Research Article

Cancer Prevention Research

Phase IIB Randomized Study of Topical Difluoromethylornithine and Topical Diclofenac on Sun-Damaged Skin of the Forearm

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Abstract

Prevention of nonmelanoma skin cancers remains a health priority due to high costs associated with this disease. Diclofenac and difluoromethylornithine (DFMO) have demonstrated chemopreventive efficacy for cutaneous squamous cell carcinomas. We designed a randomized study of the combination of DFMO and diclofenac in the treatment of sun-damaged skin. Individuals with visible cutaneous sun damage were eligible. Subjects were randomized to one of the three groups: topical DFMO applied twice daily, topical diclofenac applied daily, or DFMO plus diclofenac. The treatment was limited to an area on the left forearm, and the duration of use was 90 days. We hypothesized that combination therapy would have increased efficacy compared with single-agent therapy. The primary outcome was change in karyometric average nuclear abnormality (ANA) in the treated skin. Individuals assessing the biomarkers were blinded regarding the treatment for each subject. A total of 156 subjects were randomized; 144 had baseline and end-of-study biopsies, and 136 subjects completed the study. The ANA unexpectedly increased for all groups, with higher values correlating with clinical cutaneous inflammation. Nearly all of the adverse events were local cutaneous effects. One subject had cutaneous toxicity that required treatment discontinuation. Significantly more adverse events were seen in the groups taking diclofenac. Overall, the study indicated that the addition of topical DFMO to topical diclofenac did not enhance its activity. Both agents caused inflammation on a cellular and clinical level, which may have confounded the measurement of chemopreventive effects. More significant effects may be observed in subjects with greater baseline cutaneous damage. *Cancer Prev Res; 9(2); 128–34. ©2015 AACR.*

See related article by Tsai and Hawk, p. 125

Introduction

Skin cancer is the most common cancer in the United States, and incidence of this disease continues to rise (1). Although effective treatments for these cancers are available, individuals with a history of nonmelanoma skin cancer (NMSC) have a 10-fold increased risk for developing a second primary NMSC (2). NMSCs are associated with relatively low mortality, but the treatment can lead to considerable morbidity, and the resulting cost to society is substantial (1). Because of the high incidence, high rates of subsequent cancers, and high costs associated with this disease, prevention of NMSC should be considered a public health priority.

Difluoromethylornithine (DFMO) irreversibly inhibits ornithine decarboxylase (ODC), the rate-limiting enzyme in the synthesis of polyamines (3). Polyamines cause cell proliferation through regulation of gene expression, and elevated levels of both ODC and polyamines are associated with epithelial cancers (4). Mouse models of skin carcinogenesis have demonstrated that ODC is induced by UV light and chemical carcinogens (3). Although DFMO has not been found to be effective as a cancer therapeutic, it has been shown to decrease premalignant lesions of the skin and colon. A 6-month course of a topical DFMO preparation in individuals with moderate to severe actinic keratoses resulted in a statistically significant reduction (23.5%) in the number of premalignant lesions as compared with placebo (5). In that study, a similar reduction in cutaneous concentrations of spermidine, a polyamine, was identified. Furthermore, a daily dosing of 500 mg/m^2 of DFMO for up to 5 years in individuals with previous history of skin cancer resulted in a significant reduction of basal cell carcinomas (6).

Diclofenac is an NSAID medication that inhibits COX-1 and COX-2. Expression of COX-2 has been shown to be increased in human keratinocytes after irradiation with UV-B light, and both actinic keratoses and squamous cell carcinomas demonstrate increased staining for this protein (7, 8). A topical gel formulation of diclofenac sodium, Solaraze™, is a commercially available, FDA-approved agent for the treatment of actinic keratoses. The use of this agent for 90 days has resulted in complete resolution of actinic

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keratoses in 39% to 58% of subjects (9, 10). The reduction in number of actinic keratoses ranges from 56% to 90% with this treatment (10, 11). The effects of treatment continue after discontinuation of the drug, and the assessment of resolution is made 30 days after the completion of therapy in most studies. The activity of this agent has also been seen in the treatment of sun-damaged skin without clinically detectable actinic keratoses lesions (12).

The activity of DFMO in combination with NSAIDs was established by Meyskens and colleagues (13) with the clinical study of DFMO and sulindac in the prevention of colon polyps. This study observed that individuals taking this combination of medications had a 70% reduction in colon adenoma recurrence and a greater than 90% reduction in advanced adenomas (13). In addition, toxicity was minimal in subjects on the study drugs in comparison with placebo.

On the basis of these findings, we designed a phase IIB study of the combination of DFMO and diclofenac in the treatment of sun-damaged skin. The primary objective of the study was to determine whether a 3-month course of topical treatment with the combination of both medications had increased efficacy relative to either medication alone.

Materials and Methods

Eligibility criteria

Individuals over the age of 40 years with visible sun damage to their skin were considered eligible for participation. Women who were pregnant or nursing were excluded. Subjects could not have a history of topical medication to the forearms within 30 days of enrollment or oral diclofenac within 60 days of enrollment, and they could not use these medications while on the study. During the study, participants had to be able to limit use of NSAIDs according to specific parameters. Individuals with a history of nonmelanoma skin cancer were permitted to participate if they had undergone definitive treatment of the cancer at least 30 days prior to enrollment (for sites other than the forearm) or 6 months prior to enrollment (for sites on the forearm). Those with active inflammation on the forearms or with a history of significant immunosuppression due to medications or medical conditions were also excluded. All subjects provided informed consent prior to participation.

DFMO formulation

The original study design used a 10% (w/w) concentration cream of DFMO, which was formulated from a white powder of monohydrate monochloride (MW 236.65) from Marion Merrell Dow in hydrophilic ointment USP and was packaged as described previously (9).

However, complaints of formulation instability from early participants led to a change in formulation to the commercially available 13.9% preparation, Vaniqa™, manufactured by SkinMedica for removal of unwanted facial hair in women. The analyses reported here include only participants who used the Vaniqa™ preparation. Subjects applied DFMO to a designated 5 cm × 5 cm area of the left forearm twice a day for ninety days.

Diclofenac formulation

Commercially available Solaraze™ 3% gel was supplied by Nycomed. Subjects initially applied diclofenac twice a day to a designated 5 cm × 5 cm area of the upper left forearm that demonstrated clinical sun damage but was free of AKs. However, due to higher than expected rates of local toxicity, the dose was changed to once daily. Comparison of randomization factors showed no difference in the group dosed daily versus that dosed twice daily. Subjects from both dosing levels were included in the final analyses.

Study design

This study was reviewed and approved by the Institutional Review Board of the University of Arizona (Tucson, AZ). The trial was conducted at the Skin Cancer Prevention Annex at the University of Arizona (Tucson, AZ). All subjects underwent a careful health history, medication history, and dermatologic assessment. Qualified subjects were randomized in a 1:1:1 ratio by a computer program to one of the three treatment groups: DFMO, diclofenac, or DFMO plus diclofenac. Subjects and clinical investigators were not blinded as to treatment arm for each subject; however, investigators assessing the karyometric and other biomarkers were blinded from the treatment assignments. Initially, study medications were applied twice daily to a 5 cm × 5 cm area on the left forearm for 90 days; however, due to skin toxicity issues, the dosing for the diclofenac-containing arms was reduced to once daily. Prior to receiving medication, three 4-mm punch biopsies were taken from the skin of the left lateral forearm for assessment of histopathology, COX-2 and p53 expression, apoptosis, and nuclear chromatin karyometry. Prior to starting treatment, a blood sample was drawn for the assessment of a complete blood count and a metabolic profile (SMA-20). For premenopausal women, a urine pregnancy test was performed prior to study entry, at all follow-up visits while on the study drugs, and at the end of the study. Blood collection was repeated at the end of treatment, and biopsies were repeated one to two weeks after discontinuation of treatment. Subjects were observed monthly for adherence, toxicity, and efficacy during the treatment program. They maintained a daily diary of medication use and side effects.

We checked serum diclofenac levels at the end of the study for the first 15 subjects who were randomized to an arm containing diclofenac (alone or in combination with DFMO). None of the diclofenac levels in these first 15 subjects exceeded 50 ng/mL (approximately 1/3 of the lower end of the concentration range for recommended doses of oral diclofenac products); therefore, we discontinued monitoring of serum diclofenac levels. Our previous work (14) has established that topical 10% DFMO cannot be detected systemically.

Laboratory methods

Polyamine assays. All skin samples were placed immediately on ice and transported within 30 minutes to the laboratory, where they were cut into approximately 1-mm pieces weighing approximately 5 mg each. After weighing, the samples were stored at −80°C until processed for polyamine assays. Skin biopsies were homogenized with 0.2 mol/L perchloric acid containing 10 μmol/L 1,7-diaminoheptane as an internal standard. The homogenate was centrifuged, and 100 μL of the supernatant was transferred to a new 1.5 mL Eppendorf tube containing 100 μL of 1 mol/L sodium carbonate. To the sample tube, 100 μL of 1% dansyl chloride in acetone was added, and the sample was placed at 60°C for 1 hour. Fifty microliters of 10% glycine was added to remove excess dansyl chloride. After incubation for 30 minutes, dansyl polyamines were extracted with hexane. The extract was dried under nitrogen and redissolved with 125 μL acetonitrile. A 50-μL aliquot of the resulting solution was injected onto the high-performance liquid chromatography column. An Ultrasphere

Table 1. Study population characteristics (phase II study participants)

Groups	DFMO ($n = 52$)	Diclofenac ($n = 52$)	DFMO + diclofenac ($n = 52$)
Age (Mean ± SD)	59.3 ± 9.5	60.4 ± 11.0	61.4 ± 10.2
Gender (%; female)	71%	71%	73%
Ethnicity (%; Caucasian)	94%	98%	95%
Eye color (%; blue or blue-green)	42%	47%	37%
Hair color (%; blonde or red)	29%	25%	19%

ODC 5-μm reversed-phase column (Beckman Instruments, Inc.; 4.6 × 250 mm) was used for analysis with a gradient of acetonitrile-disodium phosphate [1.2 mmol/L (pH 5.49)], a flow rate of 2.5 mL per minute at room temperature, and a 7-minute run time. A Kratos Spectroflow 980 fluorescence detector (ABI Analytical, Inc.) provided detection with excitation at 340 nm and emission at 550 nm. The detection limit was <1 pmol, with linearity of up to 250 pmol for each polyamine injected. Recoveries for putrescine, spermidine, and spermine were 105%, 99%, and 81%, respectively. In addition, all analytes were stable in skin stored at −80°C for at least 2 months.

Immunohistochemical analysis. Immunohistochemical staining for COX-2 expression was performed using streptavidin–biotin peroxidase system with a 3,3′-diaminobenzadine (DAB) chromagen and a hematoxylin counterstain (Ventana Medical Systems, Roche) on an automated VMS 320 immunostainer (Ventana Medical Systems). A Nuance multispectral camera (CRI) and a Leica DMR microscope were used to acquire and record images at 400× magnification. Bright-field was used for analyses and spectral imaging was done from blue to red (420–700 nm). Nuance software 3.0 (CRI) was used to spectrally unmix data into distinct channels representing hematoxylin and the chromagen (NovaRED). Three separate 40× fields were evaluated for COX-2, and both staining intensity [maximum optical density (OD)] and percent-positive area were recorded and submitted for statistical analysis.

Pathologists at the University of Arizona (Tucson, AZ) have developed a system for quantifying the severity of actinic damage in skin biopsy specimens. The six criteria that are assessed include: (i) atypia (pleomorphism), (ii) inflammation, (iii) epidermotrophism, (iv) loss of granular layer, (v) parakeratosis, and (vi) dyskeratosis. Atypia and inflammation are graded on a scale from 0–2, and all other criteria are graded as absent or present (0–1). The sum of the scores for individual criteria gives the total score for the specimen. The scores for normal skin were close to zero, those for early actinic keratoses were primarily in the 1–2 range, and most of the scores for actinic keratoses ranged from 3.5 to 6. These criteria have been evaluated and found to have good intrarater reliability (15).

Karyometric analysis. Karyometry is a process by which the information on chromatin patterns is digitized and extracted from nuclei for analysis. Through this process, progression curves have been determined, which correspond to pathologic diagnoses. Karyometric analysis has been shown to detect very subtle nuclear chromatin changes in preneoplastic lesions in a variety of tissues such as prostate (16) and breast (17). By using computer analysis of digital imagery, this methodology provides reliable, highly sensitive detection of early change and novel diagnostic clues. These analyses were conducted on formalin-fixed, paraffin-embedded tissue specimens from skin biopsies. The specimens were fixed in 4% neutral buffered formaldehyde for no more than 5 days, cut in 5-μm sections, and stained with synthetic hematoxylin and eosin under controlled conditions. Digital assessment of the nuclei from these specimens generates 93 characteristics of nuclear chromatin patterns, including average nuclear abnormality. Data were recorded with a videomicrophotometer equipped with a 3 CCD Sony camera and a 100:1, N.A. 1.4 planapochromatic oil immersion objective from Nikon. Relay optics adjusted the sampling rate to 6 pixels per linear micron. A narrow band interference filter with a bandpass at 620 nm was used to enhance contrast. Approximately 100 nuclei were recorded from the basal cell layer and the immediately adjacent parabasal cell layer for each biopsy specimen.

Statistical analysis

Histograms were constructed to assess the distributions for the baseline and end-of-study polyamine levels. Non-normal distributions were tested for statistically significant differences using nonparametric methods. Changes in the polyamine levels in the skin over the course of the study were calculated by subtracting the baseline levels from the end-of-study levels, and these changes were compared among the three treatment groups using the Kruskal–Wallis test.

For the karyometry, discriminant functions were constructed for baseline and end-of-study values. Assignment to the baseline category at the end of the study indicated that no preventive effect had taken place. Response to therapy was defined as a 30% or greater reduction in the proportion of nuclei with values assigned to the baseline category at the end of the study. Classification procedures to establish efficacy of the chemopreventive intervention followed the regimen of Hastie and colleagues (18). A training set was formed from 35 cases (14 DFMO, 12 diclofenac, and 9 DFMO + diclofenac), and a first validation set was composed of 30 cases (10 DFMO, 10 diclofenac, and 10 DFMO + diclofenac). The discrimination success was evaluated by applying the discriminant rule derived from the training sets to the first validation set, with a small modification to the training set or discriminant function if needed. The first validation set is therefore not entirely independent, as it was used to suggest modifications to the training set. The resultant discriminant function was then applied, unchanged, to an additional 79 cases in an entirely independent test set to provide an unbiased estimate of the prediction error. This procedure was followed for all three treatment arms.

Results

A total of 184 participants were accrued to this open-label study from October 2007 to September 2009. Eighteen participants were accrued to the study in the initial phase; however, dermatologic adverse events reported were higher than expected, and an interim analysis was requested by the investigators. This interim analysis resulted in a change in formulation and dosing to the interventions. Participants accrued before this interim analysis are labeled as "phase I" participants. Participants accrued after these

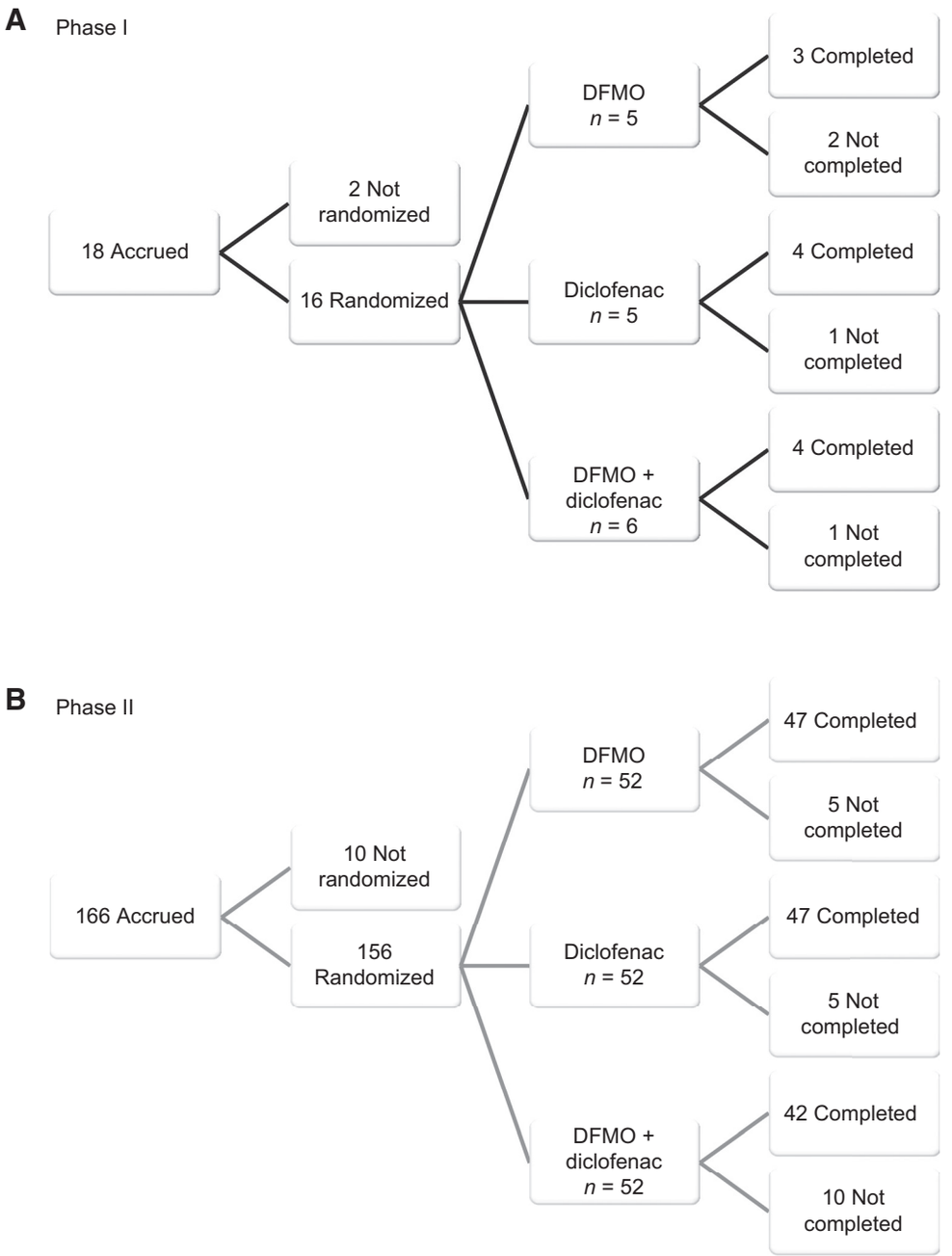

Figure 1.
A, Eighteen participants were accrued to the study in the initial phase when an interim analysis was requested due to higher than expected dermatologic adverse events. This interim analysis resulted in a change in formulation and dosing to the interventions. Subject noncompletion in this phase was due to halted participation due to the protocol and formulation changes. B, One hundred and fifty six participants were accrued after the interim analysis. Of these patients, 136 completed the end-of-study assessment. Reasons for subject noncompletion of the study included unacceptable clinical toxicity determined by patient or investigator ($n = 5$), subject decision to withdraw ($n = 9$), unrelated concurrent illness ($n = 1$), death due to unrelated causes ($n = 1$), change in eligibility status ($n = 2$), and other ($n = 2$).

analyses are labeled as "phase II" participants. A total of 156 subjects were enrolled and randomized to 90 days of topical DFMO, topical diclofenac, or use of both medications. Of these subjects, 144 had paired baseline and end-of-study biopsies (50 in the DFMO group, 49 in the diclofenac group, and 45 in the combination group), and 136 subjects completed the study through the end-of-study evaluation. The characteristics of the study population are described in Table 1, and the participant flow for each phase of the study is depicted in Fig. 1. End-of-study polyamine levels did not vary significantly among the three treatment groups, and the changes in polyamine levels over the course of the study were similar for each group as well. Testing for

Table 2. Baseline and end-of-study histologic score by treatment group

Biopsy	Treatment group	*N*	Mean	Median	SEM	SD	*P*
Baseline	DFMO	52	0.17	0	0.07	0.51	
	Diclofenac	52	0.19	0	0.083	0.59	0.58
	DFMO and diclofenac	52	0.21	0	0.063	0.46	
End of study	DFMO	48	0.50	0	0.133	0.92	
	Diclofenac	46	0.48	0	0.111	0.75	0.26
	DFMO and diclofenac	42	0.83	0	0.189	1.23	

change in polyamine levels by treatment group yielded *P* values of 0.687, 0.751, and 0.809 for putrescine, spermidine, and spermine, respectively. Similarly, COX-2 expression, as measured by percent DAB expression and maximum OD staining intensity, changed over time in all groups; however, this effect did not significantly vary by treatment group (data not shown).

Average baseline histologic scores and average histologic scores by DFMO/diclofenac treatment group are summarized in Table 2. There are no statistically significant differences in the histologic scores between the treatment groups at baseline ($P = 0.58$) or at end of study ($P = 0.26$).

A total of 26,653 nuclei were recorded for karyometric analysis: 9,507 in the DFMO arm, 8,989 in the diclofenac arm, and 8,157 in the combination treatment arm. The baseline average nuclear abnormality (ANA) was 0.75, which was lower than expected for sun-damaged skin based on historical specimens. This value reflects a nuclear abnormality only one standard deviation (averaged over all 93 chromatin features) above the value expected for a population of normal nuclei (19). The effect of the intervention was therefore expected to be confined to a small scale.

In the course of the study, the average nuclear abnormality increased rather than decreased, and the higher values were correlated with clinical inflammation of the skin. In addition, a preliminary discriminant analysis of baseline versus end-of-study datasets demonstrated an unexpected bimodality in the discriminant function score distribution (in contrast to the modest decrease in deviation from normal values as would be expected for a chemopreventive agent). These changes in the nuclear chromatin pattern were more consistent with an inflammatory reaction rather than a chemopreventive response. Because of the potential effects of inflammation on the ANA analysis, karyometric features that were not sensitive to inflammation were also evaluated in order to assess change in actinic damage. Total OD, relative nuclear area, and the number of densely stained pixels are elements of the ANA that have been shown to increase in value with increasing actinic damage and to decrease as an effect of chemopreventive intervention. For the karyometric analysis on a per-case basis, responders were identified as those who had a reduction in the proportion of nuclei assigned to the baseline category by at least 30%, which was statistically significant with $P < 0.05$. Table 3 lists the proportions of "responders" obtained from the first 65 cases (the training set + the first validation set) and from the full dataset. The second group (the final validation set) included fewer responders by this definition, possibly due to lower levels of actinic damage in this group.

Figure 2 shows the distribution of cases according to the change in the proportion of nuclei assigned to the baseline category, comparing the end-of-study values with the initial values. Significant decreases in the proportion assigned to the baseline category were present in 24% of subjects in the DFMO arm, 32% of those in the diclofenac arm, and 29% of the combination treatment arm, with $P < 0.05$.

Adverse events were carefully monitored throughout the study. Nearly all adverse events that were possibly or probably associated with medication use were local effects to the skin. In one subject, these effects were severe and required discontinuation of the treatment. One subject died while on the study due to causes unrelated to the treatment. No incidental diagnoses of skin cancers were made during the course of the study. Dermatologic adverse events are summarized in Table 4. Overall, significantly more adverse events were seen in the groups taking diclofenac. DFMO alone showed a low rate of adverse events and did not show additive adverse effects when taken in combination with diclofenac. All adverse events were resolved by the end of follow-up.

Discussion

Our study indicated that the addition of topical DFMO to topical diclofenac did not enhance its activity to treat cutaneous sun damage. The use of diclofenac also did not appear to decrease the toxicity associated with DFMO, as more toxicity was observed in subjects enrolled on the study arms that contained diclofenac as part of the treatment regimen. Both agents were found to cause inflammation on a cellular and clinically apparent level, which may have played a role in confounding the measurement of potential chemopreventive effects of these drugs. Unfortunately, a placebo arm was not included in this trial for practical reasons; however, further study with a group receiving the topical vehicle alone might help in quantifying the inflammatory effects associated with these drugs.

Although previous work demonstrated a change in skin polyamine levels associated with DFMO use in individuals with evidence of actinic keratoses (14), we did not observe a significant change in polyamine levels over the 3-month course for any of the treatment groups in this study. This result may be due to the use of DFMO in a population with less sun damage in the skin at

Table 3. Proportions of responders in evaluable patients undergoing baseline and end-of-study biopsies

	DFMO	Diclofenac	DFMO + diclofenac
Partial dataset[a] ($n = 65$)	11/24 (46%)	10/22 (45%)	9/19 (47%)
Full dataset ($n = 144$)	12/50 (24%)	16/49 (33%)	13/45 (29%)

[a]The partial dataset was composed of the combination of a training set and the first validation set. The training set was formed from 35 cases (14 DFMO, 12 diclofenac, and 9 DFMO + diclofenac), and a first validation set was composed of 30 cases (10 DFMO, 10 diclofenac, and 10 DFMO + diclofenac). The discrimination success was evaluated by applying the discriminant rule derived from the training sets to the first validation set, with a small modification to the training set or discriminant function if needed. The first validation set is therefore not entirely independent, as it was used to suggest modifications to the training set. The resultant discriminant function was then applied, unchanged, to an additional 79 cases in an entirely independent test set to provide an unbiased estimate of the prediction error.

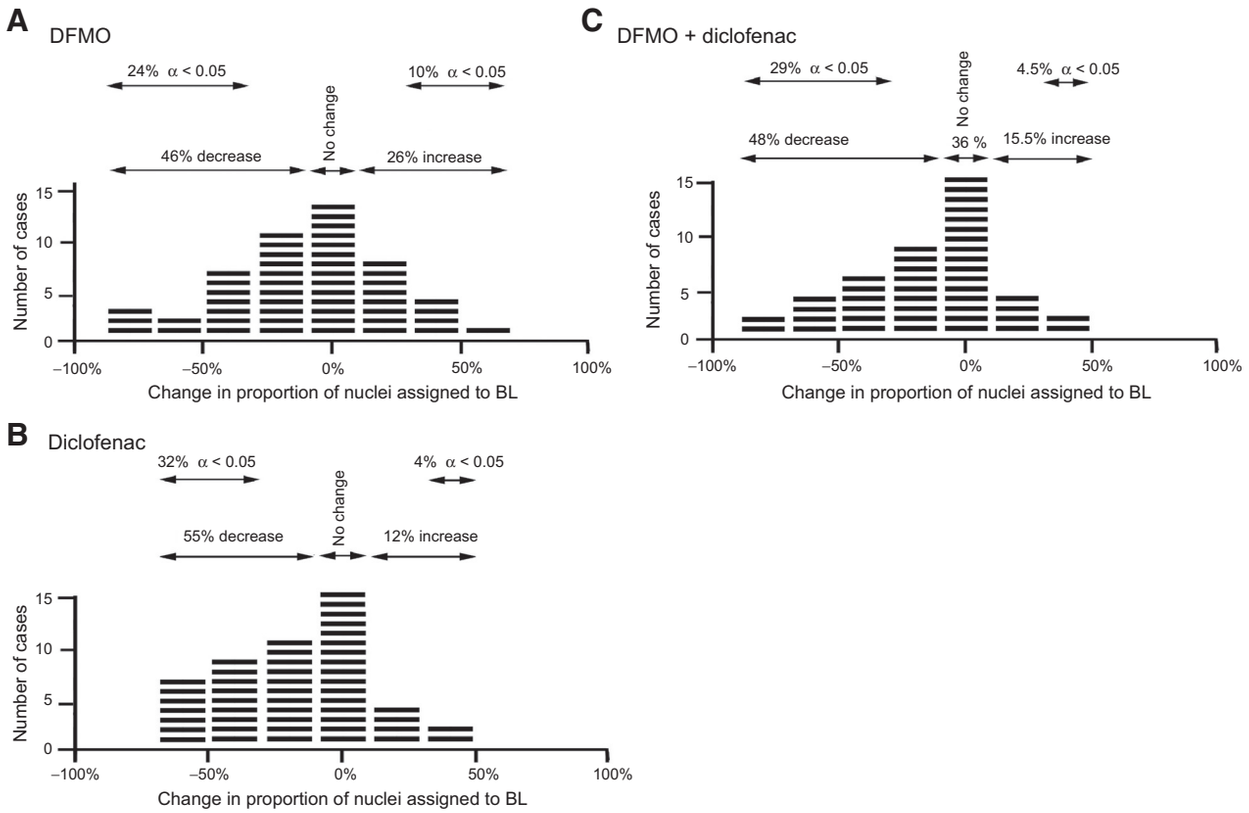

Figure 2.
Distribution of participants according to the change in the proportion of nuclei assigned to the baseline (BL) category, comparing the end-of-study values with the initial values for DFMO (A), diclofenac (B), and DFMO and diclofenac treatment arms (C).

baseline and the limited 3-month exposure, as compared with the 6-month duration studies previously reported (5). As the baseline levels of polyamines in our study were significantly lower than those seen in our previous studies in actinic keratosis patients, the lack of baseline skin damage is the more likely explanation for this result.

To our knowledge, the effects of topical diclofenac on levels of COX-2 protein in human skin have not been reported to date. On the basis of the cross-talk between the polyamine pathway and the COX-2 pathway, we hypothesized that diclofenac and DFMO may also potentially affect COX-2 levels and added this assessment as an exploratory aim. However, although cutaneous COX-2 levels varied over time, there was no evidence of a greater effect in one treatment arm as compared with the others.

The relative lack of sun damage at baseline for many subjects made it difficult to assess significant improvement with any of the courses of therapy. Karyometric analysis did allow for the detection of statistically significant reductions in skin biopsy nuclear abnormality related to the topically administered chemoprevention agents in all three study arms. The proportion of responding participants in each group was similar to that achieved with 6 months of topical DFMO (10%) in patients with severe actinic keratosis in a previously reported study (5).

Nevertheless, there was no evidence of additivity between DFMO and diclofenac in relation to a reduction in nuclear abnormality in sun-damaged skin. As the combination of DFMO and a nonsteroidal anti-inflammatory drug has been effective in the prevention of cancer precursor lesions at other sites, such as colon adenomas, study of this combination of agents for a greater

Table 4. Dermatologic adverse events by group and severity

Symptom/Sign	Severity	DFMO ($n = 52$)	Diclofenac ($n = 52$)	DFMO + diclofenac ($n = 52$)
Burning/Stinging	None	48 (92%)	44 (85%)	48 (92%)
	Mild	4 (8%)	8 (15%)	3 (6%)
	Moderate	0 (0%)	0 (0%)	1 (2%)
Pruritus	None	42 (81%)	33 (63%)	40 (77%)
	Mild	9 (17%)	12 (23%)	6 (12%)
	Moderate	1 (2%)	6 (12%)	6 (12%)
	Severe	0 (0%)	1 (2%)	0 (0%)
Rash, redness, and erythema	None	46 (88%)	34 (65%)	40 (77%)
	Mild	5 (10%)	10 (19%)	7 (13%)
	Moderate	1 (2%)	8 (15%)	5 (10%)

duration of time in a population with actinic keratoses or a greater level of baseline sun damage may yield more promising clinical results.

Disclosure of Potential Conflicts of Interest

C. Curiel-Lewandrowski has ownership interest (including patents) in DermSpectra Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J.M. Jeter, C. Curiel-Lewandrowski, S.P. Stratton, J.G. Einspahr, D.S. Alberts, P. Bartels

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Curiel-Lewandrowski, J.A. Warneke, J.G. Einspahr, M. Yozwiak, D.S. Alberts, P. Bartels

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Curiel-Lewandrowski, J.G. Einspahr, H.G. Bartels, C. Hu, D.S. Alberts, P. Bartels

Writing, review, and/or revision of the manuscript: J.M. Jeter, S.P. Stratton, J.A. Warneke, H.G. Bartels, M. Yozwiak, C. Hu, D.S. Alberts, Y. Bermudez, P. Bartels

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.M. Jeter, P.B. Myrdal, H.G. Bartels, M. Yozwiak, C. Hu, D.S. Alberts, Y. Bermudez, P. Bartels

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