A Phase 2a Study of Topical Perillyl Alcohol Cream for Chemoprevention of Skin Cancer

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Abstract

The chemopreventive and antitumor properties of perillyl alcohol (POH) that were studied preclinically indicate that topical POH inhibits both UVB-induced murine skin carcinogenesis (squamous cell tumor models) and 7,12-dimethylbenz[a]anthracene–induced murine melanoma (transgenic models involving tyrosinase-driven Ras). A previous phase 1 clinical trial in participants with normal-appearing skin showed that topical POH cream was well tolerated at a dose of 0.76% (w/w). Here, we performed a 3-month, double-blind, randomized, placebo-controlled phase 2a trial of two different doses of topical POH in individuals with sun-damaged skin. Participants applied POH cream twice daily to each dorsal forearm. Baseline and end-of-study biopsies were taken from each participant to evaluate whether the topical application of POH was effective in reversing actinic damage as evidenced by normalization of quantitative skin histopathologic scores and change in nuclear chromatin pattern as measured by karyometric analysis. There was a borderline reduction in the histopathologic score of the lower-dose POH group compared with the placebo (P = 0.1), but this was not observed in the high-dose group. However, in the high-dose group, a statistically significant reduction in the proportion of nuclei deviating from normal was observed by the use of karyometric analysis (P < 0.01). There was no statistical significance shown in the lower-dose group. No changes were observed in p53 expression, cellular proliferation (by proliferating cell nuclear antigen expression), or apoptosis in either treatment group compared with the placebo group. These results suggest that whereas our karyometric analyses can detect a modest effect of POH in sun-damaged skin, improved delivery into the epidermis may be necessary. Cancer Prev Res; 3(2); 160–9. ©2010 AACR.

Introduction

Perillyl alcohol (POH) is a naturally occurring hydroxylated monocyclic monoterpenic that is found in the essential oils of a number of plants, including cherries and lavendin. POH, administered systemically, has shown both antitumor and chemopreventive properties that have been studied in both human and animal models. In mouse skin carcinogenesis, the mechanism of action of POH includes induction of apoptosis in tumor cells while leaving normal cells unaffected (1). It has been shown in a UVB mouse skin carcinogenesis study that topical POH inhibits the development of squamous cell carcinomas and also inhibits UVB-induced activator protein-1 activation (2). The inhibition of UVB-induced activator protein-1 activation is significant because it has been shown that activation of activator protein-1 plays a functional role in mouse skin tumor promotion and progression (3). In addition, in an in vivo murine model of 7,12-dimethylbenz(a)anthracene–induced melanoma, topical administration of POH resulted in a significant reduction in the proportion of new tumors, suggesting that POH may be a promising chemopreventive agent for the prevention of melanoma (4). Clinical trial experience with oral POH for cancer treatment has been largely unsuccessful due to gastrointestinal toxicity resulting from the extremely high doses required for systemic activity. Gastrointestinal toxicities were observed as dose limiting in a phase 1 trial of 16 participants with refractory cancer (5). Similar toxicities were observed in phase 2 trials of orally administered POH in advanced ovarian, metastatic colorectal, and metastatic breast cancers, leading to early suspension of study accrual (6–8). Thus, topical application may represent a more promising
route of administration for this drug, especially in the context of skin cancer chemoprevention.

We have previously shown in a placebo-controlled phase 1 trial that a topical POH formulation was well tolerated (9). A cream-based topical formulation of POH (10) in two strengths was developed, at 0.3% (w/w; 20 mmol/L) and at 0.76% (w/w; 50 mmol/L). No severe cutaneous toxicities, systemic toxicities, or histopathologic abnormalities were observed with a 0.76% (w/w) cream POH formulation (9). Thirty-two percent of the participants reported mild adverse events at least possibly related to topical cream, but there was no significant difference in the number or grade of skin lesions between the POH-treated forearms and the placebo-treated forearm.

The purpose of this randomized, placebo-controlled, double-blind, phase 2a study was to determine if topical administration of POH cream, applied twice daily to the forearms for 3 months, could reverse actinic damage as evidenced by normalization of quantitative skin histopathologic scores and change in nuclear chromatin pattern as measured by karyometric analysis. In addition, changes in epidermal cell proliferation indices, p53 expression, and apoptosis rates were also measured using immunohistochemistry in skin biopsies.

**Materials and Methods**

**Participant eligibility and recruitment**

We recruited 89 participants with sun-damaged forearm skin [as evidenced by at least two clinically diagnosed actinic keratoses (AK) in the treatment areas]. Participants were recruited through newspaper, radio and television advertisements, public service announcements, media stories, the Southern Arizona VA Health Care System, the Melanoma/Skin Cancer Screening Program Registry, and the dermatology outpatient clinic at the University Medical Center in Tucson. Participants were screened by the study dermatologists to verify eligibility. All participants were required to be postmenopausal or surgically sterile (Health Insurance Portability and Accountability Act) mandated cardiac monitoring via study diaries.

Participants ≥18 y of age were recruited from Pima and adjoining Southern Arizona counties. Female participants were required to be postmenopausal or surgically sterile by hysterectomy. Participants had to have sun-damaged skin as judged by the study physician and have at least two AKs on both of their dorsal forearms. Clinical criteria for determining sun damage included fine wrinkling, coarse wrinkling, mottling, hyperpigmentation, and a global assessment. Participants were eligible if at least 4 wk had passed since the last surgical biopsy, surgical excision, or cryotherapy for AK in the test area. Participants were considered eligible if at least 6 mo had passed since topical treatment for AK or any therapeutic approach for squamous cell carcinoma or basal cell carcinoma in the test area (e.g., 5-fluorouracil, imiquimod, surgical excisions). Participants were required to discontinue all topical medications to the upper extremities, except for emollients or sunscreens, at least 30 d before study entry. Oral multivitamin intake was limited to Dietary Reference Intake values. Participants also agreed to limit sun exposure as much as possible during the 3-mo study. In addition, participants who used citrus peel topically or consumed citrus peel were excluded.

**Test agent**

POH cream formulations were prepared at two strengths [50 mmol/L, 0.76% (w/w) and 20 mmol/L, 0.3% (w/w)], formulated as previously described (10, 11). POH cream and matching placebo creams were packaged in 60 mL plastic ointment tubes and participants were instructed to store tubes at room temperature and avoid exposure to high temperatures. Clinic personnel and study participants were blinded to the specific contents of tubes to ensure the integrity of the double-blinded study. Adherence to the study medication was evaluated through the weighing of returned tubes and self-monitoring via study diaries.

**Trial design**

Baseline data (blood, urine, electrocardiogram, and skin biopsies) were obtained before randomization. Participants were randomly assigned by computer to one of three treatment groups (placebo cream, 0.3% POH, or 0.76% POH). Participants were instructed to apply cream twice daily to each dorsal forearm for 3 mo. Additional efficacy evaluations included alterations in patterns of biomarker expression, including p53 expression, cellular proliferation [proliferating cell nuclear antigen (PCNA)], and apoptosis rates (morphologically apoptotic cells; Table 1). Participants were also instructed to maintain a study diary to record daily time of application and any adverse events.

Pre-study requirements included dermatologic exam, blood counts, and serum chemistry analysis to rule out any serious underlying disease. In addition, an electrocardiogram was completed at baseline, at steady state (between 4 and 6 wk), and at completion of study to comply with the Food and Drug Administration–mandated cardiac monitoring for new drug products.

**Biopsies**

From each participant at baseline, and again after 12 wk (end-of-study), a 4-mm punch biopsy was obtained from Table 1. Schedule of biopsies and end points for analysis

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Time</th>
<th>Location</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baseline</td>
<td>R or L forearm</td>
<td>p53, PCNA, apoptosis, histopathology, karyometry</td>
</tr>
<tr>
<td>2</td>
<td>End of study</td>
<td>R or L forearm*</td>
<td>p53, PCNA, apoptosis, histopathology, karyometry</td>
</tr>
</tbody>
</table>

*Consistent with biopsy #1.
sun-damaged skin on one dorsal forearm, 10 cm distal to
the lateral epicondyle, for dermatopathology, karyometry,
and immunohistochemical analysis. The matched end-of-
study biopsy was collected at a point 2 cm adjacent to the
location of the baseline biopsy, oriented using baseline
photographs of the forearm. Biopsies were formalin fixed
for analyses.

**Histopathologic analysis**

Four-millimeter skin punch biopsies were collected
from the lateral posterior forearm, approximately 6 cm
distal to the elbow joint in all randomized participants
at baseline and at the end of study. The end-of-study biop-
sies for histopathology, karyometry, and immunohisto-
chemical markers were collected 10 to 18 d after the last
dose of test article was applied to allow for resolution of
any local site reactions because any acute inflammation
could confound these results. Biopsies were evaluated
in a blinded fashion by one study dermatopathologist
(P.S.) and scored according to a histopathologic scoring
system previously described (12). Formalin-fixed biopsies
were stained by routine H&E. The total histopathologic
score was the sum of four criteria including basal or supra-
basilar pleomorphism (atypia), inflammation, hyperkera-
tosis, and parakeratosis. Change in the score for paired
biopsies was calculated by subtracting the baseline score
from the end-of-study score. The high- and low-dose treat-
ment groups were compared with the placebo-treated
group.

**Immunohistochemistry**

Immunohistochemical staining was done using peroxi-
dase (p53) or alkaline phosphatase avidin with a fast red
chromagen (PCNA) and hematoxylin counterstain (Ven-
tana Medical Systems) on an automated VMS 320 immu-
nostainer (Ventana Medical Systems) at 1:100 dilutions.
Anti-PCNA PC10 and anti-p53 (Calbiochem) were used
at 1:200 dilution in antibody diluent (Ventana Medical
Systems). Negative controls (to ensure lack of nonspecific
staining) were run on each sample by substituting anti-
body diluent for the antibody. Controls included in each
run were tonsil tissue for PCNA and T47D human breast
cancer cells for p53 to ensure proper staining and to assess
variability of staining intensity. Tissue sections were an-
alyzed with ImagePro Plus software (Media Cybernetics),
a Leica DMR microscope, and a Sony 3 charge-coupled de-
vice color video camera. The percentage positive area per
40× field was determined for each biopsy. Three represent-
ative 40× fields were used for image analysis and the en-
tire epidermis was included so that all basal and supra-
basal cells were analyzed.

**Apoptosis by morphology**

The number of apoptotic cells was assessed based on
morphology (i.e., condensed and/or pyknotic nuclei,
esinophilic cytoplasm, formation of apoptotic bodies)
on H&E per 100 basal keratinocytes. The entire biopsy
was used for each apoptosis assessment.

**Karyometric analysis of topical drug effects**

For the 27 participants treated with the higher dose of
POH, 1,330 nuclei were recorded at baseline and 1,945
nuclei at the end of study. For the 17 fully evaluable par-
ticipants receiving the placebo cream, 1,318 nuclei were
recorded at baseline and 1,394 nuclei at the end of study.
Tissue sections were cut at 5 μm and stained with H&E
under carefully controlled conditions, as previously re-
ported (13). A high-resolution digitized imagery of the
histopathologic sections was recorded on a Sony 3
charge-coupled device video-microphotometer, equipped
with a 100:1, numerical aperture 1.4, plan apochromatic
oil immersion objective (NIKON). Sampling density was
adjusted to 6 pixels per linear micron. A 620-nm interference
filter was used to enhance contrast. The nuclei in the
digitized imagery were segmented by a semiautomated
procedure with manual correction where needed. For
each nucleus, features descriptive of the texture of the nu-
clear chromatin pattern were computed. In the feature
selection, a statistical difference for two data sets at a
significance level of P < 0.005 was chosen. This was done
in view of the multiple comparisons—for 95 different fea-
tures—to keep the selection of a spuriously significant
feature at a chance of less than 1 in 200, and as a compro-
mise to not increase the chances of a type II error (i.e., un-
necessarily rejecting a useful feature). For a skin biopsy to
be evaluative, a target of 100 stained nonoverlapping nu-
clei per biopsy was set for the analysis, randomly selected
after pathologist review and demarcation of the areas to be
imaged.

**Statistical analysis**

Histopathologic scoring and biomarker analyses were
conducted using Stata version 10. To determine if histo-
pathologic scores varied by treatment group at baseline,
the Kruskal-Wallis test was used. Changes in histopatho-
logic scores for treated sun-damaged skin were compared
between any treatment group and the placebo group using
the Wilcoxon rank sum test.

The values for p53 and PCNA expression were derived
by averaging the three highest-staining 40× fields for each
biopsy. Apoptosis measures were calculated by counting
the number of apoptotic cells and basal cells and then
standardizing the results to the number of apoptotic cells
per 100 basal cells. The Wilcoxon rank sum test was used
to compare the change in each measure between the treat-
ment groups and the placebo group. Proportions of ad-
verse events in different groups were compared using
Fisher’s exact test.

In the karyometric analyses, to determine differences
for the karyometric features, the Kruskal-Wallis test (14)
and ambiguity function of Genchi and Mori (15) were used.
Wilks’ lambda was used to evaluate the difference between
the four major features selected from the stepwise linear dis-
criminant algorithm. A reduction in the proportion of nu-
clei assigned to the baseline data set was used as a measure
of efficacy of the drug. The statistical significance of such a
reduction was established based on the Fleiss tables (16).
The Kolmogorov-Smirnov statistic was used to determine the deviation from the “normal” in the end-of-study biopsy nuclei.

**Results**

**Participant characteristics**

Eighty-nine participants were accrued and 83 participants were randomized (Fig. 1), with a total of 79 participants completing the study. Table 2 shows the age and gender distribution of participants within the three groups (placebo, 0.3% POH, and 0.76% POH). There was no statistically significant difference in gender (Pearson $\chi^2 = 0.95$, $P = 0.62$) or age ($F_{(3, 79)} = 0.49$, $P = 0.69$) between the three groups. All participants had sun-damaged skin with at least two clinically diagnosed and visible AKs on each of their dorsal forearms. Baseline variables including age, gender, histologic score, and number of AKs were all well balanced (no statistically significant differences) across the three study groups. To determine adherence, the number of drug applications actually administered to the skin was calculated as a percentage of the total prescribed number of applications, and the weight of the drug actually consumed was calculated as a percentage of the total weight of drug expected to be consumed under the protocol. No statistically significant difference in either

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age (SD), y</td>
<td>63.9 (8.4)</td>
<td>68.1 (10.4)</td>
<td>66.9 (9.9)</td>
</tr>
<tr>
<td>$n$ (%)</td>
<td>8 (29)</td>
<td>20 (71)</td>
<td>28 (100)</td>
</tr>
<tr>
<td>0.3% POH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age (SD), y</td>
<td>66.1 (11.6)</td>
<td>69.7 (10.4)</td>
<td>68.8 (10.6)</td>
</tr>
<tr>
<td>$n$ (%)</td>
<td>7 (26)</td>
<td>20 (74)</td>
<td>27 (100)</td>
</tr>
<tr>
<td>0.76% POH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age (SD), y</td>
<td>68.0 (7.4)</td>
<td>67.6 (11.6)</td>
<td>67.6 (10.9)</td>
</tr>
<tr>
<td>$n$ (%)</td>
<td>5 (18)</td>
<td>23 (82)</td>
<td>28 (100)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age (SD), y</td>
<td>65.7 (9.1)</td>
<td>68.4 (10.7)</td>
<td>67.7 (10.4)</td>
</tr>
<tr>
<td>$n$</td>
<td>20</td>
<td>63</td>
<td>83</td>
</tr>
</tbody>
</table>
of these two adherence measures was observed across the three study groups (data not shown).

Histopathologic scoring
Baseline and end-of-study biopsies of sun-damaged skin were evaluated by the study dermatopathologist and scored according to a histopathologic scoring system previously described (12). This score reflects the presence or absence of two criteria (hyperkeratosis and parakeratosis) and the severity (absent, mild/moderate, or severe) of two more criteria (pleomorphism and inflammation). Scoring of the biopsies taken at baseline did not vary by treatment ($P = 0.42$). Figure 2 shows the box plot of distribution of the change in histopathologic score changes for biopsies taken from sun-damaged skin by treatment group. Table 3 contains the statistical analyses of these data. The score change in the lower-dose POH group approached a statistically significant difference versus the placebo group ($P = 0.10$), which may be meaningful given the relatively small sample size. However, there was not a statistically significant difference between the high-dose POH group and the placebo group ($P = 0.41$).

Biomarker expression
No statistically significant differences by treatment group were apparent in the sun-damaged skin for change in p53 expression, cellular proliferation (as measured by PCNA expression), or apoptosis (data not shown).

Karyometric analysis
For the samples in the high-dose POH group, the results from the analysis using the Kruskal-Wallis test (14) and the ambiguity function of Genchi and Mori (15, 17) indicated the presence of statistically significant differences for more than 20 karyometric features, at $P < 0.005$. The ambiguity function values, however, suggested poor discrimination potential for nearly all of them. Table 4 lists the best of these features, with their mean values, the percent difference between the baseline and end-of-study data sets, and the ambiguity function value. An ambiguity value of 1.0 indicates no discrimination. All features are given in relative arbitrary units. All of the features listed in Table 4 had a $P$ value of $<0.005$.

The data sets treated with the higher dose were each divided into two halves by assigning alternate nuclei to the first or the second halves. The first-half data sets from the baseline and the end-of-study nuclei were used as a training set, with 668 and 995 nuclei. These data were submitted to a stepwise linear discriminant algorithm. It selected four features from the set listed in Table 4. The values following the feature ID number represent the standardized

Table 3. Average change in histopathologic score by treatment group

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Type of skin</th>
<th>Change in histopathologic score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$n$ Mean* Median Min Max SD</td>
</tr>
<tr>
<td>Placebo</td>
<td>SD skin</td>
<td>26 0.4 0 -2.0 3.0 1.3</td>
</tr>
<tr>
<td>0.3% POH</td>
<td>SD skin</td>
<td>26 -0.1 0 -2.0 4.0 1.3</td>
</tr>
<tr>
<td>0.76% POH</td>
<td>SD skin</td>
<td>27 0.1 0 -2.0 3.0 1.4</td>
</tr>
<tr>
<td>Total</td>
<td>SD skin</td>
<td>79 0.1 0 -2.0 4.0 1.3</td>
</tr>
</tbody>
</table>

Abbreviation: SD, sun-damaged.
*Mean change from baseline.
coefficients in the discriminant function. These selected features include the run percentage (#307, 1.196), short run emphasis (#303, -0.823), the relative frequency of occurrence dn/n in the pixel absorbance histogram interval from absorbance 0.70 to 0.79 (#014, 0.271), and the relative nuclear area (#002, 0.662). Wilks’ $\lambda$ was reduced to 0.9595 (i.e., only by 4.1%). The proportion of nuclei assigned to the baseline data set (i.e., the set with presumably greater deviation from normal) was reduced by 15.3%. For the sample size of 668 nuclei in the smaller data set, this is a significant reduction at $P < 0.01$ and a power of test of 0.99. The same discriminant function was next applied to the second-half data sets. The reduction in the number of nuclei assigned to the baseline data set here was 19.1%.

For the combined data sets, of 1,330 and 1,945 nuclei, the classification resulted in a reduction from 55.9% to 39.5% (i.e., by 16.4%). For the sample sizes involved, this reduction is highly significant at $P < 0.01$ and a power of test of 0.99. The distribution of discriminant function scores is shown in Fig. 3. There is a notable shift to the left on the score axis, indicating less deviation from normal in the end-of-study biopsy nuclei. The two distributions were statistically significantly different, as shown by the Kolmogorov-Smirnov statistic. The $D_{\text{max}}$ value was 0.179288 and the $D_{\alpha}$ value was 0.0546, which resulted in a $P$ value of $<0.0001$. Application of the discriminant function to the placebo-treated end-of-study data set (of 1,394 nuclei) resulted in a reduction of the proportion of nuclei assigned to the baseline data set from 55.9% to 53.4% (i.e., by only 1.5%).

Discriminant function scores for the three treatment groups at baseline and at the end of study are shown in Table 5. As indicated above, only one discriminant function was used throughout this study, and the same function was applied in all comparisons. The function compounds, as a linear combination, the values of the four features listed above (Table 4). It is evident that there is a significant shift for the group treated with the high dose of POH, with no overlap of confidence limits

### Table 4. Karyometric features

<table>
<thead>
<tr>
<th>Feature ID</th>
<th>Mean value Baseline</th>
<th>Mean value End of study</th>
<th>%Difference</th>
<th>Ambiguity value</th>
</tr>
</thead>
<tbody>
<tr>
<td>#307 run percentage</td>
<td>783.4</td>
<td>695.3</td>
<td>-11.2</td>
<td>0.981</td>
</tr>
<tr>
<td>#001 total absorbance</td>
<td>0.740</td>
<td>0.665</td>
<td>-10.2</td>
<td>0.981</td>
</tr>
<tr>
<td>#002 rel. nuclear area</td>
<td>35.2</td>
<td>31.5</td>
<td>-10.5</td>
<td>0.988</td>
</tr>
<tr>
<td>#275 run length 5-6</td>
<td>4.2</td>
<td>3.6</td>
<td>-14.3</td>
<td>0.987</td>
</tr>
<tr>
<td>#321 no. dense-staining pixels</td>
<td>603.3</td>
<td>548.7</td>
<td>-9.1</td>
<td>0.990</td>
</tr>
<tr>
<td>#303 short run emphasis</td>
<td>0.51</td>
<td>0.52</td>
<td>+2.0</td>
<td>0.986</td>
</tr>
<tr>
<td>#014 pixel absorbance Int. 0.7</td>
<td>13.8</td>
<td>12.4</td>
<td>-10.1</td>
<td>0.981</td>
</tr>
<tr>
<td>#314 pixel absorbance clumpiness</td>
<td>0.72</td>
<td>0.71</td>
<td>-1.4</td>
<td>0.995</td>
</tr>
</tbody>
</table>

Fig. 3. Distribution of discriminant function scores for the high-dose POH samples at baseline and at the end of study.
between baseline and the end of study. The baseline and end-of-study values for the low-dose group are statistically indistinguishable, as are the values for the placebo-treated group. Also, there are no statistically significant differences between the baseline groups.

**Response in individual participants**

For the high-dose POH group, a total of 3,275 nuclei were recorded, 1,330 at baseline and 1,945 at the end of study. There were 27 cases, but a paired comparison was available for only 18 cases. For most of these cases, the full target sample size of 100 nuclei had not been reached. The average number of nuclei per case was 78 nuclei. Figure 4 shows the histogram of the relative number of cases plotted over a scale of proportion of nuclei, decreased or increased, at the end of study. The large peak around the zero mark reflects case-to-case sampling error. In the lower half of Fig. 4, the same plot is shown for the placebo-treated group. Here, no paired comparison was possible, as the high-dose baseline cases were different from the placebo-treated cases. The differences were computed from a scrambled list and represent an estimate of the difference distribution, adjusted to the same percentage as presented in the top portion of Fig. 4. In this study, the difference in proportion of nuclei assigned to the baseline data set was defined as a measure of efficacy. The large sample size of nuclei available for establishing the overall efficacy of the intervention provided significance at $P < 0.01$ and a power of test of 0.99. For the average sample size of 75 nuclei per case, a reduction in the proportion of nuclei from 55% to 55.7%, with sample sizes of 1,330 and 1,657 nuclei, respectively. In the low-dose POH baseline and the low-dose POH end-of-study data sets, the percentage of nuclei applied to the baseline data set increased by 0.6%, from 55.1% to 55.7%, with sample sizes of 1,346 and 1,657 nuclei, respectively.

**Clinical response and safety evaluation**

Participants kept a daily diary during the study, which included time of application and any adverse events. Each subject recorded the time of onset and resolution of any such events, and events were rated as mild, moderate, or severe. Diaries were reviewed by clinic personnel at each visit.

Of the total events reported in the study, 66% were “possibly” or “probably” related to study treatment, as rated by the study physician. Only one event in the high-dose group was rated “definitely” related to study treatment. This event was tenderness in the test area, which was rated as mild in severity. Table 6 shows the skin-related events by participant. The most frequent event occurring in the treatment group was “rash, redness, erythema” rated mild or moderate in 54% of participants in the high-dose group and in 56% of participants in the low-dose group. The placebo group reported the same type of event in 43% of the participants. There was no statistically significant difference in the proportion between the treatment and the placebo groups.

**Discussion**

We performed a phase 2a dose-finding and efficacy trial of topically administered POH in a cream formulation having previously documented suitable skin tolerability in a phase 1 study (9). Trial participants had sun-damaged skin with at least two clinically diagnosed AKs on each dorsal forearm to document at least a moderate level of risk for nonmelanoma skin cancer. There were multiple primary end points (i.e., normalization of quantitative skin histopathologic scores and skin nuclear chromatin pattern) and secondary end points (i.e., skin biopsy PCNA, apoptosis, and p53 biomarker indices).

Our interest in POH as a topical chemopreventive agent for skin cancer was fueled by positive preclinical studies, documenting considerable activity in both UVB-induced murine squamous cell carcinoma and melanoma (2, 4). There is increasing interest in the development of topically administered agents that could slow the progression of moderately or severely sun-damaged skin to nonmelanoma skin cancer, and the strong preclinical chemopreventive activity and excellent tolerability of topically administered POH make it a strong candidate for further clinical development.

As reported, there was a borderline difference that approached statistical significance in the change in sun-damaged dorsal forearm biopsy histopathologic score between the study participants treated with placebo cream and those treated with 0.3% (low-dose) POH; however, the high-dose (0.76%) POH group did not show a similar

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**Table 5. Discriminant function scores determined by karyometric analysis of biopsies from sun-damaged skin**

<table>
<thead>
<tr>
<th></th>
<th>Baseline, mean (95% CI)</th>
<th>End of study, mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POH (high)</td>
<td>0.2492 (0.1982-0.3002)</td>
<td>−0.1705 (−0.2145 to −0.1265)</td>
</tr>
<tr>
<td>POH (low)</td>
<td>0.2367 (0.1786-0.2948)</td>
<td>0.2747 (0.2200-0.3293)</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.2392 (0.1822-0.2962)</td>
<td>0.1498 (0.0998-0.1997)</td>
</tr>
</tbody>
</table>

Abbreviation: 95% CI, 95% confidence interval.
trend. This was surprising, given the strong preclinical results, and the apparent lack of dose dependence indicates that either the drug is not effective or the pharmacokinetic profile (i.e., the residence time of the drug at the site of action) was not suitable. However, in contrast to the results obtained using the histopathologic scoring system, a dose-dependent reduction in nuclear abnormalities was observed by karyometric analysis. This supports the hypothesis that the negative histopathologic results were due to inadequate exposure of the drug to its target, most likely due to the suboptimal pharmacokinetic properties of the formulation.

Using karyometric analysis, we observed that the higher dose of POH (but not the lower dose of POH) was associated with a statistically significant reduction in the proportion of skin biopsy nuclei deviating from normal (i.e., \( P < 0.01 \) with a power of test of 0.99). In case-by-case comparisons, it was difficult in this study to establish the statistical significance of the high-dose POH versus the placebo, due to the limited sample sizes of nuclei recorded for each participant. Of those participants treated with topically administered higher-dose POH, 31% had a statistically significant reduction in proportions of nuclei deviating from normal, versus just 17% in the placebo group. This trend to less deviation from normal may very well represent a beneficial cancer-preventive effect that will continue to be studied in future clinical trials using karyometric analysis as a biomarker end point.

Fig. 4. Histograms showing relative frequency of occurrence of cases with specific differences in the proportion of nuclei assigned to the baseline data sets, comparing the high-dose POH group (top) with the placebo group (bottom).
p53, PCNA, and apoptosis were not affected by the high- or low-dose POH concentrations administered topically twice daily over 3 months. These negative biomarker results match those observed in our prior positive skin cancer chemoprevention studies of oral vitamin A and topical difluoromethylornithine (18–20). On the other hand, karyometric analysis of nuclear chromatin abnormality in baseline and end-of-study sun-damaged skin once again has proved useful as an “integrating biomarker” of response to chemopreventive agent activity (13, 18–23).

The only adverse event that occurred frequently was a mild or moderate local reaction (termed rash, redness and/or erythema) that occurred at a rate of 56% and 54% for the low- and high-dose POH, respectively. However, this reaction may very well be due to the vehicle cream because the placebo group reported this same type of event in 43% of participants, and this proportion is not statistically significantly different from that observed in the treatment groups. Similarly, the phase 1 trial also showed a lack of any serious adverse events (9) and no significant differences in the mild, study-related adverse events between the treatment and the placebo groups.

Obviously, topical POH cannot be effective as a skin cancer chemoprevention agent if it penetrates poorly into epidermal skin, or (possibly) if it rapidly penetrates the skin transdermally. An ongoing direction in our research program includes both in vivo preclinical and clinical projects to compare the skin penetration of the parent POH topical formulation and a novel prodrug topical formulation, engineered specifically to concentrate POH in the epidermis (24, 25). Development of true pharmacokinetic profiles (concentration versus time curves) of percutaneous absorption and transdermal penetration of topically administered drugs in the context of a trial such as this one is impractical due to the excessive numbers of biopsies that would be required. Development of a prodrug of POH engineered with appropriate lipophilicity will allow for ex vivo human skin models to be developed that can compare the skin pharmacokinetics of parent POH and POH prodrugs to ensure adequate residence time of the active form of the drug.

In conclusion, the results of this study suggest that topically administered POH at 0.76% twice daily for 3 months has a modest effect in reducing nuclear chromatin abnormality in moderately to severely sun-damaged skin. Further studies of prodrug formulations of POH may result in a more active formulation of this novel topical agent that could be used in future nonmelanoma skin cancer chemoprevention studies.

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No potential conflicts of interest were disclosed.

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