Long-term Follow-up Study of Ingenol Mebutate Gel for the Treatment of Actinic Keratoses

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Importance: Ingenol mebutate is the active agent (a macrocyclic diterpene ester) in the sap of the plant Euphorbia peplus. This herb has been used as a traditional remedy for several different skin lesions, including skin cancers.

Objective: To assess 12-month recurrence rates and safety associated with ingenol mebutate gel treatment in patients who previously had achieved complete clearance of actinic keratoses.

Design and Setting: The treatment area was observed for recurrence for 12 months after the original study. Patients were treated in an outpatient setting.

Participants: Patients received ingenol mebutate gel, 0.015%, daily for 3 consecutive days for actinic keratoses on the face or scalp or ingenol mebutate gel, 0.05%, daily for 2 consecutive days for actinic keratoses on the trunk or extremities. Study participants had achieved complete clearance in a prespecified 25-cm² area at day 57 of their original trial.

Main Outcome Measures: Recurrence rates and safety were assessed.

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ACTINIC KERATOSES ARE common in light-skinned individuals who spend a significant amount of time in geographic areas with moderate to high levels of solar radiation. They are considered premalignant lesions, with a small but definite risk of transformation into squamous cell carcinoma.¹ Cryotherapy with liquid nitrogen is the most widely used therapy for actinic keratoses.² This treatment may be associated with significant recurrence rates in the area treated because therapy is aimed only at the individual actinic keratoses, so-called spot therapy. Several other therapies are applied to an entire field of sun-damaged skin, so-called field therapy. These field therapies include fluorouracil cream,³ imiquimod cream,⁴ diclofenac sodium gel,⁵ and photodynamic therapy.⁶ The advantages of field therapy include the fact that subclinical and clinically evident actinic keratoses are treated, and the recurrence rates over the treated areas are significantly lower compared with those seen following spot therapy.³⁶

Results: In total, 108 patients with complete clearance of face or scalp lesions in the original trial and 76 patients with complete clearance of trunk or extremity lesions in the original trial were enrolled in the 12-month observational follow-up study. Of these, 100 patients (face or scalp) and 71 patients (trunk or extremities) completed all 12 months. The sustained lesion reduction rates compared with baseline were 87.2% for the face or scalp and 86.8% for the trunk or extremities. The estimated median times to recurrence were 365 days (face or scalp) and 274 days (trunk or extremities). There were no safety concerns during the follow-up period.

Conclusion and Relevance: Ingenol mebutate gel applied as field therapy for 2 or 3 consecutive days to treat actinic keratoses produced clinically relevant sustained clearance and long-term lesion reduction.

Trial Registration: clinicaltrials.gov Identifiers: NCT00953732, NCT00952783, and NCT00989313


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Ingenol mebutate is the active agent (a macrocyclic diterpene ester) in the sap of the plant Euphorbia peplus. This herb has been used as a traditional remedy for several different skin lesions, including skin cancers. Preclinical investigations indicate that ingenol mebutate is a pleiotropic effector that induces rapid and direct cell death and immune responses mediated by specific activation of protein kinase C δ, including neutrophil-mediated oxidative burst and clearance of tumors.⁷
Phase 3 trials have demonstrated that short-course field therapy with ingenol mebutate led to complete clearance in 28% to 42% of patients treating actinic keratoses on the trunk or extremities (using 0.05% concentration) and in 37% to 47% of patients treating actinic keratoses on the face or scalp (using 0.015% concentration), in only 2 or 3 consecutive daily doses, respectively.8

The objective of this study was to assess the long-term recurrence rates and safety associated with ingenol mebutate gel treatment of actinic keratoses in selected treatment areas during a 12-month observational follow-up period. Study participants had achieved complete clearance of actinic keratoses at day 57 in earlier studies.

METHODS

A long-term follow-up study of patients who had achieved complete clearance of actinic keratoses in 4 studies was conducted during a 12-month period. The durations of 3 long-term follow-up studies (LEO Pharma) were as follows: (1) July 29, 2009, to September 16, 2010, for study PEP005-030; (2) July 29, 2009, to September 14, 2010, for study PEP005-031; and (3) September 9, 2009, to October 11, 2010, for study PEP005-032. In the original studies, patients were treated with ingenol mebutate gel, 0.015%, daily for 3 consecutive days for actinic keratoses on the face or scalp or with ingenol mebutate gel, 0.05%, daily for 2 consecutive days for actinic keratoses on the trunk or extremities.8 To enroll in the follow-up studies, patients had to have achieved complete clearance in a prespecified 25-cm² area at day 57 of their original trial.

Patients were then seen at 3, 6, 9, and 12 months after the day 57 visit of the previous study. During that time, field treatments were not allowed. Lesion-directed therapies, such as cryotherapy or biopsy, were allowed at the investigator’s discretion and were recorded at each of the follow-up visits. Actinic keratoses in the target treatment area were counted at each visit, and information was collected regarding concomitant treatments, adverse events in the target treatment area, and any intercurrent conditions or therapies that could have resulted in immune suppression and that could have affected the number of actinic keratoses. Written patient consent and approvals from institutional review boards or independent ethics committees were obtained before the start of each study.

END POINTS

The primary end point for this study was the recurrence of actinic keratoses in the target treatment area. Patients were said to have achieved sustained clearance if their target treatment area remained clear after 12 months. Additional end point defined post hoc was the percentage reduction in the total number of actinic keratoses (including new or recurrent lesions) at 12 months compared with the number of lesions at baseline in the previous study for patients who had achieved complete clearance at day 57. This overall percentage reduction in the number of actinic keratoses from baseline was referred to as sustained lesion reduction for patients demonstrating clearance at day 57.

STATISTICAL ANALYSIS

Actinic keratoses recurrence and time to recurrence were evaluated using Kaplan-Meier methods to handle the right censoring of patients during the follow-up period. Before the analy-

RESULTS

Data from day 57 of 2 vehicle-controlled studies of ingenol mebutate gel for the treatment of actinic keratoses on the face or scalp have been reported.8 In the first study, 50 of 135 patients (37.0%) achieved complete clearance by day 57, and in the second study, 67 of 142 patients (47.2%) achieved complete clearance by day 57. One hundred eight of those patients enrolled in the follow-up study, and 100 completed 12 follow-up months. Five of 108 patients withdrew consent, 2 violated the protocol, and 1 was lost to follow-up analysis (Figure 1).

Data from a vehicle-controlled study9 of ingenol mebutate gel for the treatment of actinic keratoses on the trunk or extremities have also been reported. In this study, 42 of 100 patients (42.0%) treated with ingenol mebutate achieved complete clearance, and 38 of them enrolled in the 12-month follow-up study. In an uncontrolled study10 of ingenol mebutate gel for the treatment of actinic keratoses on the trunk or extremities, 40 of 102 patients (39.2%) achieved complete clearance, and 38 of those patients also enrolled in the follow-up study.10 Data from the two 12-month follow-up studies on the trunk or extremities were pooled, and of 76 patients included in the analysis, 71 completed 12 follow-up months. Three patients withdrew consent, 1 was discontinued by an investigator, and 1 withdrew for other reasons (Figure 1).

The mean age of 184 patients who were followed up in the entire long-term observational study population was 63.2 years. Overall, 70.7% of the patients were male, and 94.0% had Fitzpatrick skin type I, II, or III. In the original study, 76.1% of patients had been treated for 4 to 6 actinic keratoses.

CONCURRENT PROCEDURES AND CONCOMITANT MEDICATIONS

Treatments applied to the target area and systemic therapies that could have altered the course of actinic keratoses were collected and are summarized in the Table. Two patients were discontinued in the study for protocol violations. One of the patients was treated with photodynamic therapy and the other with topical 5-fluro-
uracil for actinic keratoses outside of but overlapping with the target area. Neither of the patients had new or recurrent actinic keratoses in the treatment area at any follow-up visit.

CLEARANCE

The sustained clearance rate after 12 follow-up months was 46.1% for patients treated on the face or scalp. The sustained clearance rate after 12 follow-up months was 44.0% for patients treated on the trunk or extremities. Based on the observed number of actinic keratoses seen at each of the follow-up visits, the probabilities of sustained clearance of face or scalp actinic keratoses and of trunk or extremity actinic keratoses are shown in Figure 2. The estimated median times to new or recurrent lesions in the treatment area were 365 days for the face or scalp and 274 days for the trunk or extremities.

The percentage of reductions in actinic keratoses at 12 months from the number of lesions that existed at baseline in the previous studies were calculated. These percentages were 87.2% for the face or scalp and 86.8% for the trunk or extremities among patients with complete clearance at day 57.

SAFETY

Three patients experienced adverse events in the target area during 12 months of observation. One experienced mild sunburn, the second developed a moderate hematoma, and the third had a mild rash. None of the 3 events were considered by investigators to be related to the study drug applied in the previous trial; all 3 events resolved without sequelae.

An ideal topical treatment for actinic keratoses not only must be effective in clearing patients of their actinic keratoses but also must result in sustained clearance. This is indeed one of the primary benefits of field therapy for actinic keratoses and is most relevant to our patients in clinical practice.

In this study of patients whose actinic keratoses had completely cleared with ingenol mebutate by day 57 of the previous studies, the sustained clearance rate, which is typically used to convey long-term clearance effects, was 46.1% for face or scalp lesions. The best estimate of the complete clearance rate among all patients at 14 months is the product of the 2 probabilities of being cleared (42.2% × 46.1% = 19.5%), which is considerably better than the 12-month cryotherapy complete clearance rate of 4% found by Krawtchenko et al.¹¹

### Table. Concurrent Procedures and Concomitant Medications

<table>
<thead>
<tr>
<th>Procedure or Medication</th>
<th>No. (%)</th>
<th>Face or Scalp (n = 108)</th>
<th>Trunk or Extremities (n = 76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any concurrent procedure or AK treatment</td>
<td></td>
<td>12 (11.1)</td>
<td>17 (22.4)</td>
</tr>
<tr>
<td>Cryotherapy</td>
<td></td>
<td>10 (9.3)</td>
<td>15 (19.7)</td>
</tr>
<tr>
<td>Photodynamic therapy</td>
<td></td>
<td>1 (0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Fluorouracil</td>
<td></td>
<td>1 (0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Micrographic skin surgery</td>
<td></td>
<td>0</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Biopsy</td>
<td></td>
<td>0</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Any concomitant medication²</td>
<td></td>
<td>1 (0.9)</td>
<td>2 (2.6)</td>
</tr>
<tr>
<td>Aloe vera</td>
<td></td>
<td>1 (0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Diflorasone diacetate</td>
<td></td>
<td>0</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Ivermectin</td>
<td></td>
<td>0</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td></td>
<td>0</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Lidocaine-epinephrine</td>
<td></td>
<td>0</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>

Abbreviation: AK, actinic keratosis.
²An individual patient could have used more than 1 medication.
On the other hand, a more clinically relevant measure of long-term effectiveness of an actinic keratosis therapy is the number of lesions in the treatment area at 12 months compared with the number of lesions at baseline. For patients who had achieved complete clearance in the original study, the proportions of lesions at 12 months compared with baseline totals were 12.8% of those on the face or scalp and 13.2% of those on the trunk or extremities. Therefore, at 12 months after treatment with ingenol mebutate, there was an approximately 87% reduction in the original number of actinic keratoses present in the selected pretreatment area for this patient population. This result indicates that there were far fewer actinic keratoses present in the treatment area at 12 months compared with the number of lesions seen at baseline. In the study by Krawtchenko et al\textsuperscript{11} among immunocompetent patients with actinic keratoses, fluorouracil was applied twice daily for 4 weeks (24 patients), and 1 or 2 courses of topical imiquimod, 5%, were administered 3 times per week for 4 weeks each (26 patients). The complete clearance rates at 12 months after initial evaluation of the field were 33% for fluorouracil and 73% for imiquimod, but the number of patients in each group was small.\textsuperscript{11} In another open-label study\textsuperscript{12} of fluorouracil cream, 0.5%, participants were treated with two 4-week courses of topical imiquimod, 5%, were administered 3 times per day for 4 weeks (24 patients), and 1 or 2 courses of ingenol mebutate were applied twice daily for 2 or 3 consecutive daily doses (27 patients). The absence of related safety issues during the 12-month follow-up period confirms that ingenol mebutate was well tolerated. Ingenol mebutate gel applied as field therapy for 2 or 3 consecutive daily doses was effective when treating head or body actinic keratoses, producing clinically relevant sustained clearance and long-term reduction in the number of lesions in the selected field by virtue of its dual mechanism of action that combines rapid direct cell death with a neutrophil-mediated immune response.

The long-term data on diclofenac sodium to treat actinic keratoses are limited to the results of one uncontrolled study,\textsuperscript{13} which showed no difference compared with ingenol mebutate (18% complete clearance compared with 19.5% \([42.2\% \times 46.1\%]\) for all patients). With regard to the 12-month sustained clearance rate in the subgroup of patients initially cleared, imiquimod, 5%, using a 12-week \((4 + 4 + 4)\) regimen showed 61% sustained clearance, while the corresponding value for ingenol mebutate on face and scalp locations was 46.1\%.\textsuperscript{14} In contrast to the present analysis, the imiquimod data included patients from the vehicle group with an initial complete clearance rate of 14.6% vs 53.7% for imiquimod. Fourteen patients from the vehicle group and 59 patients from the imiquimod group continued in the follow-up study, with a sustained clearance rate of 43% for the vehicle group.\textsuperscript{14} The corresponding data for initial complete clearance were 42.2% for ingenol mebutate and 3.7% for vehicle (vehicle-adjusted complete clearance rate of 38.5%).\textsuperscript{10} which is similar to that for imiquimod (vehicle-adjusted complete clearance rate of 39.1%). Sustained clearance 12 months after initial clearance using a 16-week schedule of imiquimod was comparable to that of the 12-week \((4 + 4 + 4)\) regimen,\textsuperscript{13} whereas the sustained clearance with ingenol mebutate, 3.75%, was in the range of 41% to 48% in the subgroup of patients initially cleared.\textsuperscript{4}

Early trials demonstrated that actinic keratoses of the trunk or extremities are more difficult to treat than those on the face or scalp. By applying ingenol mebutate gel, 0.05%, once daily for 2 consecutive days on the trunk or extremities, a reduction in actinic keratoses was achieved similar to that obtained by applying ingenol mebutate gel, 0.015%, once daily for 3 consecutive days on the face or scalp. Once clearing occurred, the percentage of reduction in actinic keratoses and the proportion of patients who sustained clearing were comparable between the application sites. However, the median time to recurrence of 274 days on the trunk or extremities was shorter compared with 365 days on the face or scalp.

The absence of related safety issues during the 12-month follow-up period confirms that ingenol mebutate was well tolerated. Ingenol mebutate gel applied as field therapy for 2 or 3 consecutive daily doses was effective when treating head or body actinic keratoses, producing clinically relevant sustained clearance and long-term reduction in the number of lesions in the selected field by virtue of its dual mechanism of action that combines rapid direct cell death with a neutrophil-mediated immune response.

**Figure 2.** Probability of sustained actinic keratosis (AK) clearance on the face or scalp (A) and the trunk or extremities (B) across months (Kaplan-Meier estimate).

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**Author Contributions:** Dr Lebwohl had full access to all the data in the study and takes responsibility for the in-

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tegrity of the data and the accuracy of the data analysis. Study concept and design: Lebwohl. Acquisition of data: Lebwohl, Shumack, Stein Gold, and Tyring. Analysis and interpretation of data: Lebwohl, Shumack, Stein Gold, Melgaard, Larsson, and Tyring. Drafting of the manuscript: Lebwohl, Shumack, Stein Gold, Melgaard, and Tyring. Critical revision of the manuscript for important intellectual content: Lebwohl, Shumack, Stein Gold, Melgaard, Larsson, and Tyring. Obtained funding: Larsson. Administrative, technical, or material support: Melgaard and Larsson.

Conflict of Interest Disclosures: Dr Lebwohl has been a consultant and investigator for Graceway/Medicis and for LEO Pharma. Dr Shumack is an advisory board member for LEO Pharma. Dr Tyring has been a consultant and investigator for LEO Pharma. Dr Melgaard and Dr Larsson are employees of LEO Pharma. Dr Tyring has been an investigator for LEO Pharma.

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