

Imiquimod 5% Cream Reverses Histologic Changes and Improves Appearance of Photoaged Facial Skin

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Ten healthy women with moderate signs of facial photodamage (fine lines, wrinkles, dyspigmentations, hyperkeratotic prominent pores, and poor skin texture) applied imiquimod 5% cream once daily for 5 days each week for 4 weeks. None of the subjects had actinic keratoses or had received previous treatment for basal or squamous cell cancers. Histology, hydration, coloration, and imaging assessments were conducted before and after treatment to determine the effects of imiquimod therapy. Global assessments of the improvement in skin appearance were evaluated by subjects and a dermatologist. Histologic analysis revealed that the structurally regressive changes of the epidermis—atrophy, atypia, hyperchromatic nuclei, disorderly differentiation, and loss of polarity—were completely reversed after imiquimod treatment. The dermal matrix was unaffected by imiquimod therapy. Global assessments of skin appearance revealed that imiquimod treatment yielded appreciable reductions in wrinkles, dyspigmentations, and hyperkeratotic pores. Clinical improvements in skin appearance were confirmed by imaging, coloration, and hydration assessments that demonstrated a smoother surface, more uniform color, improved texture, and elimination of hyperkeratotic pores. The correction of epidermal dysplasia, a characteristic feature of photoaged skin in which epithelial tumors arise, suggests that imiquimod exerts a prophylactic action in the prevention of cutaneous tumors. Imiquimod provides an alternative to topical retinoids in reversing the clinical and histologically regressive changes of photoaged facial skin.

Excessive exposure to ultraviolet light results in the development of a prematurely aged and photodamaged face characterized by wrinkles, dyspigmentations, laxity, poor texture, and roughness.¹ The most serious long-term consequences of photodamage include the development of common cutaneous epithelial tumors, such as actinic

keratoses (AKs), basal and squamous cell cancers, and lentigo maligna melanoma. The prevalence of these tumors is steadily increasing as the population ages.² Furthermore, cutaneous epithelial tumors are occurring more frequently in younger patients, and many of these tumors exhibit aggressive behavior. AKs and other cutaneous tumors are characterized by atypia (ie, variation in the size, shape, and staining properties of epidermal keratinocytes), hyperchromatic nuclei, loss of polarity, poor differentiation, dyshesion, and parakeratosis.³

Topical drugs used in the treatment of the photoaged face include retinoids, tretinoin, tazarotene, and adapalene.^{4,5} These agents frequently cause scaling,

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redness, and stinging,⁶ which may result in early treatment discontinuation. The presence of a number of visible AKs may be indicative of numerous subclinical microscopic AKs throughout the entire field of photodamaged skin, a process termed *field cancerization*. In such instances, it may be more appropriate to direct therapy to the whole tumor-bearing field rather than to use destructive methods such as cryotherapy or surgical excision to treat individual AKs.⁷ Examples of field therapy include chemical peels, lasers, dermabrasion, and photodynamic therapy. Treatment with 5-fluorouracil (5-FU) is the most familiar form of field therapy, owing to its ability to unmask occult, microscopic subclinical lesions.⁸

In a recent study conducted by our group, 5-FU 5% cream was administered to young adults who exhibited mild signs of photodamage but without existing AKs or other carcinomas.⁹ Treatment with 5-FU resulted in the development of multiple red, crusted lesions known as flares of subclinical AKs. Subsequent treatment with tretinoin 0.1% cream effectively reduced or eliminated the clinical signs of photodamage. Reapplication of 5-FU did not result in flares of subclinical AKs in a single subject. Biopsies of previously flared sites were negative for any signs of AKs but, more importantly, showed a normal epidermis with acanthosis, normal differentiation, restoration of polarity, and the absence of atypia and parakeratosis.

Imiquimod, a potent stimulator of both the innate and the acquired immune response, is a new entry in the category of field therapy. Imiquimod works by binding to toll-like receptor 7 on the surface of dendritic cells, macrophages, and monocytes and inducing the synthesis and release of proinflammatory cytokines and chemokines, interleukins, interferons, and cytotoxic killer T cells that enhance acquired immunity by establishing a type 1 helper T-cell immune response.¹⁰ Imiquimod 5% cream has received US Food and Drug Administration approval for the treatment of anogenital warts, superficial basal cell cancers, and AKs. The efficacy of imiquimod in eradicating AKs was recently demonstrated in a number of double-blind, randomized, vehicle-controlled studies.^{11,12}

The objective of the current study was to determine the ability of imiquimod to reverse histologic changes and improve the appearance of photoaged facial skin. Furthermore, the ability of imiquimod to unmask flares of subclinical AKs in subjects with moderate photodamage but without visible AKs was also assessed.

METHODS

Subjects

Ten healthy white female subjects with moderate signs of facial photodamage—fine lines, periorbital wrinkles,

dyspigmentations, mild roughness, and laxity—were selected for study participation based on their histories of unprotected sun exposure. None of the subjects had visible AKs, a previous history of skin tumors, or had received medical or surgical treatment for photoaged skin. All cosmetic products were prohibited for 4 weeks before and after the study, with the exception of lipstick and eye makeup.

Study Design and Treatment

This was a single-center, open-label study. The study protocol was approved by an institutional review board, and written informed consent was obtained from subjects before study participation. Subjects were instructed to evenly apply a pea-sized amount of imiquimod to the face, including the eye area, on each of 5 mornings (Monday through Friday) for 4 weeks. The application technique was demonstrated to each subject, and self-applications were monitored for the first 2 days of treatment. After allowing the medication to dry for at least 30 minutes, the subjects were instructed to apply a thin layer of daily facial moisturizer with a sun protection factor of 25.

Assessments of Global Improvement in Skin Appearance by Subjects and a Dermatologist

Poststudy surveys were completed by the subjects and a dermatologist to assess impressions of global improvement in skin appearance after treatment with imiquimod. Visual changes in skin characteristics were rated on a scale of 0 to 3, with 0 representing no improvement, 1 representing slight improvement, 2 representing moderate improvement, and 3 representing great improvement.

The following procedures were performed on 5 subjects who exhibited the greatest degree of photodamage. Except for digital photography, measurements were conducted in an environmental chamber at $70^{\circ} \pm 0.5^{\circ}\text{F}$ with a relative humidity of $40 \pm 5\%$ after an acclimatization period of 20 minutes.

Hydration Assessments—Hydration of a deeper layer of the stratum corneum was assessed by capacitance using a corneometer. Conductance or hygroscopicity, defined as the ability to take up water, was measured using a Skicon-200 device with a 3.5-MHz signal according to the method of Tagami et al.¹³ To assess the ability to retain applied water, measurements were made every 30 seconds for 2 minutes after wiping a water drop off of the skin.¹³

Coloration Assessment—A chromameter was used to quantitatively measure skin color. The measurement was given in the $L^*a^*b^*$ system of colorimetric notation. L^* describes brightness or reflectance from black to white. Fine surface scales, a characteristic feature of

photodamaged facial skin that signifies dryness, scatter and reflect light. Hence, a decreased L* value signifies decreased scaling and increased surface brightness. Similarly, a* describes color from green to red. An increased a* signifies greater redness, an indirect measure of increased blood flow or inflammation. Color from blue to yellow is described by b*.

Histologic Assessments—Full-thickness 3-mm punch biopsies were removed from the medial cheek with the patient under local anesthesia. Each biopsy was fixed in 10% buffered formalin, embedded in paraffin, and processed for histochemistry. Posttreatment biopsies were removed from an area adjacent to pretreatment biopsies. Various staining techniques were used to determine general architecture and very fine cytologic details (hematoxylin and eosin stain), elastin content (Luna stain), glycosaminoglycan content (Hale stain [colloidal iron]), melanin content (Masson-Fontana stain), and collagen content and morphology (Mallory trichrome stain).

Imaging Assessments of Improvements in Skin Appearance

Digital Photography—To visualize surface microtopographic features, a standardized table unit enabled flash photography using 2 heads positioned at a 30° angle to the subject’s cheek. Subsurface features, especially vascularity, were visualized by means of a cross-filtered polarized light using glass linear polarizers rotated to 90°.

Videomicroscopy—A hand-held high-resolution videomicroscope with a half-inch color charge-coupled device was used to record skin images.

Fringe Projection—Microtopographic details such as texture, smoothness, fine lines, and pore size were studied using a fringe projection device, which provides a 3-dimensional image of the skin’s surface.

Safety Measurements

Subjects were examined at baseline and at 1, 2, and 4 weeks regarding adverse reactions such as inflammation, scaling, and subjective sensory discomforts (itching, burning, and stinging). If a subject experienced local skin irritation, treatment was discontinued if necessary.

Statistical Methods

Descriptive statistics were used to summarize subject demographics. Results from quantitative measurements were expressed as means plus or minus SD.

RESULTS

Subject Demographics

Ten healthy white female subjects (median age, 45 years [range, 33–55 years]) were enrolled in this study. According to the Fitzpatrick skin type classification, 3 subjects

(30%) were skin type I, 6 subjects (60%) were skin type II, and 1 subject (10%) was skin type III, signifying that they were lighter-skinned persons with increased susceptibility to photodamage. All 10 subjects completed 4 weeks of imiquimod treatment.

Safety

None of the 10 subjects experienced adverse events on the face such as inflammation, scaling, erythema, or neurosensory discomfort (stinging, burning, and itching). Three subjects experienced mild erosions of the lower lip. Subsequent questioning revealed that each subject suffered from recurrent attacks of cold sores (herpes simplex virus).

Assessments of Global Improvement in Skin Appearance by Subjects and a Dermatologist

The majority of subjects reported a moderate to great improvement in skin appearance (Table 1). Subjects believed that their skin was smoother and more uniform in color and that blotchiness and wrinkles were reduced. However, the dermatologist rated global

TABLE 1
Assessment of Global Improvement After 4 Weeks of Imiquimod Treatment

Patient	Patient Assessment Score*	Dermatologist Assessment Score*
1	2	2
2	2	1
3	3	2
4	2	1
5	3	2
6	1	0
7	3	2
8	2	1
9	2	1
10	3	1
Mean	2.3	1.3

*0 = no improvement; 1 = slight improvement; 2 = moderate improvement; 3 = great improvement.

improvement in skin appearance and improvements in the smoothness of the skin surface lower than the subjects did. Subjects expressed favorable remarks relating to the cosmetic attributes of imiquimod, including ease of application, agreeable fragrance, no residue, and adequate moisturization. Nearly all subjects said they would purchase imiquimod.

Hydration Assessments—Imiquimod therapy had no effect on skin capacitance, indicating that the hydration level below the skin surface was not altered after treatment (Table 2). Although not statistically significant, conductance (hygroscopicity), water-holding capacity, and the mean water-desorption rate constant increased after imiquimod treatment. An increase in each of these parameters is suggestive of a higher water content in the superficial desquamating portion of the stratum corneum. The ability to retain imbibed water made the surface smoother and less palpably dry, features that were affirmed by the subjects.

Coloration Assessments (Colorimetry)—After imiquimod treatment, a small decrease was observed in mean reflectance (L*); this decrease is indicative of reduced scaling and is consistent with self-assessments of a smoother, less dry skin surface (Table 3). Similarly, small increases in the mean a* recording (red-shift) and the mean b* recording (yellow-shift) were evident after imiquimod treatment. Although an increase in mean a* signifies greater redness and is an indirect measure of increased blood flow, the dermatologist noted no signs or symptoms of inflammation. Histology confirmed the lack of any inflammatory changes. Three of the 5 subjects thought that treatment had produced a brighter, rosier complexion.

Histologic Assessments—Hematoxylin and eosin stain indicated that the epidermis was atrophic and displayed marked atypia before treatment (Figure 1A). Numerous enlarged clear cells (melanocytes) were present. After imiquimod treatment, the epidermis was acanthotic, epidermal atypia was completely abolished, differentiation was orderly, and polarity was restored (Figure 1B). Melanocytes, while still numerous, were smaller.

Masson-Fontana stain performed prior to treatment revealed that melanin granules (melanosomes) were abundant and irregularly deposited not only in basilar keratinocytes, but throughout the epidermis, including the stratum corneum. Some segments of the epidermis had dark clusters of hypermelanized keratinocytes even though intervening segments had little or no pigment, thereby giving rise to the mottled appearance of photodamaged skin. After imiquimod treatment, the entire epidermis became uniformly hypopigmented, the quantity of melanosomes decreased in basilar keratinocytes, and melanosomes were not present in the stratum corneum. Decreased mottling was noted in 3 of 5 subjects.

Mallory trichrome stain showed the epidermis was atrophic, keratinocytes were atypical, and there was a virtual lack of subepidermal collagen bundles before treatment. After treatment, the epidermis thickened greatly, and keratinocytes became larger and more uniform. However, there was no evidence of new collagen deposition in the subepidermal dermis.

Based on Luna stain and Hale stain, imiquimod had no effect on elastin morphology; varying degrees of elastosis and an increase in thickened, curled, and branched abnormal elastic fibers were evident before and after treatment. Similarly, the amount of glycosaminoglycans in the papillary dermis of subjects with moderate photodamage

TABLE 2
Epidermal Hydration Before and After 4 Weeks of Imiquimod Treatment

	Mean ± SD	
	Baseline (n=5)	After Treatment (n=5)
Capacitance	61±8	59±9
Conductance	899±148	1011±67
Water-holding capacity	339±221	355±193
Water-desorption rate constant	1.3±0.4	1.5±0.5

TABLE 3
Colorimetry Before and After 4 Weeks of Imiquimod Treatment

	Mean ± SD	
	Baseline (n=5)	After Treatment (n=5)
Reflectance (L*)	63±2	61±4
α-Scale (a*)	12±2	15±3
β-Scale (b*)	12±2	15±2

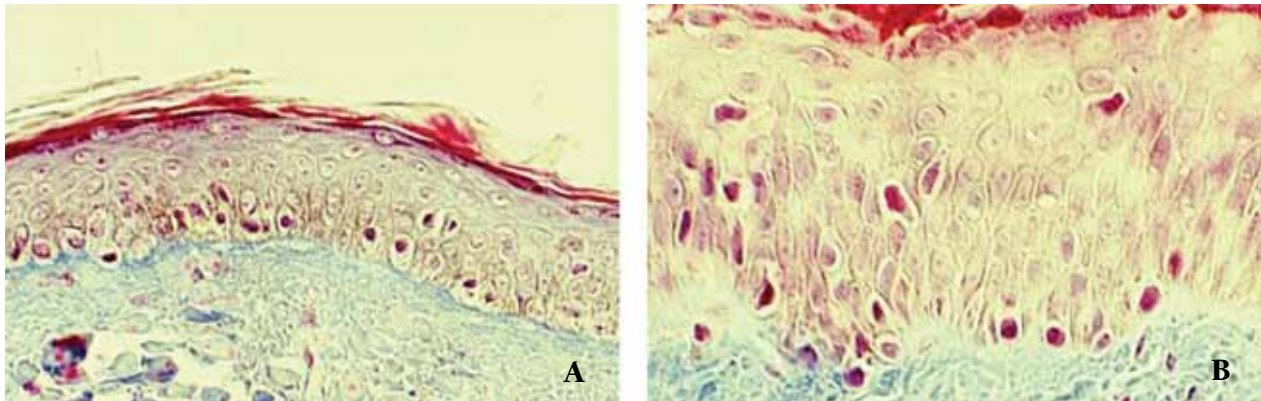


Figure 1. Photodamaged skin biopsies before (A; H&E, original magnification $\times 30$) and after (B; H&E, original magnification $\times 30$) 4 weeks of treatment with imiquimod 5% cream. After treatment, epidermal atrophy and atypia were eliminated.

remained unchanged after imiquimod treatment. Inflammatory cells and vascular changes were absent after imiquimod treatment. Therefore, the corrective effects of imiquimod were entirely confined to the epidermis, and no changes were observed in the dermal matrix.

Imaging Assessments of Improvements in Skin Appearance

As documented using digital photography, numerous periorbital wrinkles (crow's feet) were evident in the eye region and on the cheeks of untreated subjects (Figure 2A). Hyperkeratotic follicular pores (small solar comedones) were prominent, the skin surface was rough, and the texture was uneven. After imiquimod treatment, periorbital wrinkles were effaced, there was a

gross reduction in hyperkeratotic pores, the skin surface became smoother, and the texture improved (Figure 2B). Fringe projection analysis confirmed the imiquimod-mediated reduction in hyperkeratotic pores and fine lines (Figure 3). Imiquimod treatment also resulted in a decrease in enlarged pores caused by the accumulation of horny material in sebaceous follicles (Figure 4). Elimination of the horny impactions was confirmed visually by subjects. An undesirable shiny gloss, produced by greater reflectance from slightly dry, rough skin, was also eliminated after imiquimod treatment.

Assessment of Subclinical AKs

None of the subjects experienced flares of subclinical AKs after imiquimod treatment.

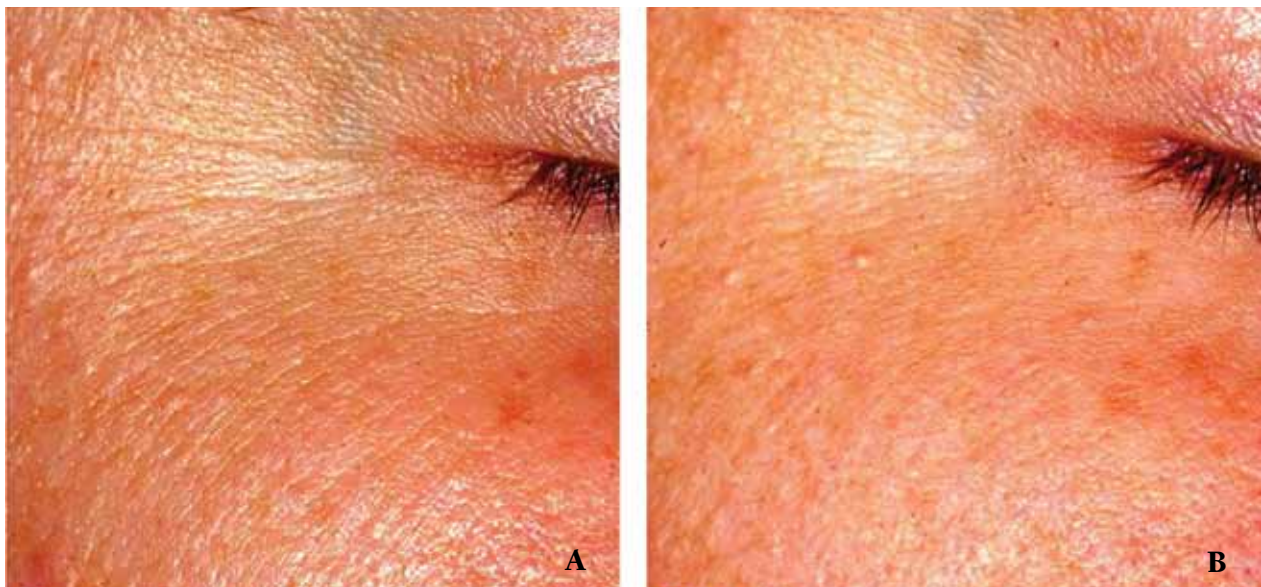


Figure 2. Digital photographs illustrate photodamaged skin before (A) and after (B) 4 weeks of treatment with imiquimod 5% cream. Treatment resulted in effacement of periorbital wrinkles, reduction in the number of hyperkeratotic pores, and improvement in the smoothness and texture of the skin.

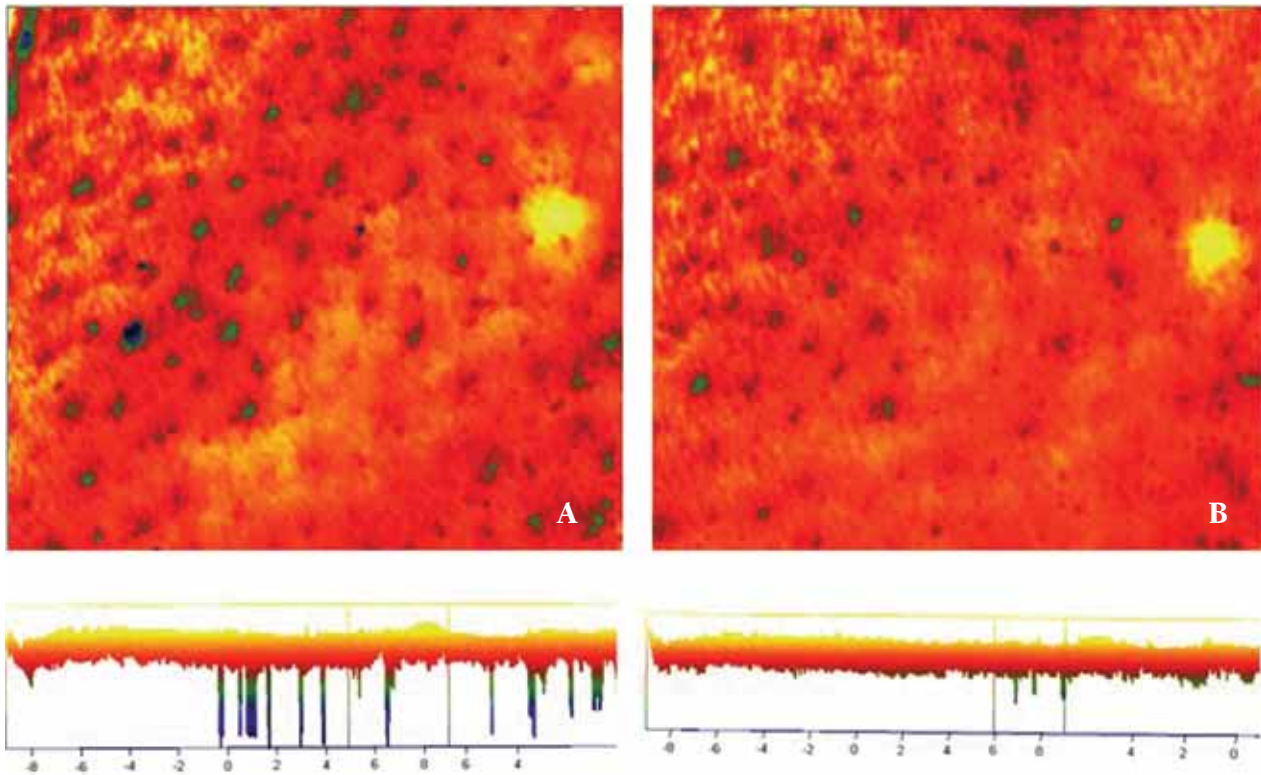


Figure 3. Fringe projection analysis illustrates the improvement in the appearance of photodamaged skin induced by imiquimod 5% cream. Before treatment (A; original magnification $\times 30$), reddened bands corresponding to valleys or depressions and numerous prominent bluish spots representing hyperkeratotic pores (solar comedones) are evident. The intervening yellowed areas are plateaus in which hyperkeratotic pores are fewer and smaller. After treatment (B; original magnification $\times 30$), the surface is more even, and fewer hyperkeratotic pores are visible. The figures below the images are cross-sectional views that, after treatment, show far fewer, shallower downward projections, reflecting the elimination of hyperkeratotic pores.

COMMENT

This study demonstrated that imiquimod treatment completely corrected epidermal atrophic dysplasia in subjects with moderate facial photodamage. The thickness of the epidermis increased (acanthosis), atypia of keratinocytes was eliminated, differentiation was orderly, and polarity was restored after imiquimod treatment. Collagen deposition, elastosis, and glycosaminoglycan content were not affected, indicating that the effects of imiquimod were confined to the epidermis. Improvements in epidermal dysplasia were accompanied by a marked improvement in the physical appearance of photodamaged skin; the skin was smoother and less dry, fine lines and wrinkles were effaced, and the complexion was brighter and less mottled. Each of the improvements in skin appearance occurred as a result of increased hydration of the epidermal layer and removal of the dry, scaly skin surface.

Subjects in this study were satisfied with the improvement in the appearance of their skin. Indeed, subject ratings of improvement in skin appearance were generally higher than those reported by the dermatologist. This

discrepancy is a common finding in studies that examine the efficacy of skin care products. Subjects may perceive subtle changes not visible to the observer and may also be unduly influenced by pleasant cosmetic attributes. Subjects expressed favorable opinions in relation to the cosmetic attributes of imiquimod, and nearly all subjects said that they would purchase imiquimod, an important finding in studies of antiaging cosmetic products. Skin products that can be shown to have appreciable antiaging effects may be rejected by patients if they have disagreeable cosmetic attributes, especially greasiness and perceptible residues.

A previous study revealed that punch biopsies of uninvolved skin of patients with advanced photodamage and visible AKs on the face contained microscopic subclinical foci, which showed the typical hallmarks of incipient AK and flared from the application of 5-FU.⁹ However, the current study demonstrated that the application of imiquimod to patients with moderate photodamage and no visible AK lesions did not result in flares of subclinical AKs, possibly because these subjects had not progressed sufficiently through the series of changes that result in the development of cutaneous tumors.



Figure 4. Videomicroscopic imaging illustrates skin texture before (A) and after (B) 4 weeks of treatment with imiquimod 5% cream. The shiny gloss on the skin surface disappeared, and enlarged pores were virtually eliminated.

A number of recent studies have demonstrated that imiquimod was highly effective in eradicating AKs and in situ lentigo maligna.^{11,12,14} Imiquimod treatment of immunosuppressed renal transplant recipients resulted in a complete reversal of epidermal dysplasia.¹⁵ Reversal of dysplastic epithelial changes exerted a prophylactic effect on the development of new squamous cell tumors. Coincidentally, an additional benefit of treatment was an improvement in skin quality. The skin surface became smoother, less pigmented, and less dry, and had greatly improved texture. These histologic and clinical changes were similar to those reported in the current study of healthy women with photoaged skin. Improvement in cosmetic outcomes may be related to the known ability of imiquimod to promote wound healing and tissue remodeling.¹⁶ Although cosmesis is certainly not the primary goal of treatment of the photoaged face with imiquimod, it is an added benefit that should be mentioned to patients, at the very least, to promote adherence.

Treatment of the photoaged face not only provides a cosmetic benefit, but represents a strategy to prevent cutaneous nonmelanoma carcinomas, whose prevalence has now reached epidemic proportions in the United States, with more than 1 million new cases per year.¹⁷ Until recently, AKs were classified as premalignant lesions; however, AKs have already undergone the earliest changes in a continuous spectrum of progressive transformations that culminate in the development of squamous cell carcinomas. Treating AKs and skin cancers after they have appeared represents prevention failure. Dermatologists should take a leading role in warning patients with facial photodamage that these changes warrant initiation of a program to prevent cutaneous tumors. This counseling applies

particularly to individuals with Fitzpatrick skin type I. These are generally light-skinned persons of Celtic (Irish, Scottish, Welsh, Breton) ancestry whose relative risk for developing skin cancers is high.¹⁸

Imiquimod was safe and well tolerated in this study. Subjects did not experience even mild local inflammatory reactions or subjective symptoms such as itching, burning, or stinging. Although previous studies reported that local adverse effects such as ulcerations, weeping, scabbing, scaling, and erythema may occur in imiquimod-treated patients with AK, treatment was well tolerated, even in patients with the most severe reactions, and local adverse events ceased after stopping treatment.^{11,12} Treatment of immunosuppressed renal transplant recipients with imiquimod resulted in the development of severe skin reactions such as soreness, exudation, and ulceration; however, these patients experienced notable improvements in skin atypia.¹⁵ The skin surface became smoother, less dry, and more uniformly pigmented. The improvements in skin atypia noted in immunosuppressed patients presaged the improvements in the appearance of photoaged facial skin in healthy subjects in the current study.

This pilot study demonstrates that topical imiquimod 5% cream reverses the histologic changes and improves the appearance of photoaged facial skin. An additional study, involving a 3-month treatment period, is currently being undertaken with the expectation that imiquimod will provide enhanced clinical and histologic benefits in the treatment of photoaged skin.

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