International Journal of Dermatology

Clinical Trial

A randomized, open, controlled trial of tretinoin 0.05% cream vs. low-dose oral isotretinoin for the treatment of field cancerization

Mayra Ianhez[1], MD, MSc, PhD, Sebastião A. Pinto[2], MD, MSc, Helio A. Miot[3], MD, PhD, and Ediléia Bagatin[4], MD, MSc, PhD

[1]Dermatology, Universidade Federal de Goiás, Goiânia, Brazil, [2]Pathology, Universidade Federal de Goiás, Goiânia, Brazil, [3]Dermatology, Universidade Estadual Paulista – Julio de Mesquita Filho, UNESP, Botucatu, Brazil, and [4]Dermatology, Universidade Federal de São Paulo/Escola Paulista de Medicina (UNIFESP/EPM), Sao Paulo, Brazil

Correspondence
Mayra Ianhez, MD, MSC, PHD
Rua 66, número 84
ap 2201-A, Jardim Goiás
Goiânia GO, CEP 74810-330
Brazil
E-mail: ianhez@hotmail.com

Funding: None.

Conflicts of interest: None.

doi: 10.1111/ijd.14363

Abstract

Background Sun exposure may lead to actinic keratoses (AKs), field cancerization, and skin cancer. Effective treatment of AKs and field cancerization is important. Oral and topical retinoids can be used for this purpose. To compare clinical, histological, and immunohistochemical effects of oral and topical retinoid for AKs and field cancerization on face and upper limbs of immunocompetent patients, as well as the impact on quality of life, safety, and tolerability.

Methods This study compared 10 mg/day oral isotretinoin (ISO) to 0.05% tretinoin cream (TRE) every other night, associated with sunscreen (SPF 60). Patients of both genders, aged 50–75 years, underwent cryotherapy with liquid nitrogen for AKs at baseline and after 120 days when they were randomized into two groups, TRE (n = 31) and ISO (n = 30), for 6 months. Outcome measures were: number of AKs, histological (thickness of *stratum corneum* and epithelium) and immunohistochemical parameters (p53, Bcl-2 and Bax), dermatology life quality index (DLQI), and adverse events.

Results Both treatments reduced the number of AKs (around 28%), the thickness of *stratum corneum*, and expression of p53 and Bax. By contrast, the epithelium thickness and Bcl-2 expression increased. There was no difference in the outcomes between TRE and ISO. Both treatments improved quality of life and were well tolerated with minimal side effects.

Conclusions Retinoids are effective and safe for field cancerization. Classical treatments for field cancerization (imiquimod and ingenol mebutate) are used for a short period; retinoids may be a good choice to intercalate with them and can be used continuously.

Introduction

Actinic keratoses (AKs) are the main manifestation of field cancerization and can evolve to squamous cell carcinoma.[1] Treatment is designed to reach the individual lesions as well as field cancerization.[2]

Cryotherapy with liquid nitrogen (LN) is widely used to treat lesions.[3] For field cancerization, various therapeutic methods are adopted such as 5% imiquimod cream, 0.015% and 0.05% ingenol mebutate gel,[4] photodynamic therapy, 5% 5-fluorouracil cream or as agent for series of superficial peelings,[5,6] and 3% diclofenac in 2.5% hyaluronic acid gel.[2] Another option is the use of topical or systemic retinoids.[7,8]

Clinical trials have demonstrated the effectiveness of retinoids for treating AKs.[9] Studies with oral isotretinoin and etretinate confirmed their efficacy in the regression and treatment of AKs,[10] while prevention has been suggested with oral isotretinoin.[11] The topical use of tretinoin (retinoic acid) in the treatment of cutaneous photodamage led to clinical improvement and reduction of AKs.[10,12] However, limitations of these studies are: case series with small numbers of patients, lack of randomization, high rates of dropout, lack of standardization related to doses and concentrations of oral and topical retinoids, and focus on non-melanoma skin cancer (NMSC).[9]

The aim of the study was to compare through clinical, histological, and immunohistochemical parameters the efficacy and safety of a 6-month regimen on low-dose oral isotretinoin (ISO, 10 mg/day) or 0.05% tretinoin cream (TRE), associated with broad spectrum sunscreen, for the treatment of AKs and field cancerization of face and upper limbs.

Materials and methods

Study design

Open, parallel, and randomized clinical trial comparing two treatments, including patients from the Skin Cancer Outpatient Clinic of a public institution and recruited during annual skin cancer campaigns, from September 2011 to June 2013. The

study was approved by the Institutional Review Board and registered at www.clinicaltrials.gov (NCT 02278861). All patients signed the consent form.

Block randomization was carried out for pairs of patients (TRE and ISO) (Fig. 1). A computer program generated the list for random allocation sequence (1 : 1). Only one researcher had access to the randomized list, distribution of medications, and patient follow-up. The time application of cryotherapy with LN ranged from 5 to 10 seconds, according to the thickness and extension of lesions.[4]

The dose of oral isotretinoin (Germed, Brazil) was 10 mg/day, after meals, while that of topical tretinoin was 0.05% in cream (Theraskin, Brazil) applied to face and upper limbs, every other night, for 6 months. Sunscreen, SPF 60 (Germed, Brazil), reapplied every 3 hours was associated during the study period.

For ISO group, laboratory tests (complete blood count, fasting glucose, ALT, AST, and lipid profile) were performed prior to inclusion, every 2 months, and at the end. The criteria adopted for dose reduction and/or drug discontinuation were those described by Altman *et al.*[13]

Population

Inclusion criteria: both genders, 50–75 years old, skin phototypes I to IV,[14] moderate to severe degrees of photoaging,[15] 5–60 AKs visible and/or palpable on face and upper limbs.

Exclusion criteria: non-menopausal women, pregnant women, nursing mothers, undergoing treatment for AKs, cancer, or photoaging, immunosuppression, laboratory abnormalities: total cholesterol > 230 mg/dl, triglycerides > 250 mg/dl, AST > 40 U/l, ALT > 41 U/l and blood/leukocyte count <3,000/mm^3.

The subjects excluded (not randomized) were indicated to use sunscreen (SPF 60) and were followed for 6 months. As cryotherapy can improve clinical parameters along the study, this group was used to compare the additional impact of retinoids at T120–T300. The analysis of this group was made only for clinical parameters.

Efficacy parameters

The parameters used to measure efficacy included actinic keratosis count, emergence of NMSC, histological and immunohistochemical measures (thickness of *stratum corneum* and epithelium, percentage of elastic tissue, p53 – wild type, Bcl-2 and Bax biomarkers) at T120 and T300, quality of life assessment (DLQI), adverse events and laboratory abnormalities.

The AKs were counted on face and upper limbs in demarcated areas, by the same evaluator on two different occasions, at each visit, according to the methodology described by Ianhez *et al.*[16]

Palpable, visible, and/or hypertrophic AKs were included and underwent clinical diagnosis. Contiguous lesions were considered as one. Skin cancer or any suspicious lesions were excised and submitted for histopathological analysis.

For the histological and immunohistochemical analyses, skin biopsies were obtained using a 4 mm punch from a standardized area on the back of the left forearm. The material was fixed in 10% buffered formalin for paraffin inclusion and subsequent hematoxylin-eosin (H&E) staining.

Immunostaining for p53, Bcl-2, and Bax was performed at the same time to ensure uniformity; positive tumors were used for the immunomarkers, as positive controls. The kits for p53 (clone D0-7, Dako, code M7001-1), Bcl-2 (Bcl2 oncoprotein, clone 124, Dako, code M0887-1), and Bax (Bax – Polyclonal Rabbit anti-human code A3533) were used according to EnvisonFLEX protocol, Dako, USA, 1 : 20 dilution.

The thicknesses of *stratum corneum*, epithelium (measured from the basal layer to upper granular layer), the quantity of elastic fibers at upper dermis, and immunomarkers for Bcl-2 and Bax were measured digitally using the ImageJ program 1 : 38.[17] The photographs were acquired according to a standardized procedure using a digital camera mounted on a Nikon Eclipse E200 light microscope, under 1,024 × 768 resolution, 24-bit color, ISO 80, TIFF, and speed F4.5 1/2,000. They represented interfollicular spaces and were captured in duplicate for each slide under 40× magnification.

Weigert-Van Gieson staining was used to estimate the elastic tissue percentage in the papillary dermis,[18] recorded in triplicate under 100× magnification. The initial images were filtered in the blue channel, and three rectangles were segmented in the papillary dermis.

The analysis of the p53 nuclear staining was performed by calculating the HSCORE in the most intense stained areas (hotspots) in the sampled tissue.[19]

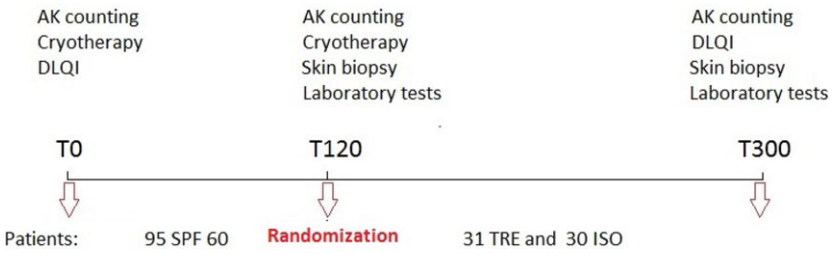

Figure 1 Timeline of the parameters evaluated in the study. SPF: sun protection factor; TRE: topical tretinoin; ISO, oral isotretinoin. *34 patients were excluded after the randomization, mostly because contraindications of the ISO group, and 33 were followed apart until T300

The intensity of cytoplasmic expression of Bcl-2 and Bax proteins in the epidermis was evaluated based on the epidermal areas most marked when viewed under a Nikon light microscope (×40). Cytoplasm of the five most stained cells were selected for digital evaluation of the staining intensity (0–255). Five fields were sampled using automated reading.

The Dermatology Life Quality Index, validated for Brazilian Portuguese – DLQI-BRA, was used to assess quality of life.[20,21]

Safety assessment considered the following side effects: retinoid dermatitis with erythema, pruritus, and scaling for TRE group;[22] mucocutaneous manifestations and laboratory alterations for ISO group; pain, blisters, ulcerations, dyschromia, and scarring for cryotherapy.

Statistical analysis and sample size

All patients included and those that remained for more than 15 days under treatment composed the ITT (intention to treat) population. Data analysis was performed for the ITT population based on a mixed-effects generalized linear model.[23,24]

The categorical variables are presented as absolute numbers and percentages. The comparison between the two groups was made using the chi-square or Fisher's exact tests. Continuous variables were tested for normality using the Shapiro–Wilk test and represented by means and standard deviations or medians and quartiles (p25–p75). They were compared using the Student's *t* or Mann–Whitney test.[25] The total DLQI scores, laboratory tests, AKs count, and analysis of histopathological data were compared in terms of time and groups using the mixed-effects linear model with robust covariance matrix and gamma adjustment or negative binomial probability, when indicated.[26] Data were tabulated in Microsoft Excel 2013, and statistical analysis was performed using SPSS 22.0.[27] The level of significance was set at $P < 5\%$.

The sample was sized so as to detect a reduction in difference of more than 20% between the groups with equal ratios and standard deviation of equivalent differences. Power of 0.9 and alpha 0.05 were adopted, resulting in 27 patients in each group.[28]

Results

The study flow chart is shown below (Fig. 2).

The average age was 64.1 years, and the gender distribution was similar (Table 1). The treatment groups were homogeneous, except for the use of acetylsalicylic acid (ASA) and anti-inflammatory drugs (NSAIDs), which was more common in TRE group.

The DLQI reduced ($P = 0.01$), with no difference between treatments. The clinical and demographic data are presented in Table 1.

There was a reduction in the total count of AKs (Table 1 and Fig. 3), related to cryotherapy and sunscreen, between T0 and T120 (−39%) as well as with treatment with oral isotretinoin (−33%) and topical tretinoin (−23%), plus cryotherapy and sunscreen, between T120 and T300.

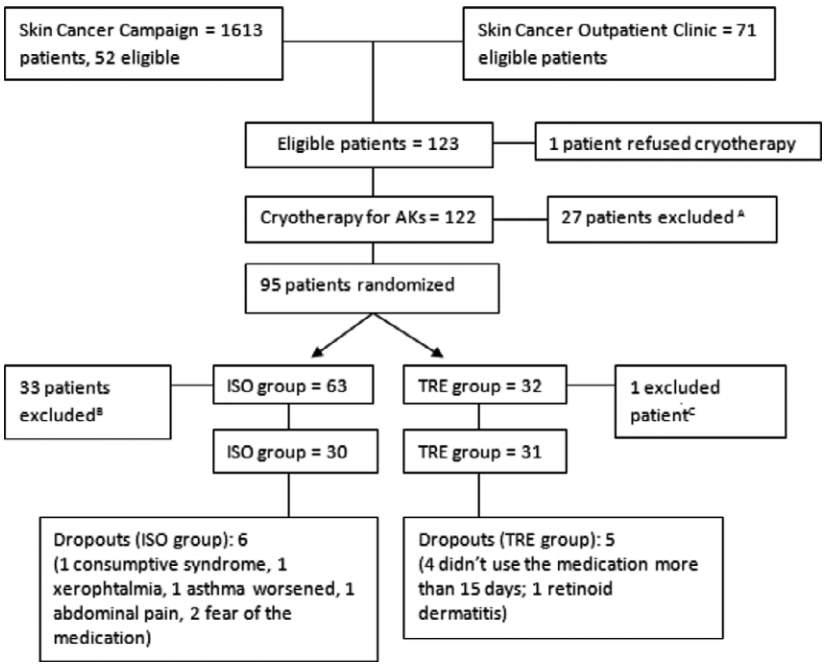

Figure 2 Flowchart of the study. ISO: isotretinoin; TRE: tretinoin; [A]: 2 skin cancer on treated area, 3 impossibility to attend appointments, 12 inexistent or wrong phone number, 5 refused to continue on the study, 4 missed the randomization, and 1 breast cancer; [B]: 25 dyslipidemia, 2 alteration of transaminases, 1 depression, 3 diabetes, and 2 refused the medication; [C]: refused the medication

Table 1 Demographic and clinical data of the study population ($N = 61$)

Variables	ISO Group ($n = 30$)	TRE Group ($n = 31$)	Total ($n = 61$)	*P*-value
Males, *N* (%)	13 (43.3)	12 (38.7)	25 (41.0)	0.71
Age (years)[#]	65.2 (6.2)	62.9 (8.2)	64.1 (7.3)	0.21
Phototype, *N* (%)				
I	2 (6.7)	2 (6.5)	4 (6.6)	0.99
II	18 (60.0)	18 (58.1)	36 (59.0)	
III	10 (33.3)	10 (32.3)	20 (32.8)	
IV	– (–)	1 (3.2)	1 (1.6)	
Previous skin cancer, *N* (%)	16 (53.3)	13 (41.9)	29 (47.5)	0.37
Current smoker, *N* (%)	5 (16.7)	3 (9.7)	8 (13.1)	0.42
ASA/Anti-inflammatory, *N* (%)	6 (20.0)	18 (58.1)	24 (39.3)	0.01
Use of ACE inhibitor, *N* (%)	5 (16.7)	7 (22.6)	12 (19.7)	0.80
DLQI SCORE T0[##]	1 (0–2)	0 (0–1)	1 (0–2)	0.65[@]
DLQI SCORE T300[##]	1 (0–1)	0 (0–2)	0 (0–1)	
AK total T0[##]	29 (14–39)	20 (16–32)	23 (16–36)	0.36[@@]
AK total T120[##]	16 (10–27)	14(7–22)	14(9–25)	
AK total T300[##]	11(7–23)	11 (7–19)	11 (7–19)	

ASA, acetylsalicylic acid; ACE, angiotensin converting enzyme; DLQI, dermatology life quality index; AK, actinic keratosis.
[#]Mean (SD).
[##]Median (p25–p75).
[@]ISO × TRE.
[@@]T120 × T300 = ISO × TRE (period of time of retinoid exposure).

Four patients developed skin cancer (basal cell carcinoma) during the study, two in each group ($P = 0.97$).

Analysis of subgroups showed no difference in AKs count reduction profile in relation to topography (face or upper limbs) ($P = 0.53$), gender ($P = 0.23$), age ($P = 0.14$), exposure to sun at work ($P = 0.26$), or schooling ($P = 0.10$).

Patients with a history of skin cancer had a lower rate of AKs reduction, regardless of the treatment group ($P = 0.01$).

NSAIDs/ASA users presented a lower reduction in AKs count with time (median: 15 to 13 vs. 14 to 10; $P = 0.03$), with better performance in TRE group (median: 14 to 13 vs. 20 to 24; $P = 0.02$). Patients with darker skin had the highest AKs reduction profile after ISO use (median: 19 to 10 vs. 14 to 11; $P < 0.01$), with no difference between treatments. Smoking was not assessed as it was reported by only eight individuals.

The excluded subjects were also followed for 6 months as an external control group using only sunscreen. These subjects have not differed from ISO and TRE regarding sex, age, skin phototype, scholarity, current smoking, previous skin cancer, and baseline AKs count, except by regular use of acetylsalicylic acid or non-hormonal anti-inflammatory medicines (data not shown). In a *per protocol* analysis, total AKs count reduction from T120 to T300 differed among sunscreen group (−27%), vs. TRE and ISO: TRE (−35%), ISO (−45%) even when adjusted by the use of acetylsalicylic acid or non-hormonal anti-inflammatory drugs ($P > 0.05$).

The histological and immunohistochemical findings (Fig. 4) demonstrated: reduction in the thickness of *stratum corneum*, expression of p53 and Bax at T120 and after retinoid use (T300). There was increase in the thickness of epithelium and expression of Bcl-2 in both groups, while no alteration of elastic

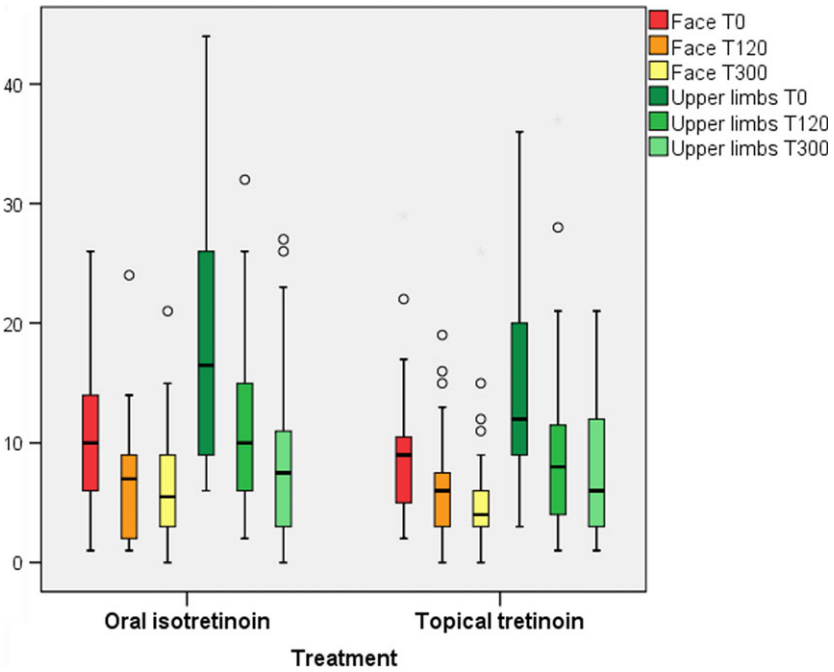

Figure 3 Actinic keratosis count at T0, T120, and T300 according to treatments and body sites

tissue was observed. Overall, the treatment groups did not differ (Table 2).

Cryotherapy caused pain and blisters in all patients; residual hypochromia occurred in 58% of the patients in one application point at least after the first session and hypertrophic scarring in one patient.

In TRE group, retinoid dermatitis occurred in 40% of the patients, usually at the beginning of the treatment, while in ISO group the mucocutaneous side effects were mild, except for one patient who presented severe xerophthalmia.

The ISO intolerance occurred in 20% of the patients leading to change in treatment regimen (10 mg every other day) and interruption due to xerophthalmia. In TRE group, side effects were less frequent and erythema was the most common (29% of the patients). Intolerance to medication occurred in 35.5% of the patients, and the cream application was reduced to one or two times a week. In ISO group, 13.3% of the patients experienced "fear" of the drug, which was stopped by two patients. By contrast, patients in ISO group had higher adherence to treatment (only 3.33% reported having forgotten to take the medication) compared to the TRE group (6.5%).

No differences were observed in the levels of hemoglobin, white blood cells, platelets, and transaminases throughout the study ($P > 0.05$). Total cholesterol levels increased in 76% of

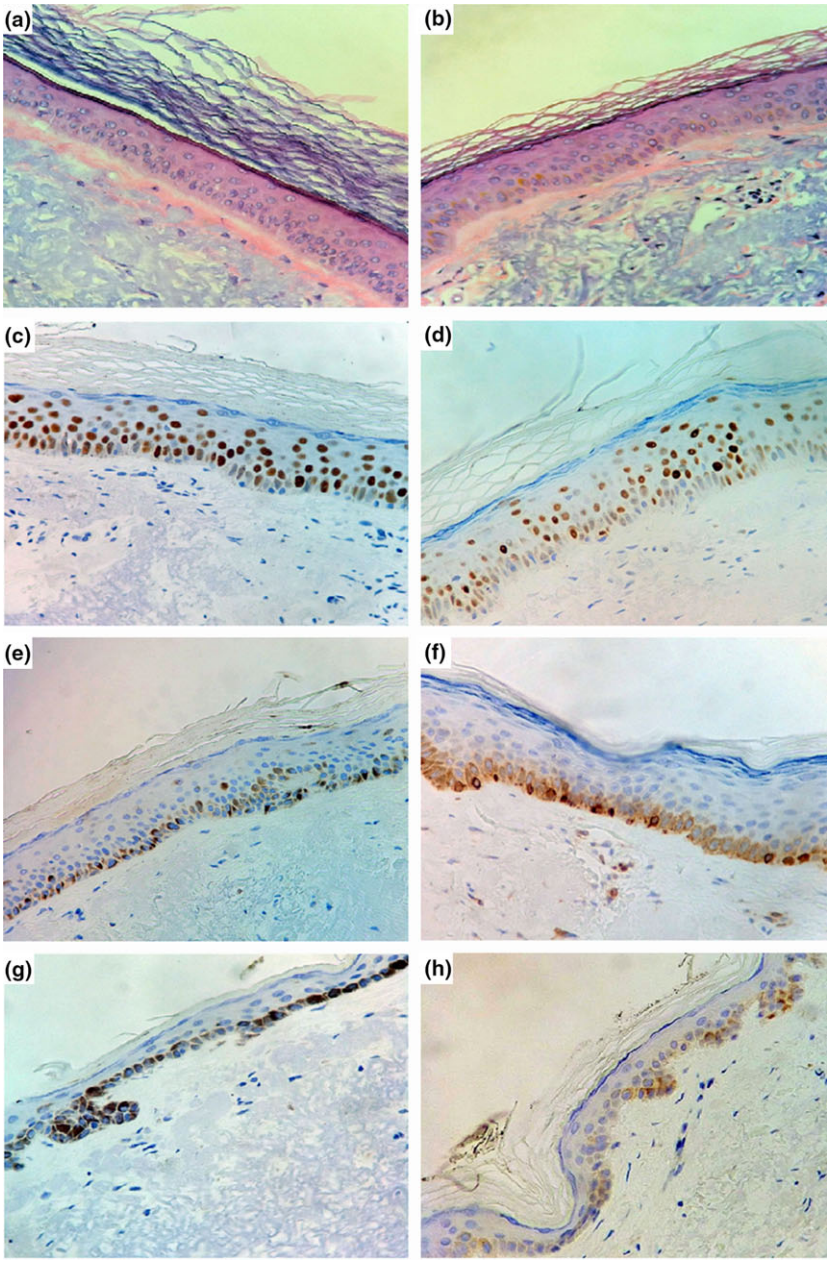

Figure 4 Histological and immunohistochemical findings before and after the use of retinoids. (a) The *stratum corneum* before and (b) after ISO 10 mg/day (reduction) – H&E staining. (c) p53 protein expression before and (d) after 0.05% TRE cream (reduction). (e) Expression of Bcl-2 before and (f) after 10 mg/day ISO (increase). (g) Expression of Bax protein before and (h) after 0.05% TER cream (reduction) (×40 magnification)

Table 2 Correlation of histological and immunohistochemical parameters between treatment groups expressed by the median of histological and immunohistochemical measures

Group	ISO		TRE			
Time	T120	T300	T120	T300	*P* (time)	*P* (time × group)
Stratum corneum (μm)	72 (58–120)	66.0 (43–85)	79 (65–105)	65 (45–82)	**<0.01**	0.26
Epithelium (μm)	96 (82–105)	137 (121–167)	90 (78–113)	120 (97–139)	**<0.01**	0.12
p53 (HSCORE)	158 (125–177)	108 (85–144)	175 (114–203)	138 (79–170)	**<0.01**	0.71
Elastic (%)	32 (28–42)	37 (30–44)	36 (26–40)	33 (29–39)	0.25	0.34
Bcl2 intensity	233 (223–239)	239 (232–247)	235 (227–242)	241 (234–244)	**<0.01**	0.79
Bax intensity	222 (204–235)	204 (169–210)	223 (205–231)	200 (173–219)	**<0.01**	0.37

Bold values indicates significance level of $p < 0.05$.

the patients and triglycerides in 61%, around 30% of the initial level, between T120 and T180, becoming normal at T300, without need to change or discontinue treatment.

Discussion

Retinoids may be used in the chemoprevention of skin cancer.[9] However, little is known regarding their safety and efficacy as a treatment strategy for field cancerization.[29]

The total AKs count, which may be used to estimate the activity of field cancerization, is the most widely used primary endpoint in observational and clinical studies.[16] Topical and systemic retinoids can reduce the number of AKs,[9] as can the application of cryotherapy[4] and the use of sunscreen.[30,31] In this study, we observed a reduction in the number of AKs both after initial treatment with LN and sunscreen and subsequently with the association of oral or topical retinoids and sunscreen.

Histologically, studies into photoaging have demonstrated thinning of the *stratum corneum* and thickening of the epidermal layer with the use of topical retinoids for 6 months,[32–35] as we have observed. Similar findings have been reported for both oral isotretinoin 20 mg every other day and for 0.05% tretinoin cream, every other night, for 6 months.[11]

Alterations in the p53 protein are described in more than 90% of the malignant neoplasms and may be indirect markers of field cancerization activity.[36,37] Retinoids reverse epidermal sun damage and probably have a chemopreventive effect on mutations of p53 protein, re-establishing the regular counterpart.[38,39] Sunscreen may also reduce the expression of p53, a fact that occurs in regular users, and noted in skin fragments collected during the winter months.[40,41] In this study, the combination of retinoids and photoprotection reduced p53 protein expression in both groups. A recent study comparing oral isotretinoin 20 mg every other day with sunscreen[42] and topical tretinoin 0.05% every other night[11] showed a reduction in the expression of epidermal p53 after 6 months, with no difference between retinoid formulations but greater than the use of sunscreen alone, demonstrating the additional effect of retinoid use.

Proteins linked to apoptosis (Bax and Bcl-2) are differentially expressed in cancer tissues and healthy skin. Studies that assessed Bcl-2 expression in AKs, seen in between 25 and 100% of such lesions, especially the hypertrophic forms, suggest an increased risk of progression to invasive disease.[43,44] Murine models indicate that the p53 tumor suppressor gene regulates the expression of Bcl-2 and Bax proteins, with a decrease in Bcl-2 and an increase in Bax.[45] The Bax protein is homologous to Bcl-2 and promotes cell death by competing with Bcl-2.[46] With the use of retinoids, normal cellular differentiation is re-established in the epithelium and the apoptotic pathways (p53 and Bax) are deactivated, thereby increasing the levels of the anti-apoptotic Bcl-2 protein, which explains the increased expression of this immunomarker and the decrease of Bax after the treatments, with no difference between groups.

Regular use of ASA and NSAIDs for more than 6 months is correlated with reduction in the number of AKs.[47] Their use should be questioned due to their influence on the number of AKs and skin tumors. In a multivariate analysis, when adjusted for the use of ASA and NSAIDs, treatment with retinoids plus sunscreen reduced the AKs count to a lesser degree among ASA/NSAIDs users, suggesting possible protection against field cancerization. We did not observe differences between treatment groups with ACE inhibitors, as previously reported.[48,49]

To date, there is no study about the use of oral isotretinoin just for AKs.[9] Only one study compared an oral retinoid to a topical retinoid for this purpose, but the number of participants was small, and the dropout rate was high.[50]

The reduction in the average DLQI scores demonstrates that both drugs may be useful in reducing the negative impact of AKs on quality of life. Another study comparing topical and oral retinoids for photoaging also showed no difference between them in terms of the reduction in DLQI scores.[11] Studies evaluating other therapeutic modalities such as PDT and imiquimod showed a similar reduction.[51]

The side effects of TRE, especially early in the treatment, were of mild or moderate intensity, as described.[12,52–54] Due to low dose of oral isotretinoin, milder mucocutaneous side effects were

observed.[55–58] Transaminases are usually altered in a dose-dependent manner by oral isotretinoin (from 8 to 11%).[57,59] However, probably due to low dose, no alterations were detected, despite the patients' higher age. Both triglyceride and total cholesterol levels showed increase but with no need to change or discontinue treatment, demonstrating that 10 mg/day of ISO can be a safe and effective alternative for AKs.

The efficacy of cryotherapy with LN varies from 39% for a single 5-second freezing cycle in light to moderate AKs, measuring up to 5 mm[60] and up to 100% with 5- to 10-second cycles in light AKs.[61] Due to the wide range of cryotherapy methods in the literature,[4] we decided to treat the AKs with a single 5–10 second cycle, according to the size of the lesion.

The dose of oral retinoid varies in different studies of NMSC. However, for AKs, the number of studies is small.[9] Thus, the dose of 10 mg/day was adopted in the expectation that it could assist in the treatment and prevention of AKs, while provoking fewer side effects than for acne, since the effects are dose-dependent and the study population was older, with drier and more sensitive skin. The concentration of retinoid cream varies among studies of AKs and NMSC, from 0.025 to 0.1%, with 0.05% being the most widely used.[9]

We observed that the reduction of total AKs count was more expressive after the first session of cryotherapy, a phenomenon observed in other studies.[62,63] This could explain why the first phase of the study (T0–T120: cryotherapy and SPF) was more effective than the second phase in reducing AKs count (T120–T300: cryotherapy, retinoids, and sunscreen). Moreover, the external control with excluded patients disclosed an inferior performance of sunscreen compared to both retinoid groups in AK count reduction, showing that the use of retinoids can benefit the treatment of field cancerization.

No differences were observed in the efficacy and safety of topical and oral retinoids, i.e., the retinoid therapy choice for the treatment of field cancerization will depend on the patient's profile, particularly in relation to the risks of adverse events.

Study limitations

Inclusion of different types of AKs (erythematous and hypertrophic); single-center, which limits the generalization of the results, despite increasing the internal validity due to the homogeneity of the groups; dropout rates in both groups (<10%), which were minimized by the ITT analysis; lack of a randomized control group using SPF alone, which inhibits the quantification of the additive effect of the retinoids; and lack of an additional ISO+TRE group.

Acknowledgments

We are grateful to the Brazilian National Council of Technology and Scientific Development (CNPq) for its financial support, to *Germed* (pharmaceutical company) for providing the 10 mg oral isotretinoin (Acnova®) and SPF 60 (Skinblock fluide Extreme®), and to *Theraskin* (pharmaceutical company) for providing the 0.05% tretinoin cream (0.05% Vitacid cream).

References

1 Schmitt JV, Miot HA. Actinic keratosis: a clinical and epidemiological revision. *An Bras Dermatol* 2012; **87**: 425–434.
2 Hoffbauer G, Anliker M, Boehncke WH, *et al.* Swiss clinical practice guidelines on field cancerization of the skin. *Swiss Med Wkly* 2014; **144**: w14026.
3 Ianhez M, Miot HA, Bagatin E. Liquid nitrogen for the treatment of actinic keratosis: A longitudinal assessment. *Cryobiology* 2014; **69**: 140–143.
4 Samorano LP, Torezan LA, Sanches JA. Evaluation of the tolerability and safety of a 0.015% ingenol mebutate gel compared to 5% 5-fluorouracil cream for the treatment of facial actinic keratosis: a prospective randomized trial. *J Eur Acad Dermatol Venereol* 2015; **29**: 1822–1827.
5 Guimarães CO, Miot HA, Bagatin E. Five percent 5-fluorouracil in a cream or for superficial peels in the treatment of advanced photoaging of the forearms: a randomized comparative study. *Dermatol Surg* 2014; **40**: 610–617.
6 Bagatin E. 5-Fluorouracil for actinic keratoses. *Exp Rev Dermatol* 2010; **5**: 131–139.
7 Marquez C, Bair SM, Smithberger E, *et al.* Systemic retinoids for chemoprevention of non-meanoma skin cáncer in high-risk patients. *J Drugs Dermatol* 2010; **9**: 753–758.
8 Campbell RM, DiGiovanna JJ. Skin cancer chemoprevention with systemic retinoids: an adjunct in the management of selected high-risk patients. *Dermatol Ther* 2006; **19**: 306–314.
9 Ianhez M, Fleury LFF Jr, Miot HA, *et al.* Retinoids for prevention and treatment of actinic keratosis. *An Bras Dermatol* 2013; **88**: 585–593.
10 Jeffs EWB, Tang EH. Actinic keratosis. Current treatment options. *Am J Clin Dermatol* 2000; **1**: 167–179.
11 Bagatin E, Guadanhim LR, Enokihara MM, *et al.* Low-dose oral isotretinoin versus topical retinoic acid for photoaging: a randomized, comparative study. *Int J Dermatol* 2014; **53**: 114–122.
12 Kligman AM, Grove GL, Hirose R, *et al.* Topical tretinoin for photoaged skin. *J Am Acad Dermatol* 1986; **15**: 836–859.
13 Altman RS, Altman LJ, Altman JS. A proposed set of new guidelines for routine blood tests during isotretinoin therapy for acne vulgaris. *Dermatology* 2002; **204**: 232–235.
14 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 1988; **124**: 869–871.
15 Glogau RG. Chemical peeling and aging skin. *J Geriatr Dermatol* 1994; **2**: 30–35.
16 Ianhez M, Bagatin E, Fleury LFF Jr, *et al.* The reliability of counting actinic keratosis. *Arch Dermatol Res* 2013; **305**: 841–844.
17 Schneider CA, Rasband WS, Eliceiri KW. NIH Image to Image J: 25 years of image analysis. *Nat Methods* 2012; **9**: 671–675.
18 Wick MR. *Diagnostic Histochemistry*. New York: Cambridge University Press, 2008.
19 Ishibashi H, Suzuki T, Suzuki S, *et al.* Sex steroid hormone receptors in human thymoma. *J Clin Endocrinol Metab* 2003; **88**: 2309–2317.
20 Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI) – a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994; **19**: 210–216.
21 Ferraz LB, Almeida FA, Vasconcellos MR, *et al.* The impact of lupus erythematosus cutaneous on the Quality of life: the

Brazilian-Portuguese version of DLQI. *Qual Life Res* 2006; **15**: 565–570.
22 Phillips TJ. An update on the safety and efficacy of topical retinoids. *Cutis* 2005; **75**: 14–24.
23 Chakraborty H, Gu H. A mixed model approach for intention-to-treat analysis in longitudinal clinical trials with missing values. 2015. Available at: https://www.rti.org/pubs/mr-0009-0904-chakraborty.pdf (accessed on 24 December 2015).
24 White IR, Carpenter J, Horton NJ. Including all individuals is not enough: lessons for intention-to-treat analysis. *Clin Trials* 2012; **9**: 396–407.
25 Norman GR, Streiner DL. *Biostatistics – The Bare Essentials*, 3rd edn. Toronto: BC Decker, 2007.
26 Azuero A, Pisu M, McNees P, *et al.* An application of longitudinal analysis with skewed outcomes. *Nurs Res* 2010; **59**: 301–307.
27 SPSS 22.0 for Windows [computer program] [cited 2015 Jul 27]. *Statistical Package for Social Science (SPSS)*. Release Version 22. Chicago, IL: SPSS Incorporation, 2013. Available from: http://www.spss.com (accessed on 24 December 2015)
28 Basagaña X, Spiegelman D. Power and sample size calculations for longitudinal studies comparing rates of change with a time-varying exposure. *Stat Med* 2010; **29**: 181–192.
29 Slaughter DP, Southwick HW, Smejkal W. "Field cancerization" in oral stratified squamous epithelium. *Cancer (Phila)* 1953; **6**: 963–968.
30 Thompson SC, Jolley D, Marks R. Reduction of solar keratoses by regular sunscreen use. *N Engl J Med* 1993; **329**: 1147–1151.
31 Ulrich C, Jürgensen JS, Degen A, *et al.* Prevention of non-melanoma skin cancer in organ transplant patients by regular use of sunscreen: a 24 months, prospective, case-control study. *Br J Dermatol* 2009; **161**: 78–84.
32 Weinstein GD, Nigra TP, Pochi PE, *et al.* Topical tretinoin for treatment of photodamaged skin. *Arch Dermatol* 1991; **127**: 659–665.
33 Bhawan J, Gonzalez-Serva A, Nehal K, *et al.* Effects of tretinoína on photodamaged skin. A histologic study. *Arch Dermatol* 1991; **127**: 666–672.
34 Olsen EA, Katz I, Levine N, *et al.* Tretinoin emollient cream: a new therapy for photodamaged skin. *J Am Acad Dermatol* 1992; **26**: 215–224.
35 Mukherjee S, Date A, Patravale V, *et al.* Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clin Interv Aging* 2006; **1**: 327–348.
36 Kim KH, Park EJ, Seo YJ, *et al.* Immunohistochemical study of cyclooxygenase-2 and p53 expression in skin tumors. *J Dermatol* 2006; **33**: 319–325.
37 Sutter C, Wersin R, Varnai M, *et al.* Elevated p53 protein expression in normal and neoplastic light-exposed epidermis. *Verh Dtsch Ges Pathol* 1994; **78**: 246–251.
38 Lens M, Medenica L. Systemic retinoids in chemoprevention of non-melanoma skin cancer. *Expert Opin Pharmacother* 2008; **9**: 1363–1374.
39 Jones E, Korzenko A, Kriegel D. Oral isotretinoin in the treatment and prevention of cutaneous squamous cell carcinoma. *J Drugs Dermatol* 2004; **3**: 498–502.
40 Ponten F, Berne B, Ren ZP, *et al.* Ultraviolet light induces expression of p53 and p21 in human skin: effect of sunscreen and constitutive p21 expression in skin appendages. *J Invest Dermatol* 1995; **105**: 402–406.
41 van der Pols JC, Xu C, Boyle GM, *et al.* Expression of p53 tumor suppressor protein in sun-exposed skin and associations with sunscreen use and time spent outdoors: a community-based study. *Am J Epidemiol* 2006; **163**: 982–988.
42 Bagatin E, Parada MO, Miot HA, *et al.* A randomized and controlled trial about the use of oral isotretinoin for photoaging. *Int J Dermatol* 2010; **49**: 207–214.
43 Nakagawa K, Yamamura K, Maeda S, *et al.* Bcl-2 expression in epidermal keratinocytic diseases. *Cancer* 1994; **74**: 1720–1724.
44 Tomas D, Kruslin B, Cupic H, *et al.* Correlation between Bcl-2 and Bax in atrophic and hypertrophic type of actinic keratosis. *J Eur Acad Dermatol Venereol* 2006; **20**: 51–57.
45 Miyashita T, Krajewski S, Krajwske M, *et al.* Tumor suppressor p53 is a regulator of Bcl-2 and Bax gene expression in vitro and in vivo. *Oncogene* 1994; **9**: 1799–1805.
46 Basu A, Haldar S. The relationship between Bcl-2, Bax and p53: consequences for cell cycle progression and cell death. *MHR* 1998; **4**: 1099–1109.
47 Schmitt JV, Miot HA. Oral acetylsalicylic acid and prevalence of actinic keratosis. *Rev Assoc Med Bras* 2014; **60**: 131–138.
48 Christian JB, Lapane KL, Hume AL, *et al.* Association of ACE inhibitors and angiotensin receptor blockers with keratinocyte cancer prevention in the randomized VATTC trial. *J Natl Cancer Inst* 2008; **100**: 1223–1232.
49 Moscarelli L, Zanazzi M, Mancini G, *et al.* Keratinocyte cancer prevention with ACE inhibitors, angiotensin receptor blockers or their combination in renal transplant recipients. *Clin Nephrol* 2010; **73**: 439–445.
50 Rook AH, Jaworsky C, Nguyen T, *et al.* Beneficial effect of low-dose systemic retinoid in combination with topical tretinoin for the treatment and prophylaxis of premalignant and malignant skin lesions in renal transplant recipients. *Transplantation* 1995; **59**: 714–719.
51 Hadley J, Tristani-Firouzi P, Hull C, *et al.* Results of an investigator-initiated single-blind split-face comparison of photodynamic therapy and 5% imiquimod cream for the treatment of actinic queratoses. *Dermatol Surg* 2012; **38**: 722–727.
52 Andreano JM, Bergfeld WF, Medendorp SV. Tretinoin emollient cream 0.01% for the treatment of photoaged skin. *Cleve Clin J Med* 1993; **60**: 49–55.
53 Barel AO, Delune M, Clarys P, *et al.* Treatment of photodamaged facial skin with topical tretinoin: a blinded, vehicle-controlled half-side study. *Nouvelles Dermatologiques* 1995; **14**: 585–591.
54 Griffiths CEM, Kang S, Ellis CN, *et al.* Two concentrations of topical tretinoin (retinoic acid) cause similar improvement of photoaging but different degrees of irritation. *Arch Dermatol* 1995; **131**: 1037–1044.
55 Barth JH, MacDonald-Hull SP, Mark J, *et al.* Isotretinoin therapy for acne vulgaris: a re-evaluation of the need for measurements of plasma lipids and liver function tests. *Br J Dermatol* 1993; **129**: 704–707.
56 Baxter KF, Ling TC, Barth JH, *et al.* Retrospective survey of serum lipids in patients receiving more than three courses of isotretinoin. *J Dermatol Treat* 2003; **14**: 216–218.
57 Brelsford M. Preventing and managing the side effects of isotretinoin. *Semin Cutan Med Surg* 2008; **27**: 197–206.
58 De Marchi MA, Maranhão RC, Brandizzi LI, *et al.* Effects of isotretinoin on the metabolism of triglyceride-rich lipoproteins and on the lipid profile in patients with acne. *Arch Dermatol Res* 2006; **297**: 403–408.
59 Vieira AS, Melchiors AC, Beijamini V. The effect of isotretinoin on triglycerides and liver aminotransferases. *An Bras Dermatol* 2012; **87**: 382–387.

60 Thai KE, Fergin P, Freeman M, *et al.* A prospective study of the use of cryosurgery for the treatment of actinic keratoses. *Int J Dermatol* 2004; **43**: 687–692.
61 Goldberg LH, Mamelak AJ. Review of actinic keratosis. Part I: etiology, epidemiology and clinical presentation. *J Drugs Dermatol* 2010; **9**: 1125–1132.
62 Foley P, Merlin K, Cumming S, *et al.* A comparison of cryotherapy and imiquimod for treatment of actinic keratosis: lesion clearance, safety, and skin quality outcomes. *J Drugs Dermatol* 2011; **10**: 1432–1438.
63 Morton C, Campbell S, Gupta G, *et al.* Intraindividual, right-left comparison of topical methyl aminolaevulinate-photodynamic therapy and cryotherapy in subjects with actinic keratosis: a multicentre, randomized controlled study. *Br J Dermatol* 2006; **155**: 1029–1036.

