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ORIGINAL ARTICLE

Treating the invisible: Subclinical actinic keratosis detected by imiquimod

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Abstract

Background: Skin in UV-exposed areas may develop UV-induced actinic damage in DNA sequences leading to proliferation of keratinocyte carcinoma, a type of non-melanoma skin cancer. Actinic keratosis (AK) represents early-stage in situ squamous cell carcinoma. It develops from the basal cell layer and may be present in subclinical stages, being reported as field cancerization, even before being clinically visible. Thus, we hypothesise that from the age of 50 + UV-exposed skin features subclinical forms of AKs without visible skin lesions.

Objectives: This study aims at clarifying the existence of the clinically invisible, early stages of AKs on chronically UV-exposed skin without showing clinically evident features of AK.

Methods: 46 volunteers (26 females and 20 males) aged 50+ with signs of moderate to severe UV-damage (photodamage) applied imiquimod 3.75% creme on their entire face every night for a 2-week period. All participants kept a diary noting onset, severity of inflammatory reactions, and unexpected side effects.

Results: More than 90% of participants without clinical evidence of AK showed inflammatory reactions on topical treatment with imiquimod on their face demonstrating an interaction between UV-damaged mutated keratinocytes and the topically applied immunomodifier, thus proofing the presence of subclinical AKs.

Conclusions: The action of imiquimod is not restricted to visible AK lesions, but often includes their vicinity, suggesting that neoplastic processes are frequent at a cellular level, not confined to clinically evident lesions, supporting the concept of field cancerization. Thus, subclinical AKs do exist in an early, macroscopically invisible state and may be targeted by imiquimod or 5-FU. At this stage, AKs are being treated before diagnosis by usual clinical means, and well before potential progression to invasive squamous cell carcinoma (SCC). Eventually, imiquimod 3.75% cream could be recommended in UV-exposed skin to prevent the presumptive development of AK and later on, early SCC.

KEYWORDS
actinic keratosis, immunomodulatory, subclinical, treatment

INTRODUCTION

Chronically UV-exposed skin bears the risk of suffering from the hazards of multiple external influences such as UV-damage, summarised under the term exposome that has been proposed as a new paradigmatic term to encompass the totality of human environmental (meaning all non-genetic) exposures from conception onwards, complementing the genome.[1–3] Over time, this exposome features a variety of age-related markers: exposed skin, for example, shows clinical macroscopic signs of accelerated skin ageing like mottled pigmentation, fine lines, wrinkles, and leathery texture.[4] Histopathologically, skin ageing is characterised by actinic elastosis and increased pigmentation at the tips of rete ridges. Exposed areas also develop UV-induced actinic damage in DNA sequences in the cellular nuclei leading to potential hyperproliferation of all types of keratinocyte carcinomas (KCs).

KCs (basal cell carcinoma and all stages of squamous cell carcinoma [SCC]) originate from UV-damaged keratinocytes.[5–7] Ackerman and Mones in 2006 clearly stated that actinic keratosis (AK) at any stage already is squamous cell carcinoma, even if this may be discussed controversially.[8] Clinically, due to the development of AK from the basal cell layer, there is no clear way to identify the boundary with healthy skin, hence it is called field cancerization.[9]

Today, various treatment options for the management of AKs are available and can be chosen according to individual case-specific requirements.

AKs developing from the basal cell layer may grow towards the skin surface, replacing the spinous layer of the epidermis, they also may proliferate into dermal layers representing invasive SCC at a stage when they are clinically still invisible.[10–14]

To prove the existence of such invisible stages of AKs in chronically UV-exposed skin, we designed a clinical study including volunteers aged 50+ with age-related UV-damaged facial skin apparently "healthy skin" without showing any clinical presence of AK and treated them with topical imiquimod.

METHODS

The study protocol was approved by local ethics committee (EK 32-618 ex 19/20) and federal institutions for security in health research.

Forty-six Caucasian volunteers, male (20) and female (26) aged 50–82 (median 60.5) (Fitzpatrick skin type 2-3) were recruited, all of them featured age dependent chronically UV-damaged facial skin but no signs of clinically visible AKs. They applied imiquimod 3.75% creme on their entire face every night for a 2-week period. During this time all participants filled in a clinical diary noting the start and severity of inflammatory reactions and unexpected side effects.

Standardised photodocumentation taking three positions, frontal view, and from left and right at a 45° angle, has been performed at baseline (Week 0), at the end of treatment (Week 2), and at follow-up (Week 6).

At baseline, all participants filled in a questionnaire on their sunburn history in their youth, daily outdoor hours, UV-protection habits, and their knowledge about the interaction of UV-light and skin cancer.

The primary endpoint of this study is to show if and to what extent chronically UV-exposed facial skin does react to topical imiquimod 3.75% treatment and investigate what percentage of participants (without clinical signs of AKs on facial skin) will show an inflammatory reaction as proof for the presence of subclinical AKs.

The secondary endpoint is to determine the eventual differences concerning the outcome of this treatment in male and female participants.

RESULTS

90% of males (18 of 20) and 92% (24 of 26) of females showed inflammatory reaction on topical treatment with imiquimod on their face, demonstrating the presence of subclinical AKs. On average, inflammation started at Day 5. There was a notable difference in men and women, as inflammation occurred earlier in males starting at Day 4 whereas in females, a reaction was observed starting at Day 6 on average. Reaction to treatment was graded in four visible stages (no reaction = 0; mild = 1; moderate = 2; severe = 3).

More specifically, a grade of 0 showed no inflammatory reaction at all, a grade of 1 showed 1–2 lesions smaller than 1 cm, a grade of 2 showed 3–5 lesions smaller than 1 cm, and a grade of 3 showed more than 5 lesions 1 cm in diameter or larger. Two independent dermatologists quoted the reactions on anonymized blinded photographs. The grade of reaction was different in males and females. There was no inflammatory

reaction in 10% (2 of 20) males and 8% of females (2 of 26).

Ratings 0–3 were summarised for each location (frontal, right, and left) and then divided by three to gain the individual severity value. The severity of reaction was lesser in females than in males. The overall severity value was 1.4 (1.6 frontal, 1.4 right, and 1.3 left) in females and 2.6 in males (2.5 frontal and 2.6 right and left) (Figure 1).

Side effects occurred in only 10% (2 of 20) of male participants. One showed flu-like symptoms and one aphthous ulcer on the lower lip. In female patients, side effects occurred more frequently: 58% (15 of 26) of females showed unwanted side effects: 46% (12 of 26) showed aphthous ulcers on the inner aspects of the lips (herpes PCR was negative), 8% (2 of 26) concomitant conjunctivitis and 4% (1 of 26) swelling of the lips. All reactions and side effects healed without sequelae within the follow-up period of 4 weeks.

The evaluation of the questionnaires revealed some interesting details and differences concerning males and females (Table 1). In general, in Central Europe, females suffer from less facial sunburns in their childhood than males, which was also confirmed with our questionnaire. In our cohort, almost 58% of women (15 of 26) use topical UV-protection on a daily basis, whereas men use it only during vacation in 50% (10 of 20) or when sporting outdoors in 40% (8 of 20). Remarkably, only 1 man (5%) of our group of 20 male volunteers uses daily sunscreen. 30% of all participants (14 of 46)—male and female equally—did not know that UV-light causes skin cancer.

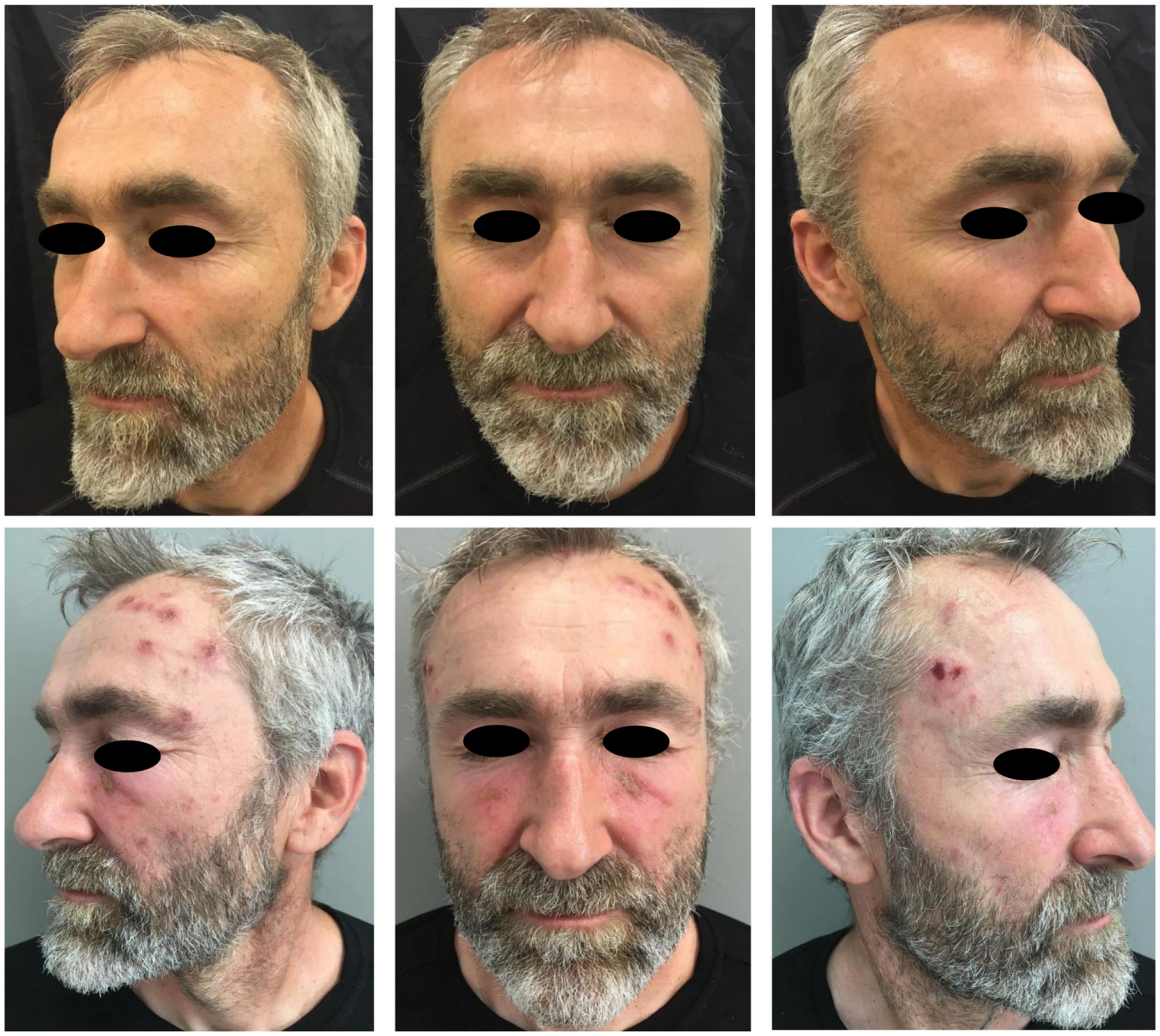

FIGURE 1 (53-year-old sports teacher) upper row: Baseline, UV-exposed skin showing no signs of actinic keratosis (AKs), lower row: Week 2, moderate to severe inflammatory reaction to imiquimod treatment of chronically UV-exposed skin likely representing subclinical AK and field cancerization.

TABLE 1 Questionnaire on sunburn history, daily outdoor hours, UV-protection habits, and knowledge about the interaction of UV-light and skin cancer.

Question	Answer	Male $n = 20$	Female $n = 26$
Did you suffer from facial sunburn when you were young?	Frequently	4 (20%)	7 (27%)
	Random	14 (70%)	13 (50%)
	Never	2 (10%)	6 (23%)
How many hours per day do you spend outdoors?	0–2	9 (45%)	11 (42%)
	2–5	8 (40%)	12 (46%)
	>5	3 (15%)	3 (11%)
Do you use topical uv-protection?	Never	1 (5%)	1 (4%)
	Every day	1 (5%)	15 (58%)
	On vacation	10 (50%)	5 (19%)
	When outdoor sporting	8 (40%)	5 (19%)
Did you know uv-light causes skin cancer?	Yes	14 (70%)	18 (70%)
	No	6 (30%)	8 (31%)

DISCUSSION

In our setting, we showed that AKs are already present even before they can be clinically diagnosed.[10] Forty-six Caucasian volunteers (Fitzpatrick skin type 2-3) applied imiquimod 3.75% cream to their apparently healthy but chronically (age 50+) UV-exposed face for 2 weeks. Over 90% (90% male and 92% of female) of them showed imiquimod-induced inflammatory reactions due to the presence of subclinical AKs.

This is not surprising because previous studies in DNA-repair-deficient mice have shown that in 60%–90% of AK and SCCs the signature mutation is in the p53 gene. Strikingly, when examining healthy-appearing skin exposed to UV light, a p53 mutation was found in 75% of cases, 15 times more often than in sun-protected skin.[15]

UV-exposed skin may present mottled pigmentation, thinning, dryness, wrinkling, and also AKs as signs of UV-damage as chronic UV exposure leads to cumulative DNA alterations overwhelming physiological DNA repair mechanisms. Consequently, carcinogenic transformation in UV-damaged skin occurs sooner or later, its extent depends on the skin type and on the amount of accumulated UV-exposure.[16]

In UV-damaged skin featuring AKs, the proapoptotic tumour inhibiting actions of imiquimod are not restricted to visible AKs, but often include their vicinity, suggesting that the neoplastic processes are, in fact, more frequent at the cellular level and not confined to clinically evident lesions, supporting the concept of field cancerization.[17–19] Field cancerisation unveiling also subclinical stages of AK has been discribed in the past in many studies [10,12] but as far as we know, this has always been a "side effect" of treating some already present clinically visible AK. In this study, we treated only patients with appearently "healthy skin" without clinically visible AK present at baseline. At this stage, the question arises: "When do AKs begin to exist?"[16,17,20] We wanted to show that practically every individual age 50+, presuming a lifelong consumed quantity of UV-light on the face, suffers from AK even before they can be clinically diagnosed. We treated "the invisible stage."

Imiquimod induces apoptosis selectively in tumour cells and does not harm normal skin.[12,13,21,22] Even if this selectivity is a main asset for choosing imiquimod treatment, it is a high-cost option among many alternative treatments at lower cost as reported in the literature. This is very important for sunny and developing countries where the prevalence of multiple AKs is high. Alternatively, 0.05% tretinoin cream or as a peel agent, 5% 5-FU cream or as an agent for sequencial and superficial peeling, or cryotherapy may be used topically.[23–28] Systemic isotretinoin has also been reported to clear AK.[29]

In our study, the lower severity of cutaneous reaction in women than in men (overall severity value 1.4 in women vs. 2.5 in men) correlates with the fact that 58% of women in this study consistently use UV-protection daily compared with only 5% of men (Table 1). Overall, it is obvious that women in the 50+ generation have habitually used skincare products and UV-protection more frequently and consistently than men for decades, and due to this prophylaxis they appear to develop less subclinical AKs. Specific gender-linked differences

concerning immune response in human skin and also the susceptibility of skin cancer development have been published earlier.[30–32] According to a Swedish Cancer registry, men are more prone to skin cancer, with squamous cell carcinomas being twice more common in older men.[33]

Concerning the side effects of imiquimod treatment in patients with subclinical AKs we found that women developed by far more unexpected side effects than men. 58% of females developed either aphthous ulcers in the oral mucosa, conjunctivitis or swelling of the lips compared with only 10% of males. Thus pointing out that the female immune system reacts differently (more sensitive?) even if there is little knowledge of the exact pathway of eventual gender-specific differences in immunologic reaction patterns.[20,30–33]

In our clinical study involving only 50+ patients with various degrees of chronic UV-damage but without visible AKs, we showed that the inflammatory response triggered by imiquimod selectively detects and eradicates clinically invisible AKs in its subclinical stage. This opens the gate to AK prevention as the use of imiquimod cream on sun-exposed areas in individuals aged 50+ every 2–3 years (interval depending on the skin type) has the potential of preventing AK and later stages of keratinocyte cancer.

AK from the angle of Shakespeare[20]**:**

"To treat or not to treat" derived from Shakespeare's monologue of Hamlet "to be or not to be", is a rather philosophical approach, leaving ample space for discussion on a "hot topic". Because only two decades ago AK was more or less an aesthetically disturbing sign of ageing even if a precursor of cancer. Today we know that AK at all stages represents skin cancer. Prince Hamlet's destiny was not „to be", thus, having a negative impact on his life. If we decide to treat AK as early as possible, we could set a positive impact for many lives.

AUTHOR CONTRIBUTIONS

Daisy Kopera: Study design and implementation. **Joachim Torrano**: Statistics and images. **H. Peter Soyer**: Editing.

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CONFLICT OF INTEREST STATEMENT

HP Soyer is a shareholder of MoleMap NZ Limited and e-derm consult GmbH and undertakes regular teledermatological reporting for both companies. HP Soyer is a Medical Consultant for Canfield Scientific Inc, Blaze Bioscience Inc, and a Medical Advisor for First Derm. J Torrano does not have any conflicts of interest. D Kopera does not have any conflicts of interest.

DATA AVAILABILITY STATEMENT

Data is available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All patients in this manuscript have provided written informed consent for their participation in the study and the use of their deidentified, anonymized, aggregated data, as well as their case details (including photographs) for publication. Ethical Approval: MedUniGraz EK 32-618ex19/20.

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