating cells assessed by immunohistochemical staining of biopsies from normal skin and AK lesions, including:

- CD3+ T-lymphocytes (presented with primary endpoint).
- CD8+ T-lymphocytes.
- CD11c+ cells (inflammatory dendritic cells).
- DC-LAMP+ cells (mature dendritic cells).
- Langerin+ cells (Langerhans cells).
- CD163+ cells (macrophages).
- Neutrophil elastase (NE)+ cells (neutrophils).

Expression of inflammatory and skin matrix modulation markers assessed by:

- Immunohistochemistry
- RNA expression.

Expression of ICAM-1 (marker of vascular endothelium activation) assessed by immunohistochemistry as area of section with positive staining.

Expression of Ki-67 and K16 (markers of cell proliferation and differentiation) assessed by immunohistochemistry as area of section with positive staining.

Expression of cleaved caspase-3 (cc3) (apoptosis) assessed by immunohistochemistry as area of section with positive staining.

Necrosis of the epidermis and dermis as assessed by haematoxylin and eosin staining (scored on a 0–3 scale).

For Group 1 only: number of TP53 gene mutations as assessed by DNA sequencing in normal skin, non-treated AK lesion, and blood at baseline, and in a treated AK lesion on Day 57.

**Statistical Methods**

All confidence intervals are presented with 95% degree of confidence.

An observed cases approach was used for tabulations of data by visit. Missing data were not imputed and no adjustments for multiplicity were made.

Categorical data were summarised using the number and percentage of subjects in each category. Continuous data were summarised using the mean, median, standard deviation (SD), minimum and maximum values.

All subjects were included in the full analysis set (FAS) and analysed for efficacy.

Demographics and other baseline characteristics, adverse events (AEs), and other safety measurements are presented using descriptive statistics.

The number of CD3+ T-lymphocytes in dermis and epidermis is presented by biopsy time in plots showing the individual subject profiles for Groups 1 and 2 separately as well as in a plot showing the mean plus/minus the standard error (±SE) for the 2 groups together. The number of CD3+ T-lymphocytes was summarised using descriptive statistics. In addition, boxplots have been produced.

With a few exceptions, the secondary endpoints are presented as the primary endpoint.

Secondary endpoint 2b: ΔCt values were plotted using dot plots by location (AK lesion/normal skin) and planned time point. The mean (±SE) was calculated and included in the plots.

Secondary endpoint 7: in Group 1, the frequency of TP53 gene mutations, as well as the frequency of TP53 gene mutations excluding mutations that were also found in the blood, was plotted using bar charts by subject, location (AK lesion/normal skin), and visit.
skin), and planned time point.

Local skin responses (LSRs) were summarised by frequency counts for each of the individual LSRs (erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation, and erosion/ulceration). A composite LSR score was calculated by summing the 6 individual LSR gradings and was summarised using descriptive statistics.

## Summary of Results

### Trial Population

All 24 allocated subjects were treated with at least one application/dose of investigational medicinal product and were included in the safety and full analysis sets; all 24 subjects completed the trial. Two major protocol deviations were recorded and they both concerned trial medication for 1 subject, who was only treated with 1 tube of IMP per day instead of 2.

The trial population of mainly males (83%) was aged 50 to 87 years (mean 68.6 years). All subjects were “White” of “not Hispanic or Latino” ethnicity and their skin type was mainly Fitzpatrick Type I (25%) or Type II (50%). The mean time since first AK diagnosis was 3.0 years ranging from 0 to 16.6 years. 21 subjects were treated on the arm including back of hand and 3 subjects were treated on the scalp.

### Pharmacodynamics Results

#### Primary endpoint

The statistics describing the number of CD3+ T-lymphocytes in AK-lesions (lesional skin [LS]) in dermis and epidermis per time point is presented in **Figure 1**. Non-lesional result at baseline (NL-Pre) is also presented.

Looking at the median values, the presence of CD3+ T-lymphocytes in dermis and epidermis was substantially elevated in AK lesions (lesional skin [LS]) at baseline (LS-Pre) compared to non-lesional biopsies at baseline (NL-Pre). In AK lesions, a slight increase in CD3+ T lymphocytes was observed at Day 4 (LS-D4) returning to LS-Pre level at 8 weeks (LS-8w), although the level remained higher than the level observed in NL-Pre biopsies.

**Figure 1** Boxplots for CD3+ T-lymphocytes, dermis and epidermis: FAS

[Boxplots for CD3+ T-lymphocytes, dermis and epidermis: FAS]

NL: non-lesional; LS: lesional skin; Pre: pre-treatment; 4h: 4 hours post first treatment; D4: Day 4 visit; D8: Day 8 visit; 8w: 8 weeks (Day 57) visit.
Secondary endpoints

Cells expressing CD3, CD8, CD11c, DC-LAMP, CD163, Ki-67 and K16 were more abundant in AK baseline biopsies compared to non-lesional biopsies.

Following treatment, cells expressing CD3, CD11c, DC-LAMP, CD163, neutrophil elastase, Ki 67 and cc3 were elevated on Day 4 and Day 8 and returned to baseline levels or slightly below at 8 weeks (Day 57).

A tendency towards increased ICAM-1 expression on vascular endothelium on Day 4 and Day 8 was observed.

Apoptosis (cc3) and necrosis (assessed on H&E stained sections) was observed in only a few biopsies, primarily on Day 4 (cc3 only) and Day 8. No clear treatment effect was observed.

Epidermal thickness was increased in AK baseline biopsies compared to non-lesional biopsies and further increased at Day 4 and Day 8.

No clear treatment effect on Langerin, TRAIL, or K16 was observed.

The expression profile of a panel of cytokines and immune-related genes showed elevated expression in AK baseline compared to non-lesional biopsies and a further increase at 4 hours post-treatment, which peaked on Day 4 and returned to or below baseline levels at 8 weeks.

No difference in the number of mutations in TP53 between normal skin biopsies, AK baseline biopsies and Week 8 AK biopsies were observed.

Safety Results

A total of 37 AEs in 19 subjects were reported (Table 1) with 3 events in 3 subjects being severe; 35 AEs in 18 subjects were judged by the investigator to be related to IMP. One not related and mild SAE of fatigue with duration less than a day was reported and 3 subjects had severe application site related AEs, which recovered after 2 to 6 days; 2 of these subjects had treatment with IMP stopped after 2 days; however, all 3 completed the trial.

Table 1  Treatment emergent adverse events by SOC and PT: safety analysis set

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<tr>
<th>Body System or Organ Class</th>
<th>LEO 43204 Gel, 0.037% (N=24)</th>
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<tr>
<td>Preferred Term</td>
<td>n (% )  E</td>
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<td>----------------------------------------------------------------</td>
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<tr>
<td>Any adverse event</td>
<td>19 (79.2) 37</td>
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<td>General disorders and administration site conditions</td>
<td>17 (70.8) 32</td>
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<td>Application site pruritus</td>
<td>12 (50.0) 12</td>
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<td>Fatigue</td>
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<td>Application site dryness</td>
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<tr>
<td>Psychiatric disorders</td>
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<td>Pruritus</td>
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<tr>
<td>Gastrointestinal disorders</td>
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<tr>
<td>Nausea</td>
<td>1 (4.2) 1</td>
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N = Number of subjects in treatment group. n = Number of subjects with event.
(%) = Percentage of subjects with data. E=Number of events. MedDRA version 18.1

The level and time course of the LSRs were as expected based on previous trials with the product and there were no clinically significant abnormal findings for vital signs, ECG and laboratory values.
Conclusions
Pharmacodynamic effects: following treatment with ingenol disoxate, a general pro-inflammatory response dominated by influx of neutrophils, macrophages, dendritic cells and T cells, and activation of vascular endothelium was observed on Day 4 and Day 8 returning to baseline on Day 57.
A similar expression profile of a panel of cytokines and immune-related genes was observed with increased expression already 4 hours after treatment.
Minimal evidence of necrosis was observed.
Safety: there were no significant safety issues observed during this trial and no new safety signals were detected.
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